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Research

Comparative assessment of induced mutants from *Solanum macrocarpon* L. (Solanaceae)

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Abstract

The mutation effects of different concentrations of colchicine (0.1 to 1.0%) on the morphology and cytology of Solanum macrocarpon were investigated. The four established mutant lines (M1, M2, M3 & M4) showed consistent variability from the natural species. Delayed germination (3-6 days) and death of embryos were observed in several treated seeds. Some radicles exhibited tumourous swellings and altered geotropic response by forming loops or rings at the tip. Cotyledons were two in M3 and M4 as in the natural species but three in M1 and M2. Leaves of mutants were generally smaller and slightly folding while the shoots were procumbent and prostrate (M1 & M2), with short and sparse internodes. Epidermal cells were irregular in shape in M3 while stomata with one guard cell were common in M1 and M4. Flowering was inhibited in M2 and fertility lowered in M1, M3 and M4. Fruits were smaller and contained fewer seeds in the mutant plants. The M1, M3 and M4 were regular diploids (2n =24) like the natural species while chromosomes doubled in the M2 producing an autotetraploid (2n = 48) with relatively stable genome.

Keywords: Mutagenesis, mutants, *Solanum macrocarpon*, autotetraploid, colchicine

Introduction

Solanum macrocarpon L. is widely cultivated in Nigeria and in other tropical regions of the world as an important vegetable and a good source of dietary vitamins (Gbile, 1985). It could also be mixed or eaten along with other vegetables to further enrich its nutritional quality (Omidiji, 1983). The species is a woody, annual and erect shrub with glabrous simple leaves. The leaves are pinnately veined, deeply lobed and obovate to oblanceolate in shape. Inflorescence is simple umbellate to subumbellate, usually subtending two to four bisexual, purple flowers. Mature fruits are in clusters of two to four on inflorescence (Gbile, 1979). This species is easily cultivated and grows as volunteer plant in gardens, roadsides and abandoned lands.

Several attempts have been made at improving the quality of the species and similar vegetables by harnessing their genetic potentials to develop resistant, better adapted and early maturing varieties of high nutritional value (Okoli, 1988; Opeke, 1962). However, artificial hybridization with related species were not very successful as many of the hybrids were either not fully fertile to ensure ready propagation or of poor quality (Ugborogho & Oyelana, 1999; Omidiji, 1983). Ugborogho and Oyelana (1999) had implicated structural chromosomal mutations for this situation. Consequently, alternative breeding techniques became inevitable in order to surmount myriads of unsuccessful breeding attempts. Crop breeding and improvement programmes were predicated on the evolution of genetic variability, either through slow natural process or induced mutagenesis (Muggleston, 1995). As a tool, mutagenesis has led to the production of a number of chemically engineered crops with high nutrient content (Barro, *et al*, 2001), unique floral and morphological adaptation to enhance pollination (Luckett, 1989), genomic and morphological variatio-

ns (Hofmann *et al*, 2004).

Induced mutagenesis equally poses a number of attendant problems. The lethal effects of gamma rays and ethyl methanesulfonate on embryos in seeds resulted in embryo death and delayed germination (Hofmann *et al*, 2004). Other observations include late flowering mutants in soybeans (Khali *et al,* 1986), dwarf mutants in wheat (Hassan, 1987) and delayed maturity (Khan *et al*, 1990). These were some of the visible evidences of the negative and unpredictable effects of mutagens in crop breeding. However, the major problem and concern is in the mode of action of these mutagens, which may create mutants and phenotypes that are usually transient and not transferable (Hoebe & Beutler, 2005).

Despite these few setbacks, mutagenesis had created a number of novel cases of positive hereditary changes, and which have formed the basis of a number of crop improvement programmes (Ugborogho & Sodipo, 1985; Pena & Sguin, 2001; Hughes *et al*., 2001 & Baro *et al*., 2001), providing viable alternatives to conventional breeding methods. Its usage will further be enhanced when a regime of predictable protocol is developed for the existing and well-known mutagens for precise application and optimal benefits.

