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Research Article

CADMIUM SULPHIDE (CDS) AND COPPER OXIDE (CUO) NANOPARTICLES (NPS) MEDIATED PHENOTYPIC MUTATION IN *CORIANDRUM SATIVUM* L. (APIACEAE)

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ABSTRACT

Chemically synthesized cadmium sulphide (CdS) and copper oxide (CuO) nanoparticles (NPs) induced phenotypic mutation (types and frequency), meiotic chromosome behavior (mutants and controls) and mutagenic effectiveness and efficiency are described in *Coriandrum sativum* L. (Family: Apiaceae; spice and leaf vegetable yielding plant species of commerce) in comparison to the conventional mutagens namely, ethyl methanesulphonate (EMS) and gamma irradiations. The objective of the work is to highlight the mutagenic potentiality of CdS- and CuO-NPs. Result suggests that both NPs can induce 9 types of phenotypic mutants at M_2 alike to that of EMS and gamma irradiations. Mutation frequency and mutagenic effectiveness and efficiency are found relatively higher in NPs than that of the conventional mutagens. Meiotic chromosome configurations (2n = 22) are nearly similar in both control and mutants. The mutants bred true at M_3.

INTRODUCTION

Experimentally induced mutation widens the gene pool of the existing germplasm, and it is a potential tool contributing to global agriculture by producing over 3248 mutants with desirable qualitative and quantitative traits in different plant species (FAO/IAEA, 2017). The conventional mutagens (both physical and chemical) are widely reported to play significant role in inducing gene mutation and crop improvement. Synthesized nanoparticles (NPs-small sized particles where one dimension ranges between 1 and 100 nm- Roco, 2003, possess unique physico-chemical properties-Remédios, 2012; Masarovičová and Kráľová, 2013) are also reported to encompass mutagenic potentiality and can induce stable, heritable phenotypic mutation in plant species (Halder *et al,* 2015a,b; Kumbhakar *et al,* 2016a,b; Ghosh *et al,* 2017) apart from their wide applications in different branches of sciences, medicine, industries, among others (Lam *et al,* 2004; Caruthers *et al,* 2007; Nowack and Bucheli, 2007; Scrinis and Lyons, 2007; Singh *et al,* 2008; Nair, 2010; Castiglione, 2011; Remédios, 2012; Masarovičová and Kráľová, 2013; Das *et al,* 2017). The present investigation is an additional endeavor to foresee the perspective of using chemically synthesized NPs like cadmium sulphide (CdS) and copper oxide (CuO) for induction of gene mutation in other plant species of commercial interest like coriander (*Coriandrum sativum* L.; Family: Apiaceae; spice and leafy vegetable yielding plant species of commerce with immense therapeutic uses-Pathak *et al,* 2011; Rajeshwari and Andallu, 2011; and cytological novelty-Pramanik *et al,* 2017). The present article describes the frequency and types of phenotypic mutants induced, meiotic chromosome configurations of the mutants and mutagenic efficiency and effectiveness of the studied NPs. This study is untaken in comparison to the conventional mutagens namely, ethyl methanesulphonate (EMS) and gamma irradiations (used as positive controls). Both CdS- and CuO-NPs are semiconductor in nature (also known as quantum dots-QDs) possessing narrow band gaps (CdS-NPs: 2.4 eV-Borovaya, 2014; CuO: 1.2 eV-Dhineshbabu, 2016) and are significantly used in biology, medicine, technology and agriculture (Naderi and Danesh-Shahraki, 2013; Al-Halafi, 2014; Safari and Zarnegar, 2014; Kokina, 2015).

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Figure 1 (A–I): Normal (A) and mutant (B–I) plant types in *Coriandrum sativum*. (A): Normal plant at flowering. (B): *chloroxantha*. (C): *thin dissected pinnae*. (D): *broad leaf*. (E): *long petiole* (➡). (F): *lax branching*. (G): *unidirectional branching*. (H–I): *thick stem*.

MATERIALS AND METHODS

Germplasm source

The mother seed stocks (moisture content: 13.60%; seed size: length-5.04 ± 0.13 mm, breadth-3.47 ± 0.08 mm; seed color assessed from RAL color chart: Sand yellow-RAL-1002-198-166-100) were obtained from Pulses and Oil Seeds Research Station, Department of Agriculture, Govt. of West Bengal, Berhampore, Murshidabad as breeder's seeds.

