BLACK CUMIN (*NIGELLA SATIVA* L.) – A REVIEW

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Abstract: Black cumin (*Nigella sativa* L., Family: Ranunculaceae) is an annual herb possessing wide range of medicinal uses apart from its commercial significance as a spice yielding plant. Black cumin seeds are used in folk (herbal) medicine all over the world for the treatment and prevention of a number of diseases. Prophet Mohammad (Peace be Upon Him) said: "Use this Black Seed; it has a cure for every disease except death" (Sahih Bukhari). The plant species is also important cytogenetically and may be used as a model plant for better understanding of gene and chromosome relationship. Despite the major advancement of modern medicine in human health-care, it is still intangible and beyond reach to ailing humanity, especially the destitute and therefore in recent years plant based system has been utilized for traditional medicine and phytotherapy. 'Medicinal plants are gift of nature' and black cumin is one such plant with potential uses, which can be explore for safe and effective herbal medicine for human benefit. Considering nearly all essential aspects of the species (synonym(s), common names, origin of the name, distribution, varieties, plant description, floral biology, pollination biology, scanning electron microscopy of seed surfaces, cultivation, economy, diseases, pest, microscopical and powdered characteristics, biochemical constituents, extraction methods of essential oils, therapeutic uses, insecticidal activity, other uses, clinical trials, biosafety, tissue culture and patents), a monograph is prepared on the laid formulation of WHO (World Health Organization) as well as on other significant parameters (cytogenetics and molecular genetics) with the following objectives: to provide an unabridged repository of references regarding the species for its effective and safe utilization as a 'Potential Medicinal Herb'; for creating awareness regarding the use of plant based medicine; understanding economic status, biosafety and patents for regulating herbal medicinal market Nationally and Internationally and exploration of cytogenetical and genetical aspects.

Keywords: Black cumin, Herbal medicine, *Nigella sativa*

INTRODUCTION

Nigella sativa L. (Family: Ranunculaceae; commonly known as Black Cumin) is an annual herb possessing a wide range of medicinal uses[1,2] not withstanding its commercial significance as a spice yielding plant[3]. Black cumin seeds are most revered (Holy herb of the Middle East – Yarnell and Abascal[4]; can heal every disease except death – Islamic prophet Mohammad; stimulates body's energy and helps recovery from fatigue and dispiritedness – The Canon of Medicine, Avicenna; included in the list of natural drugs of 'Tibb-e-Nabavi'; valuable remedy for number of diseases – Unani Tibb system of medicine) medicinally. WHO (World Health Organization) is providing emphasis on the exploration of medicinal plant species for benefit of human care system. Emphasis has been laid mainly on scientific information, on the safety, efficacy, quality control / quality assurance, dosage, toxicity description of the plant species, therapeutic uses, clinical trials, drug interactions amongst other but genetic resources and its induction must also be taken into consideration for significant utilization of a plant species under consideration. Effective utilization of *N. sativa* for therapeutic purposes as well as for trade will vastly depend upon yield (raw plant product- seeds; bioactive compounds- essential oil) and its quality. Existing germplasms may not substantiate the need for future, if not, at present. Therefore, it is of utmost essentiality to raise desirable plant type(s) in *N. sativa* through induced genetic variations and efficient breeding endeavour. Considering nearly all essential aspects of *N. sativa*, a monograph is conducted with the laid formulation of WHO as well as with other significant parameters which will provide unabridged repository of references for present and future researchers who are looking to eugenize the species as a 'potential medicinal herb' for human benefits.

Synonym(s)

Nigella indica Roxb. ex Flem., *Nigella truncata* Viv.[5]

Common names

English: fennel flower, nutmeg flower, Roman coriander, blackseed or black caraway, black sesame; India: Assamese - kaljeera or kolajeera, Bengali - kalo jeeray, Kannada – Krishna Jeerige, Tamil - karum jeerakam, Hindi/Urdu - kalaunji/mangrail; Russian: Chernushka; Hebrew: Ketzakh; Turkish: çörek out; Arabic: habbat al-barakah; Persian: siyâh dâne; Indonesian: jintan hitam; Bosnian: čurekot[6];

French: nigelle de Crète, toute épice; Germany: Schwarzkümmel; Portuguese: cominho-negro; Spanish: ajenuz, arañuel; Swedish: svartkummin[7].

Origin of the name

Originally black cumin was the common name for *Bunium persicum* and later named as *Carum bulbocastanum*, which is now near extinction and slowly *Carum carvi* graduated to the name and due to inability of the species to all over India, later *N. sativa* was adopted from Portuguese or Turkish merchants[6].

Distribution

The species is cultivated and distributed all over India especially in Punjab, Himachal Pradesh, Gangetic plains, Bihar, Bengal, Assam and Maharashtra. Apart from India, the species is also grown in Syria, Lebanon, Israel and South Europe[8] as well as in Bangladesh, Turkey, Middle-East and the Mediterranean basin[9].

Varieties

Following varieties of cultivated Kala-Zira reported with seed yield (g/plant) from Zira and Saffron Research Station, Sangla, district Kinnaur (Himachal Pradesh), India: Rarang (1.7), Pangi (1.4), Stang (2.1), Barang (1.3), Sanji (1.9), Rispa (2.0), Kanam (2.4), Kilba (1.7), Ribba (1.8), Singla (2.3), Telangi (1.4), Thangi (1.9), Lobsang (2.1), Maiber (2.4), Rogi (1.5), Kothi (1.8), Spillow (2.4), Morang (1.7), Purbani (1.8), Sharboo (1.8) and Sunam (1.8)[10]. Variety NRCSS AN 1 to different agrotechniques is also reported[11]. Cheikh-Rouhou *et al.*[12] also reported varieties namely, Tunisian and Iranian.

Plant description

The species is an erect annual herb (Fig. 1) attaining 30.0 cm to 67.6 cm (mean: 52.18 cm ± 4.42) at maturity. Number of primary branches per plant ranges from 4 to 10 (mean: 7.0±0.71); leaf arrangement alternate, leaf phylotaxy 1-2, pinnae of leaves broad, number of pinna per rachis 5-6; total branches per plant 22.5±4.1 (6-48); flower hermaphrodite with determinate flowing patterns, main axis terminate with a solitary flower (Fig. 1), delicate; flower size 2.74 cm × 2.78 cm; color (Fig. 2) - french blue (43/3 – Horticultural Color Chart); flowers without any involucre of bracts, pedunculate; peduncle long, erect; petalloid sepals broad, ovate in a single whorl, 4-6 mostly 5 and characterized by the presence of nectaries; flower fertility 89.89%; stamens in 3 to 4 whorls (Fig. 3), numerous (32 to 66; 49.6±2.7) and shed their pollen as the filament bent outward during male phase; gynoecium 5, completely united follicles, each with a long indehiscent style and composed of variable number of multi ovule carpel, developing into a follicle after pollination; fruit single partially connected to form a capsule like structure (capsule 5 to 45; mean 20.0±3.37; capsule fertility 94.5%) dehiscence through suture; fruits (length – 0.4 to 1.7 cm, mean 1.03 cm ± 0.13; seta per capsule 4 to 8, mean 5.10±0.10) with numerous seeds (59.29±3.2; average seed production/plant - 935±177.9; seed yield – 1.91 gm; seed viability 80% to 90%); seeds ovate, tetrangular, angles sharp, acute, more tapering at the end (Fig. 4), color black (000021 – British Atlas of Colour, 2007); seed size 2.33 mm ± 0.1 × 1.14 mm ± 0.02.

The quantitative data of the species were provided from plants grown in the Experimental garden of Department of Botany, University of Kalyani (West Bengal plains, Nadia, latitude 22°50' to 24°11' N, longitude 88°09' to 88°48' E, elevation 48 feet above sea level, sandy loamy soil, organic carbon 0.76%, soil pH 6.85 – Mandal *et al.*[13]) during the months of November (15th Nov – sowing; 40 cm between rows and 30 cm between plants) as rabi crop and harvested in last week of March or in first week of April[14].

Floral biology

Andersson[15] suggested that increased allocation to perianths leads to reduced allocation to direct component of fitness. Plants both with and without perianths did not differ in fecundity of total flower number. Further, perianthless plants produced heavier seeds with earlier germination dates than the control plants. No detectable effect of perianth removal was noted on seed viability or the fecundity of plants in the progeny generation. High seed mass and germination speed had positive and independent effects on progeny fecundity. The author was of opinion that it is necessary to determine whether large conspicuous perianths enhance the amount of cross pollination and in such case perianth is to be under stabilizing selection, the optimum phenotype being a compromise between pollinator-mediated selection for larger floral displays and trade off with seed size and/or germination speed. The species are capable of setting seed without being cross pollinated, an advantageous feature in seed crop which should be under strong selection for increased seed production. Finally, the author concluded that resource trade-offs with seed mass and time to germination may facilitate evolutionary reductions in flower size.

Pollination biology

Self pollinated; onset of the male stage stamen stand erect, curved outwards one by one, roughly in whorls and strictly reflecting the order of initiation, pollen grains released when anthers reach a horizontal position; male phase initiated a few days before the

stigmas became receptive and lasted for five days; anther receptivity occurred between 8.00 p.m. to 13.00 p.m. for one day only, male and female stages synchronized on the last day of the flowering; weight of pollen 0.064 mg/flower whereas the volume of nectar 0.13 µl[16]; empty anthers curved up; pollinated stigma erect and made an angle of 180º with the ovary; style and anther length nearly equal 1.73 cm; pollinator honey bee, one bee per flower, visited in morning around 7.00 a.m.; high temperature effect fertilization success by affecting stigma receptivity and accelerating ovule degeneration[17].

Scanning electron microscopy of seed surfaces

Datta and Saha[14] studied seed surface ornamentation and found that surfaces were with distinct reticulation marks; reticulation more prominently raised, pentagonal to polygonal, ovoid or irregular in outline; reticulate rows consisting of smaller tuberculate raised cells, cells either uni- or multi seriate or in aggregation along corners or junction; cells of reticulate lines showed shrinkage structure; bound area with variable number of cells (2-5), each cell comparatively larger, penta-, hexa-, polygonal or rounded in outline; lumen floor depressed or shallow glabrous (Figs. 5-10).

Cultivation

In India *N. sativa* is mostly grown once in a year as rabi crop during the months of October (late)–November to March-April in plains; while, rarely in hills in May-June[18].

1. **Area of Cultivation and Production:** Area of cultivation and annual production (source – Comparative Sales Report 2010, VDM Verlag Dr. Muller AG & Co.) were reported to be – India: 6234600 ha, 254000 t; Turkey: 8122010 ha, 689350 t; USA: 16420 ha, 11200 t; UK: 500 ha, 10 to 20 t respectively.
2. **Climate:** Grows well in cool-dry with light snowfall areas to warm-humid areas. Cool and humid weather favors flowering and seed setting[10].
3. **Soil:** Sandy, loam rich in microbial activity is the most suitable soil for cultivation. The sloppy soils of heavy rainfall areas and leveled and well drained soils of moderate rainfall areas are quite suitable for cultivation. Soil pH 7.0 to 7.5 is favorable for cultivation[10].
4. **Preparation of Land:** One ploughing followed by 2-3 harrowing and leveling will be suitable[10].
5. **Method of Sowing:** Seed sowing or by replanting previous year root stocks. Seed sowing is done during October-November by broadcasting (1.5 kg/hectare) or seed drill method or by line sowing keeping space between lines (30, 40 or 50 cm) and at the depth of 2 cm. After 20 days of sowing thinning of the plant to a distance of 20 cm is done.
   Sowing by bulbs (previous year root stock) is possible when soil moisture content of the field is favorable for deep ploughing i.e. neither too wet nor too dry[10].
6. **Manure and Fertilizer:** NPK (5:3:2) is generally applied every year along the side of the planted bulbs[10].
7. **Weed Control:** Frequent weeding reduce weed competition and produce good environmental condition for growth and development. About 3-5 weeding at an interval of 20 to 25 days is recommended by hand hoe or khurpi[10].
8. **Irrigation:** One or two irrigations at flowering and seed formation stage are helpful to increase grain size and oil content[10].
9. **Harvesting:** Black cumin grown as rabi crop in West Bengal Plains are generally harvested late March to first week of April. The crop harvested before shedding at little green stage gives high aromatic oil contents providing good market. Black cumin retains seed viability longer when it is full ripe. It is rather essential that harvesting is done before shedding (shattering of fruits is a major problem) and therefore 2 to 3 or more pickings can be done to avoid loss of seeds due to shattering of the capsules. The harvested crop is dried under sun and threshed by beating with the stick[10].
10. **Post Harvest Management:** *N. sativa* requires extensive labor in collection and harvest as the capsules (fruit) tend to shatter at maturity. Post harvest management of the fruits usually involves their harvest, one by one, by hand and dry storage till natural dehiscence. The mature fruits do not require much attention as they are self-preserving and their essential oil is a great deterrent to fungal attack, insect attack as well as rodent infestation[19].

Shelf life

The seeds of *N. sativa* store well for one year as planting material and as a spice, they are stored in airtight conditions to prevent the loss of aroma. As a spice, it is recommended to be stored away from other species as the species has a overbearing flavour and aroma and disturb the flavour of other species[10].

Economy

Reports

1. Rs. 275-300/kg in local market (Pakisthan-Mingora, Din, Peshawar, Pindi, Lahore, Gilgit and Astore), whereas in down country it cost Rs. 450-500. In Internatinal market it is sold for Rs. 850-1000/gm[20].

2. Germany: Black cumin oil 1000 ml – 23.90 EUR + shipping cost[21].
3. Black cumin MGS Heirloom Seeds; Product code: NIG02:300 seeds – 1.90€[22].
4. Black cumin USA. (i) Product No. 1130.F, 29.57 millilit. - $1.40. (ii) Product No 1130.G, 59.14 millilit. - $1.99. (iii) Product No. 1130.H, 118.29 millilit. - $2.46. (iv) Product No. 1130.I, 236.58 millilit. - $3.50. (v) Product No. 1130.J, 476.16 millilit. - $6.00. (vi) Product No 1130.K, 5lbs minimum - $5.00. (vii) Product No. 1130.N, 10lbs min. - $4.50. (viii) Product No. 1130.O, 25lbs min. - $3.95[23].
5. Black Seed 100 capsules - $9
   Black Cumin Tea (Organic) 20 Bag - $6[24].
6. In Indian market Rs. 250-300/kg. Since its cultivation fetches high income per unit area, therefore, it is highly suitable for cultivation by marginal farmers[10].

Diseases

Sinha and Singh[25] reported *Macrophomina phaseolina* infection in roots causing its deformation. Wilt (causal organism grows along the seedling and leaves and branches look light green in colour, leaves shed and plant dries up; control: spray Dithane M-45 0.2% or Dithane Z-78 or Blitox 5 w.p. at 15 days interval) and rotting of bulbs (emit a special odour; control: dipping bulbs in 0.3% bavistin for 30 mins. before planting, field kept free from stagnant water) are diseases reported[10]. Early report by McRae and Shaw[26] also suggested *Fusarium* wilt in the species. Prolonged survival of *F. udum* for upto 8 years was reported in roots.

Pest

1. Caterpillar – Makes holes in the bulbs and cut down seedlings.
   Control: Dust the soil at the sowing or hoeing with 5% Aldrine, 10% BHC at the rate of 25 kg per hectare; application of well-decomposed farmyard manure[10].
2. Armyworm and semi-looper – Feed on the flowers, seeds, and damage the crop.
   Control: Spray with 0.05% methyl parathion – 1 ml/l water or Thiodian or Endosal 35EC, 1 ml/l of water at 15 days interval[10].

Microscopical and powdered characteristics

Transverse section of seed show single layered epidermis, thick walled cells, covered externally by a papillose cuticle and dark brown contents; 2-4 layered, thick, tangentially elongated parenchymatous cells followed by reddish brown thick walled rectangular cells; endosperm thin walled, cells rectangular to polygonal filled with oil globules; powdered characteristics brownish black, parenchymatous cells and oil globules[27,28].

Biochemical constituents

Constituents of *N. sativa* seeds are fixed oil – 32 to 40% (saturated fatty acids- about 30%; palmitic acid, stearic and myristic acid; unsaturated fatty acids: arachidonic, eicosadienoic – 3%, linoleic – 50 to 60%; oleic acid – 20%; dihomolinoleic fatty acids – 10%), volatile oil- 0.4 to 0.45% (nigellone, thymoquinone, thymohydroquinone, dithymoquinone, thymol, carvacrol, α and β-pinene, d-limonene, d-citrlnellol, p-cymene), proteins 16-19.9% (arginine, glutamic acid, leucine, lysine, methionine, tyrosine, proline, threonine), minerals 1.79-3.74% (calcium, phosphorus, potassium, sodium, iron), carbohydrate 33.9%, fibre 5.50% and water 6.0%[29]. Ramadan and Morsel[30] reported that apart from physical constants: 2% w/w, foreign matter; 6% w/w, total ash; 0.2% w/w, acid insoluble ash; 20% w/w, alcohol soluble extractive; 15% w/w, water soluble extractive; 3.91% w/w organic matter; 4% w/w, loss on drying[31]. The seeds contain carotene, which is converted to vitamin A in liver[32]. Acetylated triterpene saponin (penta hydroxyl pentocyclic triterpene) has been isolated from the species[33].

Phytochemical Compounds: Categorically different phytochemical compounds of seeds are nigellone[34], nigellicine, nigellimine, nigellimine-N-oxide, avenasterol-5-ene, avenasterol-7-ene, campesterol, cholesterol, citrostadienol, cycloeucalenol, 24-ethyl-lophenol, gramisterol, lophenol, 243-methyllophenol, obtusifoliol, sitosterol, stigmastanol, stigmasterol, stigmasterol-7-ene, beta-amyrin, butyrospermol, cycloartenol, 24-methylene-cycloartanol, taraxerol, tirucallol, 3-O-[β-D-xylopyranosyl(1→3)-α-L-rhamnopyranosyl(1→2)-α-L-arabinopyranosyl]-28-O-[α-L-rhamnopyranosyl (1→4)-β-D-glucopyranosyl (1→6)-β-D-glucopyranosyl] hederagenin, volatile oil (0.5-1.6%), fatty oil (35.6-41.6%), oleic acid, esters of unsaturated fatty acids with C15 and higher terpenoids, esters of dehydrostearic and linoleic acid, aliphatic alcohol[31,35,36], nigellidine[37], carvone, d-limonene, cymene, α, β-unsaturated hydroxy ketone, steroids, hederagenin glycoside, melanthin, melanthigenin, bitter principle, tannin, resin, protein, reducing sugar, glycosidal saponin, 3-O-[β-D-xylopyranosyl-(1→2)-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl]-11-methoxy-16,23-dihydroxy-28-methylolean-12-enoate, stigma-5,22-dien-3-β-D-glucopyranoside, cycloart-23-methyl-7,20, 22-triene-3β,25-diol, nigellidine-4-O-sulfite[38], nigellamines A3, A4, A5, C[39], nigellamines A1, A2, B1, and B2[40].

Seed Oil: The seed oil contains cholesterol, campesterol, stigmasterol, β-sitosterol, α-spinasterol, (+)-citronellol, (+)-limonene, p-cymene, citronellyl acetate, carvone[41], nigellone, arachidic, linolenic,

linoleic, myristic, oleic, palmitic, palmitoleic and stearic acids. Fixed oil: linoleic acid (55.6%), oleic acid (23.4%) and palmitic acid (12.5%). Volatile oil: trans-anethole (38.3%), p-cymene (14.8%), limonene (4.3%), and carvone (4.0%)[42], 2-(2-methoxypropyl)-5-methyl-1, 4-benzenediol, thymol and carvacrol[43]. Root and shoot are reported to contain vanillic acid[44].

Extraction methods of essential oil

1. Conventional method – extraction by hexane in Soxhlet[45].
2. Enzymatic extraction[46].
3. Ultrasound assisted extraction[47].
4. Microwaves assisted extraction[48].
5. Supercritical solvent extraction[49].
6. Surfactant assisted method; based on the use of aqueous solution polyethylene glycol sorbitan monolaurate (Tween 20)[50] amongst other methods.

Oil extracted were analyzed and characterized by using classical analytical procedures, spectroscopic and chromatographic methods.

Therapeutic uses

Traditional Uses: In traditional system of medicine black cumin seeds are effective against cough, bronchitis, asthma, chronic headache, migraine, dizziness, chest congestion, dysmenorrheal, obesity, diabetes, paralysis, hemiplegia, back pain, infection, inflammation, rheumatism, hypertension, and gastrointestinal problems such as dyspepsia, flatulence, dysentery, and diarrhea[51]. It has also been used as a stimulant, diuretic, emmenagogue, lactagogue, anthelmintic and carminative[52] as well as it is applied to abscesses, nasal ulcers, orchitis, eczema and swollen joints[51]. Seed oil is considered to be local anesthetic[53,54].