The objective of this study is to establish the critical concentration(s) that will yield useful mutants and, compare features of the mutants and the natural species. Consequently, seeds were exposed to different concentrations of colchicine (0.1-1.0%), the morphological and cytological features of seedlings and mature plants scored, and the growth phenomena examined.

Material and methods

Two hundred (200) seeds of *S. macrocarpon* were presoaked in deionised water for 24hours to activate the embryos. Emission of bubbles from hilum of seed was an indication of resumption of normal metabolic activities and hence viability (Wogu & Ugborogho, 2000). Subsequently, twenty viable seeds were transferred into different concentrations of colchicine (0.1-1.0%) and soaked for 24 and 36hours. At the end of each treatment, seeds were rinsed in distilled water and placed in between folds of sterilized cotton wool in Petri dishes and intermittently moistened with deionised water. For the control experiment, twenty seeds were soaked in deionised water for 24 hours. The germinated seeds were allowed 2-3 weeks to sufficiently root in planting trays for seedlings to establish stronger adaptation needed for field performance. Seeds were considered to have germinated when the integuments were ruptured by the radicle (Ibikunle & Komolafe, 1973; Wogu & Ugborogho, 2000). Seedlings were later screened for morphological and cytological variants and the successful mutants were subsequently transferred into planting pots and cultivated alongside the control (natural species) in the field for a period of six months.

Morphometric analyses on mutants were carried out with a meter rule while details of the floral features were assessed with a hand lens and stereomicroscope. Epidermal peels taken from the central portions of the mature leaves of both natural species and mutants were mounted on glass slides in 50% glycerine and allowed to stand at room temperature for 24 hours to enhance full turgidity of both epidermal cells and stomata. The length and breadth of stomata were measured with the aid of an eyepiece graticule. The techniques of Ugborogho and Oyelana (1992) were employed for both pollen and meiotic chromosome studies while the mitotic chromosome study followed Ogunwenmo (1999). Twenty counts were made to ascertain the ploidy level of each mutant.

Results

Germination

Germination was 80% in the control. Seeds' testae ruptured from the third day of soaking, and 16 seeds germinated by the fifth day. However, germination and radicle growth were low and retarded respectively in the different concentrations of colchicine treated seeds. Table 1 shows the percentages and distribution of germination in both control and treted seeds. Germination was low (<50%) in concentrations above 0.5% in both 24 and 36h treatments. The earliest germination occurred two days after the control seeds germinated. In lower concentrations (0.1-0.4%), germination rates were similar to those of the control. Concentrations above 0.8% were lethal at 24 and 36h treatments as all the embryos died.

Concentrations above 0.5% had profound negative effects on the emerging radicles. The radicles of 24 from 400 seeds showed remarkable aberrations during growth. Tumourous swellings characterized few radicles from seeds in 0.7% (24hours) and 0.6% (36h) treatments (Fig. 1a-c). The tips of few radicles rolled into rings, coiling round the testa in two of the cases (Fig. 1d-e). These occurred in seeds in 0.5% (36hours) treatment. Interestingly, few of these aberrations were overcome and radicles resumed normal growth few hours before the testa split to release the cotyledons. This occurrence was observed among the seeds in 0.5%-0.8% at 24h. Radicles from the only two germinated seeds in 0.7% 36h treatment ceased growth, decayed and subsequently dropped off the seeds.

