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Original article

ASSESSMENT OF GENO CYTOTOXICITY EFFECTS OF DIETHYLSULPHATE ON IRISH POTATO (*Solanum tuberosum* L.)

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ABSTRACT

The study was designed to assess the effects of diethylsulphate on Geno cytotoxicity in *Solanum tuberosum* L. For this purpose, 108 healthy tubers from three varieties were obtained from Potato Research Programme, Kuru-Jos-Nigeria. These tubers were treated with six concentrations (0.0, 0.1, 0.3, 0.5, 0.7 and 0.9%) of the diethylsulphate for 3h and planted on the field at Botanical garden in randomized complete block design (RCBD). The Amplified Fragment Length Polymorphism (AFLP) technique was used to assess the effects of diethylsulphate on DNA polymorphisms and cytological analysis was carried out. Stickiness was 8.54% in RC7716-2 treated with 0.9% diethylsulphate. Mitotic index was lowest (3.73%) in Nicola treated with 0.9% diethylsulphate. A total of 93 DNA fragments, 55 polymorphic bands and 38 monomorphic bands were generated with the six different concentrations of diethylsulphate with three primers. Primer E32-M49 generated the highest percentage of polymorphic bands (64.71%). The dendrogram consists of three clusters. The control C0 was clustered in clade one. C1, C2, C3 and C4 were clustered in clade two. C5 was cladded in clade in three. The reduction in mitotic index, chromosomal aberrations and induction of DNA polymorphism is an indication that diethylsulphate has genocytotoxic effects on *S. tuberosum.* Concentrations of 0.1, 0.3 and 0.5% diethylsulphate is recommended for mutation induction for genetic variability in *S. tuberosum* L.

Key words: Diethylsulphate, *S. tuberosum*, mitotic index, AFLP, DNA polymorphism.

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INTRODUCTION

Irish Potato (*S. tuberosum* L.) belongs to the large and diverse genus *Solanum* of the family Solanaceae. Solanaceae is one of the largest and economically most important families of angiosperms. Besides *S.tuberosum*, other economically important species of the family are tomato (*Solamum lycopersicon*), egg-plant or aubergine (*Solanum melongena*), the garden peppers (*Capsicum annuum*), tobacco (*Nicotiana tabacum*) and pepino (*Solanum muricatum*). A number of ornamentals, petunia (*Petunia* hybrid) belong to the Solanaceae[1].

Mutation breeding is one of the conventional breeding methods in plant breeding [2], at genic level, when mutation causes alterations on gene on a chromosome, it is called as point mutation [3]. These alterations have greater relevance for raising superior plant types in different crops. Most of the mutations are lethal or semi-lethal and do not have any practical value possibly due to doses or concentrations used or choice of mutagens ([4]; [5]). To carry out a successful mutagenesis, selection of effective mutagens and their treatment doses are pre-requisite conditions. Apart from factors that relates to mutagenic administration, genetic variability plays an important role because it provides a fulcrum for effective and better selection which can be obtained using mutation, hybridization, recombination and selection processes ([35]; [6]). The application of molecular markers for the estimation of the variability of plant varieties and species is helpful in both detection of genetic relationships between them and making a system of plant genera [7]. Cytological analysis with respect to either mitotic or meiotic behaviour is considered one of the most dependable indexes to estimate the potency of mutagens. Investigations on mitotic aberrations and their genetic consequences form an integral part of most mutation studies [8]. *S. tuberosum* (2n =4x=48, polyploid) is one of the most important crops worldwide ranking 4th in terms of total world production behind wheat, maize and rice[9]. *S. tuberosum* is propagated by tubers and it is an exotic species with narrow genetic base as a result of reproductive isolation ([10]; [11]). Therefore, there is a need for its further improvement, which can be carry out by creating additional genetic variabilities in its genome through mutagenesis [12]. Chemical mutagens provide a good choice for selection as a tool for mutation [13]. Diethylsulphate (DES) is a highly toxic and carcinogenic chemical with formula $(C_2H_5)_2SO_4$) [14]. It occurs as a colorless liquid with a peppermint odor [14]. DES is an alkylating agents and its ability to react with DNA makes DES a good mutagen in mutation breeding programs to improve the vital characters of the floricultural crops ([15]; [16]). Genotoxicity is generally induced by DES which interferes with DNA replication particularly in heterochromatin region of chromosome [17]. The chromosomal aberrations is consider as indicators of clastogenic effects of the DES [17]. DES has clastogenic effects on plants via reactive oxygen-derived radicals ([18]; [19]). Diethylsulphate in combination with sodium azide to improve *Vicia faba* var. major [35], *Capsicum annuum* L. [15]. Mutants created by mutagenesis will become genetic resources for future crops improvement [20].