Nanoparticles

The nanoparticles (CdS- and CuO-NPs) were synthesized under laboratory condition(s) following wet chemical co-precipitation method and opto-physically characterized using UV-Visible Spectrometer (UV-Vis, Shimadzu UVPC-1601), Fourier Transform Infra-red Spectroscopy (FTIR, Jasco FT/IR-6300), X-ray Diffractometer (XRD, Shimadzu LabX, λ=1.5406 Å, 2θ from 10º-90º), Dynamic Light Scattering analyzer (DLS, Delsa^{TM} NanoC, Beckman Coulter) and Field Emission Scanning Electron Microscopy (FESEM, JEOL JSM – 7600F) for determination of nano standard quality(data unpublished). Bulk CdS and CuO are prepared identically as NPs but without the capping agent (for both Nps-Sodium dodecyl sulphate-SDS). The sizes of bulk materials (assessed from Stereo Zoom® Leica S8 APO) were measured as 13.6 ± 7.3 µm in CdS and 28.8 ± 9.8 µm in CuO.

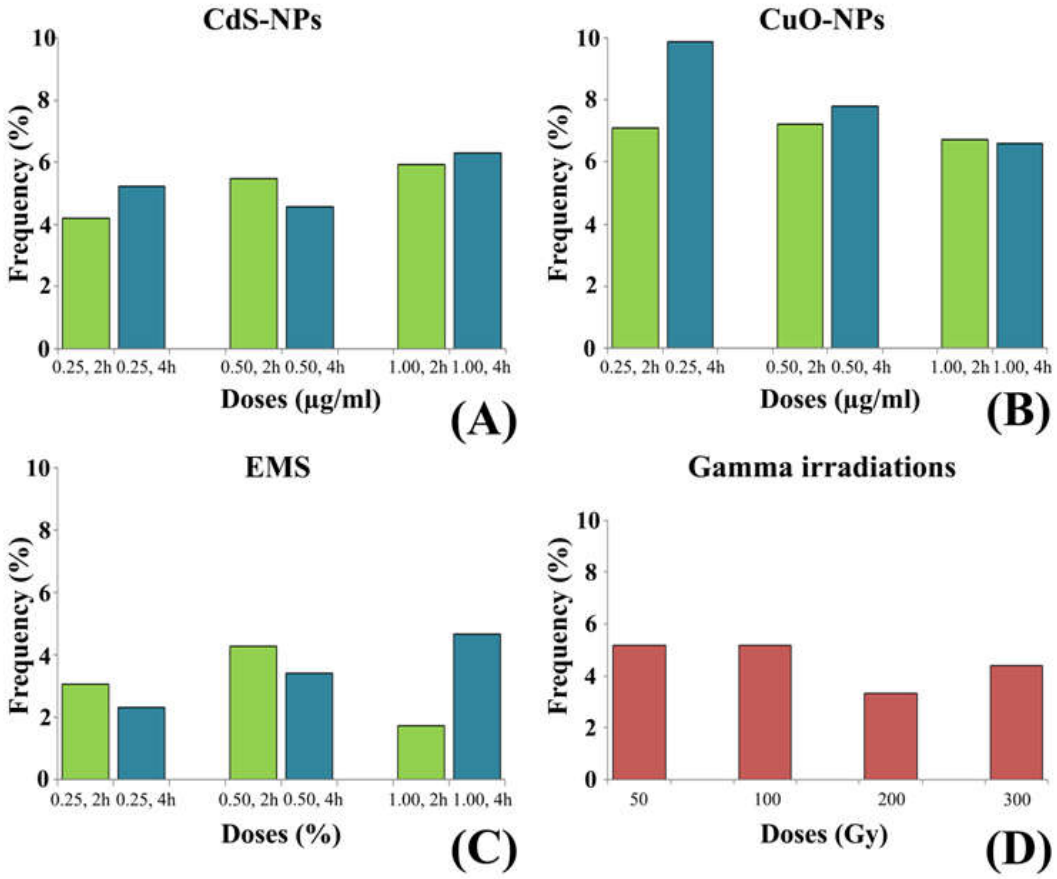

Figure 2 (A–D): Viable mutation frequency in the treating agents.

