RESEARCH ARTICLE

COPPER AND CADMIUM SULPHIDE NANOPARTICLES CAN INDUCE MACROMUTATION IN *NIGELLA SATIVA* L. (BLACK CUMIN)

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Abstract: Dry seeds(moisture content: 5.0%) of *Nigella sativa* L. (Family: Ranunculaceae; common name- black cumin, spice of commerce with immense therapeutic uses) are exposed to chemically synthesized copper (Cu) and cadmium sulphide (CdS) nanoparticles (NPs) at the doses of 0.25, 0.50 and 1.00 µg/ml for 3 and 6 h durations. EMS (ethyl methanesulphonate−0.25, 0.50 and 1.00%, 3 and 6 h durations) and gamma irradiations (25, 50, 100, 200 and 300 Gy; ^{60}Co source) are used as positive control. The objective of the work is to foresee whether NPs can induce stable phenotypic mutation. The present communication highlights macromutation types and frequency, mutagenic efficiency and effectiveness and meiotic chromosome behaviour in treated materials and suggests the efficacy if NPs in inducing mutation in *N.sativa* and crop improvement.

Keywords: Cu- and CdS-NPs, Macromutants, Meiotic analysis, Mutagenic efficiency and effectiveness, *Nigella sativa*

INTRODUCTION

The nanoparticles (NPs) are characterized by their small size and large surface and large surface area (ranging between 1 and 100 nm− Roco 2003) and possess unique physico-chemical properties (Remédios*et al.* 2012; Masarovičová and Králová 2013). NPs has global significance due to its wide application in industry, medicine, biotechnology, agriculture, different aspect of life sciences among others (Roco 2003, Lam *et al.* 2004, Caruthers *et al.* 2007, Nowack and Bucheli 2007, Scrinis and Lyons 2007, Singh *et al.* 2008, Nair *et al.* 2010, Castiglione *et al.* 2011,Remédios*et al.* 2012). Most significantly, Halder*et al.* (2015 a, b) reported that chemically synthesized copper (Cu) and cadmium sulphide (CdS) NPs can induce stable heritable changes (macromutation) in *Macrotylomauniflorum* (Lam.) Verdc (Family: Leguminosae). Furthermore, Kumbhakar*et al.* (2016) highlighted the potentiality of Cu- and CdS-NPs as mutagenic agents in *Nigella sativa* L. (Family: Ranunculaceae; common name- black cumin) as a test material. Such findings trigger the essentiality to attain further scientific knowledge from plant system on induced mutagenesis following NPs treatment. With the view to it, the present investigation has been designed and describes the mutagenic efficiency, mutagenic effectiveness and macromutation types, frequencies and their meiotic chromosome behaviour in *N. sativa*L. (spice yielding plant of commerce with immense therapeutic uses- Datta*et al.* 2012) following treatments with chemically synthesized copper and cadmium sulphide nanoparticles in comparison with the conventional mutagens namely, ethyl methanesulphonate (EMS) and gamma irradiation. The objective of the present study is to assess whether NPs can induce similar genetic variations as that of the well established mutagens under study.

MATERIAL AND METHOD

Germplasm

Seed samples of *Nigella sativa* L. (Ranunculaceae) were collected from Medicinal Plant Garden, NarendrapurRamkrishna Mission, Government of West Bengal, India. The moisture content of the mother seed stock was determined as 5.0%.

Synthesis and characterization of NPs

Cu- and CdS-NPs were prepared using wet chemical co-precipitation methods as described earlier by Chatterjee *et al.* (2012) and Halder*et al.* (2015 b) respectively.The NPs were characterized using UV− visible spectra (UV-vis), Fourier transform infra-red spectroscopy (FTIR), X-ray diffraction (XRD), Dynamic Light Scattering (DLS), Field emission scanning electron microscopy (FESEM) and Transmission electron microscopy (TEM)for assessment of their nature and size (Kumbhakar*et al.* 2016). Opto-physical studies of the prepared NPs confirm nano standard quality (Kumbhakar*et al.* 2016).