Table 1: Percentage germination of control and treated seeds of Solanum macrocarpon

Treatment		Germination in days									Total	%	Remark
Duration	Colchicine %	3	4	5	6	7	8	9	10	11	Germination		
	0.1	3	6	10	14	16	0	0	0	0	16	80	
	0.2	3	5	8	11	14	0	0	0	0	14	70	
	0.3	4	7	10	12	14	0	0	0	0	14	70	
	0.4	2	2	5	5	7	10	11	0	0	11	55	
24h	0.5	0	0	3	5	5	6	7	9	0	9	45	*
	0.6	0	0	2	3	3	5	5	7	0	7	35	*
	0.7	0	0	0	0	2	2	3	5	6	6	30	*
	0.8	0	0	0	0	1	2	2	2	3	3	15	*
	0.9	0	0	0	0	0	0	0	0	0	0	0	**
	1.0	0	0	0	0	0	0	0	0	0	0	0	**
	0.1	2	5	7	10	13	14	0	0	0	14	70	
	0.2	2	5	8	9	11	11	0	0	0	11	55	
	0.3	3	5	8	10	10	11	0	0	0	11	55	*
	0.4	1	3	5	8	8	9	0	0	0	9	45	*
36h	0.5	0	0	0	2	2	4	5	5	7	7	35	*
	0.6	0	0	0	0	1	2	4	4	6	6	30	***
	0.7	0	0	0	0	1	1	1	2	2	2	10	****
	0.8	0	0	0	0	0	0	0	0	0	0	0	**
	0.9	0	0	0	0	0	0	0	0	0	0	0	**
	1.0	0	0	0	0	0	0	0	0	0	0	0	**
Control (water)		6	11	16	16	16	16	16	16	16	16	80	

*Radicles that overcame aberrations and later resumed normal growth ***Radicles matured into roots of mutant seedlings
****Radicles later stopped growth, decayed and dropped off seed **Zero germination

Table 2: Cytomorphological characteristics of natural species and mutants from Solanum macrocarpon

Character	Natural species	Induced Mutants			
		MI	M2	M3	M4
Habit	Erect with	Procumbent	Procumbent	Shrub-like	Shrub-like
Internode	Long	Short	Short	Short	Short
Leaf	Broad	Small	Broad	Small	Small
L x B (cm)	27.2 x 15.3	12.1 x 7.6	19.7 x10.2	14. 1 x 10.7	17.3 x 11.6
Petiole L (cm)	5. 5 ± 2.4	3.6 ±1.3	3.9 ± 3.4	4.5 ± 3.2	3.9 ± 1.8
Petal L x B (mm)	18.3 x 8.4	11.7 x 7. 2	_	10.5 x 7. 7	15.7 x 7.4
Fruit Diameter (mm)	46.0 ± 1.4	28.2 ± 3.3	_	3.3 ± 4.2	3.1 ± 3.6
No of seeds/fruit	107 ± 14.8	58.0 ± 2.3	_	67.0 ± 8.9	78.0 ± 9.1
Pollen Diameter (µm)	34.2 ± 5.1	31.8 ± 3.2	_	36.1 ± 2.5	35.7 ± 4.7
Pollen fertility (%)	97.3	45.8	_	58.2	54.4
Adaxial Stomatal L x B (µm)	41.0 x 28.1	32.2 x 24.7	35.2 x 23.6	38.2 x 34.3	36.3 x 28.1
Abaxial Stomata L x B (µm)	38.9 x 26.7	34.3 x 25.8	33.2 x 22.8	37.8 x 29.5	34.9 x 26.4

- none

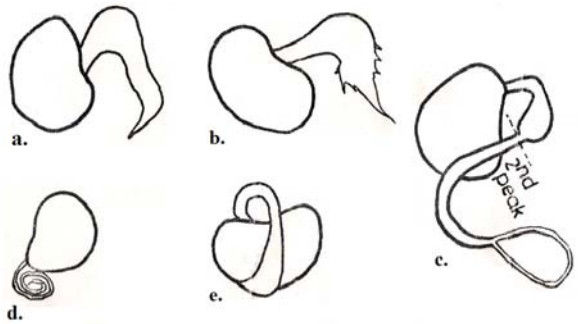

Fig. 1: Radicle growth aberration of colchicine-treated seeds of Solanum macrocarpon a-c. tumorous swellings, d. coil and e. twist formation