Pharmacological Significance: The species possesses antimicrobial (diethyl ether extract and methanol and chlorophyll extract and plant extract as well as seed oil were found to inhibit *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli* and a pathogenic yeast *Candida albicans* – Hanafy and Hatem[55], Hosseinzadeh *et al.*[56], Chaieb *et al.*[57], Khalid *et al.*[58]), anti-malarial[59], antioxidant (thymoquinine constituent of seed oil, enhance the oxidant scavenging system – Salem[60]), anti-inflammatory (the oil and thymoquinone – Salem[60]; thymoquinone has the ability to attenuate allergic airway inflammation by inhibiting Th_2 cytokines and eosinophil infiltration into the airways and exploratory effects – Isik *et al.*[61]), anticancerous (methanolic extract of plant exhibits potent inhibition of cancerous cell growth against HL-60 and U-937 cell lines with IC_{50} value 13.50 µg/ml and 28.31 µg/ml respectively – Raval *et al.*[62]), antitumerogenic (active components – thymoquinone and dithymoquinone; tymoquinone kill cancer cell by a process that involved apoptosis and cell cycle arrest with little effect in non-cancerous cells – Buyukozturk *et al.*[63]), anti-hypertensive[64], antiviral (Infections Laryngotrachietis virus – Zaher *et al.*[65]), anti-asthmatic (crude seed extracts exhibits spasmolytic and bronchodilator activities mediated possibly through calcium channel blockade – Kalus *et al.*[66]), anti-allergic (oil is an important adjuvant for the treatment of allergic disease – Dahri *et al.*[67]), anti-diabetic, antilipidemic, antiobesity[9], anticonvulsant[43,68], antitoxic[69] properties apart from having immunomodulatory (extract inhibit human neutrophil elastase activity which is mainly attributed to carvacrol – Mansi[70]), hematological (oil play role in modulating the balance of fibrinolysis/thrombus formation by modulating the fibrinolytic potential of endothelial cells – Gilani *et al.*[71], Zaoui *et al.*[72]), gastro-protective (thymoquinone protect gastric mucosa against injurious effect of absolute alcohol and promote ulcer healing – Naz[9]), nephroprotective[73,74,75], diuretic[76], cardiovascular (active ingredient thymol has shown to lower blood pressure through blockade of calcium channels - Gilani *et al.*[71], Paarakh[8]) properties as well as the species is protective against heavy metal[77,78], effects nitric acid production[79], possesses analgesic activity (volatile oil – Ramadhan *et al.*[80]) amongst others. Moreover, essential oil was found to be effective against Cr(VI) hazard and may be a promising candidate against different environmental pollutants[81,82] reported that the species is a good absorbent for the removal of cationic metals coming from wastewater. Tasawar *et al.*[82] reported that black cumin (tested on 80 subjects, divided randomly into 2 groups) is effective to change the lipid profile significantly in a way which is beneficial to heart.

Black seed has also been used externally where it is applied directly to abscesses, nasal ulcers, orchitis, eczema and swollen joints[51]. *N. sativa* is also a potential source for antidermaphytic drugs. The ether extract of seeds and its active principle thymoquinone are found to be effective after clinical trials against many species of three important genera of dermatophytes: *Trichophyton*, *Epidemophyton* and *Microsporum*[83,84]. The volatile oil inhibited the spontaneous movements of rat and guinea pig uterine smooth muscle and also the contraction induced oxytocin suggesting its anti-oxytocic potential[69]. Hot water extract of NS as well as whole seeds in large oral doses causes abortion in human pregnant females[85]. The species is also used in long term treatment of opioid defense[86]. Thymoquinone has been reported to exhibit effect on dopaminergic neurons against Parkinson's disease[87].

Insecticidal activity

Essential oil from dried fruits was isolated by hydrodistillation and tested for its repellent, toxic and developmental inhibitory activities against wheat

flour pest *Tribolium castaneum*[88]. Results indicated that the essential oil reduced the oviposition potential and increased the developmental period of *T. castaneum* in comparison to control group. Fumigation of essential oil inhibited development of larvae to pupae and the pupae to adults and also resulted in the deformities in the different developmental stages of the insects. All the responses were found concentration-dependent.

Other uses

N. sativa seed cakes in the feed of buffalo and lambs improved their body weight and reproductivity as well as seeds in the food of broiler chicks improved their immunity and feed conversion efficacy[89,90].

Clinical trials

Significance of the species has been documented from some clinical trial experiments. Al-Ghamdi[91] administered aqueous suspension of the seeds orally at two dose levels (250 mg/kg and 500 mg/kg) for five days to assess carbon tetrachloride (CCl_4)-induced liver damage. CCl_4 (250 microl/kg intraperitonelly/day in olive oil) was given to the experimental group on days 4 and 5, while the control group was only treated with the vehicles. Animals treated with CCl_4 showed remarkable centrilobular fatty changes and moderate inflammatory infiltrate in the form of neutrophil and mononuclear cells when compared to the controls. This effect was significantly decreased in animals pretreated with *N. sativa*. Histopathological or biochemical changes were not evident following administration of *N. sativa* alone. Serum levels of aspartic transaminase (AST), L-alanine aminotransferase (ALT) were slightly decreased while lactate dehydrogenase (LDH) was significantly increased in animals treated with CCl_4 when compared to control group. LDH was restored to normal but ALT and AST levels were increased in animals pretreated with *N. sativa*. In conclusion, it appeared that seeds are possible safe and protective against CCl_4-induced hepatoxicity.

Ali and Blunden[92] examined the hypolipidemic and antioxidant effects of dietary black seed in hyperlipidemic rabbits (24 male rabbits were fed with 0.5% cholesterol diet for 1 month, randomly assigned to two groups – control group received the hypercholesterolemic diet and the black seed group was fed 7.5 g/kg b.w/day crushed black seed + 0.5% cholesterol diet, each for 2 months). Fasting blood samples were obtained at baseline, after hyperlipidemia, 1 month and 2 months of treatment to determine serum lipid profile, malondialdehyde (MDA) level, total antioxidant status (TAS), superoxide dismutase (SOD) and glutathione peroxidase (GPX). Results indicated that black seed can favorably decrease serum lipid profile and lipid peroxidation levels in hyperlipidemic rabbits, thereby indicating that seeds may be considered as a useful therapy for hyperlipidemia.

Abbas *et al.*[93] reported that *N. sativa* oil possesses anti-inflammatory and bronchodilator activities. Clinical trial with mouse model suggested that *N. sativa* significantly reduced blood eosinophil count; IgG1 and IgG2a levels, cytokine profiles and inflammatory cells in lung tissue. These effects were comparable to the effects of dexamethasone except unchanged IFN-y level.

Abou-Gabal *et al.*[94] studied the effect of the oral administration of aqueous suspension of *N. sativa* (50 mg/kg.b.wt) against chromosomal aberrations and ultrastructural changes of the bone marrow cells in mice treated with carbon tetrachloride CCl_4 (two dose level: 1.9 ml/kg.b.wt and 3.8 ml/kg.b.wt). Mitotic activity decreased in bone marrow cells of animals treated with CCl_4 as well as significant increase in the number of bone marrow cells with different types of chromosomal aberrations was recorded. Ultrastructural changes were also dose-dependent including both nucleus and cytoplasm of erythroid and myeloid elements of the bone marrow cells. Treatment of the animals with *N. sativa* improved both genotoxicity and ultrastructural changes induced by CCl_4.

Al-Kubaisy and Al-Noaemi[95] reported protective role of seed oil against effect of CCl_4 on the liver cells.

Samir Bashandy[96] reported that administration of NS oil to hyperlipidemic rats improved their reproductive efficiency (increase in seminal vesicle weight, testosterone level, sperm motility and sperm count and a decrease in sperm abnormalities) and produced additional protection against hyperlipidemia induced reduction in fertility.

Najmi *et al.*[97] performed clinical study (2 groups of 30 patients each) to evaluate the adjuvant effect of seed oil on various clinical and biochemical parameters of the metabolic syndrome. Group I (standard group) patients were given Atorvastatin 10 mg once a day and tablet Metformin 500 mg twice a day along with *N. sativa* seed oil 2.5 ml twice a day for six weeks. Results indicated that Group III patients showed significant improvement with reference to total cholesterol, low density lipoprotein and fasting blood glucose, thereby indicating that seed oil is effective as an add-on therapy in patients with metabolic syndrome and also possessing therapeutic activity in diabetic and dyslipidemic patients.

Al-Sa′aidi *et al.*[98] determine the effect of alcoholic extract of black seed *N. sativa* on fertility parameters in white rat. A total of 60 mature males were divided into 3 groups – the first group (control) intake drinking water, while the other two groups (T_1 and T_2) intake the extract in two doses (0.5 and 1.5 g/kg respectively) daily for 53 days. The results revealed that treatment with alcoholic extract of *N. sativa* led to significant increase (P<0.01) in body weight gain

(g), reproductive parameters (seminiferous tubules thickness and diameters, account of spermatogonia, primary and secondary spermatocytes, spermatids, free spermatozoa, account of sertoli and Leydig cells, diameter of Leydig cells and the height of epithelial cells entirely covered epididymal caudal), hormones (testosterone and follicle stimulating hormone) as well as protein concentration and significant decrease (P<0.01) in leutinizing hormone and cholesterol concentration.

Mohammad *et al.*[99] from clinical trial experiments with male albino rats suggested that the aqueous extracts of *N. sativa* have increased spermatogenesis activity in seminiferous tubule.

Al-Attar and Al-Taisan[100] reported the preventive effects of black cumin seeds (seed extract – 300 mg/kg/day) on Spragu Dawley Rats (clinical trial performed with 50 male rats, divided into four groups) exposed to Diazinon. Results indicated that seeds can be considered therapeutic agent against hematotoxicity, immunotoxicity, hepatoxicity, nephrotoxicity and cardiotoxicity induced by diazinon and may be against other chemical pollutants, environmental contaminants and pathogenic factors.

El-Naggar[101] investigated the cytotoxicity of *N. sativa* dry methanolic extract on cultured cortical neurons and its influence on neurotransmitter release, as well as the presence of excitatory (glutamate and aspartate) and inhibitory amino acids (gamma-aminobutyric acid-GABA- and glycine). The secretion of different amino acids was studied in primary cultured cortical neurons by HPLC using a derivation before injection with dansyl chloride. NS modulated amino acid release in cultured neurons; GABA was significantly increased whereas secretion of glutamate, aspartate, and glycine were decreased.

Mohamed *et al.*[102] investigated protective role of *N. sativa* in DAB (dimethylaminoazobenzene) induced liver carcinogenesis. The study included 140 Albino mice weighing 40-50 gm divided into 4 groups: Group I - normal control; Group II - *N. sativa* treated control; Group III – treated with DAB; Group IV – treated with *N. sativa* and DAB. Biochemical investigations, flow cytometric analysis and histopathological examination of the liver tissue were performed and the results showed significant change in the DNA content, histomorphology, and antioxidant enzymes in liver tissues of the DAB treated group. These changes were restored to normal with *N. sativa* treatment. Further, it was noted that treatment with *N. sativa* only showed comparable result with control untreated group. Thus, it was inferred that *N. sativa* lonely induce no harmful effect on the liver rather it exerts hepatoprotective effect against liver carcinogens.

Al-Naqeep *et al.*[103] reported (experiment conducted on HC rabbit) that *N. sativa* seeds powder or oil showed hypocholesterolemic and antiatherogenic cardioprotective properties.

Attia *et al.*[104] performed experiment on male rats and were of opinion that omega-3 polysaturated fatty acid (ω_3) and seed oil of *N. sativa* might prevent oxidative stress and attenuate the changes in the biochemical parameters (levels of urea, creatine, total bilirubin and uric acid contents and aminotransferase, phosphatases, and lactic dehydrogenase) induced by Lindane (r-HCH-r-hexachlorocyclohexane).

El-Gohary *et al.*[105] studied the effect of carboplatin (a synthetic antineoplastic agent used for cancer treatment) and *N. sativa* oil alone or in combination on human breast cancer cell (MCF-7) *in vitro* and Ehrlich as cites tumor bearing female mice (*in vivo*). The *in vitro* experiment on MCF-7 cells illustrated that IC_{50} of carboplatin was 11.8 µg/ml, IC_{50} of *N. sativa* oil was 39 µg/ml and IC_{50} of the combination between carboplatin and black cumin oil was 3.78 and 40 µg/ml respectively. The *in vivo* experiment illustrated that carboplatin (10 mg/kg) increased the enzyme activity of aspartate amino transferase (GOT) and aniline amino transferase (GPT) by 56.52% and 51.14% respectively as compared to both healthy control (non-tumor transplanted mice) and negative control. The activity of GOT and GPT was increased by 14.75% and 19.84% respectively as compared to healthy control under the effect of *N. sativa* oil (12 ml/kg); while, the enzyme activities decreased in comparison to negative control. The combination of carboplatin and oil appeared to increase the enzyme activity of GOT and GPT by 62.41% and 49.39% respectively compared to both healthy control and negative control. Agarose gel electrophoresis revealed that carboplatin induced DNA damage of liver tissue but *N. sativa* oil showed intact DNA without any damage.

Parhizkar *et al.*[106] studied the estrogenic activity of *N. sativa* by vaginal cornification assay using an ovariectomized rat model (40 ovariectomized Sprague Dawley rats, weighing 250 to 350 g were used; NS powder given at 300, 600 and 1200 mg/kg for 21 consecutive days; compared with 0.2 mg/kg conjugated Equine estrogen as positive control). Data obtained from vaginal smear suggested that NS possesses estrogenic function which can be helpful in managing menopausal symptoms as an alternative for Hormone Replacement Therapy.

Rayan *et al.*[107] studied the effect of black cumin oil (BSO) against *Toxoplasma gondii* Me 49 strain in a murine model of infection. After clinical diagnosis with mice (35 mice were studied in 3 groups) and assessment of survival rate and brain cyst burden, brain histopathological lesions and immunohistochemical expression of inducible nitric oxide synthase (iNOS) it was noted that BSO in prophylactic or therapeutic regimens significantly enhanced protection of infected mice against death (P=0.01) and reduced brain cyst burdens at 5, 7 and 12 weeks post infection compared to the infected untreated control.

Antitumor- Ait *et al.*[108] suggested that essential oil (IC_{50}=0.6% v/v) and ethyl acetate (IC_{50}=0.75%) extracts were more cytotoxic against P8-15 cell line than butanol extract (IC_{50}=2%). The authors further suggested that TQ induced apoptosis and inhibited proliferation in pancreatic ductal adenocarcinoma cells. TQ also increased P^{21}WAF1 expression, inhibited histone deacetylase activity and induced histone hyperacetylation. TQ is reported that it acts as a novel inhibitor of pro-inflammatory pathways which combines anti-inflammatory and proapoptotic modes of action. Banerjee *et al.*[109] performed *in vitro* studies on pancreatic cancer cells preexposed with thymoquinone (25 µmol/l) for 48 h followed by gemcitabine or oxaliplatin resulted in 60 to 80% growth inhibition compared with 15 to 25% when gemcitabine or thymoquinone was used alone which suggested that the mechanism of thymoquinone could potentiate the killing of pancreatic cancer cells by down regulation of nuclear factor kappa B (NF-kappa B), Bcl-2 family, and NF-kappa B-dependent antiapoptotic genes. Breyer *et al.*[110] tested 4-acylhydrazones and 6-alkyl derivatives of thymoquinone for growth inhibition of human HL-60, leukemia, 518A2 melanoma, KB-VI/Vbl cervix and MCF-7/Topo breast carcinoma cells. The 6-hencosahexaenyl conjugate was most active in all resistant tumor cells, with IC_{50} (72 h) values as low as 30 Nm in MCF-7/Topo cells. Nagi and Almakki[111] investigated the effect of thymoquinone (TQ) *in vivo* and *in vitro* male albino rats on fibrosarcoma induced by 20-methylcholanthrene. It was found to inhibit tumor incidence and tumor burden significantly. Shafi *et al.*[112] reported methanol (IC_{50}-2.28 µg/ml), n-hexane (IC_{50}-2.20 µg/ml) and chloroform (IC_{50}-0.41 µg/ml) extracts of the seeds effectively killed HeLa cells by inducing apoptosis.

Diabetic and Cardiovascular Activities- Meddah *et al.*[113] observed improvement of glucose tolerance and body weight in rats after chronic oral administration *in vivo*, which validate the traditional use of black cumin seeds against diabetes. Chandra *et al.*[114] reported that HIV protease inhibitors, nelfinavir (5-10 µM), saquinavir (5-10 µM) and atazanavir (5-20 µM) with *N. sativa* seed extract decreases glucose stimulated insulin secretion from rat pancreatic beta-cells. Altan *et al.*[115] were of opinion that combined treatment with NS and hPTH alone in improving bone mass, connectivity, biomechanical behavior and strength in insulin-dependent diabetic rats. NS treatment alone or in combinations significantly increased the area of insulin immunoreactive beta-cells in diabetic rats suggesting that NS might be useful in the treatment of diabetic osteopenia. Kanter *et al.*[116] and Kaleem *et al.*[117] suggested that oral administration of ethanol extract of black cumin seeds (300 mg/kg body weight/day) to streptozotocin induced diabetic rats for 30 days significantly reduced the elevated levels of blood glucose, lipids, plasma insulin and improvement altered levels of lipid peroxidation products and antioxidant enzymes like catalase, superoxide dismutase, reduced glutathione and glutathione peroxidase in liver and kidney. Meral *et al.*[118] suggested that NS might be used in diabetic patients to prevent lipid peroxidation, increase in anti-oxidant defense system activity and also to prevent liver damage. al-Awadi *et al.*[119] reported the significance of NS seeds for its use in non-insulin dependent diabetic mellitus. An aqueous decoction of a plant mixture containing NS was found to lower blood glucose level after oral administration[120]. Al-Hader *et al.*[121] suggested that intraperitoneal administration of volatile oil of seeds produced a significant hypoglycemic effect in normal and alloxan induced diabetic rabbit.

Oral supplement of *N. sativa* seeds to normal rats was investigated and the results showed intrinsic cardiac properties without evidence of an increased cardiac work load or energy consumption *in vivo* which makes the seeds an isotropic agent with hemodynamic profile[77,122,123]. Shafei *et al.*[124] examined the effects of aqueous and macerated extracts from *N. sativa* on heart rate and contractility of the isolated heart. Results showed a potent inhibitory effect of both extracts on both heart rate and contractility of guinea pig heart that was comparable and even higher than that of diltazem which may be due to calcium channel inhibitory or an opening effect for the plant on potassium channels of the isolated heart. Dichloromethane extract of seeds (0.6 ml/kg/day), essential oil and unsaponifiable matter of oil, volatile oil and thymoquinone found to be cardioprotective[125,126,76,67]. Gilani *et al.*[127] reported that thymol has shown lower blood pressure through blockade of calcium channels. The effect of oral treatment of Wister albino rats with different doses of powdered seeds (100, 200, 400 and 600 mg/kg/day) for four weeks on the levels of serum lipid was investigated, and it was found that it causes significant decrease in low density lipoprotein-cholesterol levels, triglyceride levels and increase in high density lipoprotein-cholesterol level[128].

Pulmonary Activity- Nigellone was found to inhibit effectively the histamine release from the mast cells suggesting its use in asthma[129]. Padmalatha *et al.*[130] studied the antinaphylactic effect of a polyherbal formulation containing NS on mesenteric mast cells. The antinaphylactic activity was possibly due to the membrane stabilizing potential, suppression of antibody production and inhibition of antigen induced histamine release. Gilani *et al.*[127] suggested that bronchodilatory effect of NS seeds was mediated possibly through calcium channel blockade. Keyhanmanesh *et al.*[131] studied the prophylactic effect of TQ on tracheal responsiveness and WBC (white blood cell) count in lung lavage of sensitized guinea pigs. The results suggested the preventive effect of TQ on tracheal responsiveness and inflammatory cells of lung lavage of sensitized

guinea pigs. Suddek[132] was of opinion that TQ-induced relaxation of the precontracted pulmonary artery is probably by the activation of ATP-sensitive potassium channels and possibly by non-competitive blocking of serotonin, alpha-I and endothelin receptors.

Immunomodulation- Islam *et al.*[133] studied the effect of volatile oil of *N. sativa* seeds (NSVO) for its immunomodulating and cytotoxic properties in rats and it was found that there was a significant decrease in splenocyte and neutrophil counts, but a rise in peripheral lymphocytes and monocytes in rats. LC_{50} values for NSVO were 155.02±10.4, 185.77±2.9, 120.40±20.5, 384.53±12.1 and 286.83±23.3 micro g/ml respectively against the SCL, SCL-6, SCL-376, NUGC-4 cancer lines and 3T6 fibroblast line. Results indicate NSVO as a potential immunosuppressive cytotoxic agent. Swamy and Tan[134] performed *in vitro* cytotoxicity of seed extracts (in ethyl acetate fraction) in different cancer cell lines P388, Molt 4, Wehi 164, LL/2, HePG2, SW 620 and J82 as measured by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay and the ethyl-acetate column chromatographic fraction (CC-5) showed selectivity against HePG2, Molt 4 and LL/2. CC-5 was relatively non-toxic against human umbilical cord endothelial cells at 50 µg/ml. Results therefore indicated that CC-5 possesses a potent cytotoxic effect as well as a potentiating effect on the cellular immune response.

Contraceptive Activity- Hexane extract of the seeds prevented pregnancy in Sprague-Dawley rats treated orally at 2 g/kg daily dose on day's 1-10 post – coitum. The active hexane extract exhibited only mild euterotrophic activity comparable to ethinyl-estradiol, but was devoid of any estrogenicity in the immature rat bio-assay[135]. Agarwal *et al.*[136] reported that ethanolic extract of seeds possesses antifertility effect in male rats which is probably due to inherent esterogenic activity.

Nephroprotective Activity- Ali[137] investigated the effect of oil (oral treatment: 0.5, 1.0 or 2.0 ml/kg/day for 10 days) on gentamycin induced nephrotoxicity in rats. A dose-dependant amelioration of the biochemical and histological indices of GM nephrotoxicity that was statistically significant at the two higher doses. Treatments enhanced antioxidant status in plasma and also reduced glutathione concentrations in renal cortex and enhanced growth. Badary *et al.*[138] studied the effect of TQ on the nephropathy and oxidative stress induced by doxorubicin (DOX) in rats (10 mg/kg/day – supplemented with drinking water for 5 days before DOX and daily thereafter) and found that TG, TC and serum urea lowered significantly. TQ has been suggested to be protective agent for protienuria and hyperlipidemia associated with nephritic syndrome.