Treatments

Dry and filled seeds of *N. sativa* were exposed to the prepared solutions of Cu- and CdS-NPs (0.25, 0.50 and 1.00 µg/ml, 3 and 6 h durations) and EMS (0.25, 0.50 and 1.00 %, 3 and 6 h durations; solution prepared in 0.2 M phosphate buffer, pH 6.8). Dry seeds were also exposed to gamma irradiation doses (25, 50, 100, 200 and 300 Gy, source ^{60}Co,

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absorbance dose rate 47.4 Gy/min, source to distance 12 cm). Dry control and bulk Cu- and CdS controls (0.25 µg/ml, 3 h) were also kept for assessment. Each lot of treatment comprised of 100 seeds.

Assessment of biological damages

Biological damages (M_1 generation attributes) like lethality, injury and sterility were ascertained from seed germination, seedling growth and seed yield per plant respectively. The extent of lethality and injury were determined from the relative reduction of seed germination frequency and seedling growth in treated samples (under controlled Petri plate conditions- as was suggested by Konzak*et al*. 1965) as compared to controls (per cent of control). Seed sterility was assessed in each treatment (seeds of each plant was weighed on harvest) and is represented as percentage of reduction in seed weight in treatment in relation to controls (Dubey and Datta 2014).

Raising of M_1 and M_2 plant population

Fifty seeds from each treatment including controls were sown in the experimental field plots of Department of Botany, University of Kalyani (West Bengal plains, Nadia, latitude 22˚50′ to 24˚11′ N, longitude 88˚09′ E to 88˚48′ E, elevation 48 ft above sea level, sandy loamy soil, pH 6.85) in late November to raise M_1 plant population. The plants were harvested in mid-April.

Selfed seeds (first formed flower was bagged in each case) of each surviving M_1 plants were harvested separately and were used to raise M_2 (plant to row) generation. Control lines were also grown. Plants were grown having 15 cm between plants and 25 cm between rows. No fertilizer was applied during any stage of plant growth (both at M_1 and M_2).

Screening of phenotypic (Macromutants) mutants

Macromutants were carefully screened at M_2 from seedling to maturity and the frequency of the mutants was estimated as per 100 plants in accordance with Gaul (1964). Chlorophyll mutants were classified after Blixt 1961. Seedling colors were laid down with reference to RAL COLOUR CHART (UK). The mutant trait(s) were confirmed at M_3 from selfed segregation of M_2 mutants.

Mutagenic efficiency and effectiveness

The efficiency and effectiveness of NPs, EMS and gamma irradiations were calculated from viable (total) mutation frequency (Walther 1969) as proposed by Konzak*et al.* (1965). The mutagenic efficiency was calculated as Mf/L, Mf/I and Mf/S and the effectiveness as (Mf/c) × t and Mf/Gy unit converted to kR (Mf = mutation frequency, L = lethality, I = injury, S = sterility, c = concentration, t = duration, Gy = gray unit and kR = kiloroentgen).

Meiosis

Meiotic analysis was performed in controls and in mutant plant types. For the purpose, floral buds (2 to 3 in each case) of suitable sizes were fixed (5 a.m. to 6.30 a.m.) in acetic alcohol 1:3 for overnight and preserved in 70% alcohol under refrigeration. Pollen mother cells (PMCs) and pollen grains obtained from anther squash preparations were stained in 2% acetocarmine solution. Fully stained pollen grains were considered fertile (Marks 1954). Scattered metaphase (MI) and anaphase I (AI) cells were only scored for analyses. Photomicrographs were taken from suitable preparations.