Treatments

Dry seeds of *C. sativum* were treated (0.25, 0.50 and 1.00 µg/ml; 2 and 4 h durations) with CdS- and CuO-NPs as well as exposed to gamma irradiations (50, 100, 200 and 300 Gy, source ^{60}Co, absorbance dose rate 47.4 Gy/min, source to distance 12 cm) and EMS treatments (0.25, 0.50 and 1.00%, 2 and 4 h durations; solution prepared in 0.2 M phosphate buffer- using 0.2 M KH_2PO_4 49.7 ml and 0.2 M K_2HPO_4 50.3 ml; pH maintained at 6.8). Doses were employed following pilot trials. Untreated dry and CdS and CuO bulk (0.25 µg/ml, 2 h) were also kept as controls. Bulk controls were used to assess mutation frequency, if any.

In each lot, 200 seeds were exposed of which 100 seeds were given in Petri plates (under uniform conditions; temperature- 27ºC ± 1ºC) lined with moist filter papers for determination of germination frequency and seedling growth.

Lethality and injury were measured from reduction in germination frequency and seedling growth respectively as per cent of dry control.

Raising of M_1 and M_2 generations

Hundred seeds from each lot of treatment including controls were grown in lines (20 cm and 30 cm between plants and rows respectively) in the experimental field of Department of Botany, Kalyani University during the months of November to April. One inflorescence of each treated M_1 plant (as well as from few control plants) was selfed and seeds were kept in separate packets on harvest to raise M_2 plant population.

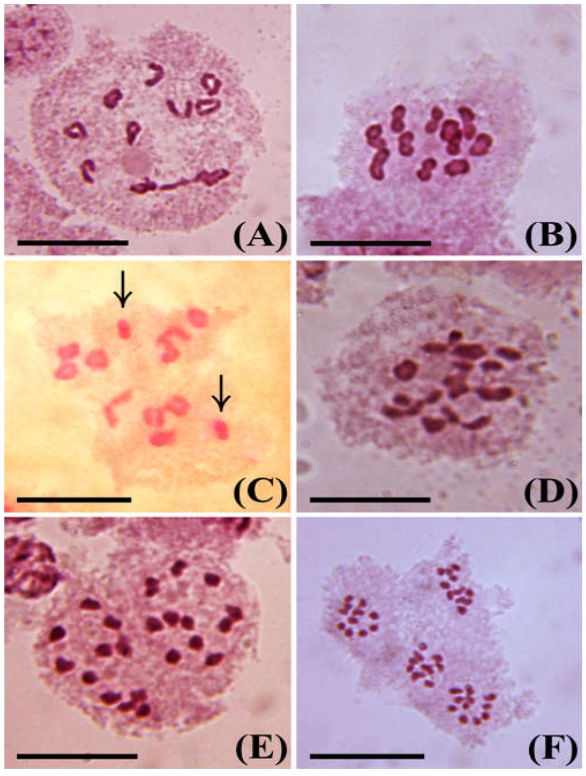

Figure 3 (A–F): Meiotic configurations at diplotene (A), metaphase I (B–D), anaphase I (E) and anaphase II (F) of normal (A) and mutant plant types (B–F). (A–B): 11II. (C): 10II+2I (➡). (D): 8II+6I. (E): 11/11 separation. (F): cytologically balanced cell. Scale bar = 10 µm.

At M_1, seed sterility was ascertained following assessment of total seed weight of each plant and pooled across treatments and represented as per cent reduction in seed weight in treatment compared to control (dry).

Selfed seeds of each M_1 treated plant were sown in lines at M_2 (plant to progeny) and macromutants (phenotypic) were screened throughout the life period (germination to harvest). Frequency of mutation was measured as per 100 M_2 plants (Gaul, 1964). The seedling color (confirmed from RAL color chart: Rap Yellow-RAL 1021-243-218-011) of chlorophyll mutant was classified as per Blixt (1961).

No fertilizer doses were employed during the growth period and standard cultural practices were performed (uniform in both generations) in relation to weeding and irrigations.

Assessment of mutagenic efficiency and effectiveness

Mutagenic efficiency and effectiveness of NPs in relation to EMS and gamma irradiations were assessed from viable mutation frequency (Walther, 1969) following the formulae proposed by Konzak *et al* (1965). The mutagenic efficiency was calculated as Mf/L, Mf/I and Mf/S and effectiveness as Mf/c × t or Gy unit converted to kR (Mf = mutation frequency, L = lethality, I = Injury and S = Sterility, c = concentration, t = duration of treatment, Gy = gray unit and kR = kiloroentgen).