Effectiveness- Qidwai *et al.*[139] performed clinical trial experiment (study design was randomized, double-blind trial) to assess effectiveness, safety, and tolerability of powdered *N. sativa* seeds in capsules on serum lipid levels, blood sugar, blood pressure, and body weight in adults (123 patients were recruited; 64 and 59 patients were randomized to the intervention and the control arms respectively; 39 patients in the intervention group and 34 in the control group completed the study). Favourable impact of powdered *N. sativa* seed in capsule was noted on almost all variables; however, larger study with adequate sample size was recommended.

Biosafety

1. Seed powder did not produce any toxic effects at very high doses (28 gm/kg orally) in rabbits[140].
2. Seed oil safe when given orally to rats (LD_{50} of 28.8 ml/kg)[72].
3. Oral thymoquinone was found safe (LD_{50} of 2.4 g/kg)[141].
4. Oral thymoquinone (LD_{50} of around 1000mg/kg) and intraperitoneal (LD_{50} of around 100 mg/kg) in mice/rat, safest[142].

Cytological and cytogenetical studies

Karyomorphology: Gregory[143] was pioneer to enumerate the number of chromosomes (2*n*=12) in somatic complement of *N. sativa*. Bhattacharyaya[144] revealed five pairs of very long (L_1) to long (L) chromosomes with median to sub median primary constrictions and a single pair of medium-sized (M) chromosomes with sub-terminal primary constrictions in the species. Secondary constrictions were located in two of the long pairs of chromosomes and karyotype formula was suggested as $2n=12=2L_1+4L^S+4L+2M$.

Saha and Datta[145] reported four morphologically distinct chromosome types (A, B, C, D) in *N. Sativa* (2*n*=12) on the basis of chromosome length (very long 15.0 to 20 µm; long 10.0 to 14.9 µm; median 5.0 to 9.9 µm), nature of primary constriction and presence or absence of secondary constriction (Figs. 11-12). The somatic complement possessed one pair AA (very long, 19.13 µm; F% 44.01), one pair BB (very long, 16.70 µm; both primary- F% 44.88 and secondary constriction were present), three pairs CC (C_1C_1- very long, 15.31 µm; C_2C_2- long, 14.86 µm and C_3C_3- long, 13.80 µm; F% 44.04 to 45.42) and one pair DD (medium 6.64 µm, F% 7.23) chromosomes (TF% 41.38, haploid chromatin length 86.50 µm ± 3.3). The somatic chromosome types could easily be marked in meiotic plates (Figs. 13-14).

Ghosh and Datta[146] karyotyped *N. sativa* through Image Analyzing System (Micro Image ™ Lite Software, Version 4.0 for windows, 47N40155 2000 0515 MAN VG MIX) and revealed four (2*n*=12=4A+4B+2C+2D; karyotype formula: $2L^{sc}_{1sm}+2L_{1m}+2L_{sm}+2S_t$) morphologically distinct chromosome types (L_1= very long≥15.0 µm, L= long

13.0 to <15.0 µm, M= medium 7.0 to <13.0 µm, S= short <7.0 µm; m= metacentric, sm= sub-metacentric, t= telocentric and sc= satellites). The somatic chromosome complements in the species formed graded karyotype which was symmetric in nature (TF% 42.90). Total haploid chromatin length was noted to be 78.62 µm ± 2.87.

Meiotic Analysis and Pollen Fertility: Saha and Datta[145] reported regular 6 bivalents formation at diplotene and metaphase I (MI) in most PMCs (Figs. 15-17); while, the rest demonstrated 5II+2I formations (175 meiocytes assessed). Frequency of bivalent and univalent per cell varied from 5.88 to 6.0 and 0.00 to 0.23 respectively. Frequency of bivalent and univalent per cell was 5.95 and 0.10 respectively. The bivalents formed rings (range- 2.85±0.51 to 3.58±0.24/cell) and rods (range- 2.41±0.24 to 3.15±0.51/cell). Average frequency of ring and rod per cell over the plant was 3.18 and 2.77 respectively. Chiasma per nucleus range between 8.87±0.21 and 9.62±0.32 (average: 9.34±0.28). Frequency of bivalents, ring and rod configurations per cell and chiasma per nucleus showed random distribution over the plants ($p>0.05$) but univalents per cell was non-random ($p<0.01$) as evidenced from χ^2 test of heterogeneity. Mostly (99.49%- pooled over the plants) anaphase I (AI) cells manifested equal 6/6 separation (Fig. 18) of chromosomes, rare often unequal separation (5/7), lagging chromosome and bridges were also noted. Pollen fertility among black cumin plants varied from 95.2% to 100.0% (average: 98.06%). Saha and Datta[145] were further of opinion that the meiotic chromosomes could easily be identified and marked in meiotic plates.

Pachytene Chromosome Analysis: Datta[147] reported that the length of pachytene chromosomes (Fig. 19) in the species ranged from 51.86 µm to 140.55 µm with mostly median primary constrictions (F%: 41.60 to 47.56; arm ratio: 0.71 to 0.91). A telocentric (F%: 12.36; arm ratio: 0.41) was also marked in the pachytene complement. Four (chromosome type A- 140.55 µm; type B- 109.75 µm and 97.89 µm; type C- 94.93 µm and 89.32 µm; type D- 51.86 µm) morphological types were suggested with 2 bivalents (B type) documenting secondary constrictions. However, further studies on the somatic complement has suggested that one pair of chromosome were with secondary constriction[145,146].

Accessory Nucleoli: Rang and Datta[148] revealed consistent presence of single nucleolus (size 8.36 µm ± 0.08) in PMCs (pollen mother cells) of *N. sativa* and it is in accordance to the number of chromosome with secondary constriction in the complement[145,14,146]; however, nucleolus is not commensurable to the number of secondarily constricted chromosomes and it has been proven that those chromosomal regions which code for 18S and 24S RNA are nucleolar organizing in nature[149].

Rang and Datta[148] found 1 (48.39% to 65.57% PMCs; size: 8.30 µm ± 0.18) to 5 (size variation between 1.67 µm and 8.30 µm) nucleoli (Figs. 20-25) in different mutant (1-2: *Lax branching* and *viridis*; 1-5: *bushy, chloroxantha, crinkle leaf, feathery leaf, narrow leaf*) lines of *N. sativa*. Nucleoli was either free or found in association to different bivalents but occasionally two nucleoli of different or same sizes were seen attached to a single bivalent. Multiple and variable sized nucleoli formation were presumed as an outcome of disturbed genetic state of the plant types caused by gene mutation and the mutant genes possibly have induced changes in the regulatory system of the cell thereby activating various latent loci capable of synthesizing tiny nucleoli. Hiko-Lchi and Chen-Hui Kao[150] attributed size variation of nucleolus on the basis of difference in the intensity of nucleolar forming power.

Mitotic and Meiotic Abnormalities Arising out of Irradiations: Kumar and Nizam[151] assessed the effect of X-rays on dry and pre-soaked seeds of *N. sativa* and noted that the frequency of mitotic and meiotic aberrations in the pre-soaked seeds was higher than that of the dry seeds. The aberrations encountered were mostly related to spindle organization and formation of dicentrics, rings, micronuclei and acentric fragments. Mandal and Basu[152] studied X-ray induced chromosomal aberration from leaf meristems, pollen mother cells and endosperm and reported that aberration percentage increased with an increase in doses and decrease with time lapse from 2 to 24 hours after irradiations. Most resistant tissue was endosperm though it had the largest Interphase Chromosome Volume (ICV).

Datta and Biswas[153] (X-irradiations to dry seeds, doses- 6, 8, 10, 20, 30 kR, LD_{50}- lie between 8 kR and 10 kR), Datta *et al.*[154] (gamma irradiations- 5, 10, 20, 30, 40, 50 and 60 kR doses, seed moisture- 1.8%, LD_{50}- lie between 20 kR and 30 kR, treatments beyond 30 kR were lethal) and Mukherjee and Datta[155] (gamma irradiations- 50, 100, 150 and 200 Grey, moisture content- 19.04%, LD_{50} lie between 50 Gy and 100 Gy) reported physiological (germination and seedling growth under petriplate conditions) and chromosomal disturbances (mitotic and meiotic including pollen fertility) in irradiated samples. Frequency of total mitotic anomalies enhanced in treatments but the percentage of dividing cells decreased with an increase in the radiation doses, and it was suggested that mitotic disturbances have affected physiological processes like germination and seedling growth. Apart from normal chromosome configuration $2n=12$ (Fig. 26), irradiations (X-irradiation as well as gamma irradiations) have induced chromosomal aberrations like fragments, ring configuration of chromosome, pseudochiasma like configurations, diplochromosomes, cells with polyploid and aneuploid chromosome number and deformed cellular configurations at metaphase (Figs. 27-31), and bridges (single, double, criss-cross, inter-

locked and incomplete) with or without fragments (2 to 4 identical sized and rare often with one fragment- Figs. 32-41), and multipolar organization of chromosomes at anaphase (Figs. 42-43). At resting cells micronuclei (1-4 variable sizes; condensed as well as uncondensed) and giant cells were also noted (Figs. 44-47). Meiotic abnormalities studied following irradiations (apart from normal 6II formation- Figs. 48-49) were univalents (2-8, Figs. 50-52), fragments (paired identical sized- Fig. 53), multivalents (Figs. 54-57), stickiness (Fig. 58) and cell fusion (Fig. 59) at metaphase I (MI); while, fragments, bridges with or without an accompanying fragment were observed in anaphase I and II cells irrespective of normal segregation of chromosome at AI[153,154,155] (Figs. 60-65). Mukherjee and Datta[155] noted enhanced frequency of quadrivalents (mostly ring- 89.79%, rest were of chain configuration) was noted in higher doses of treatments. Most of the ring quadrivalents were of adjacent orientation (63.64%); while, the rest were alternate (34.09%) and rare often non co-oriented (2.27%). A PMC at 200 Gy was observed to posses 6II + two nearly identical sized (2.93 µm and 2.59 µm) fragments (1.21%) thereby suggesting localized breakage in chromosome due to irradiation. Paired identical sized fragments (5.38 µm) at AI was also studied in one of the two telocentric chromosomes (one telocentric is marked intact at one pole). Pollen sterility and meiotic anomalies studied have shown dose dependent increasing tendencies thereby indicating that former is an outcome of the latter.

Rang and Datta[156] exposed dry, pre-soaked (12 hours in distilled water), totally dehydrated and stored (one year six months stored under desiccation; one season stored seed) seed samples (moisture content: 7.5%) of *N. sativa* to gamma irradiations (5, 10 and 20 kR doses) and also that some amount of the dry irradiated materials were treated with ethyl methane sulfonate (EMS) and hydrogen peroxide (H_2O_2) for six hours at 0.25 percent to evaluate the cytogenetic changes that might occur due to gamma-irradiation influenced by the physical and chemical factors. Assessment of radio-sensitivity has been made from attributes like seed germination, rate of seedling growth, mitotic index, frequency and spectrum of chromosomal aberrations in root tip cells and pollen and seed sterilities of M_1 plants as well as M_2 mutation (macromutants) frequency. Results indicated that the factors (physical and combined treatments) have influenced gamma radiation sensitivity in inducing cytogenetical and genetical changes along with M_2 mutation frequency.

Mitotic Abnormalities Induced by Chemical Treatments: Biswas and Bhattacharyaya[157] studied the effect of some mutagenic chemicals like mateic hydrazide (MH), acridine orange (AO), ethyl urethane and ethylene-diamine-tetracetic acid (EDTA) at variable concentrations and durations on the root tip mitosis of the species. The chemicals induced cytological aberrations viz., fragments, laggards, micronuclei, grouping and stickiness of chromosomes and reduced mitotic index in prolonged treatments and in higher concentrations. The authors were of opinion that the chemicals possibly affect nucleic acid synthesis in differential manner which ultimately causes hazards in replication thereby inducing chromosome breakage.

Kumar and Nizam[158] studied induced somatic pairing of homologous chromosomes from root tip mitosis following treatment with mitomycin C. It was observed that the homologous chromosomes become juxtaposed to each other with remarkable regularity in the prometaphase cells following treatment for 40 minutes, whereas the untreated cells showed no such associations. It was presumed that these movements may be due to kinetochore activity which normally causes congregation of chromosomes towards the equatorial plate of the spindle but which does not occur contemporaneously in all chromosomes. In view of the observation, the authors were inclined to believe that kinetochores were responsible for placing homologues near each other and stickiness has been attributed to be a factor for association of homologous chromosomes.

Chand[159] reported that pentachlorophenol (PCP) inhibited mitosis in shorter duration of treatments and cytological abnormalities were formed. Incorporation studies revealed that PCP inhibited DNA synthesis. The chemical was found to affect nuclear membrane cycle, chromosome division cycle, spindle organization and chromosome movement, condensation and spiralization of chromosomes and DNA and protein synthesis.

Induced mutagenesis

Variants in M_1 Generation: Datta and Biswas[160] reported that as compared to the erect nature of the stem in untreated control plants, stem anomalies including bifurcation (Fig. 66), trifurcation (Fig. 67-68), twisting (Fig. 69), unbranched (Fig. 70) and twining nature of stem (Fig. 67) were observed at 4, 10 and 30 kR of X-ray doses and 2 and 4 hours treatment with 0.75% and 0.50% EMS respectively. Interesting floral anomalies were found to occur in all treated doses of EMS and only 20 kR X-irradiation. In relation to control flower (Fig. 71) interesting floral variations like adnation of sepals, elongated and strap shaped petals, two gynoecium in the same flower and presence of bract like structures (incompletely forked) similar to that of the petalloid sepals were observed (Figs. 72-78). The abnormalities studied at M_1 have not recurred in M_2 generation and these were non-inheritable changes (chimeric in nature) possible arising out of somatic mutation.

Macromutants and Their Inheritance Pattern: Kumar and Nizam[161] induced (X-rays and gamma rays) few viable mutants such as multicolor capsular

fruits and color fruit coat with ornamentations including mutation affecting branching pattern and fertility at M_2. Datta and Biswas[160] induced (X-ray and EMS) several chlorophyll (*albina* > *xantha* > *chlorotica* > *chloroxantha* > *albescens* > *albino-terminals* > *xantha-terminals* > *lutea* > *viridis* = *marginata* = *coeruleovirens*) and morphological (13 different types; 9 viable – *lax branching* - Fig. 80, *feathery leaf, bushy, male sterile, crumpled leaf, dwarf, early flowering, prostrate* - Fig. 81, and *brown seed coat*; 4 non-viable types– *cup* - Fig. 85, *needle leaf, crinckle leaf* and *cotyledonary leaf*) mutants in relation to normal trait (Fig. 79). Threshold doses were effective and efficient and 0.5% EMS, 2 hours treatment was the best among all the treated doses. Chlorophyll mutatuions occurred predominantly than other types and among them *viridis* and *chloroxantha* were the viable types and were found to be controlled by two pairs of recessive genes; while, the mutant trait(s) of *bushy*, *dwarf*, *feathery leaf*, *lax branching* and *early flowering* mutants were controlled by a single pair of recessive gene. Datta and Biswas[162] assessed different mutants (*lax branching, feathery leaf, bushy, early flowering, prostrate, dwarf, brown seed coat* and *viridis*) for different quantitative traits at M_2, M_3 and M_4 generations (ANOVA performed in mutant lines with control at M_4) and were of opinion that the mutants have exhibited superiority over the control plants in some of the characters only but not in all the parameters. This observation was significant as it offered scope of improvement through hybridization and selection.

Mitra and Bhowmick[163] induced ten different types of chlorophyll mutation in two cultivars of *N. sativa* following treatments with gamma irradiation and EMS. Higher doses of gamma-rays and lower concentration and duration of EMS were reported to be most efficient. Mitra and Bhoumick[164] studied the mutagenic effects (gamma irradiation and EMS) of some biological parameters in M_1 generation and suggested that gamma irradiations were more effective than EMS and the cultivar KS-1 was more sensitive to mutagens under the tested doses and concentrations.

Datta and Rang[165] screened seven viable morphological mutants (*lax branching, feathery leaf, bushy I* - Fig. 82, *bushy II* - Fig. 83, *lax pinnae* - Fig. 84, *needle leaf* and *crumpled leaf*) from 7956 treated plants at M_2 following mutagen treatments (gamma-rays, EMS and H_2O_2 and their combined treatments) to dry seeds (moisture content: 7.5%). F_2 segregation (control × mutant, F_1 normal) revealed that *lax branching, feathery leaf, bushy I* (associated traits: synchronous flowering, compact habit, thick dark green pinnae of leaves), *bushy II* (thick dark green pinnae of leaves) and *lax pinnae* (pinnae elongated) mutant traits were controlled by a single pair of recessive genes; while, selfed lines of *needle leaf* and *crumpled leaf* mutants showed that the mutant traits were controlled by two pairs of recessive genes.

Datta and Rang[166] spotted a viable *chloroxantha* mutant in EMS treated population at M_2. The seedlings of *chloroxantha* (Fig. 86) were pale greenish yellow in color (2012 – "Dictionary of Colour" by Maerz and Paul 1950) and the mutant could be easily marked at the very seedling stage. The mutant plants showed delayed flowering (17 to 29 days from control plants) and maturity, which indicates that the mutant being deficient in chlorophyll content might have utilized their buffering capacity to maintain the photosynthetic efficiency by increasing the number of branches (consequently pinnae of the leaves increased in the mutant) and duration of the crop to complete their life cycle successfully. The inheritance of the mutant trait was recessive and was under the control of two gene loci. The mutant was compared with control at M_4 and results indicated that *chloroxantha* possessed higher number of primary branches and capsules per plant and had smaller seed (length) than normal; although, other traits were more or less comparable to normal plants. The authors presumed that the color of *chloroxantha* may be exploited as genetic marker for efficient breeding.

Rang and Datta[167] spotted five dark reddish brown (color code - 3/2), one yellowish brown (5/4) and one peach (512/1) color (colors were confirmed from Horticultural Color Chart 1968 and Munsell Soil Color Chart 1975) seeded plants at M_2 following different treatments of gamma irradiations and EMS. Mutation frequency of dark reddish brown color (Fig. 89), yellowish brown color (Fig. 88) and bicolor (peach color was associated with blackish tinge at the base and the apical region – therefore designated as 'bicolor' - Fig. 90) was estimated to be 1.92, 0.055 and 0.54 percent respectively (7956 plants scored). Dark reddish brown and yellowish brown seed-coat color traits were monogenic recessive to black seeds (Figs. 87-90); while, the inheritance of bicolor trait of seeds was under the control of two pairs of recessive genes (mutant × normal – reciprocal crosses were performed, F_1 – black and F_2 segregation analyzed following χ^2 – test analysis). Crossing experiments suggested that black coloration of seeds is dominant over other seed colors and gene symbols assigned were B for black, b^{dr} for dark reddish brown and B^y for yellowish brown colors and p for peach color of seeds, and the dominant form (P) of this gene has no effect on B or on any allelic forms of B (b^{dr}/b^y) and the mutation involving both the dominant genes (B-P) results to bicolor seeds. Following genotypes were proposed for the seed-coat colors – BBPP, b^{dr} b^{dr} PP, $b^y b^y$PP and bbpp for black, dark reddish brown, yellowish brown and bicolor seeds respectively. The true breeding mutant plants were evaluated at M_4 in comparison to control for several agronomic traits and it was noted that dark reddish brown seed coat

mutant was as productive as normal; while the bicolor and the yellowish brown seed coat mutants were sort sized and small seeded plants.

Polygenic Mutation: Datta and Biswas[168] analyzed variations for quantitative characteristics (plant height, number of primary and total branches per plant, total capsules, capsule chamber/fruit, capsule length and seed per capsule) from 10 randomly selected plants of each of the M_2 (X-irradiated and EMS treated) lines and computed mean and coefficient of variations and also determined student t-test between control and treatment. The magnitude of variability released (as evidenced from C.V.) through induction of mutation was both positive as well as in negative direction, thereby suggesting random nature of mutation.

Biochemical Studies on Induced Mutants: Electrophoretic characterization and evaluation of seed protein in control and EMS induced mutant line of the species were performed from seed samples[169] and the qualitative as well as quantitative variations in banding pattern among the plants were noted. The authors were of opinion that electrophoretic characterization of the mutant lines may be used as an additional parameter to supplement cytogenetic data in understanding genetic variations. Das *et al.*[170] extracted protease from germinating seeds of wild type and seven EMS induced mutant lines of *N. sativa* and the activity was assayed with casein as substrate in the pH range 3.5-8.0. Results indicated that most protease types showed pH 3.6-7.0 and more than one protease enzyme in the plant types tested. Amylase activity and variation of amylase isozyme pattern were also studied and it was reported that gene(s) controlling enzyme production/activity have been affected differentially in different mutants.

Cytogenetical consequences of induced mutagenesis

Translocation Heterozygosity: Datta and Biswas[171] isolated a cytologically marked plant (phenotypically indistinguishable) from the R_1 population of gamma irradiations, which showed ring or a chain quadrivalent in 49.38% meiocytes at MI (241 cells scored). Although normal 6II formation was noted predominantly (50.72%) at MI, the most common type of configuration studied in the marked plant was 4II+1IV (34.25%); while in the remaining meiocytes the quadrivalent appeared in association with 2 univalents. PMCs with ring of four chromosomes (41.08%) occurred more frequently than those with chain quadrivalent (8.3%). Among the meiocytes showing interchanged configurations, 65.55% were alternate and 34.45% were with adjacent orientations. Anaphase I separation was mostly (82.0%) balanced (6/6) although pollen sterility was high (55.8%) with extremely poor seed setting (12.2±5.7) per capsule as compared to normal (pollen sterility – 2.2 to 3.6%; seed setting 65.6±4.2/capsule) plants.

Datta and Biswas[172] screened four extremely dwarf plants at M_3 having identical leaf phenotype as their progenitor from the selfed M_2 *feathery leaf* mutant (0.50%, 2 hour EMS treatment). Meiotic studies revealed the characteristic presence of paired fragments in the parent (M_2) and multivalents in the *dwarf* mutant plants (M_3 as well as M_4). The dwarf plants were designated as *telescopic* mutants as the leaves were found to be clustered around the stem forming a crown-like appearance. Out of four *telescopic* mutant (Fig. 91), one of which showed prevalence of ring quadrivalent. The cytogenetically marked *telescopic* mutant was semisterile and the possible origin of the mutant lines has been ascribed due to deficiency of genes as an outcome of chromosomal deletion in the parent.