RESULT AND DISCUSSION

Mutation types andfrequencies

In comparison to normal trait(s) (Fig 1a), a total of 14 macromutant types (Table 1) are screened at M_2 mutagenized population (3357 plants scored). Out of 14 types, 9 in EMS, 6 in gamma irradiations, 13 in Cu-NPs and 14 in CdS-NPs are spotted. The mutants comprise of two non-viable types namely, *chloroxantha* (the plants dried at flowering stage: 51-57 days from sowing, bright yellowish green colored seedlings−Fig. 1b) and *viridis* (medium green colored seedlings, slow growing with reduced height, dried at flowering stage). The viable mutants are*sparsely arranged pinnae* I (associated with broad elongated pinnae−Fig. 1c) and II (Fig. 2d−e), *narrow pinnae*, *crumpled pinnae* (upward folding of pinnae to form cup like structure), feathery leaf, heterophyllous leaf (broad and narrow pinnae both present − Fig. 1f), cluster pinnae top, long petiole (Fig. 1g), thick stem (with associated bushy trait − Fig. 1h), dwarf, early flowering (37 to 39 days from sowing compared to 47 to 60 days in control plants) and synchronous maturity (Fig. 1i). Of all the mutants, synchronous maturity plant type is most significant as it will minimize seed loss at harvest. This mutant type appeared only in NPs treatments. The mutant *cluster pinnae top* is found specific to CdS-NPs treatments. The predominant mutant plant types recorded in all treated materials are *heterophyllous leaf*, *long petiole*, *sparsely arranged pinnae* I and II and *narrow pinnae*. No mutant was scored in controls. The mutant trait(s) in comparison to normal is presented in Table 2.

Across doses of treatments it seems that EMS has induced higher mutation frequency (viable: 9.95%, total: 11.44% than gamma irradiations (viable: 6.32%, total: 6.32%), Cu-NPs (viable: 5.95%, total: 6.44%) and CdS-NPs (viable: 3.92%, total: 4.26%). Higher mutation frequency in EMS than other treating agents may be due to low number of M_2 plant scored. Total failure of germination is recorded in 1.00% EMS, 3 and 6 h durations. Over the M_2 population, the macromutants (Table 1) occurred in the following order: EMS−*sparsely arranged pinnae* II >*chloroxantha* = *long petiole* = *dwarf*>*early*

flowering = narrow pinnae = sparsely arranged pinnae I >*heterophyllous leaf = thick stem*; gamma irradiation− *heterophyllous leaf*>*sparsely arranged pinnae* II >*long petiole*>*dwarf = narrow pinnae = sparsely arranged pinnae* I; Cu-NPs− *sparsely arranged pinnae* II>*heterophyllous leaf = synchronous maturity*>*dwarf*>*narrow pinnae = long petiole*>*sparsely arranged pinnae* I = *feathery leaf*>*viridis*>*chloroxantha = crumpled pinnae = thick stem = early flowering*; CdS-NPs−*sparsely arranged pinnae* II >*narrow pinnae* >*heterophyllous leaf* >*crumpled pinnae = cluster pinnae top = early flowering*>*sparsely arranged pinnae* I = *dwarf*>*viridis = feathery leaf = long petiole*>*thick stem*>*synchronous maturity*>*chloroxantha*. Apart from EMS (0.50%, 6 h−16.67%), maximum mutation (viable) frequency is mostly found in initial doses of gamma irradiations (25Gy− 8.82% and 50 Gy− 6.61%), Cu-NPs (0.50 µg/ml, 3 h−11.11%; 0.50 µg/ml, 6 h−16.37% and CdS-NPs (0.25 µg/ml, 3 h− 4.33%). The M_2 mutants segregated at M_3 in accordance with Mendelian patterns (data not given in the present communication) suggesting that the altered trait(s) than normal are genetically controlled.

Mutagenic effectiveness and efficiency

The mutagenic effectiveness (Tables 3 and 4) relates doses to mutation and is found to be maximum in EMS− 0.50%, 6 h, 25 Gy gamma irradiations, 0.50 µg/ml, 3 and 6 h Cu-NPs, 0.25µg/ml, 3 h CdS-NPs treatments.

On comparative basis it can be suggested that NPs are effective as mutagens like EMS and gamma irradiations. The mutagenic efficiency defined as the relation of number of mutational events to undesirable effects (lethality, injury and sterility) and is found to vary in relation to treating agents, and it seems that mostly threshold doses are efficient.

