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CYTOGENETICAL STUDIES OF MACROMUTANTS INDUCED BY NANOPARTICLES (Cu, CdS, CuO and ZnO-NPs) IN *SESAMUM INDICUM* L.

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Abstract

A total of 23 phenotypic mutants were screened in M_2 plant population (7112) of Sesamum indicum L. (Family: Pedaliaceae) affecting seedling colour, leaf traits, stem characteristics, branching nature, floral colour, fruit characteristics and maturity following dry seed (moisture content – 7.40%) treatments with nanoparticles (copper, cadmium sulphide, copper oxide and zinc oxide – 1.00, 2.00 and 4.00 µg/ml, 3 and 6h durations), ethyl methanesulphonate (EMS – 0.25, 0.50 and 1.00%, 3 and 6h) and gamma irradiations (50, 100, 200, 400 and 600 Gy). Viable mutation frequency is found higher in gamma irradiation followed by ZnO-NPs, CuO-NPs, Cu-NPs, CdS-NPs and EMS. A total of 9 different macromutants types are found in Cu-, CuO- and ZnO-NPs, 11 in EMS and 13 in CdS-NPs and gamma irradiations. Excepting viridis (non-viable), all macromutants bred true at M_3. Reciprocal crossings performed between mutant and normal plant types suggest that the mutant traits are controlled by nuclear genes (monogenic recessive and digenic) and they segregated in accordance to Mendelian pattern of segregations. Meiotic analyses reveal that normal and mutant plants were cytologically alike (2n=26) suggesting that mutation is not the consequence of chromosomal disturbances. Results highlight mutagenic potentiality of NPs alike to that of the conventional mutagens used as positive controls.

Key words: *Sesamum indicum, macromutation, nanoparticles, EMS, gamma irradiations, cytogenetical studies*

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INTRODUCTION:

Nanoparticles (NPs, atleast one dimension <100 nm) are reported to interact with DNA of plant species [1] causing DNA lesions (Ag-NPs) and mispairing during replication and bringing about mutagenic changes [2] or fixed into mutation [3]. Nel et al. [4] also highlighted that carbon nanotubes (CNTs) can induce DNA damage and mutagenesis. Kumbhakar et al. [5,6] opined that Cu- and CdS-NPs inducing chromosomal alterations in meiotic cells can bring about heritable consequences in subsequent generations. Halder et al. [7,8] pioneerly reported stable, heritable phenotypic changes in *Macrotyloma uniflorum* as the consequences of Cu- and CdS-NPs treatments. Therefore, NPs interaction with plant DNA is significant for genome alteration which may lead to gene mutation. If so, it will be opening up new dimension in agriculture science and crop improvement. The present endeavour highlights the potentiality of engineered nanoparticles (copper – Cu, cadmium sulphide – CdS, copper oxide – CuO and zinc oxide – ZnO) in inducing stable, heritable phenotypic mutation in *Sesamum indicum* L. var. B67 (Family: Pedialiaceae, common name – sesame, plant species yielding oil of commerce along with immense pharmaceutical importance – [9,10]) in comparison to conventional mutagens ethyl methanesulphonate (EMS) and gamma irradiations. Cytogenetical aspects of the induced mutants are described.

MATERIALS AND METHODS:

Germplasm

Sesamum indicum L. var B67 (moisture content: 7.40%) seeds were collected from Pulse and Oil Seed Research Station, Govt of West Bengal, Berhampore, India as breeder stocks.

Nanoparticles

Copper, cadmium sulphide, copper oxide and zinc oxide nanoparticles were synthesise using wet chemical co-precipitation techniques and opto-physically characterised (data published elsewhere)

Treatments

Seeds of variety B-67 were exposed to synthesized nanoparticles (1.0, 2.0 and 4.0 µg/ml, 3 and 6 h duration) and to conventional mutagens (EMS: 0.25, 0.50 and 1.00%, 3 and 6 h, diluted in 0.2 M KH_2PO_4:0.2 M K_2HPO_4 :: 50.3:49.7, pH 6.8; Gamma irradiations: 50, 100, 200, 400 and 600 Gy, source: Co^{60}, source to distance 10 cm, dose rate 35.5 Gy/ min). Treated seeds (NPs and EMS) were washed in ddH_2O for 2 h. Untreated seeds and seeds treated with bulk materials (4.0 µg/ml, 6h) were kept as controls. Two hundred seeds were exposed in each lot.