Saha and Datta[145] induced 5 translocation heterozygotes (P-14 and P-26 from 5 kR and P-32, P-36 from 10 kR) following gamma irradiations (5, 10 and 20 kR) to dry seeds (moisture content 7.5%). P-14 (possessing long drooping floral shoot), P-32 (lax branching) and P-36 (semi-dwarf with thick and non-shattering capsules) were viable translocations; while, P-26 and P-37 yielded only abortive seeds at R_1 following selfing and on open or controlled pollination. The translocation heterozygotes exhibited the formation of either a ring or a chain of 4 chromosomes in 38.7% to 77.7% meiocytes apart from 6II formation (Figs. 92-101). Predominance of rings occurred in all translocation heterozygotes excepting P-26 where rings and chains were nearly equal. P-14 and P-26 had more adjacent orientation of quadrivalents than alternate; while, P-32, P-36 and P-37 demonstrated random orientations. The quadrivalent behavior was found to be persistent in all generations (R_1, R_2 and R_3) of P-14, P-32 and P-36. The rings showed preponderance of adjacent orientation and the chains demonstrated frequent alternate orientation. Though normal 6/6 separation of chromosomes at AI was observed in 85.8, 83.3, 69.4, 82.3 and 86.4% cells of P-14, P-26, P-32, P-36 and P-37 respectively (rest showed unequal separation of chromosomes and bridge formation with a lagging fragment - Figs. 102-103), pollen fertility was reduced in the heterozygotes (8.2 to 37.5%). F_1's raised from intercrossing of P-14, P-32 and P-36 were meiotically assessed and the results indicated that same 2 non-homologus chromosomes were involved in translocation and the 2 longest pairs were suggested to be associated.

Desynapsis (Synaptic Mutants): Datta and Biswas[173] noted desynaptic behavior of chromosomes in a *bushy* mutant (M_2 generation, 0.5% EMS, 2 hour treatment) and the mutant trait was reported to be controlled by a single pair of recessive genes. The *bushy* mutant plant could always be characterized by their delayed germination, flowering and maturity, high frequency of sterile pollen grain formation, poor seed setting and univalent formation in the meiocytes. Desynapsis

studied in the mutant was partial or weak because of high frequency of bivalents per cell (4.70 to 5.24) than univalent per cell (1.53 to 2.59). Compared to controls (5.24 ± 0.41 chiasma/cell), frequency of chiasma has been found to be decreased in M_2 *bushy* mutant (4.57 ± 0.49 /cell). Univalents formed in the mutant line were found to be distributed randomly in most of the cases, which were not affected by the number of bivalents per cell. Less frequently, however, occurrence of univalent in close proximity to each other could be marked, which may be an indication of their belonging to same pair and their very recent separation. Anaphase I separation was irregular (34.44% to 44.23%) in the mutant line leading to the formation of laggards and unequal separation of chromosomes. Lagging chromosomes at anaphase II and unequal size of microspores in tri- and polysporous condition were also noted.

Saha and Datta[174] reported two synaptic mutants (DS-1, 5 kR gamma irradiations; DS-2, 10 kR) at M_2 (screened from 6582 M_1 plant progenies) possessing distinctive phenotypic marker trait (lax branching). The synaptic mutants (medium strong type) demonstrated fuzzy appearance of chromosomes at early prophase I (Figs. 104-105) along with univalent frequency ranging from 0 to 12 (enhanced frequency - Figs. 106-112) per cell (control: 0.10, DS-1: 2.47, DS-2: 3.50), reduced number of chiasma and bivalent per nucleus (control: 5.95 II/cell, chiasma 9.34 ± 0.3; DS-1: 4.77 II/cell, chiasma 7.40 ± 0.3; DS-2: 4.25 II/cell, chiasma 5.59 ± 0.4), few meiocytes with unequal separation (5/7, 5-1-6 and 4/8) at AI (control: 0.5%, DS-1: 15.6%, DS-2: 22.5%), cytologically balanced AII cells and high pollen fertility (control: 98.06%; DS-1: 96.57%; DS-2: 93.95%).

Male Sterility: Male sterile mutants with distinctive phenotypic marker traits (*bushy*- EMS treatment; Datta and Biswas[175]; chlorophyll deficiency- 6 hours 0.25% EMS - Fig. 113; Rang and Datta[176]; *dwarf*-chlorophyll deficiency - Fig. 116, *crumpled pinnae* - Fig. 115, *bushy* - Fig. 114 and *lax pinnae*, gamma irradiation- 5, 10 and 20 kR and EMS- 0.25, 0.50 and 1.00%, 3 hours; Datta and Saha[177]) were isolated from M_2 mutagenized population. Concomitant association of phenotypic marker trait(s) with male sterility was unique as it not only give selective advantage but will also be of immense value in the breeding behavior of the crop.

Datta and Biswas[175] isolated a male sterile mutant which was indistinguishable at earlier stages of growth, but the mature plant could be recognized by its characteristics dark green, thick and leather like pinnae of the leaves and synchronous flowering. Although the mutant demonstrated normal behavior of meiotic chromosome with 6 bivalents in MI cells and usual formation of tetrads, none of the pollen grains could be scored in the mutant which is an indication of complete inhibition of pollen grain development leading to male sterility. Post tetrad developmental disturbances might be responsible for arrestation of pollen formation.

Rang and Datta[176] reported a male sterile plant which exhibit broad elongated lax pinnae along with yellowish green pinnae in the shoot apex of the primary axis at the onset of floral bud initiation (Fig. 113), and non-dehiscent and pollenless anthers at anthesis. The male sterile plant showed desynaptic behavior of chromosomes and the chromosomal association studied at diplotene, diakinesis and MI (168 PMCs scored) were 6II (4.76%) - Fig. 117, 5II+2I (9.52%), 4II+4I (4.76%) and 12I (80.95%) - Fig. 118-119. Mean frequency of univalents and bivalents per cell was estimated to be 10.10 and 0.90 respectively, chiasma frequency per nucleus observed in the male sterile plant was 0.31±1.2 as compared to 9.9±0.74 in normal plants. Unequal separation of chromosomes was studied in AI (71.43%) and AII (42.62%) from 82 and 122 cells respectively. The male sterile plant produced tetrads mostly with unequal spory (Fig. 120) followed by near complete degeneration of microspores (Fig. 121) compared to normal oval shaped fertile pollen grains in control plants (Fig. 122).

Datta and Saha[177] categorized male sterile mutant plants into five (I to V) types on the basis of sterility and morphology. The mutants were type I: mutant *dwarf*, pollen grains 100.0% sterile and showed sign of degeneration, pollen grains were round and small sized 27.2 µm × 24.8 µm (Figs. 128-129) as compared to oval shaped pollen grains (Fig. 130), 39.0 µm × 38.08 µm; type II: mutant yellowish green color, 100.0% sterile pollen grains, small roundish with thick wall; type III: mutant with crumpled and deformed pinnae of leaves, anther small sized 3.74 mm ± 0.05, brownish, shrunken and indehiscent and were completely pollenless at maturity. Meiotic analysis revealed no clear bivalent formation, rather the chromatin agglutinated into unequal masses (Figs. 123-125). Agglutination of microspores was also evident which consequently degenerated (Figs. 126-127); type IV: *bushy*, normal cytological behavior, 100.0% sterile pollen grains; type V: the mutant plants were with long elongated and dissected pinnae of leaves and the pinnae were lax in nature. The mutant plants demonstrated normal meiotic chromosomal behavior and formed tetrads but the pollen grains were completely sterile. The male sterile mutants showed monogenic recessive (IV to V) as well as digenic recessive (II) mode of inheritance pattern. Type I and III were both male and female sterile. The mutants arising out of gene mutation and the mutant genes have favoured the continuation of meiosis and thereafter they have acted on microspores and on pollen grains. The mutants were non-structural nuclear type as per classification proposed by Gottschalk and Kaul[178] and Johns *et al.*[179].

Trisomic: Datta and Biswas[180] isolated a trisomic (detected after male meiotic studies) plant from the

selfed progenies of M_2 *lax branching* mutant at M_3. Morphologically the trisomic plant was weak with slender stem and drooping lamina at the seedling stage. At maturity the plant attained a height of 19.7 cm. Flowering in the trisomic was delayed by 10-11 days as compared to normal plants. Only four flower buds of the trisomic bloomed, while the rest dried up. Flowers were smaller in size and at maturity the stamens turned brownish in contrast to yellowish green color in the control and ultimately rudimentary capsules with abortive seeds were formed. The trisomic showed 6II+1 (87.8%) and 5II+ 1III (12.2%) chromosomal associations in 72 and 10 PMCs respectively (Figs. 131-133). At AI, either the extra chromosome appeared as laggard or has been incorporated in any of the two poles (Fig. 134). The aneuploid plant appeared to be a primary trisomic, with 58.17% pollen sterility and it was completely seed sterile.

Cytomixis: Datta and Biswas[180] studied transfer of nuclear materials from one PMC to another at prophase I and MI while performing male meiotic analysis in M_2 mutants (observed in *lax branching* mutant). Chromatin transfer between adjacent meiocytes occurred through cytoplasmic links and the migration was at random within a group of PMCs (Figs. 135-137). The phenomenon of cytomixis was restricted between/among few clusters of meiocytes of a single microsporophyll squash preparation. Cytomixis resulted in hypo- and hyperploid variation in chromosome numbers (19.87%) in meiocytes, thereby producing aneuploid and polyploid PMCs. The nucleolus of the meiocytes, undergoing chromatin transfer, in most cases remained in the donor cell; rarely it passed to the cytoplasm of the recipient cell along with the chromatin materials. Clumping and sticky nature of the nuclear materials were also noted in certain PMCs.

Meiotic Instability: Datta and Biswas[181] identified a phenotypically aberrant and sterile plant at M_3 in the selfed progeny of EMS-induced M_2 mutant (*lax branching*), which showed aneuploid variation in chromosome numbers. Phenotypically, the aberrant plant exhibited lax branching nature (Fig. 138) attaining a relatively shorter height (32.7 cm) at maturity as compared to rather erect (43.65 cm ± 1.72) and compact habit of the normal plants. During the initial growth period of the plant the pinnae of lamina were represented by linear, thicker appendage like structures and at the latter stages few normal leaves developed. Most of the flower buds terminated in rudimentary flowers excepting a few which bloomed after 121 – 137 days after sowing instead of 70 – 98 days in control plants. The flowers had only 1–2 normal looking stamens, while rest of the microsporophylls were represented as leafy projections. These flowers produced only rudimentary capsules with abortive seeds. Meiotic analysis revealed distinct chromosomal instability - Figs. 139-143 (2II+5I – 2.6%, 5II – 5.3%, 11I – 2.6%, 5II+1I – 7.9%, 4II+3I – 7.9%, 6II – 26.3%, 1IV+ 4II+2I – 5.3%, 7II – 5.3%, 12 II – 36.8%, 78 PMCs could only be analyzed) with remarkably higher pollen sterility (78.5%). Both stained and unstained pollen grains were considerably smaller sized (10.05 µm ± 0.2; normal – 39.8 µm ± 0.6) than control pollen grains (Figs. 144-145). Moreover, functional instability of the stained (fertile) pollen has been evidenced by the formation of only rudimentary seeds in the marker plant. The aberrant has been ascribed as the outcome of cytomixis noted in *lax branching* M_2 mutant.

Induced polyploidy

Biswas and Chatterjee[182] induced tetraploid plants following seed and seedling treatments with various concentrations of colchicine and the plants were with increased number of branches, enhanced size and frequency of stomata, increase in the number of flowers, variation in pollen size, fruit setting and the rate of germination of seeds, increase in the number of septa per fruit and seeds per septum and delayed flowering. Biswas and Datta[183] performed meiotic analysis in colchicine induced (seedling treatments) autotetraploid plants and found prevalence of chromosome irregularities producing varying number of quadrivalents (0-4), trivalents (0-2) and univalent (0-10). The tetraploids were seed sterile.

Saha and Datta[184] induced one autotetraploid (C_0-1; 5 hour treatment with 0.5% aqueous solution of colchicine for 3 consecutive days) following treatment with colchicine at the apical meristematic tips of young seedlings bearing only two cotyledonary leaves. The autotetraploid at maturity yielded 37 healthy seeds, 30 seeds were sown in C_1 generation and 11 plants were obtained of which 4 were cytologically confirmed to be autotetraploids. The seeds of C_1 tetraploids were bulked and 25 randomly selected healthy seeds were sown in C_2 generation from which 5 plants were obtained and all were meiotically confirmed to be tetraploids. The most prominent morphological changes of C_0-1 tetraploid and its progenies at C_1 and C_2 were increase in flower and capsule sterility and reduction in seed number per capsule and seed fertility (expressed as per cent of control). Seed set in the tetraploid plants varied from 0.64 to 12.62% of control, thereby demonstrating negative selection value of induced autotetraploids. However, one autotetraploid (C_2-2) possessed some useful traits compared to the diploid and other tetraploids (Figs. 146-147). The C_2-2 plant yielded 118 good seeds (12.62% of control) and the flowers (significantly larger than those of diploids) of the plant (synchronous flowering) remained in blooming stage for a considerably long period (25 to 32 days) than the flowers of diploids (4 to 5 days) and the other tetraploids (8 to 12 days) studied over two generations. Compared to normal (Fig. 148) diploids ($2n$=12) the induced tetraploids ($2n$=$4x$=24) formed

quadrivalents (0-4), bivalents (1-12) and univalent (0-14) in varying proportion at MI (Figs. 149-151). Trivalents (0-2) were only observed in C_0-1 plant. The induced tetraploids formed 0.80 to 2.08 quadrivalents per cell and the coefficient of quadrivalent realization was low. Chi-square test of heterogeneity revealed that the frequency of bivalents and quadrivalents per cell among the tetraploids was random ($p>0.05$) but number of univalent per cell was non-random ($p<0.001$). The mean chromosomal association in C_2-2 was 1.37IV+9.00II+0.50I (32 PMCs scored). The univalent frequency among tetraploids demonstrated significant positive correlation with abnormal AI (laggards, bridges, groupings and unequal separation - Figs. 152-158) cells ($r=0.81$; $p<0.05$). Anaphase II cells showed unequal and multisporic conditions (Figs. 159-160).

The abnormal AI cells showed significant negative correlation with pollen fertility ($r=-0.99$, $p<0.001$). However, the correlation between frequency of abnormal AI cells and seed set and between pollen fertility and seed yield and between pollen fertility and seed fertility were non-significant. Cytological examination of induced autotetraploids leads to the conclusion that reduction in pollen fertility was the result of chromosomal disturbances arising from pairing irregularities. Seed sterility seems to have a genetical rather than cytological basis.

Genetic variability

Datta[147] studied relationship between yield and its attributes (plant height, number of primary branches/plant, total capsules/plant and seeds/capsules) and found significant positive correlation in all cases excepting for seed/capsule. Plant height was positively associated with primary branches/plant ($r=0.71$, $p<0.01$) and total capsules/plant ($r=0.69$, $p<0.01$); while, number of primary branches/plant was significantly associated with capsules/plant ($r=0.80$, $p<0.01$). However, capsules/plant showed insignificant relationship with seeds/capsule ($r=0.04$, $p>0.05$). Path coefficient analysis revealed that the direct contribution of total number of capsules/plant ($P_{35}=0.7460$) was very high and the trait indirectly contributed in high amount through plant height and number of primary branches. Direct contribution of plant height ($P_{15}=0.2886$) and seeds/capsule ($P_{45}=0.1296$) to yield was relatively low. Primary branches/plant ($P_{25}=-0.3374$) showed negative contribution to yield. Results indicated that capsules/plant is the most important trait for selection and crop improvement.

Iqbal *et al.*[185] studied 34 accessions with 2 check genotypes of black cumin for assessment of mineral nutrients. High variation was recorded for Fe, Ca, Cu, Mg, Pb, Zn, Co, Mn, Na, P, B, K and N amongst genotypes suggesting sample selection based on the composition of mineral nutrients. Correlation studies revealed significant association between Cu and Ca, and between Mg and Ca and Mg and Cu. Based on principle component analysis (PCA) six clusters were observed and it was suggested that the genotypes may be utilized in various combinations for genetic improvement of the species. Iqbal *et al.*[186] recorded genetic variation for plant height, days to first flower, days to 50% flowers, days to maturity, biomass, capsule weight, yield, seed weight and harvest index while studing 31 genotypes under field conditions with 3 replications. Three accession (MP00023, MP00111 and MP00120) were found better for more than one character and are expected to be a potential for improvement of *N. sativa*.

Tissue culture

Callus Induction: Banerjee and Gupta[187] raised calluses from leaf tissues and were of opinion that induction of calluses depended on the balance between auxin and kinetin in the medium and coconut milk factor as a source of kinetin. Chand and Roy[188] used different concentrations of 2,4-D, NAA and IAA to explore maximum callusing in the species. The concentration of kinetin has been kept constant throughout the experiments. The calluses grown in medium containing NAA, have been found to be friable, soft and green in color than in media containing IAA and 2,4-D. It was suggested that NAA was most favorable for producing callus tissue. Ghosh and Gadgil[189] initiated callus culture from excised hypocotyl segment when cultured in MS agar medium supplemented with IAA, NAA, IBA and 2,4-D. Chand and Roy[190] observed that in presence of GA in the media the seeds as explant grew into plantlet and there was no callus formation; while in the presence of NAA in the media seeds first produced calli from which plantlet developed. In the presence of IAA seeds grew into plants but at the base callus formation took place. In all cases amount of kinetin and coconut milk were kept constant. It was also pointed out that in presence of 2,4-D, kinetin and coconut milk seed proliferated into callus tissue without formation of plantlets. Datta *et al.*[191] reported calli formation from hypocotyl segment in MS medium supplemented with 2,4-D (2 mg/l) and kinetin (1 mg/l), and they were creamy white, compact ones. Youssef *et al.*[192] reported that 0.05 per cent casein hydrolysate promotes callus growth; however, growth was reduced by increasing salinity. On the contrary, it was also suggested that accumulation of primary products in callus cultures is enhanced by salt stress. Al-Ani[193] cultured roots, hypocotyls and leaves in MS medium supplemented with 2,4-D (0.0, 1.0, 2.0, 3.0, 4.0 mg/l) and kinetin (0.0, 1.0, 1.5, 2.0, 2.5, 3.0, 5.0 mg/l) and best callusing was obtained from leaf explants with 1 mg/l 2,4-D and 1.5 mg/l Kin. Such callus yielded higher thymol concentrations after 75 days by HPLC.

Suspension Culture: Banerjee and Gupta[194] reported that in suspension culture 91% free cells of *N. sativa* was obtained in WHITE's medium supplemented

with casein hydrolysate, inositol and adenine. Ploidy distribution pattern was similar in cell clumps of different sizes and free cells. Chromosomal irregularities were more in free cells. A number of globular embryoid were formed when casein hydrolysate, inositol and adenine were added in the medium after subsequent omission of auxin and coconut milk.

Embryogenesis: Banerjee and Gupta[195] noted embryogenesis in leaf callus (MS media supplemented with casein hydrolysate; coconut milk replaced). Casein hydrolysate suppressed the differentiating capacity at a concentration of 100mg/l after fifth subculture. It was reported that 2,4-D and kinetin have inhibiting effect on morphogenesis. On the histological examination of differentiated tissue, it was observed that roots, shoots, buds and leaves have originated from group of meristematic cells whereas embryoids have initiated by the repeated division of single cell.

Elhag *et al.*[196] with an objective of inducing and isolating somatic embryos for biosynthetic studies callus cultures were initiated from leaf, stem and root explants of axenic seedlings on MSB5 basal medium supplemented with kinetin (0.46 µM) and 2,4-D (4.5 or 13.5 µM) or NAA (5.4 or 16.2 µM) in the dark. Cultures initiated and subcultured on medium containing NAA produced friable callus with numerous roots regardless of explant type. These cultures differentiated into somatic embryos on medium containing NAA. The embryos developed into leafy structures on basal medium devoid of growth regulators. When the embryogenic callus was transferred to liquid medium containing NAA, numerous embryos and clusters of embryos were released into the liquid medium but, in contrast to solid medium, development remained arrested at the early embryonic stages.

Chromosomal Instability: Chand and Roy[188] reported very high number of chromosomes in media containing 2,4-D and kinetin; while NAA resulted very minor chromosomal variations. Ghosh and Gadgil[189] found shift in ploidy level from diploid to higher polyploids in presence of 2,4-D and when kinetin was mixed with 2,4-D or 2,4-D mixed with coconut milk factor. Bansal and Sen[197] reported that polyploidy has been a common feature of occurrence in calluses induced from root, shoot and leaf tissues and their appearance did not show marked difference in the tissues. Datta *et al.*[191] studied numerical variations in chromosome number including polyploidy, aneuploidy and haploidy as well as structural anomalies (Figs. 161-166) from callus tissues raised from hypocotyl segment. Frequent chromosome elimination in different cell lines was noted; however, the marker chromosomes (telocentric) were found constantly at different ploidy level. Kumar and Roy[198] were of opinion that apart from occurrence of high frequency of aneuploid and polyploid cells in callus tissues, structural anomalies like binucleate cell, micronuclei, diplochromosomes, multipolarity, sticky bridges and ring chromosomes formation was also observed. Anomalies might be due to endoduplication and various mitotic disturbances.