Meiotic analysis and pollen grains fertility

Meiotic chromosome behaviour is nearly normal and comparable in controls as well as in macromutants,PMCs regularly show 2n=12 chromosomes (Fig. 2 a−f) always. Controls show 6II formation in 100% meiocytesand it varies from 97.78% (*chloroxantha*) to 58.28% (*thick stem*) in mutants. Most of the univalents scored are rather due to precocious separation of chromosomes as mostly AI cells in mutants are balanced (92.45% to 100.0%). Rarely 1 to 2 laggards are observed at AI (Fig. 2g−h) and AII (Fig. 2i). Pollen grain fertility is assessed in controls (dry− 95.80%; bulk Cu− 95.79% and bulk CdS− 89.87%) as well as in mutants (70.25% in *viridis* to 89.81% in *heterophyllous leaf*).

The present investigation reveals the potentiality of Cu- and CdS-NPs in inducing macromutation in *N. Sativa*, which is corroborating to earlier reports in *Macrotylomauniflorum* (Halder*et al.* 2015 a, b). Thus, NPs inducing genetic changes can be explored for crop improvement.

Table 1. Macromutant types and frequency across doses in treatments.

Mutant types	Frequency (%)			
	EMS	Gamma irradiations	CdS-NPs	Cu-NPs
Chloroxantha	1.49	0.00	0.10	0.20
Viridis	0.00	0.00	0.24	0.29
Sparsely arranged pinnae I	0.99	0.55	0.34	0.39
Sparsely arranged pinnae II	1.99	1.65	0.63	1.37
Narrow pinnae	0.99	0.55	0.53	0.49
Crumpled pinnae	0.00	0.00	0.39	0.20
Feathery leaf	0.00	0.00	0.24	0.39
Heterophyllous leaf	0.50	2.20	0.48	0.78
Cluster pinnae top	0.00	0.00	0.39	0.00
Long petiole	1.49	0.82	0.24	0.49
Thick stem	0.50	0.00	0.19	0.20
Dwarf	1.49	0.55	0.34	0.68
Early flowering	0.99	0.00	0.39	0.20
Synchronous maturity	0.00	0.00	0.15	0.78
Total plants scored	201	364	2067	1025

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

Mutants	Attributes	t-value	DF	Probability
Sparsely arranged pinnae I	Interpinnae distance Control-2.17±0.053	10.77	16	>0.001
(associated trait)	Mutant-3.49±0.110			
	Broad and elongated pinnae Length: control-2.22±0.029 mutant-3.06±0.040	18.78	18	>0.001
	Width:	5.52	16	>0.001

	control-0.23±0.017 mutant-0.36±0.018			
Sparsely arranged pinnae II	Interpinnae distance Control-2.16±0.047 mutant-2.83±0.128	4.90	16	>0.001
Narrow pinnae	Pinnae Length: control-2.22±0.290 mutant-0.76±0.037	29.68	18	>0.001
	Width: control-0.23±0.016 mutant-0.16±0.018	2.97	16	>0.01
Long petiole	Petiole length Control-5.62±0.084 Mutant-14.04±0.318	24.99	20	>0.001
Thick stem	Stem thickness Control-0.21±0.023 mutant-0.52±0.025	9.12	18	>0.001
Dwarf	Height Control-31.38±1.632 mutant-4.45±0.132	16.45	18	>0.001

Table 3. Mutagenic efficiency and effectiveness of EMS and gamma irradiation treatments in M_2 generation.