Meiotic analyses

For meiotic analyses, inflorescences (umbel) from mutants and controls were fixed in acetic alcohol (1:3 v/v) at 5.30 am to 6.30 am (suitable time for obtaining divisional plates assessed from trials) and subsequently preserved in 70% alcohol under refrigeration. Another squash preparations were performed and PMCs and pollen grains were stained using 2% aceto-carmine solution. Fully stained pollen grains were determined as fertile (Marks, 1954). Metaphase I (MI), anaphase I (AI) and anaphase II (AII) cells were scored. Well scattered meiocytes were photomicrographed.

RESULTS AND DISCUSSION

Mutation frequency and types

A total of 9 macromutant types (Fig 1B-I) are screened (8 viable; *Chloroxantha*-non-viable) from the mutagenized population (4776 plants scored) at M_2 (Table 1). The mutant trait(s) are identified in relation to corresponding normal trait(s) (Fig 1A) in control plants.

Table 1 Macromutant types and frequency across doses of treatments.

Mutant types	Frequency (%)			
	CdS-NPs	CuO-NPs	EMS	Gamma irradiations
Chloroxantha	0.38	0.44	0.29	0.29
Thin dissected pinnae	0.82	0.96	0.81	0.29
Broad leaf	0.44	0.79	0.22	0.58
Long petiole	0.57	0.87	0.44	0.14
Thick stem	1.14	1.66	0.44	0.87
Lax branching	0.95	1.40	0.52	0.72
Unidirectional branching	0.38	0.70	0.15	0.58
Early flowering	0.63	0.52	0.37	0.87
Dwarf	0.38	0.44	0.22	0.58
Total plants scored	1577	1146	1360	693

All the treating agents (CdS-NPs, CuO-NPs, EMS and gamma irradiations) induce 9 types of macromutants. Mutation seems to affect seedling color (*Chloroxantha*-Fig 1B), leaf traits(s) (*thin dissected pinnae*-Fig 1C, *broad leaf*-Fig 1D and *long petiole*-Fig 1E), stem characteristics (*thick stem*-Fig 1H-I), branching pattern (*lax branching*-Fig 1F and *unidirectional branching*-Fig 1G) and maturity (*early flowering* and *dwarf*).

Total and viable mutation frequencies are 5.71% and 5.33% (viable: 4.19% to 6.29%) in CdS-NPs, 7.77% and 7.33% (viable: 6.57% to 9.85%) in CuO-NPs, 3.46% and 3.16% (viable: 1.75% to 4.68%) in EMS and 4.91% and 4.62% (viable: 3.33% to 5.18%) in gamma irradiations respectively (Fig 2A-D). Mutation frequency is not dose dependent in any of the treating agents. Across doses of treatments mutants appear in the following order: CdS-NPs - *thick stem>lax branching>thin dissected pinnae>early flowering>long petiole>broadleaf>chloroxantha=unidirectional branching=dwarf*; CuO-NPs - *thick stem>lax branching>thin dissected pinnae>long petiole>broad leaf>unidirectional branching>early flowering>dwarf=chloroxantha*; EMS - *thin dissected pinnae>lax branching>thick stem=long petiole>early flowering>chloroxantha>broad leaf=dwarf>unidirectional branching*; gamma irradiations-*thick stem=early branching>lax branching>broad leaf=unidirectional branching=dwarf>thin dissected pinnae=chloroxantha>long petiole*. It is remarkable that both NPs, EMS and gamma irradiations yield similar types of mutant.

The predominant mutants namely, *thick stem*, *lax branching* and *thin dissected pinnae* are found to occur in all the treating agents. The mutant types are statistically differentiated in relation to normal trait(s) in untreated control plants (Table 2). The *chloroxantha* mutant flowered but failed to set seeds. No mutation is scored in controls (untreated control and bulk controls).

Table 2 Mutant trait (s) in comparison to control.