Morphometric Analyses of the mutants

Morphometric analysis of matured seedlings from radicles that eventually resumed normal growth did not express any phenotypic change from the natural species. However, four of the six seedlings that emerged from seeds in 0.6% 36h treatment consistently showed distinct morphological and cytological variations from the natural species. The morphometric data are presented in Table 2. The mutants were designated as M1, M2, M3 and M4 (Fig. 2a-d). The M1 and M2 produced three cotyledons (Fig. 3a), a deviation from the normal two Fig. 3b). Growth of the main axes and foliages were retarded in the mutants with slightly folding and smaller leaves. The M1 and M2 were procumbent to pros-

Fig. 2: Mutants and control of Solanum macrocarpon a. M1, b. M2, c. M3 d. M4, e. control/natural sp., Scale bar: a-d = 5cm; e = 8cm

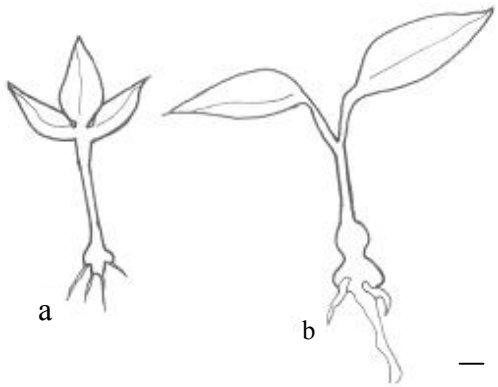

Fig. 3: Cotyledons (pleiocotyl) of mutants of Solanum macrocarpon a. tricotyl, b. dicotyl, Scale bar=2mm

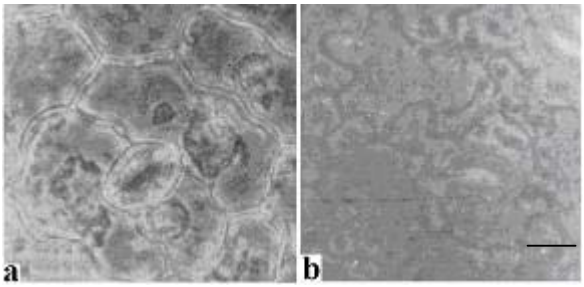

Fig. 4: Leaf epidermis and stomata of Solanum macrocarpon a. control – isodiametric cells, b. M3 – irregular cells, Scale bar=20µm

trate (Fig. 2a & b). The internodes in the M3 and M4 were short, fewer and with few emerging branches,

hence, their shrub-like appearance (Fig. 2c & d). The leaf epidermal cells were smaller in the mutants and extensively irregular in shape in M1 and M4 (Fig. 4a-b). Stomata were anomocytic as in natural species and were characterized by one or no guard cells in M1 and M3.

The M2 did not flower throughout the duration of the study. Fertility was lowered in M1, M3 and M4. Fruits were smaller and with fewer seeds.

