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Research Article

MUTATIONAL BIOASSAY AND AMELIORATIVE ACTION OF CAFFEINE FOR INDUCTION OF EARLY MATURING MUTANT IN *NIGELLA SATIVA* L.

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ABSTRACT

With the unfolding of mutational studies, chemical mutagenesis is widely used for developing induced mutants in plants. The efficiency of this technique using one of potent chemical mutagen i.e., Caffeine is accounted in the present piece of work. The existing germplasm (seeds) of *Nigella sativa* was mutagenised with 0.10%, 0.25% and 0.50% dose of caffeine to develop a viable mutant at M_2 generation level. Different qualitative and quantitative traits of mutant plant was screened time to time and contrasted with control (untreated/normal plant). There observed a considerable deviation in phenotypic characters like shape of vegetative leaf, number of petals, capsule and seed size. Wide array of macro- mutations altered the overall morphology of plant which became matured 18 days earlier than its wild type; thereby induced an elite line called "Early Maturing Mutant" at 0.25% dose of caffeine. Thus, on assaying different morphological mutations induced, it is concluded that Caffeine at its optimal dose (0.25%) is ameliorative for medicinal herb- *Nigella sativa*. Moreover, the same concentration not disturbed the cell cycle of mutant as studied through cytology (Meiosis I and II) of PMC.

Key Words: Early mutant, chemical mutagenesis, caffeine, pollen mother cell (PMC), meiosis.

INTRODUCTION

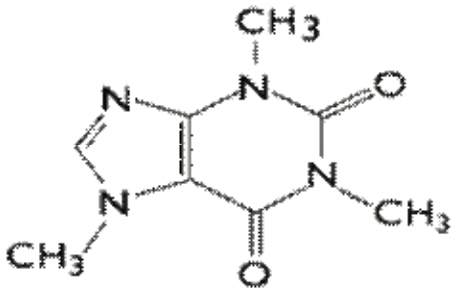
The medicinal Arabic herb Habat-ul-sauda or a Linnean species *Nigella sativa* (diploid, 2n=12) is an annual aromatic plant belongs to buttercup family. The habit bears erect branching stem, pinnatisect leaves and terminal solitary white flowers. The fruit is inflated capsule formed by the union of follicles, encasing numerous angular black seeds. Small fennels represent the main useful part of the plant having malifarious uses, not only employed as spice and food preservative but have tremendous medicinal potential. It is a panacea for all ailments except death-Prophet Mohammad (PBUH). It is listed as natural drug of Tibb-e-Nabvi being cytostatic, anticarcinogenic[1], antioxidant[2], antipyretic[3], antidiabetic[4], anti-inflammatory[5], immunostimulant[6]. The aqueous and n-hexane extract of *Nigella* have protective effect on liver against paracetamol toxicity[7]. The various properties of kalonji are due to vast chemical diversity it possesses. It is a rich pool of alkaloids, fixed and volatile oil. Besides these important constituents, the seed extract is also a good source of proteins, amino acids, vitamins, minerals, sterols, saponins, flavonoids, etc. Thymoquinone is the most abundant constituent of the seed[8].

Development of mutants using chemical mutagenesis broader the boundaries of improvement of one or more vegetative and reproductive traits, which synergistically can enhances the yield and quality. Use of chemical mutagens in mutation breeding programmes have become an inherent component for the development of determinate plant type with improved yield, seed size, grain quality, non-shattering plants[9], disease and lodging resistance, early maturity and others. Usually mutagens cause chromosomal instability which in several cases leads to apoptosis. But chemical mutagens may not always be harmful. They may stimulate DNA repairing at their lower and moderate doses. Caffeine is among various mutagens which posses safe threshold at its optimal concentration. It holds vital mutagenic effect in number of biological systems. It posses significant ability to improve qualitative and quantitative traits in plants[10] and reduces carcinogenic risk due to dietary and environmental effect[11].

Aromatic and medicinal plants are soully associated with mankind. These are being the natural source of pharmaceutical and pharmacological products have gained the attention for plant based medicaments. The widespread use of kalonji throughout the world due to its valuable chemical diversity, has led to the need to raise such mutant line which could serve at its earliest, the increasing medicinal demand of present sky-high population. The early maturing mutant in this aspect can serve better over its wild type. Thus, the prime strategy in the present work is to achieve potential mutations using optimal doses of Caffeine via chemical mutagenesis to serve mankind through this important medicinal herb.