Mutation induction brings about alterations in the genome to create variability of characters. Therefore, this work was undertaken to study the diethylsulphate (DES) induced genocytotoxicity in *S. tuberosum* and to assess the role of DES as an agent for creation of additional genetic variability for crop improvement.

MATERIALS AND METHODS

Healthy tubers (108) of *S. tuberosum* from three varieties (Nicola, BR 63-18 and RC 7716-2) were obtained from the Potato Research Programme, Kuru-Jos-Nigeria. Six concentrations of diethylsulphate (0.0, 0.1, 0.3, 0.5, 0.7 and 0.9 % as w/v) were prepared according to [5] and used for the mutagenic inductions. 108 healthy tubers from three varieties were taken and washed in running tap water for 10 minutes and 18 tubers per concentrations of DES for 3hr at room temperature 28±2°C with intermittent shaking to provide uniform treatment to the dipped tubers. At the end of the treatment time, the tubers were thoroughly washed in the running tap water for 30 minutes to remove the residual mutagens. The treated tubers were put in a plastic container and taken to Botanical garden of Ahmadu Bello University, Zaria (lat. 11º 9^{i} N, long. 7º 42^{i} E and altitude 660m above sea level) and kept for three days for acclimatization. The treated tubers were planted on the field at the garden in complete randomized block design (CRBD) with six replications during 2013 experimental season for 6months. Changes in molecular and cytological parameters were investigated as affected by DES.

Genomic DNA extraction

Extraction and purification of DNA was done using ZR Plant/Seed DNA MiniPrep™ following the manufacturer's instructions. The quality and quantity of DNA were measured by comparison of band-intensity on ethidium bromide stained agarose gels with a standard DNA molecular weight of 1000bp and by the absorbance at 260nm and 280nm using Nano-Drop Spectrophotometer (Model ND1000).

Amplifies Fragment Length Polymorphism

Amplified fragment length polymorphism (AFLP) procedure (AFLP-PCR) reactions were conducted using three primer combinations E32-M49, E35-M49 and E35-M48. The AFLP analysis was conducted as described by [21] at IITA Bioscience Laboratories, Ibadan, Nigeria. The amplified fragments were detected by silver staining method as described by [22]. The resulting gels were scored manually.

Cytological studies

Root tips were harvested from 72 genotypes of the sixty (60) treated samples and 12 from control of three varieties and fixed in 3:1 Absolute ethanol and glacial acetic acid for 24h and washed and preserved in 70% alcohol [23]. The root tips were hydrolyzed for 10min in normal hydrochloric acid solution (1NHcl) at 60°C. The root tips were smeared in 2% aceto-orcein [23] and temporary slides were prepared and examined under microscope for mitotic stages. Mitotic stages where seen were identified, both normal and abnormal stages were counted and photographed at x400 and

x1000 magnifications using Sony DSCW800 digital camera.

Data analyses

The experiment was arranged in a randomized complete block design with six replications of 108 tubers for each chemical mutagen. Data collected were analyzed using Image Studio Lite software from Li- Cor Inc. version 5.2., Power maker Version 3.25 Statgen.ncsu.edu, PopGene Version 1.32 [24]. Statistical analysis was performed using analysis of variance (ANOVA) RCBD format, Duncan's Multiple Range Test (DMRT) was used to separate the means using software from Statistical Analysis System Institute Inc.2012.

RESULTS

The AFLP process was successful and DNA banding patterns on polyacrylamide gel on E32- M49 and E35- M49 (Plate 1).

DNA Polymorphism

Total number of polymorphic loci per *S. tuberosum* genotype for three primer combinations ranges from 0.0 to 25.0 polymorphic loci. Total number of loci for three primers (TL) was 93 loci, total number of polymorphic loci for three primer combinations were 57 and Percentage polymorphic loci (PPL) was 57.58% (Tables 1 and 2).

Phenogram

Phenogram for relationship between control and treated *S. tuberosum* genotypes consists of three clusters. The control C0 in clade one (1). Clade two (2) hosted C1, C2, C3 and C4, while C5 alone in clade three (3) (Figure 1).