Mutants	Attributes	Plant types		t-value at 18 DF	Probability level
		Control	Mutant		
Thin dissected pinnae	Length (cm)	2.78±0.04	1.06±0.11	84.95	>0.001
	Width (cm)	0.24±0.03	0.48±0.05	4.03	>0.001
Thick stem	Diameter (cm)	0.26±0.01	0.47±0.05	4.27	>0.001
Broad leaf	Length (cm)	3.19±0.05	4.45±0.05	18.44	>0.001
	Breadth (cm)	2.95±0.01	4.21±0.03	49.41	>0.001
Long petiole	Length (cm)	8.71±0.14	12.11±0.07	22.47	>0.001
Lax branching	Angle of divergence from main axis	38.60º±1.03	63.50º±1.36		
Early flowering	Days	58-60	42-45		
Dwarf	Height (cm)	40.70±1.18	18.00±1.02	15.38	>0.001

Mutagenic effectiveness and efficiency

Mutagenic effectiveness (Tables 3 and 4) related to mutational events is found higher in CuO-NPs (14.18 to 1.68) followed by CdS-NPs (8.38 to 1.57), EMS (6.16 to 1.17) and gamma irradiations (1.14 to 0.15). In all cases, initial doses are most effective. NPs seem to be more effective than EMS and gamma irradiations. Mutagenic efficiency (Tables 3 and 4) is defined as the number of mutational events to undesirable effects (lethality, injury and sterility) and is found variable in relation to treating agents; however, threshold doses are most efficient in all cases. Mutagenic efficiency is also higher (highest in CuO-NPs) in NPs than the conventional mutagens.

Table 3 Mutagenic effectiveness and efficiency in NPs treatments.

NPs	Treatments		Per cent of control			Viable mutation frequency (%)	Mutation effectiveness	Mutation efficiency		
	Concentration (µg/ml)	Duration (h)	Lethality (L)	Injury (I)	Sterility (S)		Mf/c×t Mf/kR	Mf/L	Mf/I	Mf/S
CdS-NPs	0.25	2	7.23	15.27	32.89	4.19	8.38	0.58	0.27	0.13
	0.50	2	15.66	39.65	42.62	5.49	5.49	0.35	0.14	0.13
	1.00	2	28.92	48.60	46.64	5.94	2.97	0.21	0.12	0.13
	0.25	4	12.05	21.59	40.60	5.22	5.22	0.43	0.24	0.13
	0.50	4	26.51	40.38	50.34	4.58	2.29	0.17	0.11	0.09
	1.00	4	40.96	53.60	53.69	6.29	1.57	0.15	0.12	0.12
CuO-NPs	0.25	2	5.00	32.79	19.40	7.09	14.18	1.42	0.22	0.37
	0.50	2	22.50	49.55	29.31	7.23	7.23	0.32	0.15	0.25
	1.00	2	35.00	65.73	46.55	6.72	3.36	0.19	0.10	0.14
	0.25	4	10.00	33.83	32.76	9.85	9.85	0.99	0.29	0.30
	0.50	4	27.50	54.30	39.66	7.78	3.89	0.28	0.14	0.20
	1.00	4	37.50	74.78	51.72	6.57	1.64	0.18	0.09	0.13

Table 4 Mutagenic effectiveness and efficiency in EMS and gamma irradiated samples.

Mutagens	Treatments		Per cent of control			Viable mutation frequency (%)	Mutation effectiveness	Mutation efficiency		
	Concentration(%)/ Gy	Duration (h)	Lethality (L)	Injury (I)	Sterility (S)		Mf/c×t Mf/kR	Mf/L	Mf/I	Mf/S
EMS	0.25	2	14.77	15.20	55.18	3.08	6.16	0.21	0.20	0.06
	0.50	2	28.41	39.40	64.15	4.27	4.27	0.15	0.11	0.07
	1.00	2	34.09	65.20	72.83	1.75	0.88	0.05	0.03	0.02
	0.25	4	25.00	26.55	66.39	2.34	2.34	0.09	0.09	0.04
	0.50	4	40.91	38.74	71.99	3.41	1.71	0.08	0.09	0.05
	1.00	4	51.14	69.89	77.87	4.68	1.17	0.09	0.07	0.06
Gamma irradiations	50	–	15.91	44.37	35.01	5.70	1.14	0.36	0.13	0.16
	100	–	20.45	49.81	24.93	5.16	0.52	0.25	0.10	0.21
	200	–	7.95	55.16	47.06	3.33	0.17	0.42	0.06	0.07
	300	–	10.23	38.56	6.72	4.41	0.15	0.43	0.11	0.66

Table 5 Meiotic configurations and pollen grains fertility in control and mutant plant types.

