Copper Oxide Nanoparticles Induced Fertile Desynaptic Mutant Line in Coriandrum sativum L. (Apiaceae)

Ankita Pramanik1, Animesh Kumar Datta1*, Sudha Gupta2 and Bapi Ghosh1

1 Department of Botany, Cytogenetics, Genetics and Plant Breeding Section, University of Kalyani, Kalyani–741235, West Bengal, India
2 Department of Botany, Pteridology-Paleobotany Section, University of Kalyani, Kalyani–741235, West Bengal, India

Received August 9, 2017; accepted October 28, 2017

Summary A desynaptic plant (2n=22) is screened from copper oxide nanoparticles (CuO-NPs) treated (dry seeds exposed to 0.25 µg mL−1, 4 h) M1 population (one from 47 plants) of Coriandrum sativum L. (moisture content: 13.60%). Selfed seeds of the marked plant also yield a desynaptic plant (one plant out of five raised; 50 seeds sown) at M2. The mutant plants show enhanced univalent frequency (diplotene-diakinesis: 0–14/cell, metaphase I (MI): 0–20/cell) and reduced chiasma/cell (diplotene-diakinesis: 12.58±0.95 to 12.20±1.34; MI: 12.11±1.13 to 11.64±0.97) than dry and bulk controls (diplotene-diakinesis: univalent frequency 0–4/cell, chiasma/cell 14.77±1.20 to 13.00±0.92; MI 0–4/cell, chiasma/cell 13.85±0.93 to 12.91±0.49). The marked plants document unequal chromosome segregation at anaphase I (AI) (10/12, 9/13, 8/14 and 6/16) and anaphase II (AII) (unequal groups) compared to cytologically balanced separation in controls. Pollen grains fertility is also reduced in the mutant plants (83.36 to 77.76%) than controls (96.91 to 89.88%). Both the M1 and M2 desynaptic plants yield seeds. Results suggest that CuO-NPs can alter meiotic synchrony of genes controlling retention of chiasma formation and maintenance, leading to the development of desynaptic mutant line.

Key words Coriandrum sativum, Copper oxide nanoparticle, Desynapsis, Meiosis, Fertile.

The process of meiosis is genetically conditioned and programmed by set of genes which function coordinately to control the phenomenon of synapsis, recombination, reduction division, isolation of cross over chromatids during gamete formation, fertility among others (Zickler and Kleckner 1999, Armstrong and Jones 2003, Pankratz and Forsburg 2005). As and Ds are two groups of meiotic genes controlling homologous chromosome pairing behavior (synapsis), and under homozygous recessive conditions (as as/ds ds) they cause pairing failure (Gottschalk and Klein 1976). The ‘ds ds’ genes acting on diplotene-diakinesis bring about failure of chiasma formation or inability to generate or retain chiasmata leading to the formation of univalents, and the phenomenon is coined as desynapsis (Li et al. 1945, Reiger et al. 1968) or dys-synapsis (Kaul and Nirmala 1993). A better alternative term for desynaptic is proposed by Riley and Law (1965). Male meiotic mutant like desynapsis is reported both from natural (vide Koduru and Rao 1981, Poddar et al. 1998, Armstrong and Jones 2003, Jackson et al. 2002, Sosnokhina et al. 2002, Maity and Datta 2009, Mandal and Datta 2011) and mutagen induced (Bozzini and Martini 1971, Gottschalk and Baqur 1971, Palmer 1974, Gottschalk and Kaul 1980, Datta and Biswas 1985, Bhattacharya and Datta 2012, Gulfishan et al. 2013, Naseem and Kumar 2013, Bhat and Wani 2015) population of different plant species. Apart from cytogenetical significance, desynaptic mutants can potentially induce aneuploids (Soost 1951, Burnham 1962, Jackson et al. 2002).

Nanoparticles (NPs) is small atomic aggregates, possessing unique opto-physical properties, size at least one dimension ranging from 1 to 100 nm (Roco 2003, Remédiós et al. 2012). They are reported to induce chromosomal variations in mitotic (Nair et al. 2010, Castiglione et al. 2011, Shaymurat et al. 2012, Raskar and Laware 2014, Nagaonkar et al. 2015, Ghosh et al. 2017) and in meiotic (Kumbhakar et al. 2016) cells of different plant species. Kumbhakar et al. (2017) first reported a sterile desynaptic mutant in Nigella sativa following treatment with chemically synthesized cadmium sulphide nanoparticles (CdS-NPs). The present article describes the meiotic configurations of fertile desynaptic mutant plants screened at M1 following copper oxide nanoparticles (CuO-NPs) treatment and M2 from the progenies of M1 plants of Coriandrum sativum L. (Apiaceae). The objective of the work is to highlight the potentiality of chemically synthesized CuO-NPs to induce cytological mutant(s) alike to that of conventional mutagens.