Mitotic analysis

Normal mitosis was observed in control *S. tuberosum* genotypes. In the treated *S. tuberosum* genotypes, five different types of chromosomal aberrations were observed with the following percentage occurrence at concentration of 0.9% Diethylsulphate. Sticky chromosomes 9.88% in RC7716-2, 7.00% in BR63-18 and 6.91% in Nicola, Fragmentation 3.97% in BR63-18, 3.96% in RC7716-2 and 2.96% in Nicola. Bridge formation 3.93% in BR63-18, 3.09% in Nicola and 2.94% in RC7716-2. Ox-Bow chromosomes 6.72% in RC7716-2, 2.95% in Nicola and 2.78% in BR63-18. Cyclic chromosomes 6.87% in RC7716-2, 2.96%in BR63-18 and 2.41% in Nicola (Tables 3, 4, 5 and Plate 2)

Mitotic Index

Mitotic index ranges from 4.09 to 10.17% in RC7716-2, 3.51 9.56% and 3.00 to 10.33 in Nicola. Mitotic abnormality ranges from 0.0 to 14.50% in BR63-18, 0.0 to 12.04% and 0.0 to 11.0% in Nicola (Tables 3, 4, 5 and Plate 2).

Table 1. Total number of amplified fragments, monomorphic bands and polymorphic bands generated by AFLP using three primer pairs.

S/N	Name of primer	Sequences	TF	MB	PB	%Poly
1	E32-M49	5'-GACTGCGTACCAATTCAAC-3' 5'-GATGAGTCCTGAGTAACAG-3'	51	18	33	64.71
2	E35-M49	5'-GACTGCGTACCAATTCACA-3' 5'-GATGAGTCCTGAGTAACAG-3'	26	11	15	57.69
3	E35-M48	5'-GACTGCGTACCAATTCACA-3' 5'-GATGAGTCCTGAGTAACAC-3'	16	09	07	43.75
Total			93	38	55	

TF: Total fragment, MB: Monomorphic band, PB: Polymorphic bands, %Poly: Percentage Polymorphism

TABLE 2. Total Number of Polymorphic Loci per Genotype per primer Combination

Sample code.	Genotype	Mutagen	Conc. (%)	E32M49	E35M49	E35M48	Total
1	Nicola	Control	0.0	0	1	0	1.0
4	Nicola	DES	0.9	6	1	0	7.0
5	RC7716-2	Control	0.0	0	0	0	0.0
6	RC7716-2	DES	0.3	7	5	2	14.0
9	BR63-18	Control	0.0	1	0	1	2.0
11	BR63-18	DES	0.5	12	1	1	14.0
14	Nicola	DES	0.1	0	0	1	1.0
19	RC7716-2	DES	0.5	5	5	1	11.0
22	BR63-18	DES	0.1	2	2	1	5.0
Total				33	15	7	55

DES: Diethylsulphate, O: 0.0%, A: 0.1%, B: 0.3%. C: 0.5%, D: 0.7%, E: 0.9%

Table 3. Effects of Diethylsulphate on Mitotic Index and Chromosomal Aberrations in *S. tuberosum* L. var, Nicola

	Mitotic cycle				Chromosomal aberrations				
	TCE	C M	TAC	MI	Stck	Fr	BF	OB	CC
Conc. (%)									
0.0	1374.8± 0.56^{a*}	156.33±0.22^{a}	0.00±0.00^{f}	10.33±0.22^{a}	0.54±0.68^{d}	0.86±0.61^{c}	0.71±0.83^{c}	0.33±0.45^{b}	0.48±0.54^{c}
0.1	1396.2± 0.23^{a}	123.33±0.14^{b}	2.83±0.11^{e}	7.66±0.14^{b}	0.79±0.21^{d}	1.33±0.68^{c}	0.79±0.47^{c}	0.44±0.33^{b}	0.88±0.50^{c}
0.3	1423.7± 0.38^{a}	99.41±2.83^{c}	6.64±0.11^{d}	5.47±0.12^{c}	3.77±0.53^{c}	2.35±0.31^{b}	1.03±0.34^{c}	2.32±0.44^{a}	1.70±0.38^{b}
0.5	1423.7±0.39^{a}	74.16±0.47^{d}	8.33±0.18^{c}	4.66±0.11^{c}	5.08±0.53^{b}	2.52±0.17^{b}	2.27±0.42^{b}	2.45±0.46^{a}	2.02±0.62^{b}
0.7	1423.5±0.39^{a}	70.66±0.18^{e}	10.00±0.24^{b}	4.16±0.16^{c}	5.80±0.55^{b}	2.86±0.33^{b}	2.47±0.42^{b}	2.51±0.33^{a}	2.63±0.48^{a}
0.9	1445.8±0.26^{a}	54.66±0.11^{e}	11.00±0.19^{a}	3.00±0.14^{d}	7.00±0.61^{a}	3.97±0.83^{a}	3.93±0.47^{a}	2.78±0.20^{a}	2.96±0.55^{a}
LSD	23.22	10.77	0.88	0.99	1.07	1.01	1.01	0.64	0.60