Molecular genetics

Al-Huqail and Al-Saad[199] performed DNA fingerprinting in 4 accessions from Saudi Arabia, Ethiopia, Egypt and Syria with an objective of genotypic characterization between/among black cumin taxa. Inter Simple Sequence Repeat (ISSR) method was employed in the PCR technique to detect genetic polymorphism. The scored bands of the DNA fingerprints (17 primers representing 3 types of intermicrosatelites – di, tri and tetra of short tandem repeats) were 108 in Saudi Arabia, 106 in Ethiopia, 100 in Egypt and 81 in Syria and the percentage of dissimilarity was computed to be 21.5-36.3%. Twenty four genes representing 24 different enzymes and isozymes were selected and scanned via PCR technique using suitable SSR primers and the obtained results showed some changes in the genetic structure of some of these genes. Iqbal *et al.*[200] carried out investigation to explore genotype specific fingerprinting of 32 germplasms based on randomly amplified polymorphic DNA markers. From 58 random primers used, 15 primers generated 249 reproducible and scorable amplification products across all the genotypes, out of which 164 (66.0%) fragments were polymorphic revealing a high level of polymorphism among the genotypes. The proportion of common bands was low (34.0%). In 13 genotypes, 27 bands of different masses (kilobases) were recorded and were considered specific. The specific/amplified PCR products were reported to be used as molecular markers for identification of germplasms and resource protection. The result of genetic polymorphism was validated from UPGMA and PCA.

Genes

1. APETALA 3 – like protein (AP3-3) mRNA, 746bp, linear, partial cds, accession – HQ694794[201].
2. APETALA 3 – like protein (AP3-2) mRNA, 865bp, linear, partial cds, accession – HQ694795[201].
3. PISTILLATA – like protein (PI-2) mRNA, 809bp, linear, partial cds, accession – HQ694796[201].
4. PISTILLATA – like protein (PI-1) mRNA, 840bp, linear, partial cds, accession – HQ694797[201].
5. microsatellite NIG_HSP 70 sequence, DNA, 345bp, linear, accession – HM803244.1[202].
6. *Nigella sativa* voucher A. Guener, M. Vural and H. Sagban 9189 internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal

transcribed spacer 2, complete sequence, DNA, 621 bp, linear, EU699463[203].

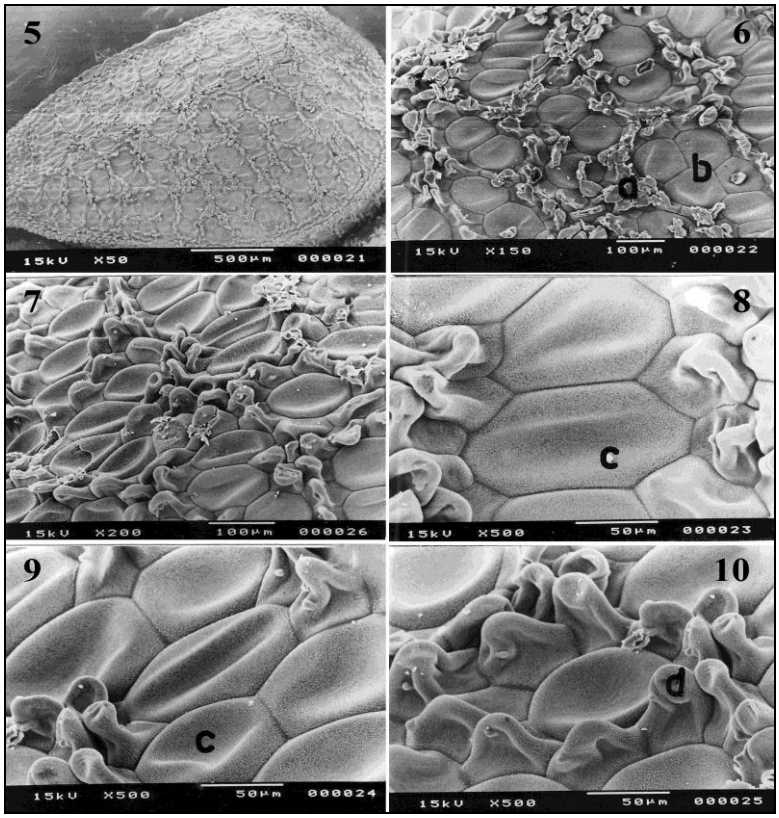
7. *Nigella sativa* internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence, DNA, 621 bp, linear, EU699464[203].
8. *Nigella sativa* beta-amyrin synthase (basl) mRNA, complete cds, mRNA, 2430 bp, linear, FJ013228[204].
9. *Nigella sativa* beta-amyrin synthase (basl) gene, complete cds, DNA, 4444 bp, FJ013229[204].
10. *Nigella sativa* squalene epoxidase 1 (seq 1) mRNA, complete cds, mRNA, 1566 bp, linear, FJ232947[205].

Patents

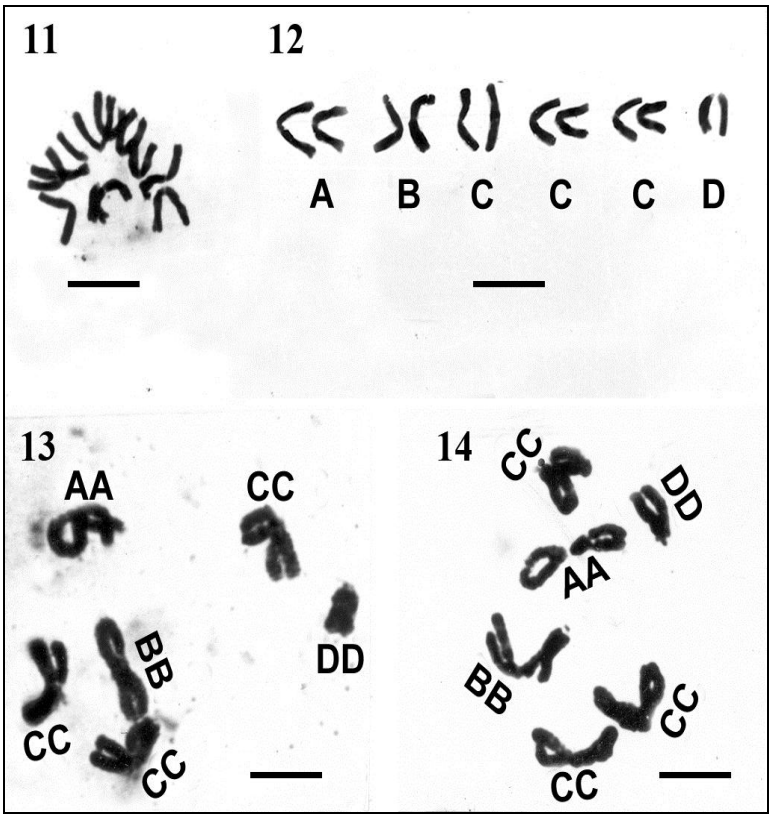
Nigella sativa currently has five FDA (Food and Drug Administration) separate patents in the U.S.A. for the treatment of:

1. Inhibition of cancer cell growth, Patent no.- US 5,653,981, Inventor- R. D. Medenica.
2. Diabetes, No.-US 6,042,834, Inventor – Wasif Baraka.
3. Improvement of the Immune System, No.- US 5,482,711, Inventor – R. D. Medenica.
4. Viral Infections, No.- US 6,841,174, Inventor – S. I. A. Shalaby and E. M. A. H. Allah.
5. Psoriasis, No.- US 6,531,164, Inventor – H. H. R. Credé.

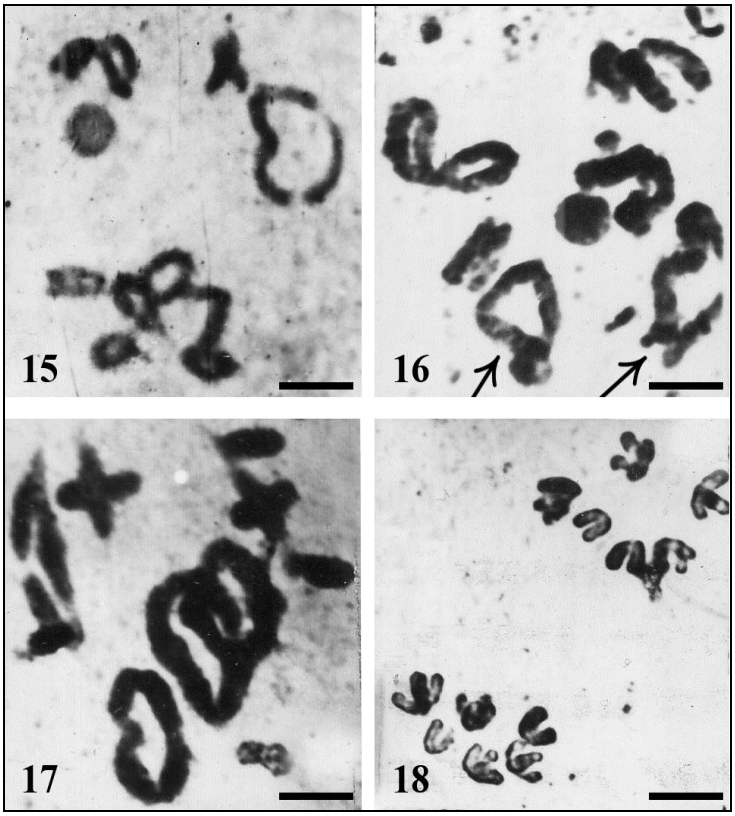
Figs. 1-4. 1) Normal *N. sativa* plant. 2) Flower before pollination. 3) Flower after pollination. 4) Seeds of black cumin.



Figs. 5-10. Scanning Electron Microscopy of seed surfaces of black cumin. [Source: Cytologia 68, 2003]



Figs. 11-14. Chromosomes (2*n*=12) in *Nigella sativa*. 11) Mitotic chromosome. 12) Photoplate ideogram showing 4 (AA, BB, CC, DD) chromosome types. 13-14) Diplotene plates where the bivalents are marked. Bar=15 µm. [Source: Cytologia 67(4), 2002]



Figs. 15-18. Meiotic configurations (2*n*=12). 15-16) 6II at diplotene. 17) MI showing 6II. 18)6-6 separation of chromosomes at AI. Bar=15 µm.

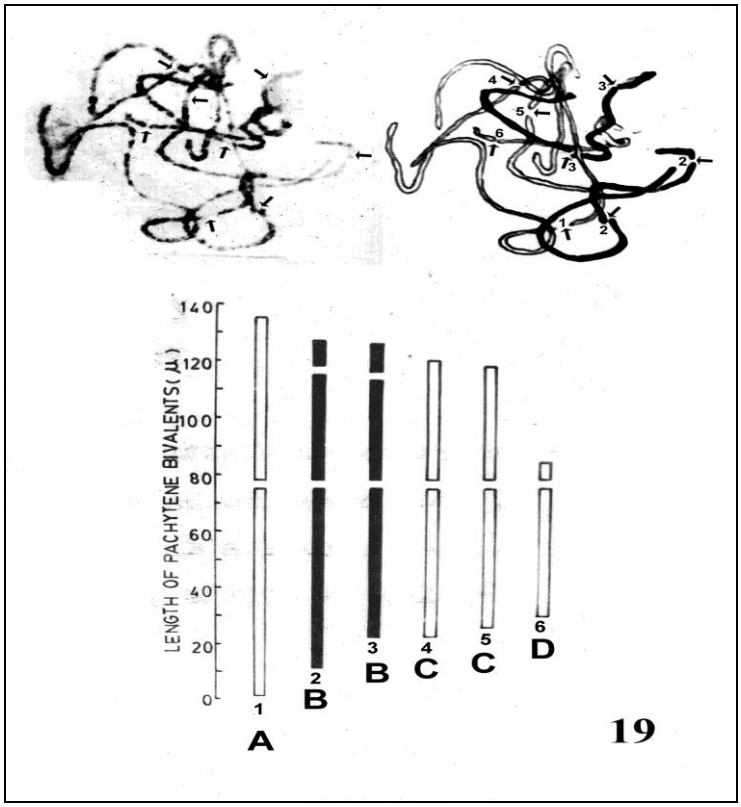
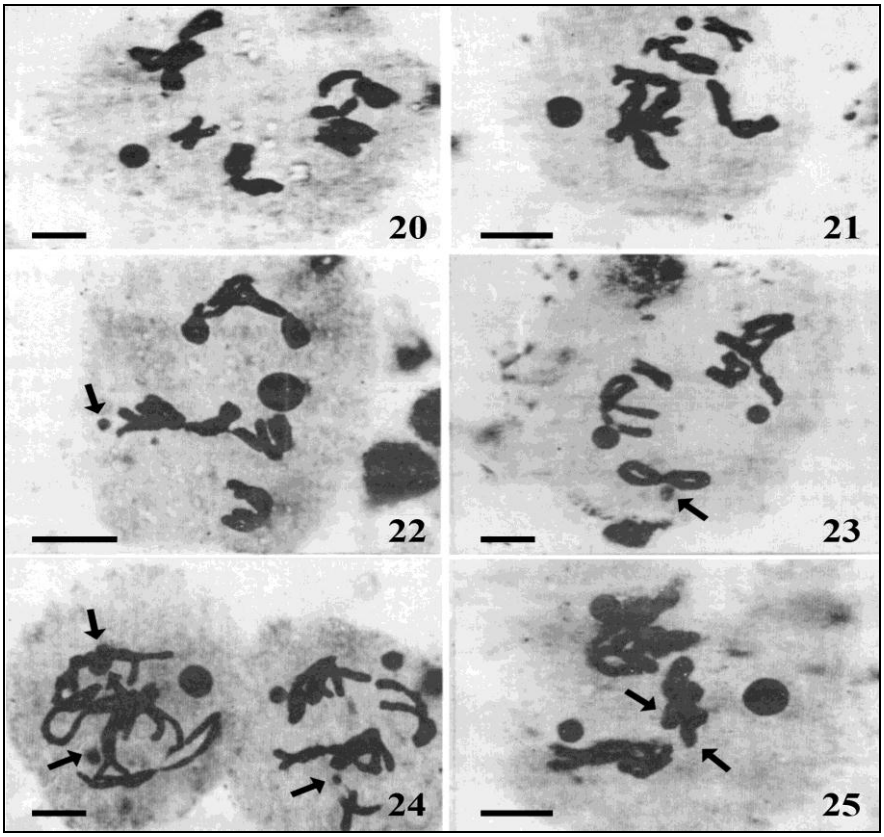
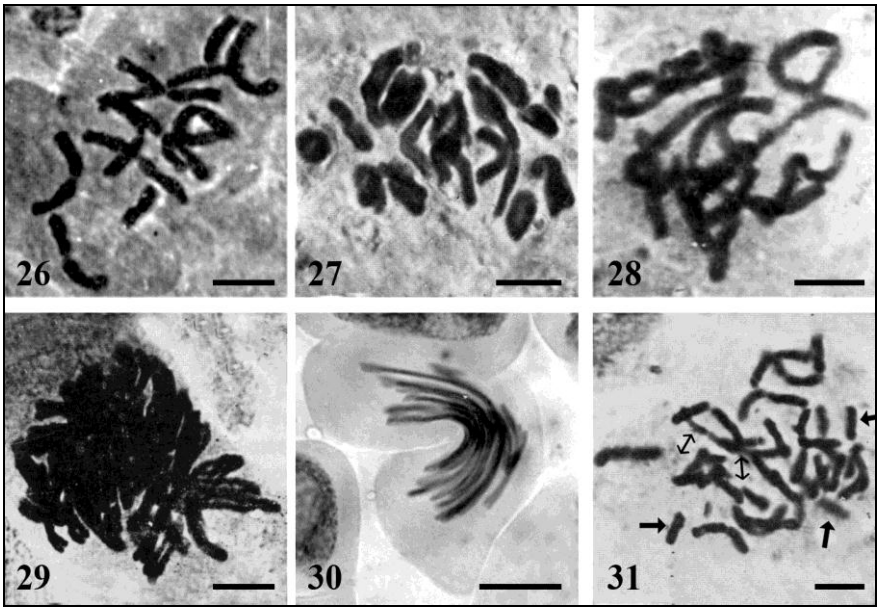


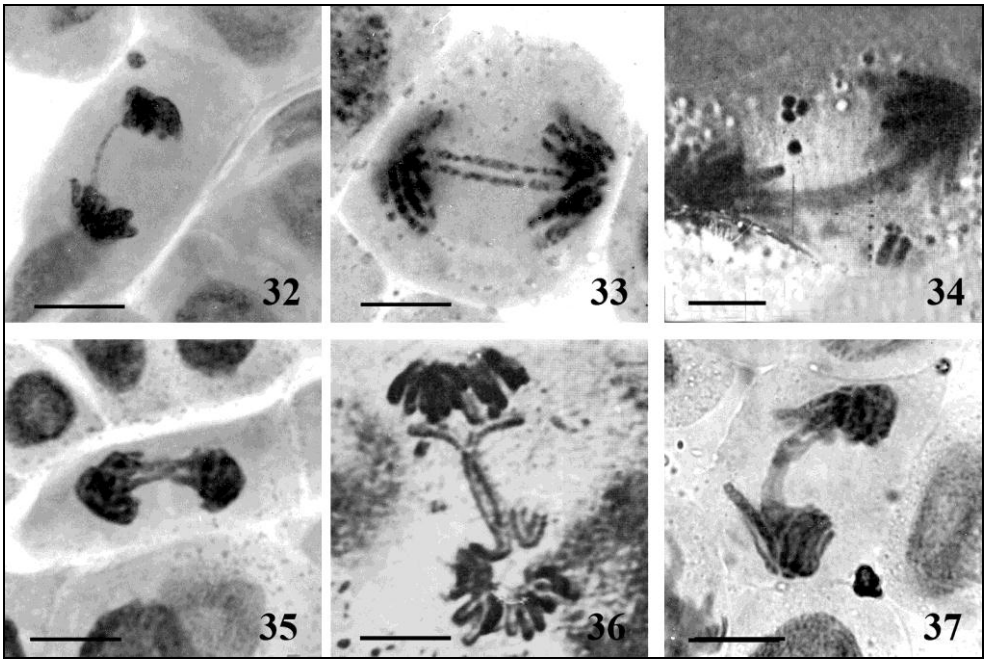
Fig. 19. Pachytene chromosome configurations.



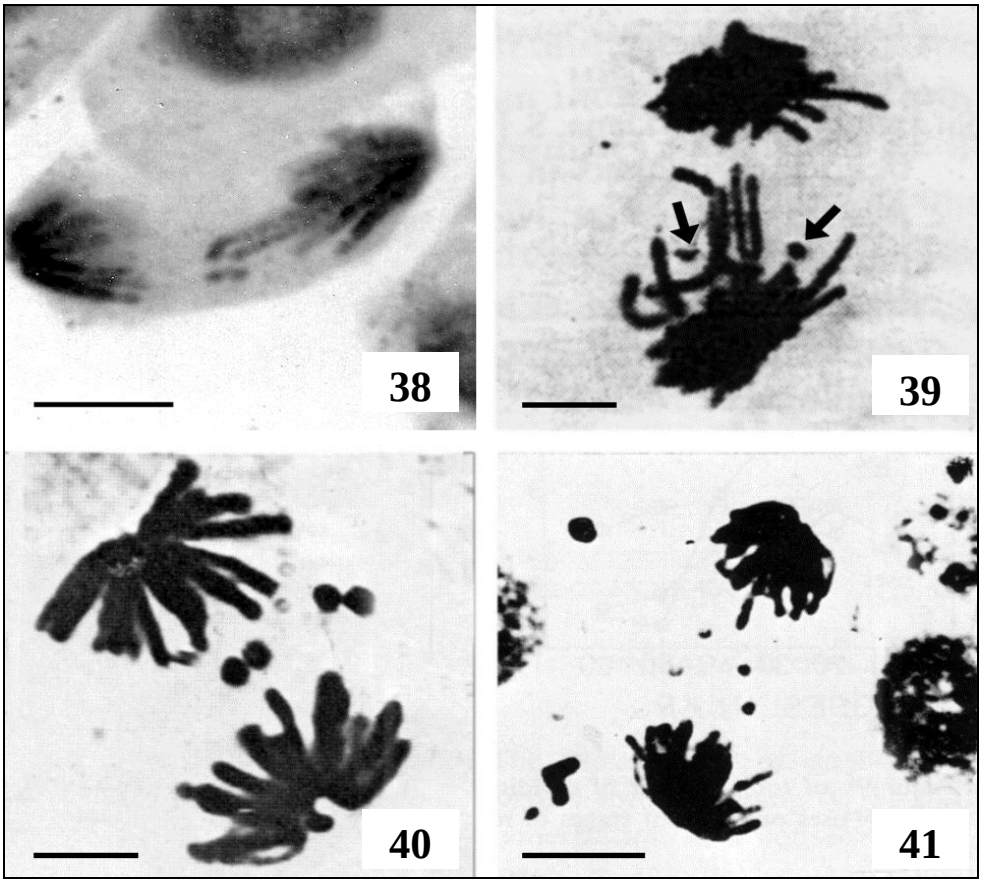
Figs. 20-25. PMCs at prophase I showing variation in number and size of nucleoli in control and in mutant lines of *N. sativa*. 20) One nucleolus. 21-22) Two unequal sized nucleoli, unattached to bivalents. 23) Three nucleoli. 24) Four nucleoli of which two are attached to a bivalent (a) and three unequal sized nucleoli (b). 25) Five nucleoli. Bar=15 µm. [Source: J. Phytol. Res. 12(1-2), 1999]