Raising of plant population

Treated (NPs, EMS and gamma irradiations) and control (dry and bulk CdS) seeds (100 seeds from each treatment) were sown in experimental field plot of the Department of Botany, Kalyani University (West Bengal plain) in 4th week of February 2013 to raise M_1 population (February to July as irrigated crop). Selfed seeds (first formed floral buds were bagged) of each surviving M_1 plants were collected in separate zipped polythene packets and stored in a desiccator. M_2 population was grown from selfed M_1 eeds in plant to row progenies in the year 2014. Distance between plants and rows were maintained uniformly as 15 cm and 30 cm respectively.

Detection of macromutants and its inheritance

Phenotypic variants [distinctive phenotypic trait(s) variation from normal trait(s)] were screened from seedling to maturity of M_2 plants. The phenotypic variants were confirmed as macromutants at M_3 from selfed segregation and from reciprocal crossings. Macromutant frequency was estimated as per 100 M_2 plants [11]. No fertilizer application was made during plant growth period.

M_2 mutants (used as female parent) were crossed with pollen grains (90% to 98% pollen grain fertility) from control plants (possessing normal phenotypic traits) and F_1 plants were raised. All F_1 were phenotypically normal. F_1 plants were selfed and F_2 progenies were grown. Segregation pattern obtained were computed following χ^2-test analysis for study of inheritance of trait(s).

Meiotic chromosome behaviour

Meiotic chromosome behaviour of mutants and as well as of control plants were assessed at M_3. Floral buds of suitable sizes of each plant type were fixed (5 am–6 am; pilot trials reveal best time for detection of divisional plates) in Carnoy's fixative (ethanol: acetic acid: chloroform::6:3:1) for a period of 72 h. Three changes were given in the fixative at an interval of 24 h. Floral buds were subsequently preserved in 70% alcohol under refrigeration (16˚C±1˚C). Pollen mother cells (PMCs) and pollen grain obtained from anther squash preparations were stained in 2% aceto-carmine solution. Uniformly stained pollen grains were considered as fertile [12]. Metaphase I (MI) and anaphase I (AI) cells were observed. Suitable cytological preparations were photomicrographed.

RESULT AND DISCUSSION:

Macromutant types and frequency

A total of 23 distinct phenotypic variants are screened from 7112 plant population at M_2 following NPs and conventional mutagen treatments and the variants are confirmed as mutants at M_3. Out of the 23 types, only one (*viridis*) mutant type is found non-viable as it dried up at the seedling stage. The mutant types recorded are 9 in Cu-, CuO- and ZnO-NPs, 13 in CdS-NPs, 11 in EMS and 13 in gamma irradiations. Calculated viable mutation frequency across doses

(Figure 1) is 1.06% (toytal-1.06%) in Cu-NPs, 0.96% (total-0.96%) in CdS-NPs, 1.17% (total-1.17%) in CuO-NPs, 1.30% (total-1.30%) in ZnO-NPs, 0.61% (total-0.70%) in EMS and 1.51% (total-1.51%) in gamma irradiations. Mutation frequency is not dose dependent. Compared to normal trait(s) (Figure 2a), the macromutants noted are found to affect seedling colour (*viridis*), leaf trait (*broad leaf I* and *II*, *coarse leaf*, *narrow leaf*, *ovate leaf*, *crumpled leaf*, *elongated pinnae I* and *II* and *quadri-petiolar* leaf), stem characteristics (*thick stem* – Figure 2f), branching pattern (*lax branching*, *branching from base*, *bushy* and *unbranched* – Figure 2e), floral characteristics (*multichambered fruit* – Figure 2d, *tri-* and *quadri-axillary fruit* – Figure 2b,c and *globular fruit*) and maturity (*early flowering*). All the treating agents show predominance of mutation affecting leaf traits.