*Means ± SE followed by different letters along columns iffers significantly at 5% level of probability using LSD test, Conc.: Concentration, TAC: Total Abnormal Cells, MI: Mitotic Index, TCE: Total Cells Examined, SE: Standard Error, CM: Cells in mitosis, Stck: Stickiness, Fr: Fragments, BF: Bridge Formation, OB: Ox-Bow chromosomes, CC: Cyclic chromosomes,

Table 4. Effects of Diethylsulphate on Mitotic Index and Chromosomal Aberrations in *S. tuberosum* L. var, BR63-18

	Mitotic cycle				Chromosomal aberrations				
Conc. (%)	TCE	CM	TAC	MI	Stck	Fr	BF	OB	CC
0.0	1363.2±0.25^{a}	65.50±1.28^{a}	0.00±0.00^{f}	9.56±0.21^{a}	0.67±0.67^{e}	0.00±0.00^{c}	0.00±0.00^{bc}	0.00±0.00^{f}	0.00±0.00^{c}
0.1	1376.0± 0.20^{a}	123.7±0.15^{b}	3.04±0.58^{e}	7.21±0.16^{b}	2.17±0.31^{d}	1.67±0.33^{b}	0.33±0.33^{b}	0.50±0.34^{e}	2.00±0.17^{b}
0.3	1387.0±0.17^{a}	113.1±0.25^{c}	5.72±0.84^{d}	6.09±0.10^{bc}	3.96±0.48^{c}	2.56±0.22^{b}	0.50±0.50^{b}	2.67±0.61^{d}	2.80±0.68^{b}
0.5	1386.2±7.67^{a}	88.6±0.16^{d}	7.98±1.04^{c}	5.33±0.14^{bc}	6.11±0.13^{b}	2.60±0.36^{b}	1.91±0.45^{a}	3.55±0.50^{c}	3.57±0.49^{b}
0.7	1421.5±0.19^{a}	73.5±0.10^{e}	10.45±0.93^{b}	4.42±0.10^{bc}	6.57±0.72^{b}	3.09±0.31^{a}	2.30±0.35^{a}	4.60±0.40^{b}	4.54±0.31^{b}
0.9	1422.8±0.30^{a}	61.2±0.25^{f}	12.04±0.96^{a}	3.52±0.19^{bc}	9.88±0.10^{a}	3.96±0.41^{a}	2.94±0.37^{a}	6.72±0.41^{a}	6.87±0.48^{a}
LSD	22.08	11.84	1.06	1.24	1.04	1.06	0.64	0.66	1.01

*Means±SE followed by different letters along columns differs significantly at 5% level of probability using LSD test, Conc.: Concentration, TCE: Total Cells Examined, SE: Standard Error, CM: Cells in mitosis, Stck: Stickiness, Fr: Fragments, BF: Bridge Formation, OB: Ox-Bow chromosomes, CC: Cyclic chromosomes, TAC: Total Abnormal Cells, MI: Mitotic Index

Table 5. Effects of Diethylsulphate on Mitotic Index and Chromosomal Aberrations in *S. tuberosum* L. var, RC7716-2