Treatments	(%/Gy)	Duration (h)	Per cent of control			Viable Mutation frequency (%) (Mf)	Mutagenic effectiveness (Mf/conc.)×duration (Mf/kR)	Mutagenic efficiency		
			Lethality (L)	Injury (I)	Sterility (S)			Mf/L	Mf/I	Mf/S
EMS	0.25	3	13.89	34.38	77.41	9.09	12.12	0.26	0.15	0.36
	0.50	3	13.89	50.91	75.51	8.77	5.84	0.11	0.05	0.07
	1.0	3	33.33	73.82	–	–	–	–	–	–
	0.25	6	8.33	41.87	75.04	6.67	2.73	0.74	0.16	0.18
	0.50	6	22.22	59.93	82.46	16.67	0.54	–	0.36	0.03
	1.0	6	94.44	94.16	–	–	–	–	–	–
Gamma irradiations	25	–	–	10.07	44.08	8.82	3.53	–	0.88	0.20
	50	–	22.22	6.91	67.14	6.61	1.76	0.30	0.96	0.09
	100	–	5.56	32.34	78.36	4.62	0.46	0.83	0.14	0.06
	200	–	52.78	62.84	100.00	–	–	–	–	–
	300	–	83.33	84.82	100.00	–	–	–	–	–

Table 4. Mutagenic efficiency and effectiveness of Cu- and CdS-NPs treatments in M_2 generation.

Treatments (%/Gy)		Duration (h)	Per cent of control			Viable Mutation frequency (%) (Mf)	Mutagenic effectiveness (Mf/conc.)× duration	Mutagenic efficiency		
			Lethality (L)	Injury (I)	Sterility (S)			Mf/L	Mf/I	Mf/S
CdS-NPs	0.25	3	16.67	28.51	11.91	4.33	5.77	0.26	0.15	0.36
	0.50	3	22.22	47.29	36.86	2.55	1.70	0.11	0.05	0.07
	1.0	3	8.33	0.49	17.01	5.42	1.81	0.65	11.06	0.32
	0.25	6	5.56	25.04	21.93	4.09	2.73	0.74	0.16	0.18
	0.50	6	–	4.55	40.68	1.62	0.54	–	0.36	0.03
	1.0	6	13.89	42.48	41.13	9.25	1.54	0.67	0.22	0.22
Cu-NPs	0.25	3	13.88	11.63	16.26	1.93	2.57	0.14	0.17	0.12
	0.50	3	2.77	32.09	25.35	11.11	7.41	4.01	0.35	0.44
	1.0	3	13.88	14.01	32.52	1.92	0.64	0.14	0.14	0.06
	0.25	6	5.55	6.31	31.47	5.70	3.80	1.03	1.03	0.18
	0.50	6	2.77	–	44.23	16.37	5.46	5.91	5.91	0.37
	1.0	6	5.55	10.07	43.53	2.88	0.48	0.52	0.52	0.06

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Figure plate 1 (a−i) showing control **(Fig. a)** and macromutant plant types **(b−i)** of *N. sativa*. **(Fig. a)** control; **(Fig. b)***chloroxantha*; **(Fig. c)***sparsely arranged pinnae* I-associated trait broad and elongated pinnae; **(Figs. d−e)***sparsely arranged pinnae* II; **(Fig. f)***heterophyllous leaf*; **(Fig. g)***long petiole*; **(Fig. h)***Thick stem* with bushy trait; **(Fig. i)** mutant showing synchronous maturity.