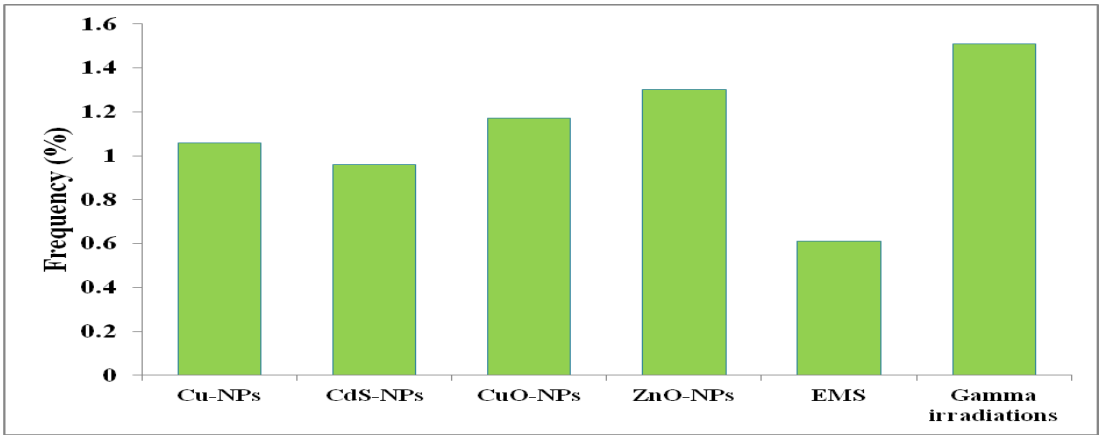

Fig 1: Viable mutation frequency in different treating agents.

Fig 2: Normal (a) and mutant (b–f) plant types of *Sesamum indicum*. (a) Normal; (b) *quadra-fruits per node*; (c) *tri-fruits per node*; (d) *multilocular fruits*; (e) *unbranched*; (f) *thick stem with long petiole*.

Table 1: Overview of the occurrence of macromutants in different treated materials across doses

Plant type	EMS	Gamma irradiation	Cu-NPs	CdS-NPs	CuO-NPs	ZnO
Viridis	0.15	-	-	-	-	-
Broad leaf I	0.08	-	0.15	0.09	0.16	0.24
Broad leaf II	-	-	0.15	-	0.16	-
Elongated petiole I	-	0.43	-	0.13	-	0.18
Elongated petiole II	-	-	-	0.11	0.30	0.24
Quadripetiolar node	-	-	-	-	-	0.03
Coarse leaf	0.08	0.12	0.15	0.09	0.14	0.09
Thick stem	-	0.27	0.15	0.02	0.19	0.21
Crumpled leaf	-	0.04	-	-	-	-
Ovate leaf	-	0.04	-	-	-	-
Small leaf	0.04	-	-	-	-	-
Narrow leaf	-	-	-	0.09	-	-
Branching from base	0.04	-	-	0.09	0.04	0.03
Unbranched	0.04	0.04	-	-	-	-
Bushy	0.04	0.12	-	-	0.07	-
Dwarf	0.08	0.12	-	-	-	-
Pigmented flower	0.08	-	0.06	0.02	-	-
White flower	0.04	0.08	0.03	0.02	-	0.12
Early flowering	-	-	-	0.07	-	-
Triaxillary fruits	-	0.04	-	0.02	-	-
Quadriaxillary fruit	-	0.19	0.15	0.02	-	-
Globular fruit	-	-	0.03	-	0.04	-
Multichambered fruit	0.11	0.04	0.06	0.09	0.07	0.18
Total	11	12	09	13	09	09

Table 2: Male meiotic configurations and pollen grain fertility in normal and mutant plant types at M_3