	Mitotic cycle			Chromosomal aberrations					
Conc. (%)	TCE	CM	TAC	MI	Stck	Fr	BF	OB	CC
0.0	1363.2±0.25^{a}	65.50±1.28^{a}	0.00±0.00^{f}	9.56±0.21^{a}	0.67±0.67^{e}	0.00±0.00^{c}	0.00±0.00^{bc}	0.00±0.00^{f}	0.00±0.00^{c}
0.1	1376.0± 0.20^{a}	123.66±0.15^{b}	3.04±0.58^{e}	7.21±0.16^{b}	2.17±0.31^{d}	1.67±0.33^{b}	0.33±0.33^{b}	0.50±0.34^{e}	2.00±0.17^{b}
0.3	1387.0±0.17^{a}	113.16±0.25^{c}	5.72±0.84	6.09±0.10^{bc}	3.96±0.48^{c}	2.56±0.22^{b}	0.50±0.50^{b}	2.67±0.61^{d}	2.80±0.68^{b}
0.5	1386.2±7.67^{a}	88.56±0.16^{d}	7.98±1.04^{c}	5.33±0.14^{bc}	6.11±0.13^{b}	2.60±0.36^{b}	1.91±0.45^{a}	3.55±0.50^{c}	3.57±0.49^{b}
0.7	1421.5±0.19^{a}	73.52±0.10^{e}	10.45±0.93^{b}	4.42±0.10^{bc}	6.57±0.72^{b}	3.09±0.31^{a}	2.30±0.35^{a}	4.60±0.40^{b}	4.54±0.31^{b}
0.9	1422.8±0.30^{a}	61.22±0.25^{f}	12.04±0.96^{a}	3.52±0.19^{bc}	9.88±0.10^{a}	3.96±0.41^{a}	2.94±0.37^{a}	6.72±0.41^{a}	6.87±0.48^{a}
LSD	22.18	11.84	1.06	1.24	1.04	1.06	0.64	0.66	1.01

*Means±SE followed by different letters along columns differs significantly at 5% level of probability using LSD test, Conc.: Concentration, TCE: Total Cells Examined, SE: Standard Error, CM: Cells in mitosis, Stck: Stickiness, Fr: Fragments, BF: Bridge Formation, OB: Ox-Bow chromosomes, CC: Cyclic chromosomes, TAC: Total Abnormal Cells, MI: Mitotic Index

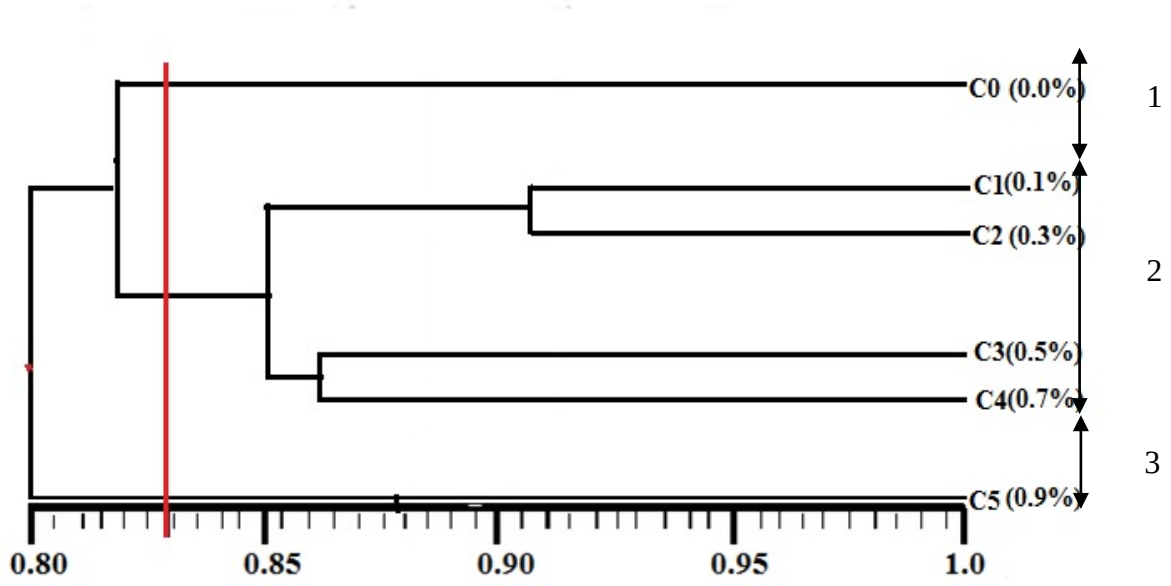

Figure 1. Dendrogram of S. *tuberosum* L. treated with six different concentrations of Diethylsulphate based on AFLP data.