* Corresponding author, e-mail: dattaanimesh@gmail.com
DOI: 10.1508/cytologia.83.103
Materials and methods

Seed samples

Seed stocks as breeder’s seeds of Coriandrum sativum L. were obtained from Pulses and Oil Seeds Research Station, Department of Agriculture, Govt. of West Bengal, Berhampore, West Bengal.

Nanosuspension and treatments of seeds

CuO-NPs were prepared by wet chemical co-precipitation methods and were opto-physically characterized using different instrumentations for determination of nano standard quality. The nanosuspension used in the present investigation had a size range of 25.7 to 95.5 nm (mean: 28.7 ± 7.64 nm, polydispersity index=0.31) assessed from Dynamic light scattering with spherical to ellipsoidal particle geometry as evinced from Field emission scanning electron microscope.

Dry seeds of C. sativum were exposed to different doses of CuO-NPs (0.25, 0.50 and 1.00 µg mL−1, 2 and 4 h durations) treatments. Dry control, and bulk CuO (prepared identically as NPs but without the capping agent-sodium dodecyl sulphate) as control were also kept for assessment. In each lot, 200 seeds were treated and out of which 100 seeds were sown (20 and 30 cm distance between plants and rows, respectively) in the experimental field plots of Kalyani University to raise M1 generation during the months of November (3rd week) and April (4th week). No fertilizer application was made during the growth period of the plants.

Screening of the mutant

During routine male meiotic study of CuO-NPs treated M1 plant population, a semi-dwarf (23.4 cm height at maturity, control–39.6 ± 1.12 cm) plant without any marked phenotypic trait was screened (0.25 µg mL−1, 4 h; one out of 47 plants) to possess desynaptic behavior of chromosomes. The marked plant yielded 71 seeds (0.87 g) compared to 320 seeds/plant (4.93 ± 3.8 g) in dry control. Fifty selfed seeds of the M1 marked plant were sown in M2 generation and out of five raised plants only one plant was meiotically confirmed to possess desynaptic behavior of chromosomes.

Meiosis

Inflorescences (umbels) of the marked M1 plant and its progenies (M2) were fixed in acetic alcohol 1:3 (v/v) during 5:30 a.m. and 6:30 a.m. for 24 h. The inflorescences were subsequently preserved in 70% alcohol and kept under refrigeration for further uses. Anther squash preparations were performed and PMCs and pollen grains were stained in 2% aceto-carmine solution. Well-scattered meiocytes were evaluated at diplotene-diakinesis, MI, AI and AII and data were scored. Pollen grains fertility was assessed based on stainability (Marks 1954). Fully stained pollen grains were considered fertile. Photomicrographs were taken from suitable squash preparations.

Table 1. Meiotic attributes and seed yield in controls and in desynaptic mutant plants at M1 and M2.

<table>
<thead>
<tr>
<th>Attributes</th>
<th>Controls</th>
<th>Mutant</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dry</td>
<td>Bulk CuO</td>
</tr>
<tr>
<td>Diplotene:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of cells scored</td>
<td>35</td>
<td>39</td>
</tr>
<tr>
<td>Bivalent/cell</td>
<td>10.89</td>
<td>10.92</td>
</tr>
<tr>
<td>Univalent/cell</td>
<td>0.23</td>
<td>0.15</td>
</tr>
<tr>
<td>Chiasma/cell</td>
<td>13.91±0.56</td>
<td>14.77±1.20</td>
</tr>
<tr>
<td>PMCs with 11II (%)</td>
<td>91.43</td>
<td>92.31</td>
</tr>
<tr>
<td>MI:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of cells scored</td>
<td>65</td>
<td>60</td>
</tr>
<tr>
<td>Bivalent/cell</td>
<td>10.86</td>
<td>10.85</td>
</tr>
<tr>
<td>Univalent/cell</td>
<td>0.28</td>
<td>0.30</td>
</tr>
<tr>
<td>Chiasma/cell</td>
<td>13.09±0.62</td>
<td>13.85±0.93</td>
</tr>
<tr>
<td>Meiocytes with 11II (%)</td>
<td>87.69</td>
<td>85.00</td>
</tr>
<tr>
<td>AI:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cells scored</td>
<td>60</td>
<td>127</td>
</tr>
<tr>
<td>Balanced (11/11) AI cells</td>
<td>100.00</td>
<td>100.00</td>
</tr>
<tr>
<td>Unequal segregation (%)</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>AII:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PMCs analyzed</td>
<td>68</td>
<td>92</td>
</tr>
<tr>
<td>Cytologically balanced cells (%)</td>
<td>100.00</td>
<td>100.00</td>
</tr>
<tr>
<td>No. of pollen grains analyzed</td>
<td>886</td>
<td>551</td>
</tr>
<tr>
<td>Pollen grains fertility (%)</td>
<td>96.14</td>
<td>96.91</td>
</tr>
<tr>
<td>Seed set/plant</td>
<td>320±4.90</td>
<td>357±4.20</td>
</tr>
<tr>
<td>Seed yield (g)</td>
<td>4.93±3.80</td>
<td>5.21±4.10</td>
</tr>
</tbody>
</table>
Results and discussion