Plant types	No. of PMCs scored at MI	Mean/cell		No. of cells scored at AI	PMCs with 13/13 segregation (%)	No. of pollen grains scored	Pollen grain fertility (%)
		II	I				
Normal	77	12.93	0.14	28	100.0	773	87.58
Broad leaf I	70	12.90	0.20	37	100.0	360	77.78
Broad leaf II	83	12.89	0.22	52	100.0	396	75.42
Coarse leaf	66	12.79	0.42	21	90.5	563	67.50
Ovate leaf	93	12.91	0.18	17	100.0	910	65.71
Narrow leaf	82	12.88	0.24	34	100.0	838	74.34
Elongated petiole	85	12.90	0.20	29	100.0	202	78.70
Thick stem	87	12.93	0.14	19	100.0	565	31.86
White flower	62	12.90	0.20	33	96.9	382	78.70
Pigmented flower	61	12.89	0.22	16	100.0	272	64.10
Bushy	96	12.80	0.40	43	90.7	502	81.48
Unbranched	71	12.87	0.26	28	100.0	267	62.27
Branching from base	51	12.92	0.16	31	100.0	462	90.46
Triaxillary fruit	67	12.87	0.26	29	100.0	218	72.17
Quadriaxillary fruit	81	12.95	0.10	27	100.0	466	76.40
Tripetiolar node	89	12.92	0.16	21	100.0	577	79.40
Multichambered fruit	69	12.88	0.24	37	100.0	455	84.36
Globular fruit	58	12.93	0.14	16	100.0	182	59.86
Dwarf	61	12.71	0.58	36	83.3	269	4.12
Early flowering	74	12.91	0.18	22	100.0	773	87.58

Broad leaf II, *narrow leaf*, *elongated petiole II*, *quadri-petiolar node*, *early flowering* and *globular fruit* mutants are scored specifically in NPs treated population. No mutants are spotted specific to Cu- and CuO-NPs whereas narrow leaf and early flowering plant types are identified only in CdS-NPs treated population. *Quadri-petiolar* node mutant plant type is specific to ZnO-NPs. Few mutants are also found to occur only in conventional mutagen treatments (EMS- *small leaf* and *dwarf*; gamma irradiation-*crumpled leaf*, *ovate leaf* and *unbranched*). *Tri-* and *quadri-axillary fruit per node*, *unbranched* (provide enhance plantation per unit area), *bushy* and *branching from base* are most significant mutants in the plant species which closely correspond to the plant ideotype being looked for in the species. Furthermore enhance yield in the mutants will be significant for pharmacological practices. No macromutants are scored in dry control and bulk controls.

Across doses of treatments, macromutants appeared in the following order; Cu- *thick stem> broad leaf I = broad leaf II = coarse leaf = multichambered fruit> pigmented flower = white flower =globular fruit> quadra-axillary fruit*, CdS- *thick stem> elongated petiole I = triaxillary fruit> elongated petiole II> broad leaf I = narrow leaf = coarse leaf = multichamberd fruit> early flowering> white flower> pigmented flower = branching from base = quadra-axillary fruit*, CuO- *elongated petiole I> thick stem> broad leaf I = broad leaf II> coarse leaf> multichambered fruit = globular fruit> bushy> branching from base*, ZnO-NPs - *broad leaf I = elongated petiole I> thick stem> elongated petiole I = multichambered fruit> white flower> coarse leaf> quadri-petiolar node = branching from base*, EMS- *viridis> multichambered fruit> broad leaf = coarse leaf = dwarf> small leaf = branching from base = unbranched = bushy = white flower* and gamma irradiations - . In the present study, most of the identified mutant plant types are distributed uniformly irrespective of nature of the treating agents thereby suggesting the existence of possible genomic hotspot prone to treating agents. Thus, the NPs possesses mutagenic potentiality and can bring about similar phenotypic alterations as that of the conventional mutagens.

Meiotic analysis of the macromutants

Male meiotic analysis reveals 2n=26 always in control and mutant plant types (Figure 3a-d). Meiotic study from M_3 plant types suggests that mean chromosomal association per cell is 12.93II+0.07I in untreated control plant and it ranges from 12.71II+0.29I to 12.95II+0.05I in mutants. Most of the mutants show balanced anaphase I (Figure 3d) segregation (13/13) excepting coarse leaf (90.5%), white flower (96.9%), bushy (90.7%) and dwarf (83.3%) plant types. Pollen grain fertility is 87.6% in control and it reduces in mutants (87.6%-early flowering to 4.1%-dwarf) excepting branching from base (90.5%). Meiotic studies reveal that the mutants are the consequence of gene mutation rather than chromosomal anomalies.