1: Cluster one, 2: Cluster two, 3: Cluster three

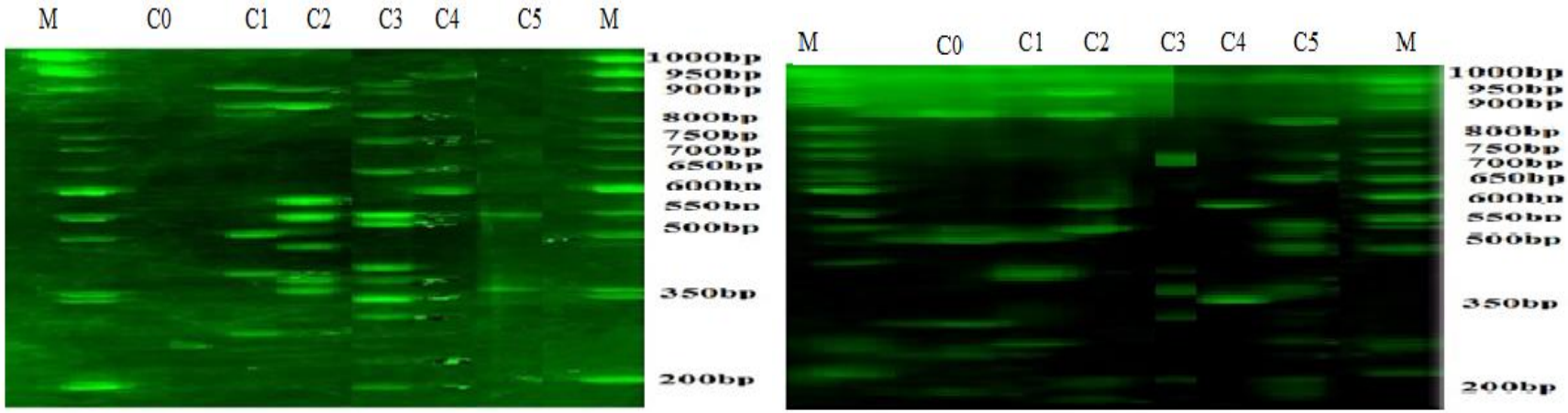

Plate 1. DNA Band Profile of six concentrations of Sodium azide in *S. tuberosum* L. using AFLP marker with primer E32-M49 and E35-M49.

a: Primer E32-M49, b: Primer E35-M49, M:Molecular Ladder, C0 : Control, C1:0.1%, C2 :0.3%, C3 : 0.5%, C4 : 0.7%, C5 : 0.9%.