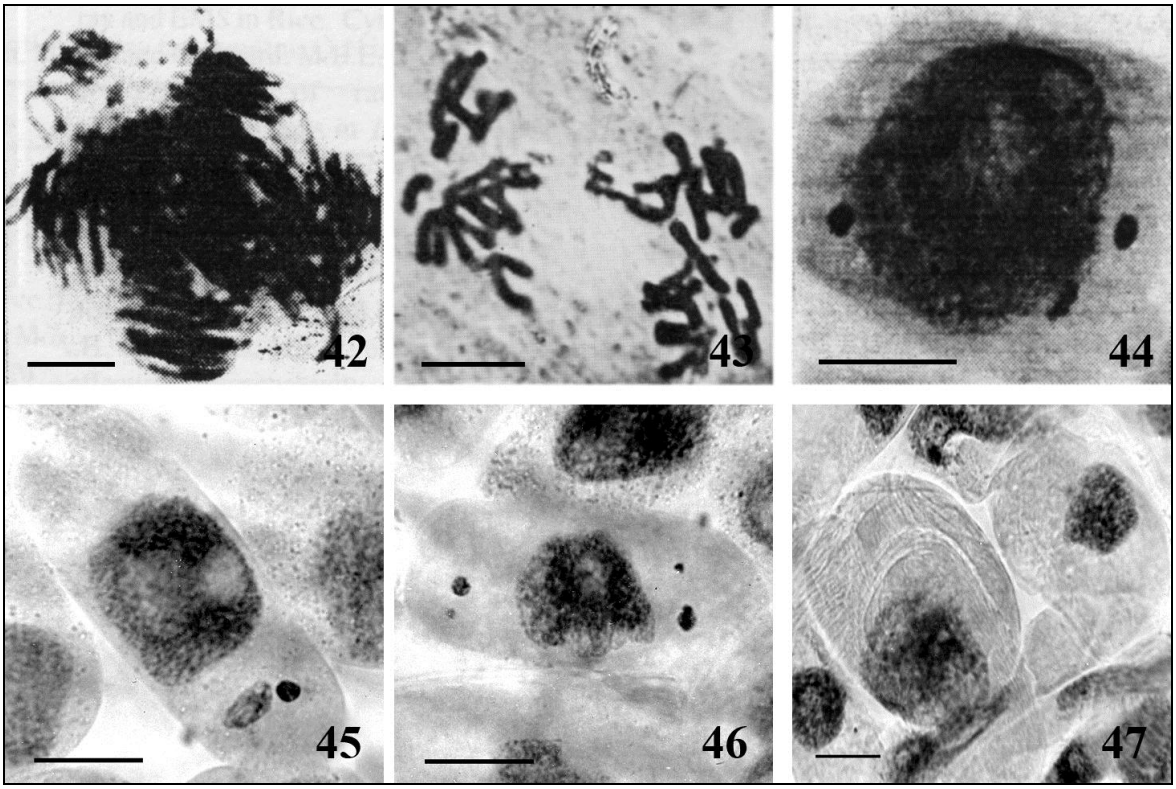
Figs. 26-31. Mitotic consequences following irradiations at metaphase. 26) 2n=12 – normal configuration. 27) Pseudochiasma like configuration. 28) Ring chromosome. 29) Diplochromatic nature of chromosomes in a polyploid cell. 30) Abnormal shaped cell with chromosome bending. 31) Aneuploid cell with fragments and unequal chromosome length. Bar=15 µm. [Source: Cytologia 48, 1983; Cytologia 51, 1986; J. Plant Dev. Sci. 3(1), 2011]



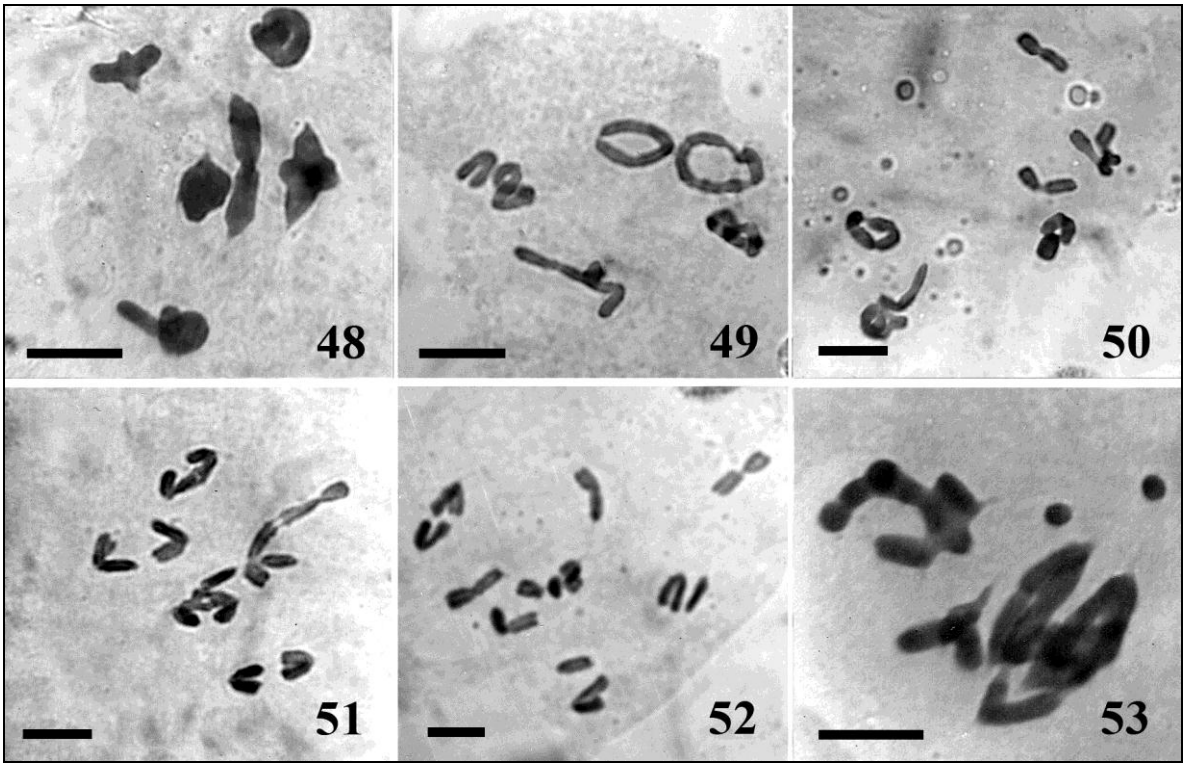
Figs. 32-37. Anaphase bridge formation in irradiated samples. 32) Single bridge with a round globular fragment. 33) Double bridge. 34) Double bridge with equal sized round and rod fragments. 35-36) Criss-cross bridge. 37) Interlocked bridge. Bar=15 µm. [Source: Cytologia 48, 1983; Cytologia 51, 1986; J. Plant Dev. Sci. 3(1), 2011]



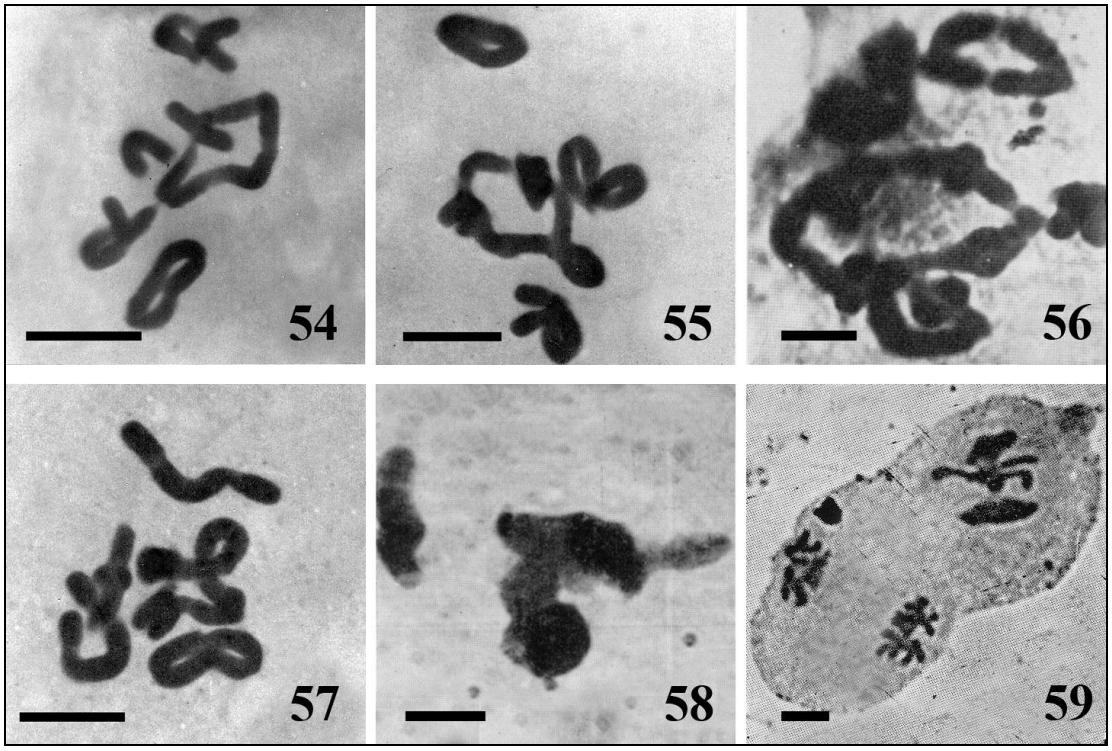
Figs. 38-41. Anaphasic events following irradiations. 38-39) Incomplete bridge with two identical sized fragments. 40) Paired fragments. 41) Four fragments. Bar=15 µm. [Source: Cytologia 48, 1983; Cytologia 51, 1986; J. Plant Dev. Sci. 3(1), 2011]



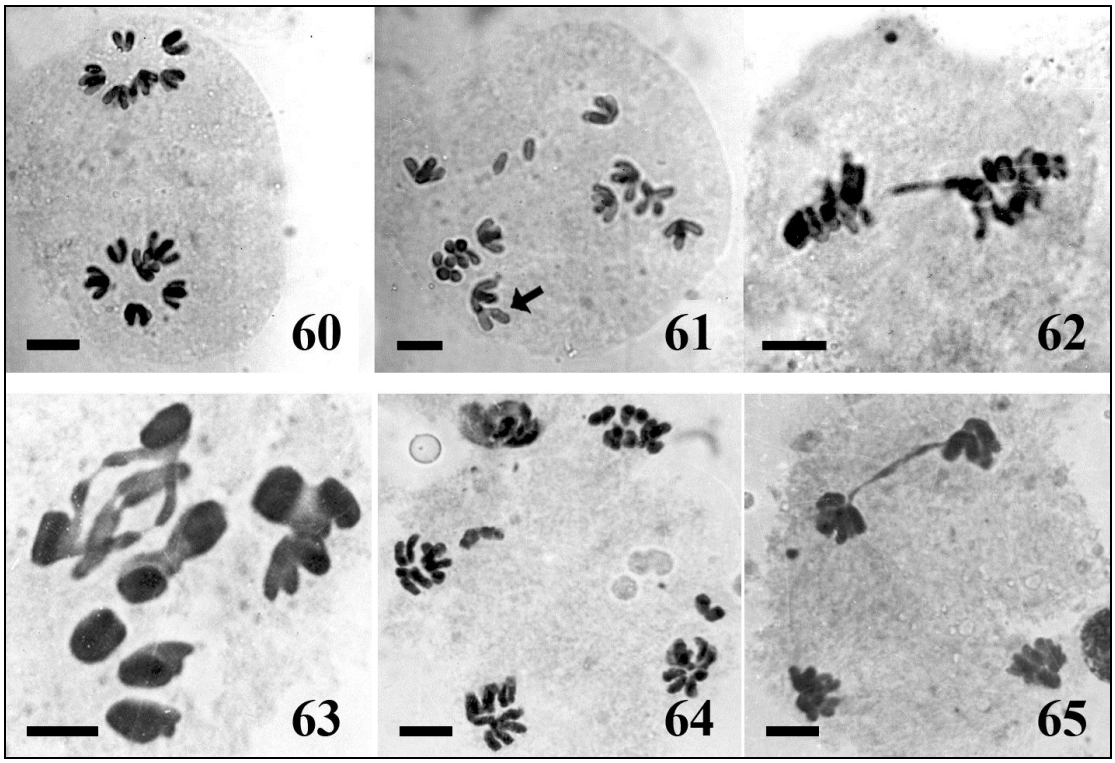
Figs. 42-47. Mitotic events following irradiations. 42) Polyploid cell at anaphase showing multipolar organization. 43) Multipolarity at anaphase. 44) Two condensed nearly identical sized micronuclei in resting cell. 45) Condensed and uncondensed micronuclei. 46) Four unequal sized micronuclei. 47) Giant cell. Bar=15 µm. [Source: Cytologia 48, 1983; Cytologia 51, 1986; J. Plant Dev. Sci. 3(1), 2011]



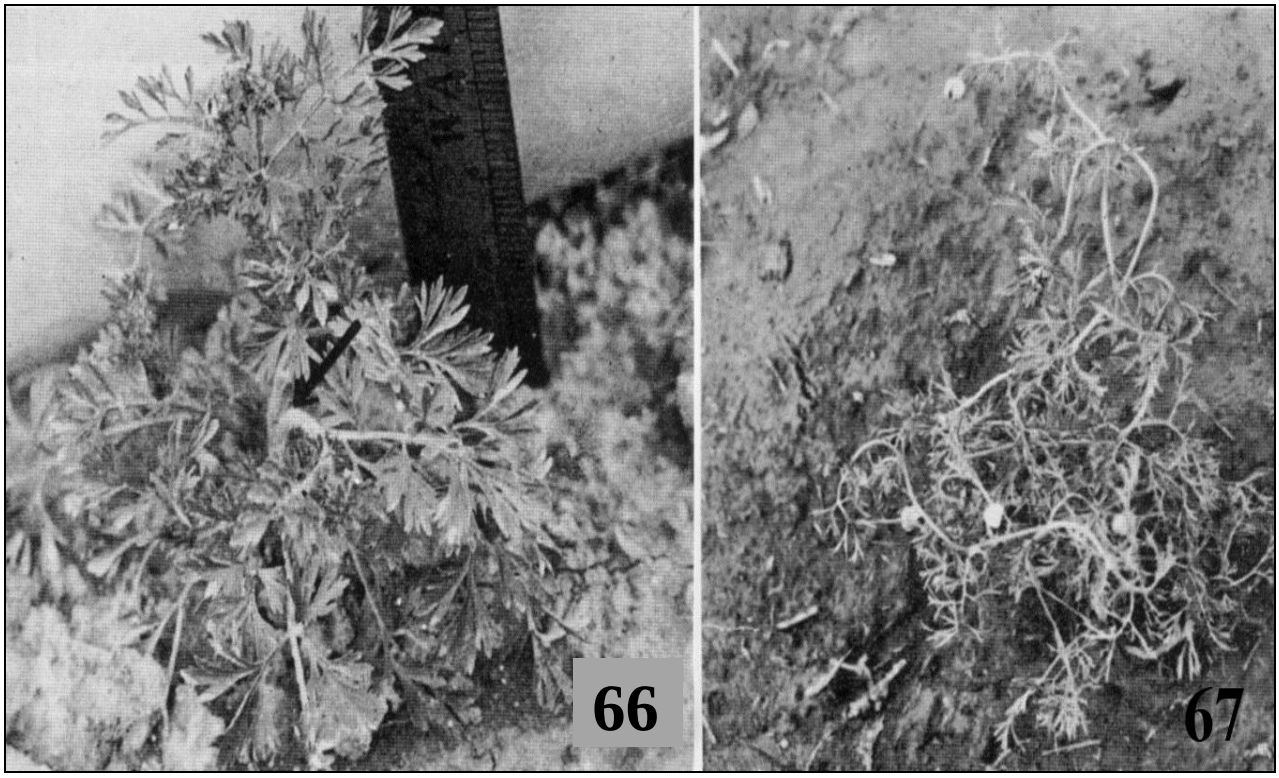
Figs. 48-53. Meiotic consequences of irradiations at metaphase I. 48-49) 6II. 50-51) 3II+6I. 52) 2II+8I. 53) 6II+2 identical sized fragments. Bar=15 µm. [Source: J. Plant Dev. Sci. 3(1), 2011]



Figs. 54-59. Meiotic events at MI. 54)1IV (adjacent orientation) + 4II. 55) 1IV (alternate) + 3II+2I. 56) 1IV (adjacent) + 4II. 57) 1IV (non-co oriented) + 4II. 58) Sticky configuration of chromosomes. 59) Fusion of two PMCs. Bar=15 µm. [Source: Cytologia 48, 1983; Cytologia 51, 1986]

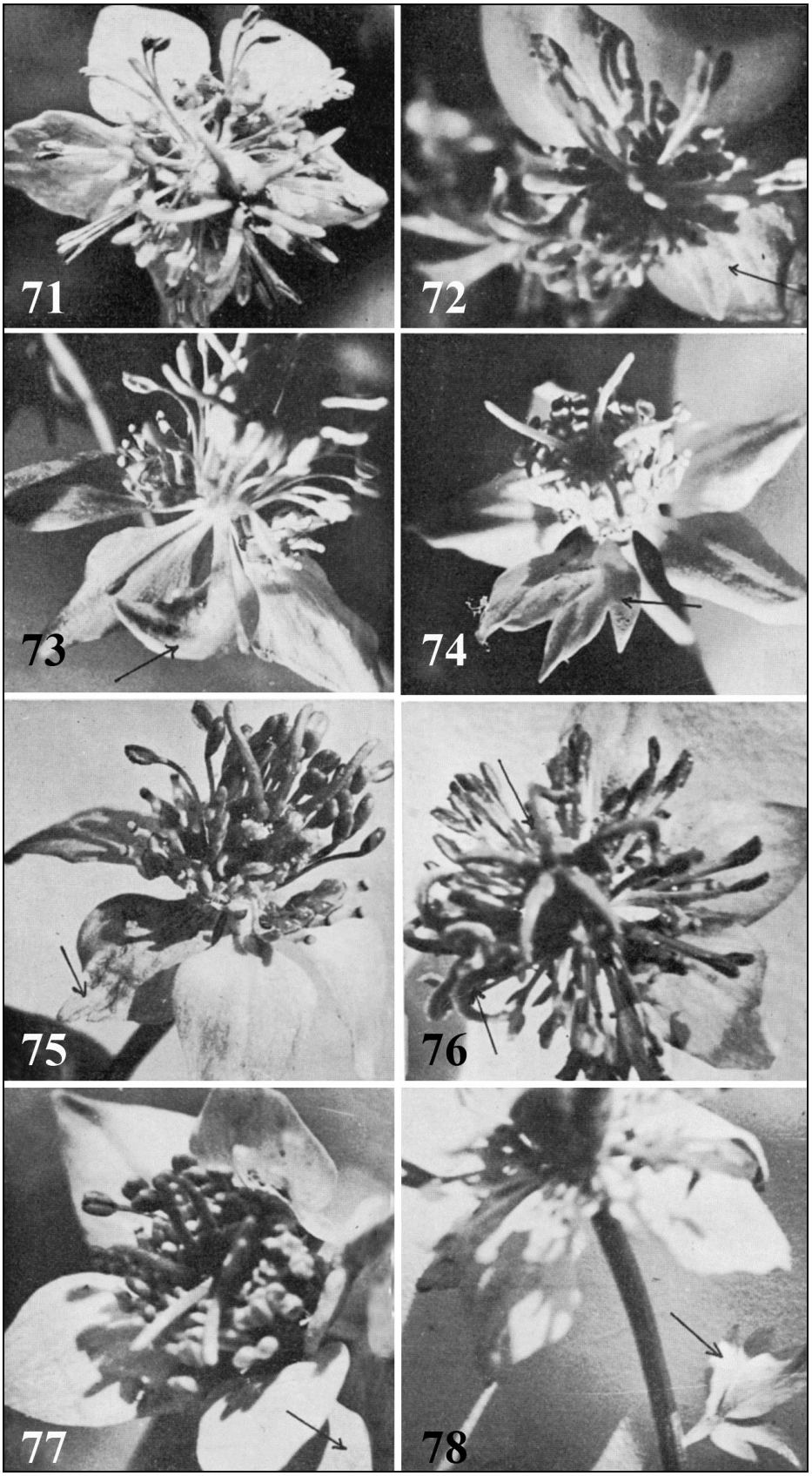


Figs. 60-65. Meiotic configurations in irradiated samples at AI and AII. 60) 6-6 separation at AI. 61) Two fragments at AI. 62) Dicentric chromatid bridge with an acentric fragment. 63) Double bridge formation at AI. 64)Two lagging chromosomes at AII. 65) A bridge with a fragment at AII. Bar=15 µm. [Figs. 26-65. Ref.: Cytologia 48, 1983; Cytologia 51, 1986; J. Plant Developmet Sci. 3(1-2), 2011]