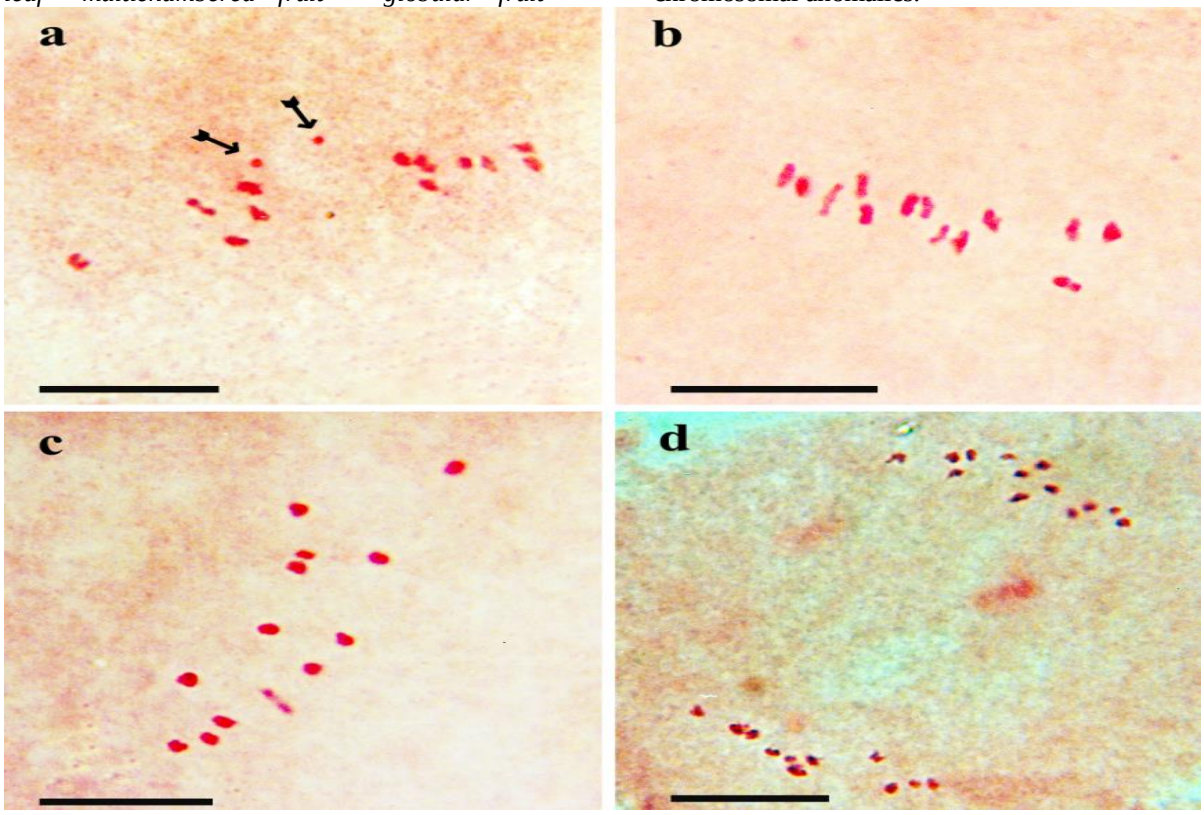

Fig 3:Meiotic configurations of *S. indicum* showing 2n=26 chromosomes at metaphase I (a–b) and anaphase I (c) (a) 12II+2I (↦); (b–c) 13II; (d) 13:13 separation.