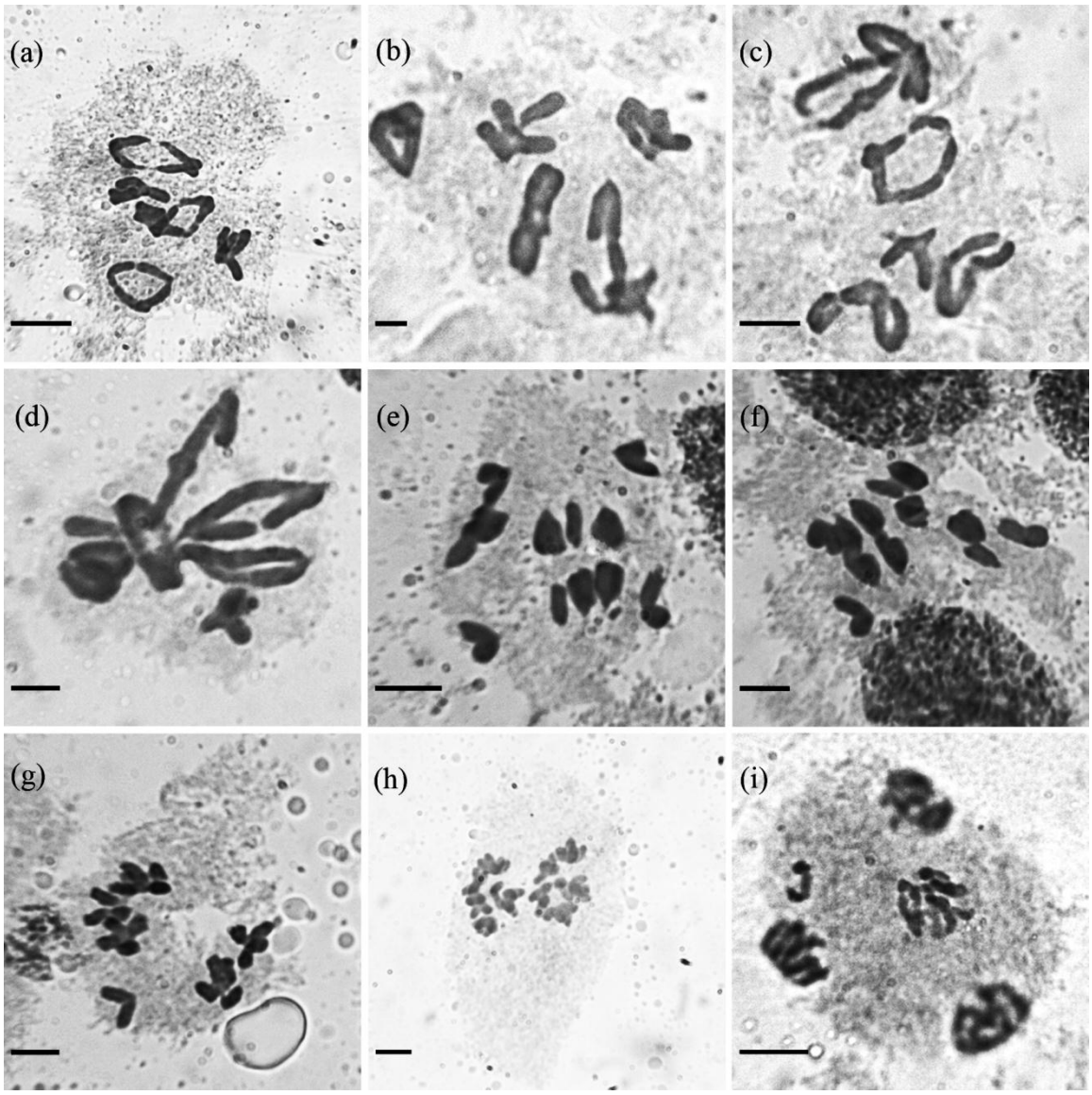

Figure plate 2 (a−i) showing meiosis configuration in mutants at MI **(a−f)**, AI **(g−h)** and AII **(i)**. **(Figs. a−d)** 6II (2n=12); **(Fig. e)** 1II+10I; **(Fig. f)** 2II+8I; **(Figs. g−h)** 5-1-6 separation of chromosomes; **(Fig. i)** a laggard. Scale bar=10µm.

REFERENCES

Blixt, S. (1961).Quantitative studies of induced mutations in peas. V. Chlorophyll mutations.*Agric. Hort. Genet.Landskrona.***19:**171-183.

Caruthers, S.D., Wickline, S.A. and Lanza, G.M. (2007).Nanotechnological applications in medicine.*Curr.Opin. Biotechnol.***18** (1): 26–30.

Castiglione, M.R., Giorgetti, L., Geri, C. and Cremonini, R. (2011). The effects of nano-TiO_2 on seed germination, development and mitosis of root tip cells of *Vicianarbonensis* L. and *Zea mays* L. *J. Nanopart. Res.* **13**: 2443–2449.

Chatterjee, A.K., Sarkar, R., Chattopadhyay, A.P., Aich, P., Chakraborty, R. and Basu, T.A. (2012).Simple robust method for synthesis of metallic copper nanoparticles of high antibacterial potency against E. coli.*Nanotech.***23** (085103): 1−11.