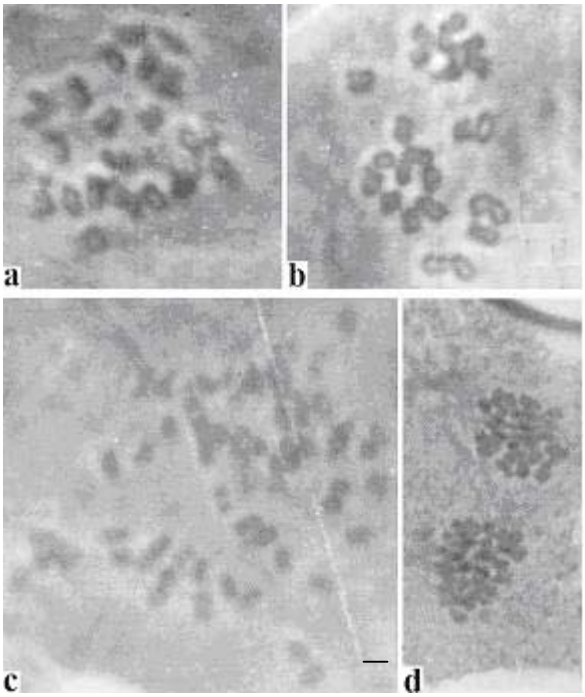

Fig. 5: Mitotic and meiotic chromosomes of Solanum macrocarpon a. control 2n=24, b. M3 2n=24, c. M2 2n=48, d. M2 anaphase n=24, Scale bar: a-c=3µm

Cytological Analysis

The M1, M3 and M4 possessed diploid chromosomes (2n = 24, Fig. 5b) as in natural species though few aneuploid cells (2n = 28, 32) were encountered in M3. The M2 was transformed into a tetraploid (2n = 48, Fig. 5c). Meiosis was generally irregular in all the mutants. Configurations revealed clumps and multivalencies while chromosome bridges were prevalent in M3 (Fig. 6a-d).

Discussion

The differential germination response of seeds in the various concentrations of colchicine was evident. The percentage and rate of germination, and overall growth pattern of radicles and shoots differ between the control and mutants. The length of soaking in colchicine had profound structural manifestation on the emerging radicles than dosage. This correlated significantly with the number and patterns of aberrations observed on the radicles. Evidently, the degree and extent of tumourous swellings, coiling and twisting of radicles were profound in seeds from concentrations above 0.5% at 36h treatment. In the same vein radicles of seeds from much lower concentrations but same time duration (36h) had almost similar aberrations and occurrences.

The potency and negative metabolic effects

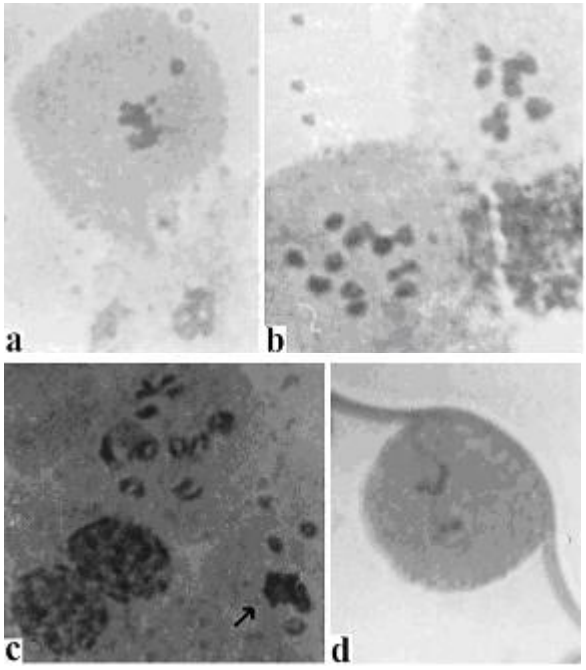

Fig. 6: Meiotic chromosomes of Solanum macrocarpon a. M3 clump and isolated chromosome, b. control bivalents n=12, c. M1, multivalent (arrow), 2 isolated univalent and a bivalent, d. M3 anaphase bridge

of colchicine on the developing embryos reflected significantly on seeds at 36h soaking duration. Several embryos died within the seeds before the testa ruptured. Hofmann *et al* (2004) observed similar correlation between high concentration of mutagen and embryo lethality. They discovered that the mean survival rate of embryogenic culture decreased from 74% (1mM Ethyl methanesulfonate (EMS) to 43% (30mM EMS) treatment.