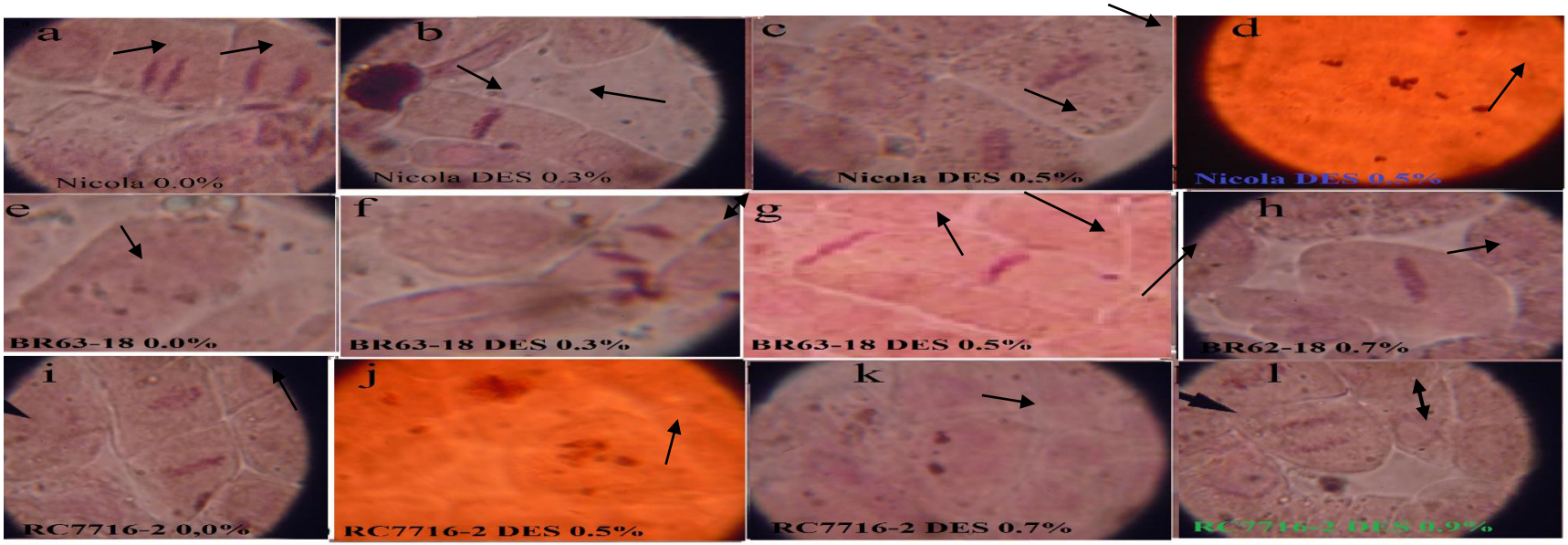

Plate 2. Selected Photomicrographs of *S. tuberosum* L. genotypes treated with different concentrations of Diethylsulphate

a: Normal post metaphase chromosomes movement to poles, b: Sticky metaphase chromosomes c: Ox-Bow metaphase chromosomes movement and metaphase chromosomes clumping. d: Late prophase, chromosomes movement to metaphase plate, e: Late prophase, chromosomes in groups, f: Normal post metaphase chromosomes movement to poles. g: Sticky metaphase chromosomes and late group of chromosomes, h: Normal metaphase i: Rectangular bridge formation, j: Late prophase. k: Lost fragment l: Normal post metaphase chromosomes movement to poles

DISCUSSION

S. tuberosum L. treated with DES analyzed with three AFLP primer pairs detected high number of fragments. [25] reported lower fragments per primer in mucunna. The high number of fragment detected by AFLP is an indication that AFLP is suitable marker for the tested genotypes. [26] reported mean of 35.25 fragments per primer combination higher than mean value for this work. [27] reported mean value of 411.75 fragments per primer pair in Sphenostylis stenocarpa Hochst ex. A Rich. Harms, [28] reported 63 polymorphic bands in *Pinus sylvestris.* [29] reported 505 bands in Potato genotypes. [30] reported variations in varietal responsess to different concentrations of mutagen in *S. tuberosum in* percentage polymorphisms. Polymorphic information content value for this study was high. [31] reported lower PIC value for SA induced mutation in common beans. The high the PIC index for DES treated *S.tuberosum* is indicative of high genetic diversity of the tested *S. tuberosum* genotypes.

The size of the AFLP fragments for this study were high (900bp). [25] and [28] reported a highest fragment size of 400bp in Mucuna and 550bp in *P. sylvestris* lower than reported value for this work. [32] reported that various molecular markers shows different efficiency for evaluating DNA polymorphism from different species.

Phenetic diagram based on Jaccard's coefficient and by UPGMA clustering method formed three clusters. Similarly, [30] reported formation of three clusters in *S. tuberosum* treated with different concentrations of sodium azide. The distribution of treatment groups into different clusters showed that *S. tuberosum* genotypes treated with diethylsulphate were different from the control genotypes. The absence of polymorphic bands from control genotypes in the primer E32-M49 and E35-M49 is an indication that differences exist between treated and the control genotypes. The C5 that was cladded alone is as result of difference in the concentrations of the diethylsulphate. The percentage polymorphism and DNA banding patterns on the polyacrylamide gels also is an indication that differences existed among treated *S. tuberosum* genotypes according to different concentrations of diethylsulphate. Similar result was reported by [3] in different concentrations of Cadmium in *Capsicum annuum* L. DNA banding patterns.

Mitotic analysis

The behaviour of mitotic chromosomes at prophase and metaphase and their separation at anaphase and telophase was normal in the control genotypes of *S. tuberosum*. [30] reported normal mitotic chromosomes behaviours in control *Solanum* species. RC7716-2 treated with 0.5% diethylsulphate resulted in multivalents clumping at prophase stage, this result is in agreement with ([33]; [34]; [30]) who reported bivalents and multivalents clumping at both prophase and metaphase stages in their studies. Diethylsulphate concentration of 0.5% resulted in stickiness, clumping of chromosomes that were observed in Nicola. Similarly, [30] reported stickiness, clumping and cyclic chromosomes in Nicola treated with 0.9% sodium azide.

These chromosomal abnormalities brings about weakening and disturbance of growth processes which

resulted in stunted growth of some genotypes of *S. tuberosum* at higher concentrations of the diethylsulphate. This is result is in agreement with [35]; [30]) who reported stunted growth in Lens culinaris L. and S. *tuberosum* at high concentrations of chemical mutagens.