Datta, A.K., Saha, A., Bhattacharya, A., Mandal, A., Paul, R. and Sengupta, S. (2012). Black cumin (*Nigellasativa* L.) – a review.*J. Plant Dev. Sci.***4** (1): 1–43.

Dubey, P.K. and Datta, A.K. (2014).Induced mutagenesis in *Abelmoschusmoschatus* (L.)Medik.*Int. Res. J. Pharm.*, **3** (5): 432–435.

Gaul, H. (1964). Mutation in plant breeding.*Rad. Bot.* **4**: 155−252.

Halder, S., Mandal, A., Das, D., Gupta, S., Chattopadhyay, A.P. and Datta, A.K. (2015a). Copper nanoparticle induced macromutation in *Macrotylomauniflorum* (Lam.) Verdc. (Family: Leguminosae): a pioneer report. *Genet.Resour. Crop Evol.***62** (2): 165−175.

Halder, S., Mandal, A., Das, D., Datta, A.K., Chattopadhyay, A.P., Gupta, S. and Kumbhakar, D.V. (2015b). Effective potentiality of synthesisedCdS nanoparticles in inducing genetic variation on *Macrotylomauniflorum* (Lam.) Verdc.*BioNanoSci.***5** (3): 171−180.

Konzak, C.F., Nilan, R.A., Wagner, J. and Foster R.J. (1965). Efficient chemical mutagenesis.*Rad. Bot. (Suppl.)*. **5**: 49–70.

Kumbhakar, D.V., Datta, A.K., Mandal, A., Das, D., Gupta, S., Ghosh, B., Halder, S. and Dey, S. (2016). Effectivity of copper and cadmium sulphide nanoparticles in mitotic and meiotic cells of *Nigella sativa* L. (black cumin) – can nanoparticles act as mutagenic agents? *J. Exp. Nano. Sci*. 1− 17.doi: 10.1080/17458080.2016.1149236.

Lam, C.W., James, J.T., McCluskey, R. and Hunter, R.L. (2004). Pulmonary toxicity of single-wall carbon nanotubes in mice 7 and 90 days after intratracheal instillation.*Toxicol. Sci.***77** (1): 126−134.

Marks, G.E. (1954). An aceto-carmine glycerol jelly for use in pollen-fertility counts. *Stain Technol.*, **29:** 277.

Masarovičová, E. and Kráľová, K. (2013).Metal nanoparticles and plants.*Ecol. Chem. Eng. S.* **20** (1): 9−22.

Nair, R., Varghese, S.H., Nair, B.G., Maekawa, T., Yoshida, Y. and Sakthi D. K. (2010). Nanoparticulate material delivery to plants. *Plant Sci.***179** (3): 154–163.

Nowack, B. andBucheli, T.D. (2007).Occurrence, behavior and effects of nanoparticles in the environment.*Environ. Pollut.***150** (1): 5−22.

Remédios, C., Rosario, F. and Bastos, V. (2012). Environmental nanoparticles interactions with plants: morphological, physiological, and genotoxic aspects. *J. Bot.*Article ID 751686,1−8.

Roco, M.C. (2003). Broader societal issues of nanotechnology.*J. Nanopart. Res*., **5** (34): 181−189.

Scrinis, G. and Lyons, K. (2007). The emerging nano-corporate paradigm: nanotechnology and the transformation of nature,food and agri-food systems. *Int. J. Sociol. Agric. Food.* **15** (2): 22−44.

Singh, M., Singh, S., Prasada, S. and Gambhir, I.S. (2008).Nanotechnology in medicine and antibacterial effect of silver nanoparticles. Digest *J. Nanomater. Biostruct.***3** (3): 115−122.

Walther, F. (1969).Effectiveness of mutagen treatments in ionizing radiation in barley.Induced mutation in plants.*Proc. symp*.On the nature, induction and utilization of mutations in plants.IAEA-FAO.Pullman wash; 261−270.