The time between the rupturing of seeds' testae and initiation of cellular activities by embryos was about 48h after presoaking. Therefore, the 36h soaking duration seems appropriate for optimal random effects of inducing specific or point mutation in the developing embryo. Thus, the 36h duration allowed for appropriate action of colchicine on specific sites of chromosomes. The consistent low ge-

rmination rate and percentage of seeds in higher concentrations of colchicine suggest a significant interference with specific enzyme system essential for normal embryonic growth. Luckett, (1989) attributed the unequal and complete ceassation of growth in the apical meristem in seedlings of cotton treated with 1-5% colchicine to the malfunctioning of specific enzymes responsible for cell division at this region. Alteration of complex enzyme system through mutagenesis had led to altered metabolic processes, giving rise to unique phenotypes. This technique had been employed to manipulate combinations of group of enzymes or evolve new enzyme complex for specific purposes. Velasco *et al.* (2004) succeeded in creating a profile of variations in quantity and quality of α tocophenol in seeds of sunflower through induced mutagenesis. Specific alterations of the biochemical functions of genes and group of gene products had also been discussed by Ostergaard and Yanofsky (2004). Conklin *et al.* (1999) described weak point mutant alleles of VTC 1 generated by ethyl methanesulfonate (EMS) mutagenesis which resulted in reduced vitamin C levels in *Arabidopsis.*

There was ceassation of radicle growth in higher concentrations of colchicine, and few radicles decayed and dropped few days after emergence while some radicles resumed normal growth, having overcome the initial shock. The interaction between seeds' genotypes (composite genes) and medium would have led to resumption of growth in these radicles. The genotype through constant genetic recombination may have provided the platform for the synthesis of required cellular substances for the repairs of specific enzyme pathways. Torrey (1961) affirmed the basic importance of genomes in the response of roots to various chemical mutagens. Similarly, Barro *et al.* (2001) highlighted the multicellular and heterogeinity nature of seeds and their unlimited potential to produce tissues that can express a mixture of mutated and non-mutated (chimera) cells after mutagenesis. For these radicles, the non-mutated cells may have proliferated new cells and tissues for normal growth resumption.

Morphometric analysis of the mutants showed a significant difference in a number of features from the natural species. The leaves were smaller and slightly folding, dimensions of the floral whorls and sizes of fruits were also smaller. Growth of the main axes and foliages were not as vigorous as in natural species. The M3 and M4 appeared shrub-like with short and fewer internodes. Similar observations by Yagoob and Rashid (2001) showed a significant reduction in height and number of branches in mutants after exposure to high doses of gamma rays. Velasco *et al.* (2000) identified a dwarf Safflower genetic stock described as 'Enana' developed from the Spanish cultivar 'Rancho' through chemical mutagenesis. The dwarf safflower (*Carthamus tinctorius* L.) mutant 'Enana' was c. 60cm tall while the pure cultivar 'Racho' was c. 100cm tall.

The M1 and M2 were both procumbent to prostrate. The change in growth habit may have been due to abnormal branching of the main axes. A spontaneous maize mutant that was characterized by irregular branching of the inflorescence was reported by Irish (1997) to explain the unpredictability of mutated genes. The M2 did not flower throughout the duration of study. It was presumed sterile as flowering normally takes 2 - 3months. Delayed flowering and irregularity in the length and duration of flowering had been observed in cultivars of mungbean exposed to high doses of gamma rays (Rashid, 2001).

The ceassation of spindle fibres due to colchicine treatment may have contributed to the different degrees of abnormalities observed in mitosis. Changes in configuration of chromosomes were evident in all the mutants. Chromosome number was doubled in the M2 while the other mutants expressed different degrees of aneuploidy. Colchicine had been found potent in inducing polyploids and creating high level of genetic variability among species (Muggleston, 1995; Lukett, 1989). Some colchicine-induced-tetraploids have earlier been reported by Ramesh (1983) and, Ugborogho and Sodipo (1985). However, the M2 induced-autotetraploid did not express any gigas features contrary to the suppositions of Seetharami-Reddi and Reddi (1985). The configurations of chromosomes at meiosis in the mutants equally affirmed the homogeinity of the natural species. Meiotic configurations showed clumpiness, multivalency and chromosome bridges in the mutants.

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