Plant Types	No. of PMCs scored at Metaphase I	Mean chromosome/cell		No. of meiocytes scored at Anaphase I	Frequency of balanced AI cells (11/11)	No. of meiocytes scored at AII	Frequency of cytologically balanced AII (%)	Pollen grains fertility	
		II	I					No. of pollen grains scored	Frequency (%)
Control (untreated dry)	60	10.85	0.30	127	100.00	92	100.00	551	96.91
Chloroxantha	65	10.06	1.88	90	93.33	66	92.42	310	55.16
Thin dissected pinnae	62	10.21	1.58	112	96.43	89	95.51	473	92.39
Broad leaf	53	10.17	1.66	106	92.45	78	94.87	630	91.11
Long petiole	32	10.19	1.63	98	94.90	70	95.89	566	89.05
Thick stem	46	10.61	0.78	103	96.12	94	97.87	583	93.48
Lax branching	55	10.80	1.60	87	96.55	79	92.41	684	93.42
Unidirectional branching	86	10.17	1.65	114	93.86	90	96.67	610	83.11
Early flowering	60	10.12	1.77	103	95.15	94	98.94	572	90.73
Dwarf	46	10.09	1.83	91	98.90	64	96.88	413	86.20
χ^2 test of heterogeneity *p* value at 9 DF		>0.05	<0.05						

Meiotic analyses

Meiotic chromosome configurations studied in mutant and control plant types (Fig 3A-F) reveal 2n = 22 (n = 11) chromosomes always (Table 5) with predominant of 11 bivalent formation (control: 85.00%; mutant: 48.88%-*thin dissected pinnae* to 87.21%-*lax branching*). Average chromosome association per cell at MI is 10.85II ± 0.30I in untreated dry control and it ranges from 10.61II ± 0.78I (*thick stem*) to 10.06II ± 1.88I (*chloroxantha*) among the mutants. Bivalents frequency is randomly distributed ($p>0.05$); while, univalent show non-randomness ($p<0.05$) in distribution among the plant types. The meiocytes show cytologically balanced chromosomes segregation at AI (100.00%) and AII (100.00%) in control and it ranges 98.90% to 92.45% in AI and 98.94% to 92.42% in AII among the mutants (Table 5). Occasionally, mutants. Pollen grains fertility is higher in untreated control (96.19%) than the mutants (93.48% to 55.16%). Meiotic analyses highlight that possibly the mutants are the consequences of genetic outcome rather than cytological disturbances.

The mutants bred true at M_3. The mutants raised are significant for direct uses (*thick stem*, *broad leaf*, *lax branching*, *unidirectional branching*, *early flowering*) as well as for efficient breeding (*long petiole*, *dwarf*). Under proper agronomic managements and selection practices, the mutants can be more effectively explored for crop improvement in the species.

NPs inducing phenotypic mutation corroborates with earlier reports in *Macrotyloma uniflorum* (Halder *et al,* 2015a,b),

Nigella sativa (Kumbhakar *et al,* 2016b) and *Indigofera tinctoria* (Ghosh *et al,* 2017). Possibility of gene mutation in plant species following NPs treatments is predicted earlier (Singh *et al,* 2009; Khanna *et al,* 2015; Langie *et al,* 2015). Vidal *et al* (2001) highlighted that Ag-NPs cause DNA lesion which mispaired with normal bases during replication inducing gene mutation. Nel *et al* (2006) opined that carbon nanotubes (CNTs) counteracting with DNA cause damage or fixed into mutation.

CONCLUSION

This article highlights the following: 1) CdS-NPs and CuO-NPs can act as mutagens and can induce similar types of mutation as that induced by the conventional mutagens, 2) mutant types reveal that CdS- and CuO-NPs can target the identical hotspot in the genome alike to EMS and gamma irradiations and 3) enhanced mutagenic effectiveness and efficiency of NPs over EMS and gamma irradiations is most promising for their use in any mutation breeding programme. Thus, it may be inferred that NPs due to their operational simplicity and cost effectivity can be an alternative source of conventional mutagens.

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Conflict of Interest

Authors declare that there is no conflict of interest.

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