MATERIAL AND METHOD

Mutagenic treatment: Four sets each of 50 healthy and mature seeds were maintained separately. One set of germplasm was kept at zero level (control, no treatment). Caffeine as mutagen was buffered at pH 7 and treatment was given independently to remaining 3 sets at 0.10%, 0.25% 0.50% dosage level for 24 hours, after 12 hours of presoaking in distilled water.



Chemical structure of Caffeine ($C_8H_{10}N_4O_2$)

Morphological screening: Control and treated seeds were sown for M1 plant population during the month of October-2014 in the Net house of Department of Botany, Aligarh Muslim University Aligarh, India. After seed germination the preliminary screening for phenotypic characteristics was done regularly to notice any mutated character in the plant morphology. On maturity, M1 seeds were collected. M2 generation was raised from all harvested seeds during October-2015 and different qualitative and quantitative traits were recorded from control and treated plants that were showing maximum deviation in characters.

Cytological screening: For cytological examination, appropriate sized flower buds from control and treated plant were fixed separately in Carnoy's fluid for 24 hours and preserved in 70% alcohol. Anthers were compacted in 1% propionocarmine and permanent slides were made through an alcohol (NBA series). Different meiotic stages were recorded through photographs, using a high resolution (Dsx 100 Olympus) Microscope.

RESULTS

Analysing the effect of different concentrations (0.10%, 0.25% and 0.50%) of Caffeine on various morphological characters, qualitative and quantitative traits, an "Early maturing mutant" was recovered in M2 generation at 0.25% dose of Caffeine. The mutant plant was matured 18 days earlier than control.

Qualitative and quantitative traits: Early mutant took 5-6 days for germination, 116 days for flower initiation, 133 days for reaching to 50% flowering and 158 days for maturity which is comparable with all these quantitative traits (Table1) in control plant as 8-9 days for germination, 134 days for flower initiation, 151 days for 50% flowering and 176 days for maturity respectively. The mutant was also found superior over control on an account of Qualitative traits, showing vigorous growth, bearing yellowish white hexapetalous flowers as compare to white pentapetalous flowers in control (Figure:1-III). The capsule and seed size was also big (Figure: 1-IV&V). However, the seeds were black in both the cases.

Morphological parameters: Different morphological parameters were contrasted between control and mutant plant (Table 2). The mutant was taller increasing the height to 69.10 cm as compare to 66.20 cm in control. Number of branches per plant was 7.00 while it is decreased to 5.00 in control plant. Yield related characters were also enhanced in mutated plant. The mutant was vigorous bearing 9.00 capsules per plant which is significantly comparable with that of control (only 5 capsules/plant). Increased number of seeds per capsule (54.00) was observed in mutant against 49.00 in control plant. The yield was also markedly increased from 1.62g (control) to 2.11g (mutant).

Meiotic studies: On examination of microsporogenesis, cell cycle (Meiosis I & II) was observed to be normal in control as well as early maturing mutant. All the meiotic stages were normal without any chromosomal lesions. 6 bivalents were observed at Metaphase-I (Figure. 2A). Normal anaphasic separation (6: 6) was observed at Anaphase-I (Figure 2B). Similarly at Metaphase II, the chromosomes in two groups were normally arranged at two equatorial plate (Figure.2D) and normally passing to two poles during Anaphase II (Figure.2E). Also no aberration was detected at Telophase I/II (Figure.2C&F) having normal chromosomal groups at the poles showing regular divisional phase of the cell cycle.

Table 1: Salient qualitative and quantitative characters to distinguish between control and early maturing mutant

Qualitative Traits			
S. No.	**Traits**	**Control**	**Mutant**
1	Plant Habit	Erect	Erect
2	Growth	Normal	Vigorous
3	Flower	Pentapetalous	Hexapetalous
4	Flower Color	White	Yellowish White
5	Capsule	Pentalocular	Hexalocular
6	Seed Size	Normal	Big
7	Seed Color	Black	Black
Quantitative Traits			
8	Days to germinate	8-9	5-6
9	Days to flower initiation	134	116
10	Days to 50% flowering	151	133
11	Days to maturity	176	158

Table 2: **Morphological parameters of control and early mutant**

S. No.	Parameters	Control	Mutant
1.	**Plant Height (cm)**	66.20	69.10
2.	**Number of Branches/Plant**	5.00	7.00
3.	**Number of Capsules/Plant**	5.00	9.00
4.	**Number of Seeds/Capsules**	49.00	54.00
5.	**Yield/Plant (g)**	1.62	2.11