Meiotic chromosome configurations of control and mutants are presented in Table 1. Meiotic analysis documents $n=11$ chromosomes always in the studied (control and mutant plants) plant types (Figs. 1–16). Meiocytes predominantly form 11 II (Figs. 1 and 3) in diplotene-diakinesis (dry control: M1-91.43%, M2-92.31%; bulk control: M1-85.42%, M2-93.62%) and MI (dry control: M1-87.69%, M2-93.00%; bulk control: M1-91.18%, M2-94.74%) cells of controls. The mutant plants (M1 generation-diplotene: 11 II-44.71% to 5 II+12 I-2.35%; MI: 11 II-54.88% to 4 II+14 I-1.87%; MI: 1II-49.07% to 1II+20 I-0.93%) show variable chromosomal associations in their meiocytes (Figs. 1–11). Univalent frequency per cell is found more enhanced in the marked plants (0–14/cell at diploten-diakinesis; 0–20/cell at MI) than controls (0–4/cell at diplotene-diakinesis; 0–4/cell at MI).

Mean chromosome association per cell is 9.56 II+2.87 I (M1) and 9.30 II+3.40 I (M2) at diplotene and 9.63 II+2.73 I (M1) and 9.27 II+3.46 I (M2) at MI of the marked plants compared to 10.89 II+0.23 I (M1) and 10.92 II+0.15 I (M2) at diplotene-diakinesis and 10.85 II+0.30 I (M2) at MI of dry control plants. Univalents formed at diplotene-diakinesis in both M1 and M2 marked plants are found to lie in close proximity to one another suggesting residual attraction between homologues and their very recent separation. Furthermore, the univalents in the mutant plants are found both random (Figs. 4, 6, 8, 10, 11) as well as polar (Figs. 5, 7, 9) in orientations irrespective of the number of bivalents per cell corroborating earlier report of John and Lewis (1965). Chiasma frequency is reduced in mutants (diplotene-diakinesis: 12.20±1.34 at M1, 12.58±0.95 at M2; MI: 12.11±1.13 at M1, 11.64±0.97 at M2) than dry control (diplotene-diakinesis: 13.91±0.56 at M1, 14.77±1.20 at M2; MI: 13.09±0.62 at M1, 13.85±0.93 at M2) and the
reduction in all cases on comparison (on computation of Student’s t-test) is significant (p<0.05). Thus, enhanced univalent frequency along with reduced chiasma frequency in diplotene-diakinesis and MI cells of M₀ and M₁, mutant plants compared to controls suggest failure of synopsis due to impairment of homologues or due to non-retention of chiasmata. Such phenomenon of desynapsis occurring in a M₁ plant and its descendant is rather non-retention of chiasmata. Such phenomenon of desynapsis is of medium-strong type (based on univalent frequency/cell) as per classification proposed by Prakken (1943).

As compared to 100.00% cytologically balanced AI (Fig. 12) and AII cells, the desynaptic plants manifest 71.21% (M₁) and 74.36% (M₂) AI cells with equal (11/11) separation of chromosomes. The mutant plants show unequal segregation (M₁: 10/12–16.67%-Fig. 13, 8/14–3.03%-Fig. 14, 6/16–1.52%-Fig. 15; M₂: 10/12–8.97%, 9/13–5.13%, 8/14–2.56%, 6/16–2.56%) of chromosomes, bridge (Fig. 16) formation (M₁: 4.55%; M₂: 1.28%) and presence of 1–2 laggards (M₁: 3.03%; M₂: 5.13%) at AI. About 31.15% (M₁) and 26.56% (M₂) AII cells in the mutant plants are found abnormal (irregular groups, tripolarity and laggards). Pollen grain fertility ranges from 96.91 to 89.88% among the control plants and it is 83.36 to 77.76% in mutants. The mutant plants set seeds.

The desynaptic mutant is the outcome of基因 mutation in Ds group of genes leading to homozygous recessive condition (Sharma and Reinbergs 1974, Gottschalk and Klein 1976) which affects genetic control of chiasma formation and maintenance (Kitada and Omura 1983), and subsequently forms univalents in meiocytes.

Acknowledgements

The authors are thankful to Pulses and Oil Seeds Research Station, Department of Agriculture, Govt. of West Bengal for generous help for supplying of the germplasm.

References

Copper Oxide Nanoparticles Induced Fertile Desynaptic Mutant Line in Coriandrum sativum L. (Apiaceae)

Cell 16: 1651–1660.