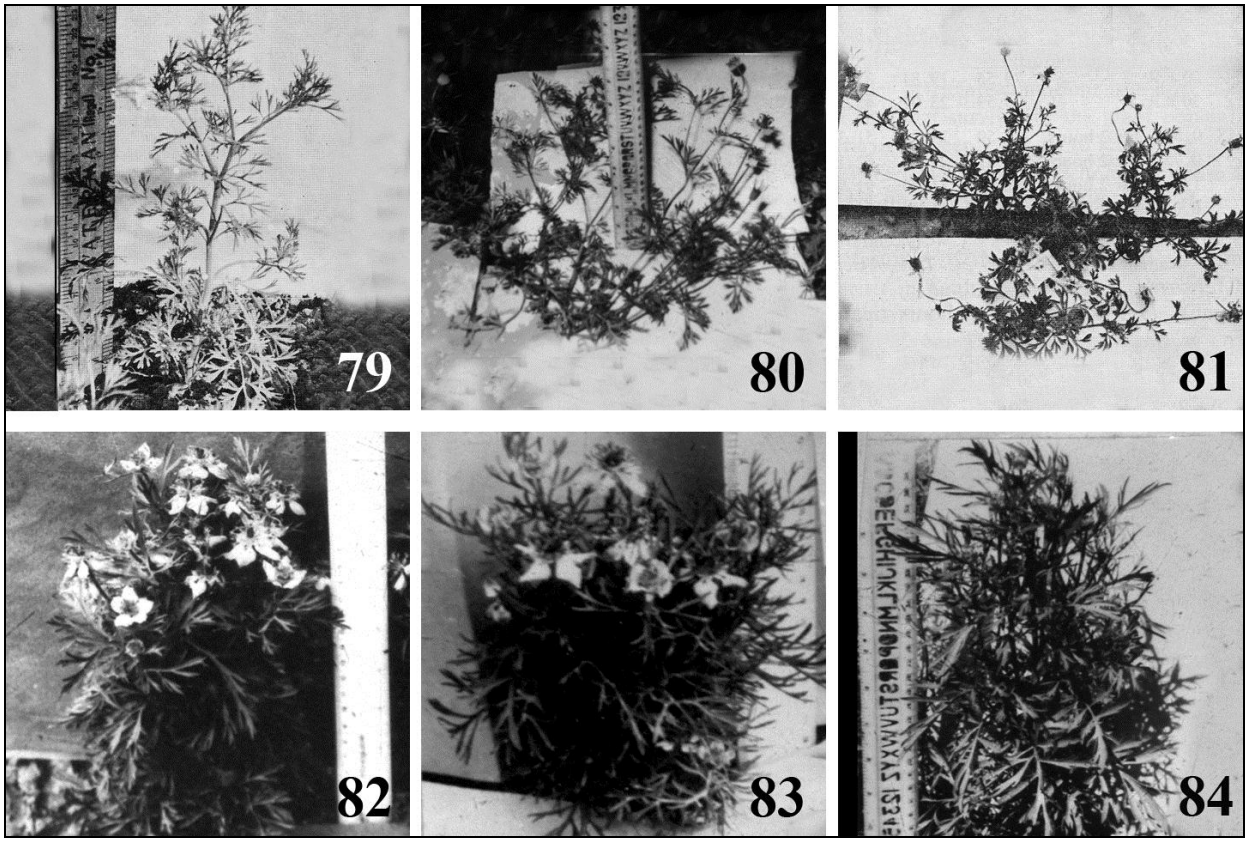


Figs. 66-67. Stem anomalies. 66) Bifurcation. 67) Twining nature. [Source: Cytologia 50, 1985]

Figs. 68-70. Stem abnormalities. 68) Trifurcation. 69) Twining. 70) Unbranched. [Source: Cytologia 50, 1985]



Figs. 71-78. 71) A normal flower. 72-78) Floral abnormalities. 72) Unequally dissected petaloid sepal. 73) Shield shaped sepal. 74) Triforked sepal. 75) Elongated and strap shaped petal. 76) Presence of two gynoecium in a same flower. 77) Small sized sepal in addition to the normal complement. 78) Incompletely forked bract like structure. [Source: Cytologia 50, 1985]



Figs. 79-84. Control and mutants of N. sativa. 79) Normal plant. 80) *Lax branching*. 81) *Prostrate*. 82) *Bushy I*. 83) *Bushy II*. 84) *Lax pinnae*. [Source: Cytologia 50, 1985]

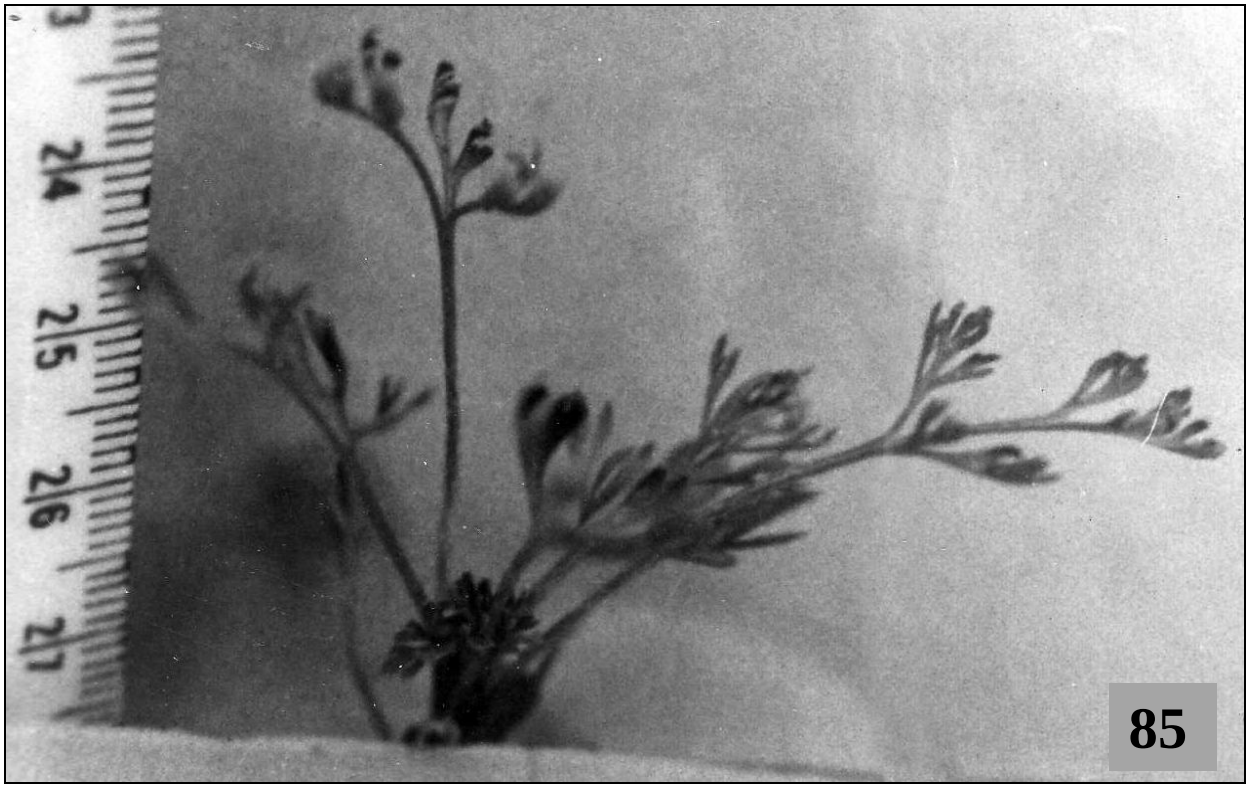


Fig. 85.*Cup leaf* mutant.

Fig. 86. *Chloroxantha* with normal plants in field condition. [Source: Ind. J. Genet. Pl. Breed. 61, 2001]

Figs. 87-90. Seed-coat color in *N. sativa*. 87) Black in normal. 88) *Yellowish brown*. 89) *Dark reddish brown*. 90) *Bicolor*.

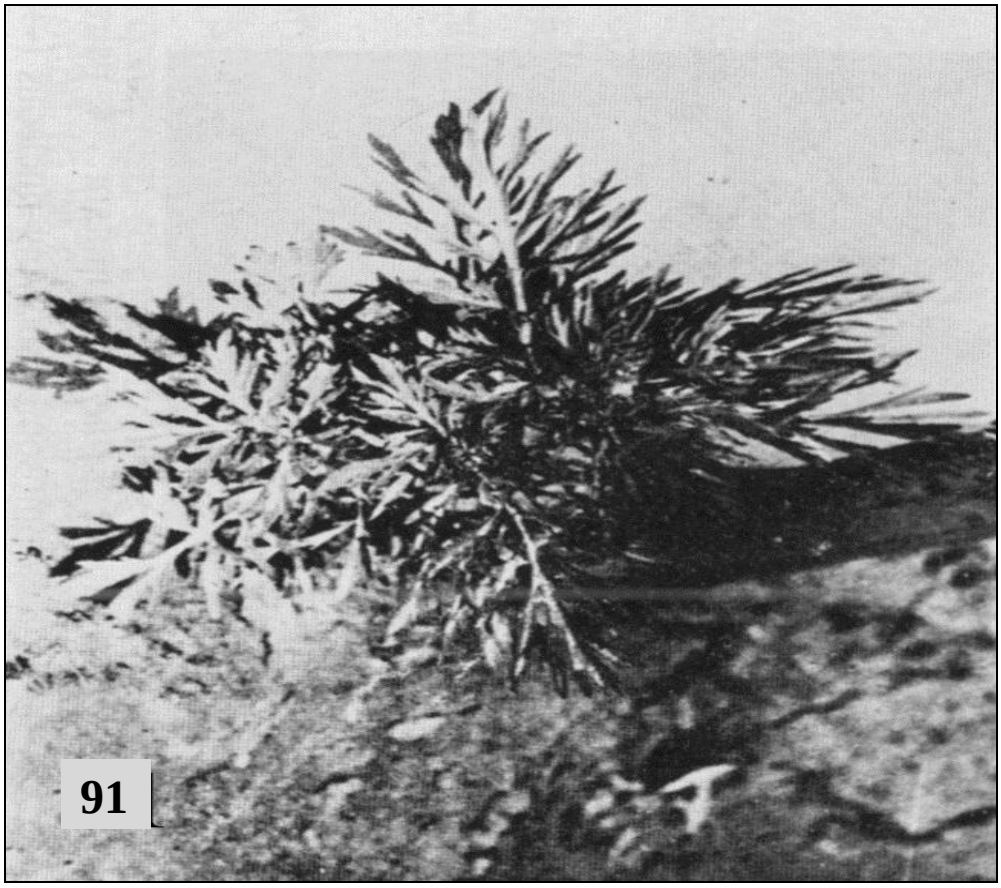
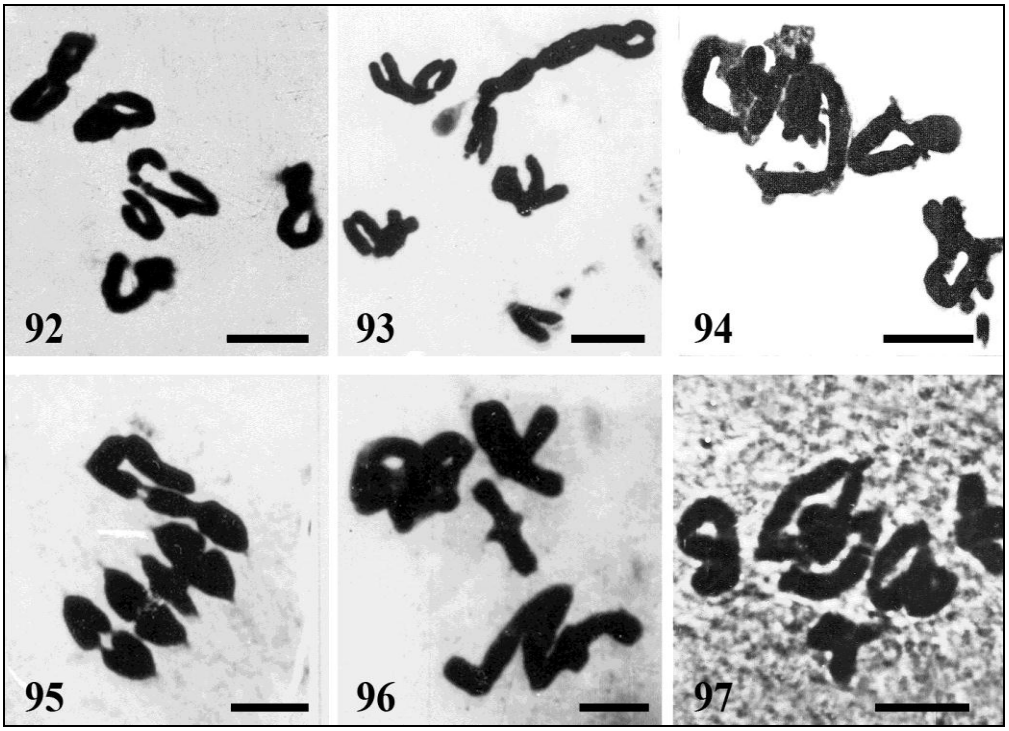
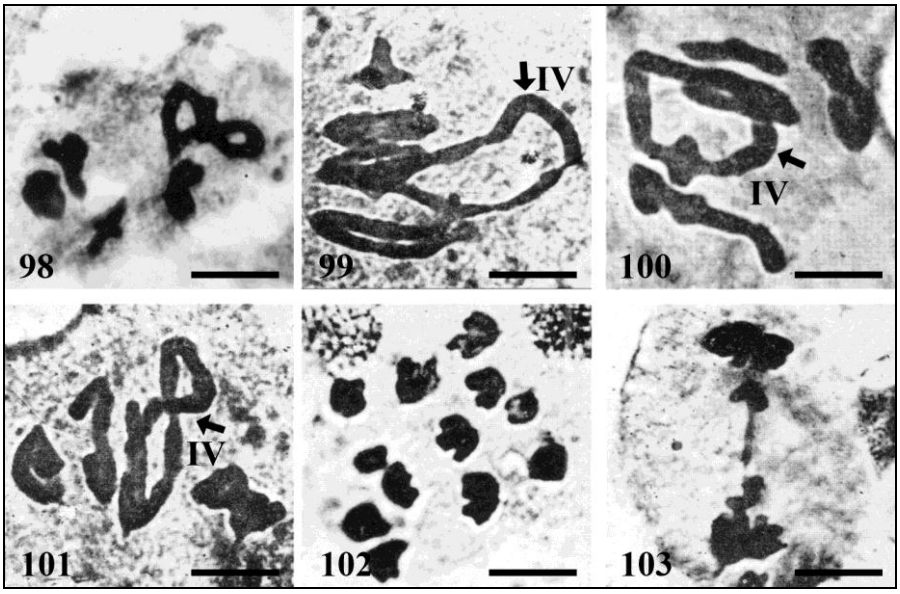


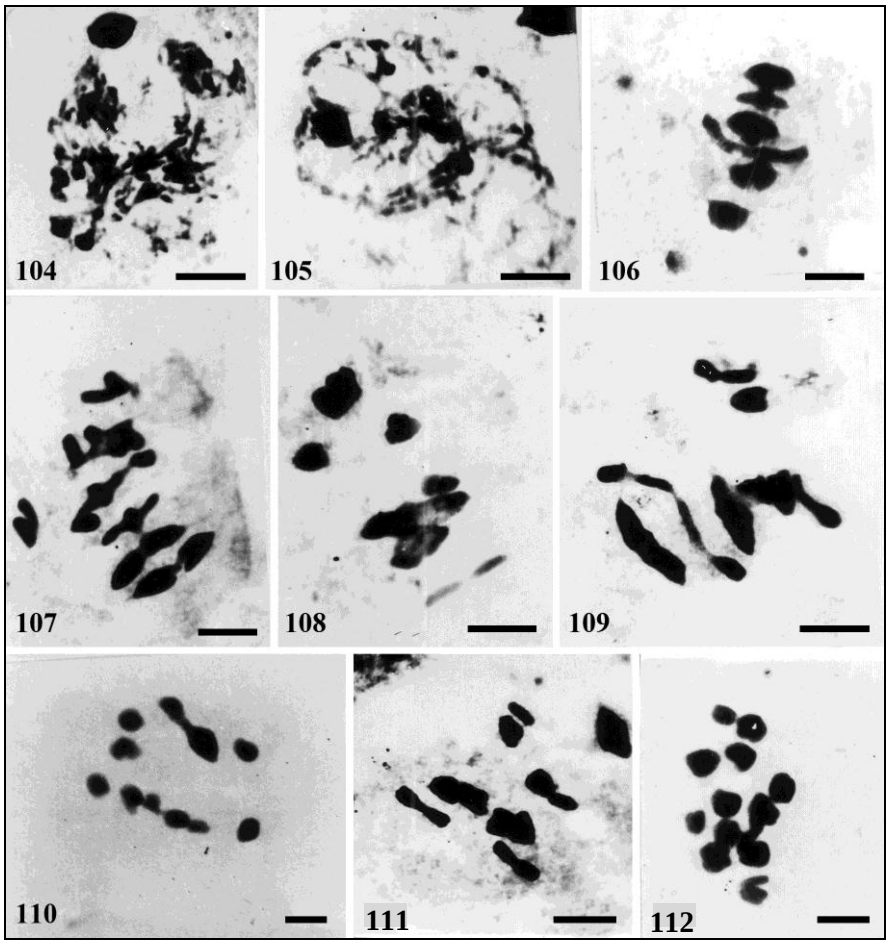
Fig. 91. *Telescopic* mutant in *N. sativa*. [Source: Cytologia 51, 1986]



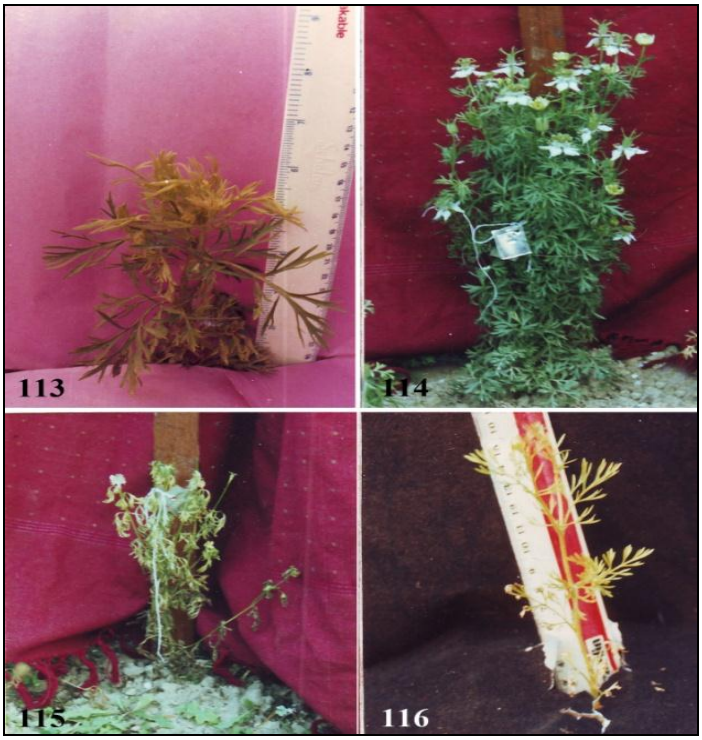
Figs. 92-97. Meiotic configurations at MI (92, 94-97) and diplotene (93) in translocation heterozygotes. 92) 6II. 93)1IV+4II. 94) 1IV (chain, alternate) + 4II. 95) 1IV (chain, adjacent) + 4II. 96) 1IV (chain, alternate) + 4II. 97) 1IV (ring, alternate) + 4II. Bar=15 µm. [Source: Cytologia 67, 2002]



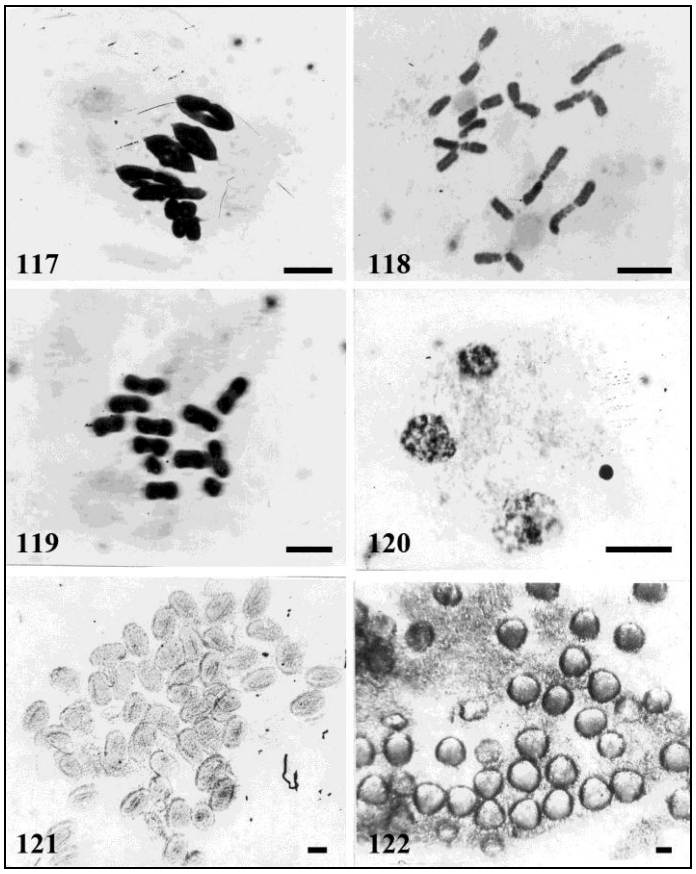
Figs. 98-103. Meiosis in translocate heterozygotes. 98 and 101) 1IV(ring, alternate) + 4II at MI. 99-100) 1IV (ring, adjacent) + 4II at MI. 102) 5-7 separation of chromosomes at AI. 103) Dicentric chromatid bridge with an acentric fragment at AI. Bar=15 µm. [Source: Cytologia 51, 1986]