Inheritance patterns of the mutant traits

Reciprocal crossing performed between control and mutant plant types yield all F_1 plants with normal phenotype(s). F_1 plant types are selfed which segregated at F_2 into normal and mutant phenotypes. Segregation analysis using χ^2 test suggests mutant trait(s) inheritance are in accordance with Mendelian pattern. Mutant traits are either monogenic (3:1−*coarse leaf*, *ovate leaf*, *elongated petiole I*, *thick stem*, *white flower*, *pigmented flower*, *bushy*, *unbranched*, *globular fruit*, *dwarf*, *early flowering* and *narrow leaf*) or digenic (9:7−*broad leaf I* and *II*, *triaxillary fruit*, *quadraxillary fruit*, *tripetiolar node*, *multilocular fruit*; 15:1− *branching from base*) recessive in nature. Significantly, the mutant namely *broad leaf I* and *elongated petiole I* (Figure 2f) are associated with *thick stem* trait always. On selfing the mutants with associated traits bred true at M_3. No segregants between the associated mutant traits is noted neither at F_1 reciprocal crossing (between normal and mutant plant type) nor selfed progenies at F_2 suggesting possible pleiotropic gene action controlling the mutants with associated traits. Furthermore, reciprocal crossings yielding similar results in all cases indicate that the mutant traits are controlled by nuclear genes.

CONCLUSION:

The present investigation highlights that NPs (Cu, CdS, CuO and ZnO) can induce stable, heritable (in accordance with Mendelian segregation pattern) phenotypic mutations like that of conventional mutagens. However, experimental novelty using next generation sequencer (NGS) can through more light on nano-bio interaction specifically in understanding the role of NPs interacting with DNA for genomic alteration.

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REFERENCES:

1. Atha DH, Wang H, Petersen EJ, Cleveland D, Holbrook RD, Jaruga P, Dizdaroglu M, Xing B Nelson BC. Copper oxide nanoparticle mediated DNA damage in terrestrial plant models. Environ. Sci. Technol. 2012;46: 1819–1827.

2. Vidal AE, Hickson ID, Boiteux S, Radicella JP. Mechanism of stimulation of the DNA glycosylase activity of hOGG1 by the major human AP endonuclease: bypass of the AP lyase activity step. Nucl. Acids Res. 2001;29: 1285–1292.

3. Langie AS, Azqueta A, Collins AR. The comet assay: past, present, and future. Front. Genet. 2015;6: 1−3.

4. Nel A, Xia T, Madler L, Li N. Toxic potential of materials at the nanolevel. Science. 2006;311: 622–627.

5. Kumbhakar DV, Datta AK, Das D, Sahu VP, Barbuddhe SB, Sharma S, Ghosh B, Pramanik A.Nanoparticle (metallic copper and cadmium sulphide) application: photocatalytic potentiality and antimicrobial effectivity. Indian J. Sci. Technol. 2017;10: 1–17.

6. Kumbhakar DV, Datta AK, Das D, Ghosh B, Pramanik A, Saha A. Copper and cadmium sulphide nanoparticles can induce macromutation in *Nigella sativa* L. (black cumin). J. Plant Development Sci.2016b; 8: 371−377.

7. Halder S, Mandal A, Das D, Gupta S, Chattopadhyay AP, Datta AK. Copper nanoparticle induced macromutation in *Macrotyloma uniflorum* (Lam.) Verdc. (Family: Leguminosae): a pioneer report. Genet. Resour. Crop Evol. 2015a;62: 165−175.

8. Halder S, Mandal A, Das D, Datta AK, Chattopadhyay AP, Gupta S, Kumbhakar DV. Effective potentiality of synthesised CdS nanoparticles in inducing genetic variation on *Macrotyloma uniflorum* (Lam.) Verdc. BioNanoSci. 2015b;5: 171−180.

9. Bedigion D. (ed) 2011. Sesame, the Genus Sesame. Econ. Bot. A book review CRC Press, 65: p 335.

10. Nweke FN, Ubi BE, Kunert K. Application of microsatellite polymorphisms to study the diversity in seed oil content and fatty acid composition in Nigerian sesame (*Sesamum indicum* L.) accessions. Afr. J. Biotech. 2012;11: 8820–8830.

11. Gaul H. Mutations in plant breeding. Rad. Bot.1964; 4: 155–232.

12. Marks GE. (1954) An aceto-carmine glycerol jelly for use in pollen-fertility counts. Stain Technol. 29: 277.