The stickiness of chromosomes was the most common chromosomal aberrations observed throughout this investigation. Similarly, [36] and [30] in *T. foenum-graecum* L and *S. tuberosum* treated with maleic hydrazide and sodium azide all reported chromosomes stickiness as a major chromosomal aberration in their studies. [37] reported that chromosomes stickiness is as result of partial dissociation and altered pattern of organization of nucleoprotein. Chromosomal stickiness makes separation and free movement chromosomes difficult and incomplete and thus chromosomes remain attached to other chromosomes a phenomenon called bridge formation or cut to be seen as lost chromosome. [30] reported similar results in *S. teberosum.* Bridge formation as chromosomal abnormality was highest at 0.9% diethylsulphate. This finding is in agreement with [30] who reported bridge formation as chromosomal abnormality highest at 0.9% sodium azide treated *Solanum species.* This may also be due to the defective formation of the spindle apparatus ([38];[39]).

Fragmentation was as result of stickiness of chromosomes and consequently failure of movement of chromosomes to poles as result breakage was also observed in this study. [40]; [41]; [30]) reported chromosomes fragmentation in their studies. Fragments may also be acentric chromosomes that are formed as a result of inversion. Fragmentation as chromosomal aberration was highest at 0.9% diethylsulphate. Similarly, [30] reported fragmentation *S. tuberosum* treated with sodium azide. These chromosomal aberrations can produce different kinds of aneuploids, hypoploidy, hyper ploidy and structural aneuploids [42].

Mitotic index is an acceptable measure of cytotoxicity for all living organisms [43]. A decrease of mitotic index below 50% usually has lethal effects [44]. If mitotic index decreases below 22% of control, it causes sub lethal effects on test organism [45]. It is the measure of cytotoxicity that make some chemicals to be used as mutagens in the improvement of agronomic traits of crops. The reactions of chemical mutagens with DNA result in base changes and create mutations ([46]; [47]). If the mutants have good agronomic traits, such mutants are then bred with existing cultivars.

The application of DES, on all *S. tuberosum* genotypes resulted in decrease in mitotic index and increase in mitotic abnormality. The decrease in mitotic index by DES resulted in delay growth initiation because the enzyme O-acetyl serine sulphydrylase modifies the mutagens in the cells into a mutagenic metabolite azido alanine [48]. The inhibitory effects of DES on the mitotic index as in this study is an indication that DES have genotoxic and mutagenic effects on the organisms. Similar effect of DES on mitotic index was observed in barley seedlings and *S. tuberosum* [49]. [30] reported genocytotoxicity effect of sodium azide in *S. tuberosum.* The reduction in mitotic index by DES was dose dependent. This result is in agreement with [30] who reported dose dependent effect of sodium azide treated *Solanum* varieties. The *S.*

tuberosum genotypes shows a decrease in mitotic index from lowest in control and highest in the treated genotypes at 0.9% DES. There was an increase in mitotic abnormality from control *S. tuberosum* genotypes to treated genotypes at 0.9% concentrations of the DES. This result is in agreement with [49] and [30] who reported effects of DES and SA in in barley seedlings and *S. tuberosum* varieties, DES induced dysfunctions are registered at the level of genetical, Cytological and morphological. Chromosomal aberrations, mitotic inhibition, decrease of tuber sprout faculty, reduction of plant growth rate, a greater sterility degree in pollen germination, survival rate reduction and morphological mutations.[50] reported similar work in *Cicer arietinum* L. var. K. 850.

CONCLUSION AND RECOMMENDATIONS

This study revealed that *S. tuberosum* genotypes treated DES generated a total of 93 DNA fragments, 55 polymorphic bands and 42 monomorphic bands with six different concentrations of diethylsulphate with three primer combinations. Primer E32-M49 generated the highest percentage of DNA polymorphic bands (64.71 %). Chromosome stickiness was 9.8% in RC7716-2 treated with 0.9 % SA. Mitotic index was lowest (3.0 %) in Nicola treated with 0.9% DES. The reduction in mitotic index, chromosomal aberrations, and induction of DNA polymorphism is an indication that diethylsulphate has genocytotoxic effects. Concentrations of 0.1, 0.3 and 0.5 % of diethylsulphate is recommended for mutation induction for genetic variability in *S. tuberosum* L.

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