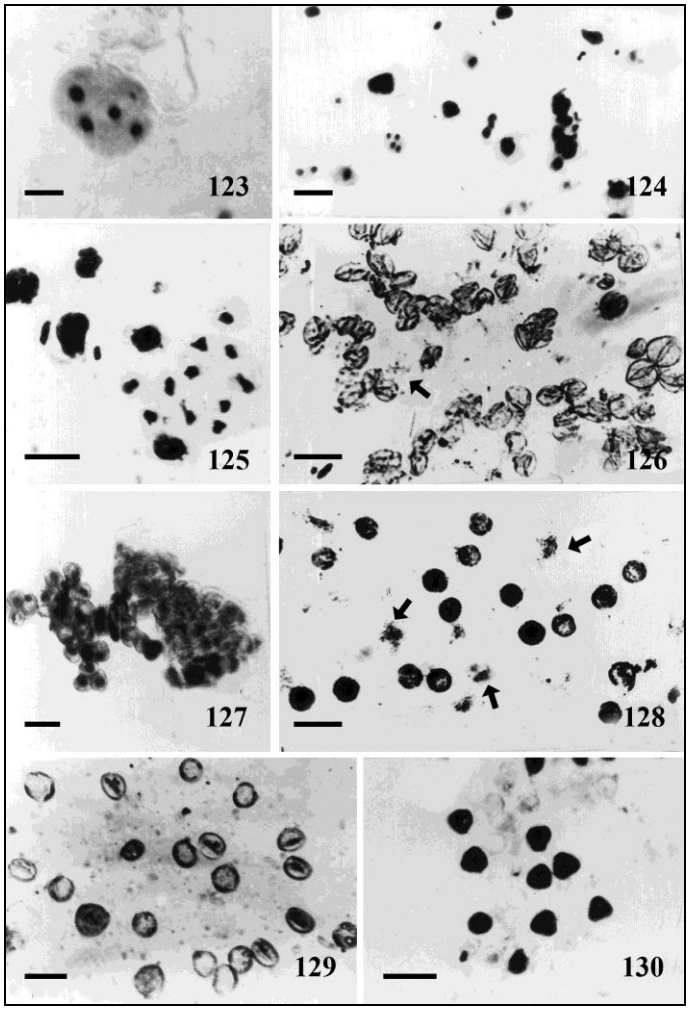
Figs. 104-112. Meiosis in synaptic mutants. 104-105) Early prophase I cells showing fuzzy chromosomes and lack of pairing. 106-112) MI chromosome associations. 106) 6II. 107) 5II+2I. 108-109) 4II+4I. 110) 2II+8I. 111) 1II+10I. 112) 12I. Bar=15 µm. [Source: Plant Archives 2, 2002]



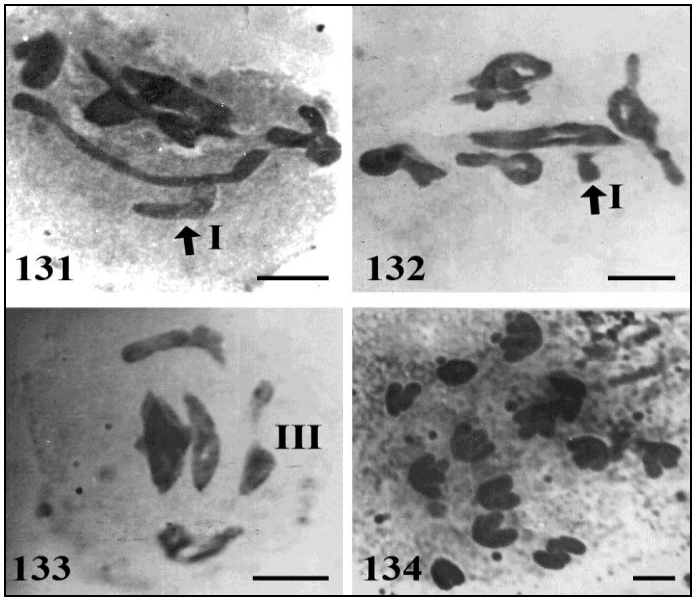
Figs. 113-116. Phenotype of male sterile mutants. 113) Mutants showing chlorophyll deficiency in pinnae of the apical part. 114) Bushy. 115) Crumpled pinnae. 116) Chlorophyll deficiency. [Source: Plant Archives 1, 2001]



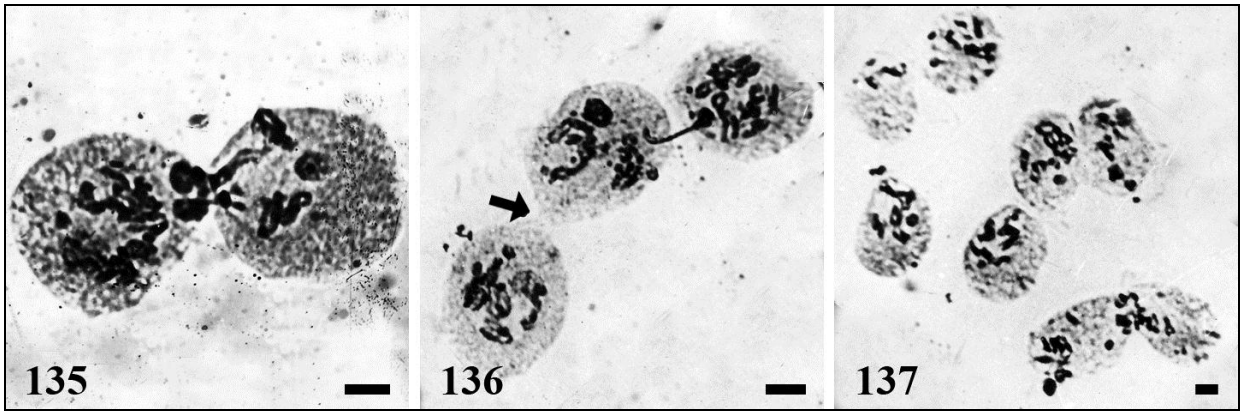
Figs. 117-122. Meiosis in a male sterile mutant (117-121). 117) 6II at MI. 118-119) 12I at MI. 120) AII with unequal spory (near complete degeneration of one pole). 121) Degenaration of microspores. 122) Fully stained round to oval shaped pollen grains in normal plants. Bar=15 µm. [Source: Plant Archives 2, 2002]



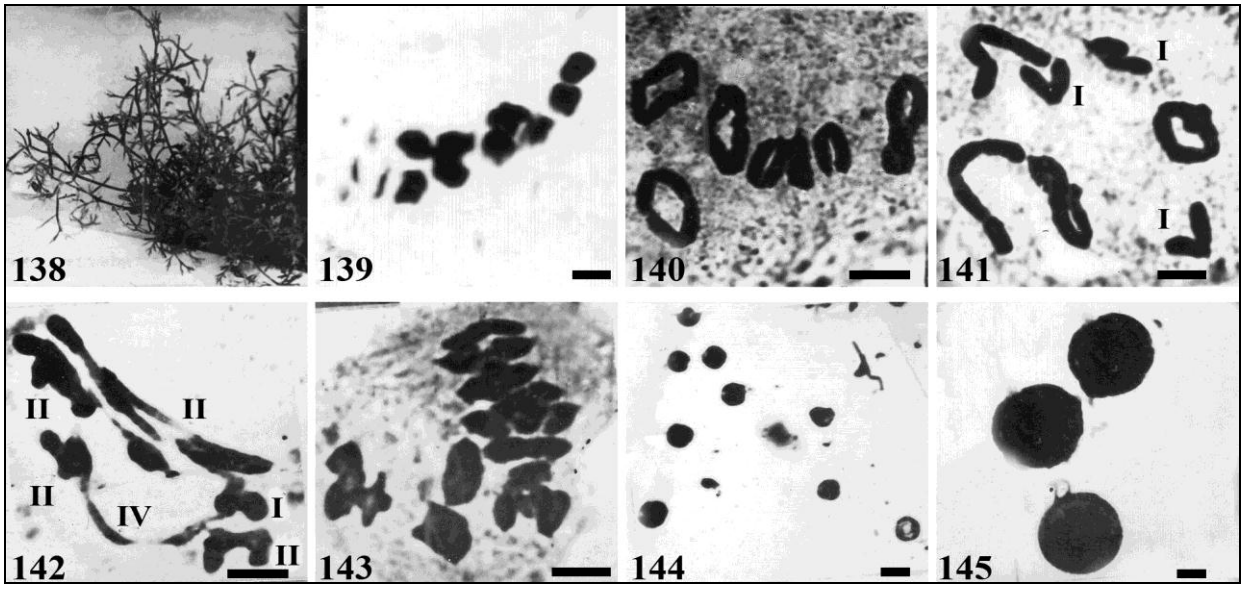
Figs. 123-130. Meiosis in male sterile mutants. 123-125) Agglutination of chromatin into unequal masses. 126 and 128) degenerative pollen grains. 127) Agglutinated pollen grains. 129) Small sized round unstained pollen grains. 130) Fertile pollen grains in normal plants. Bar=15 µm. [Source: Plant Archives 2, 2002]



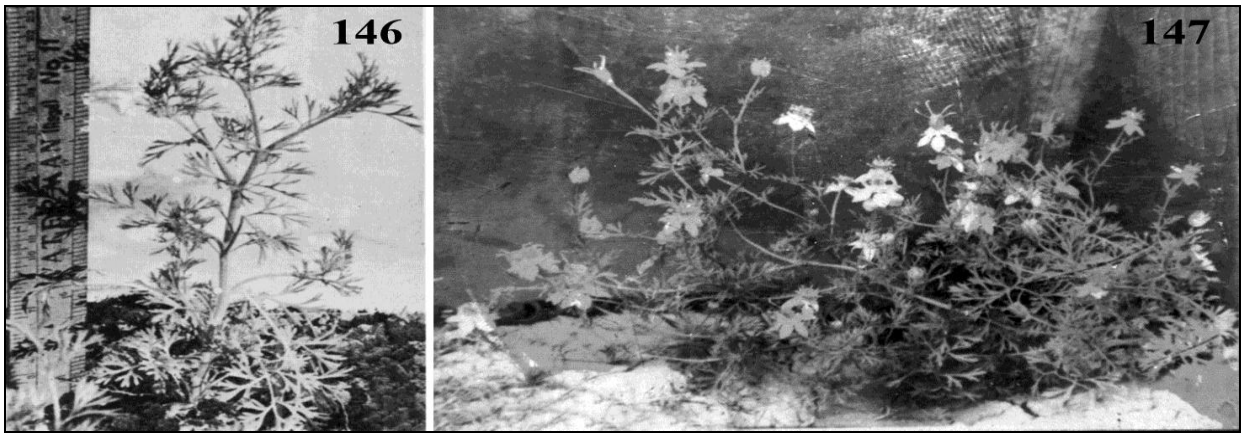
Figs. 131-134. Meiosis in a trisomic plant (2*n*= 13). 131-132) 6II+1I at MI. 133) 5II+ 1III at MI. 134) 9-7 separation of chromosomes at AI. Bar=15 µm. [Source: Cytologia 49, 1984]



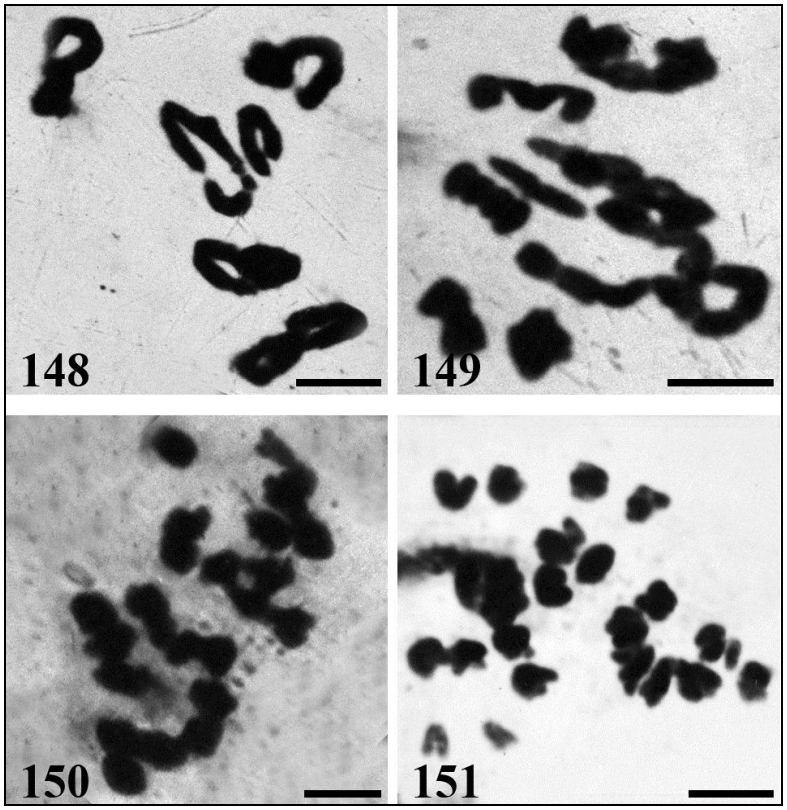
Figs. 135-137. Chromatin bridge (Fig. 136) and fusion of meiocytes (Figs. 135 and 137) in chromosome/ chromatin transfer. Bar=15 µm. [Source: Cytologia 49, 1984]



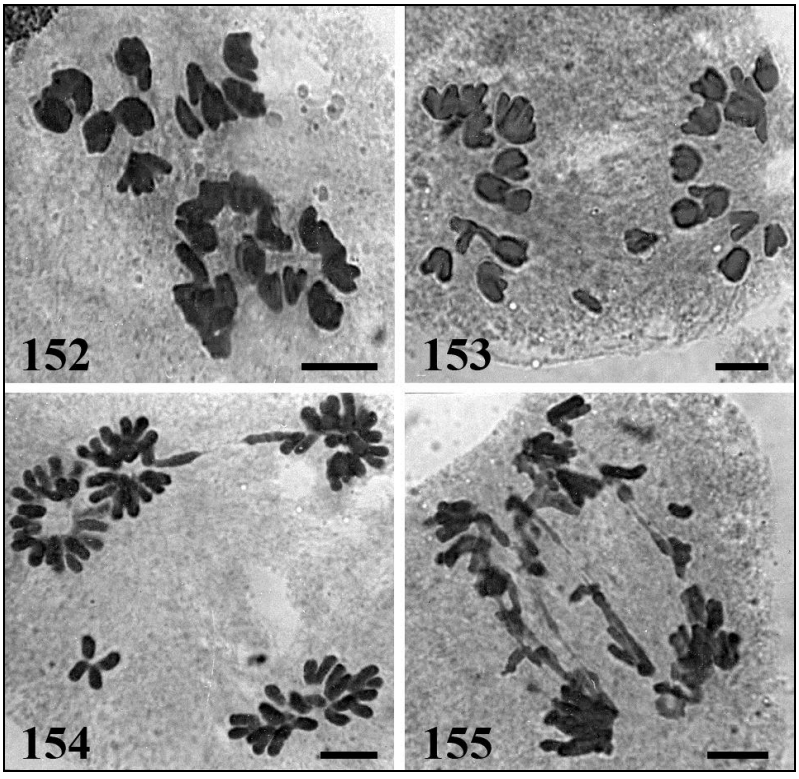
Figs. 138-145. 138) Aberrant plant showing lax branching nature and leaf deformity. 139-143. Meiosis in the aberrant plant. 139) 2II+5I (2*n*=9) at MI. 140) 6II (2*n*=12) at MI. 141) 4II+3I (2*n*=11) at MI. 142) 1IV+4II+1I (2*n*=13) at MI. 143) MI showing 12II (2*n*=24). 144-145. Pollen grains. 144) Stained and unstained small sized pollen grains in the aberrant plant. 145) Normal stained pollen grains in control. Bar=15 µm. [Source: Cytologia 50, 1985]



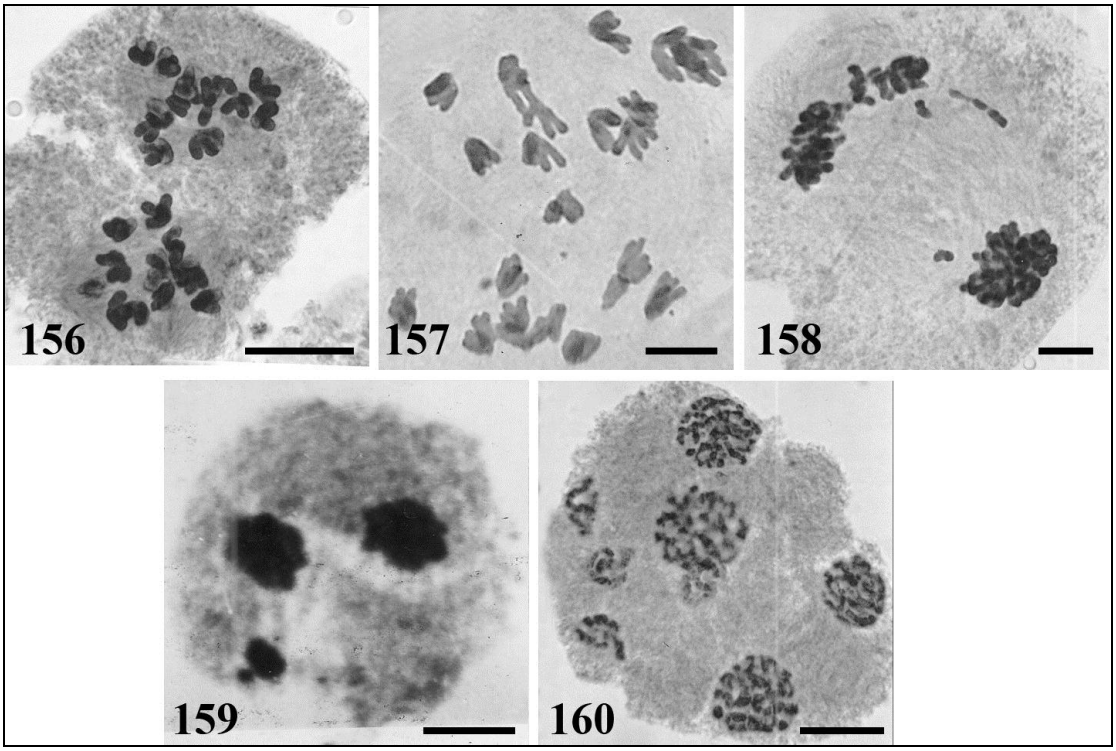
Figs. 146-147. 146) Normal diploid. 147) Autotetraploid showing synchronous flowering. [Source: Indian J. Genet. Plant Breed. 62, 2002]



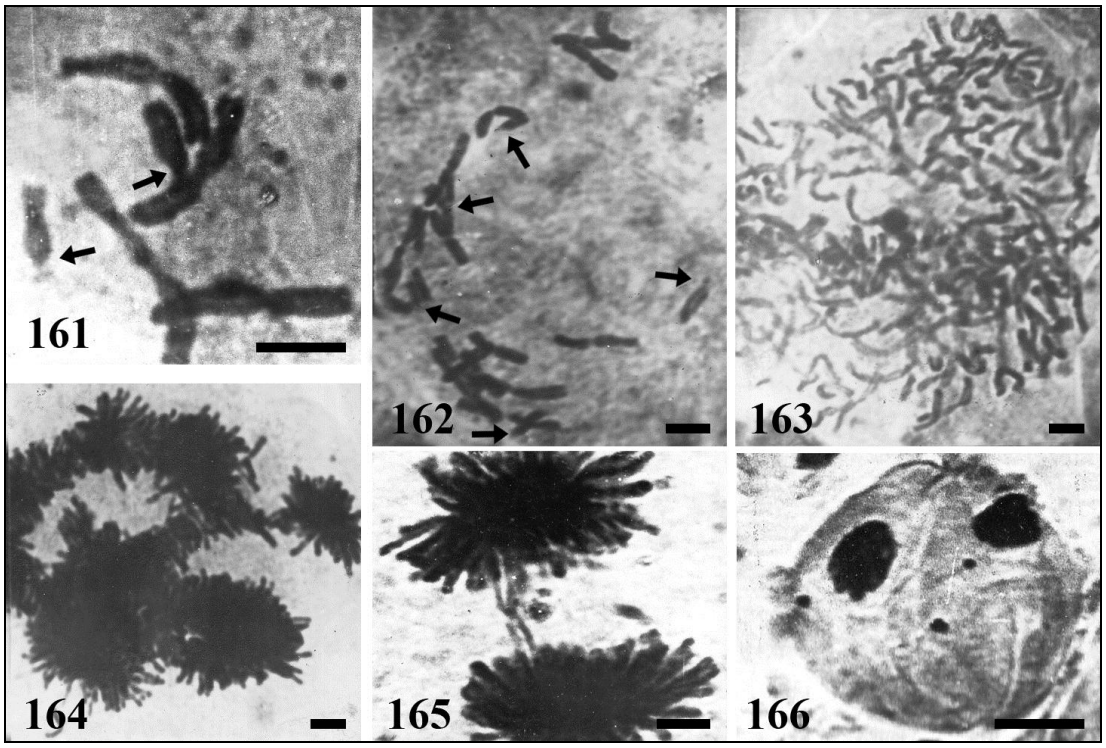
Figs. 148-151. Meiotic configuration at MI and AI (151). 148) 6II in diploid. 149-151. Meiosis in autotetraploid. 149) 1IV+9II+2I. 150) 2IV+4II+8I. 151) 8-16 separation of chromosomes. Bar=15 µm. [Source: Indian J. Genet. Plant Breed. 62, 2002]



Figs.152-155. AI configurations in the autotetraploid. 152) 11-13 separation. 153) 11-13 separation associated with a fragment. 154) Tripolarity along with a lagging chromosomes. 155) Multiple bridges with fragments. Bar=15 µm.



Figs. 156-160. AI (156-158) and AII (159-160) configurations in autotetraploids. 156-157) Unequal (11-13) separation of chromosomes. 158) Tripolarity with laggards. 159) Unequal spory. 160) Multiple spory. Bar=15 µm.



Figs. 161-166. Chromosome variations and abnormalities in callus tissue. 161) 2*n*=6. 162) 2*n*=24. 164-165) Enhanced ploidy level. 166) Bridge formation with higher ploidy. 167) Five extremely variable chromatin masses. Bar=15 µm.

CONCLUSION

Despite the major advancement of modern medicine in human health-care, it is still intangible and beyond reach to ailing humanity, especially the destitutes. In recent years plant based systems has been utilized for traditional medicine and phytotherapy. Medicinal plants are 'Gift of Nature' and *N. sativa* is one such plant with potential uses which can be explored for safe and effective herbal medicine for human benefit. Cytogenetical studies also revealed that the species can also be used as a model plant for better understanding of gene and chromosome relationship.

ACKNOWLEDGEMENT

This monograph is dedicated to those individuals who believe in herbal medicine and also to researchers working in the field of Cytogenetics.

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