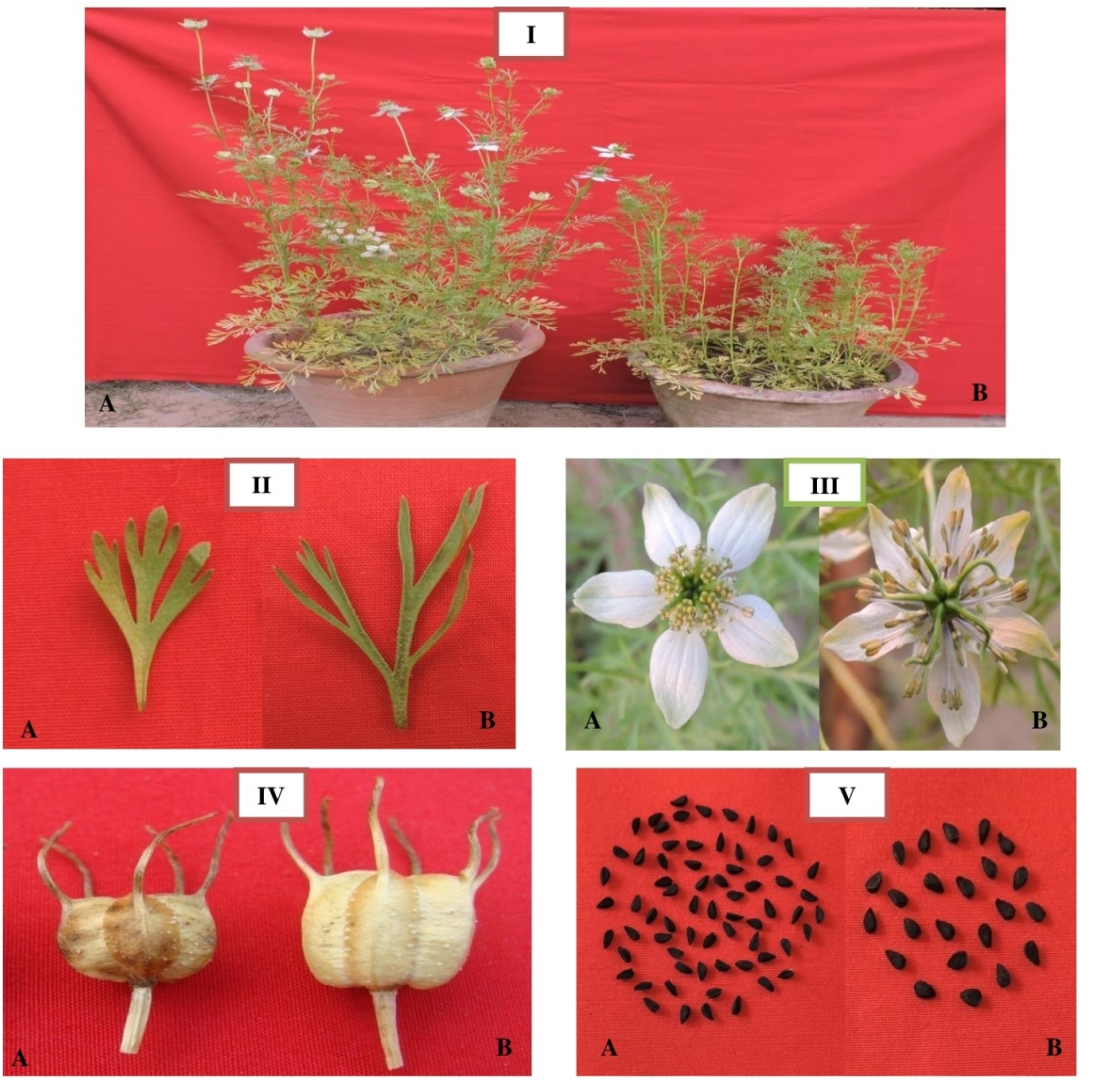


Figure 1: Morphological variations between Control and Early Maturing Mutant of Nigella sativa

I:	A - Early Maturing Mutant Plant	B - Control Plant
II:	A - Vegetative Leaf Control	B - Vegetative Leaf Mutant
III:	A - Flower Control	B - Flower Mutant
IV:	A - Capsule Control	B - Capsule Mutant
V:	A - Seeds Control	B - Seeds Mutant

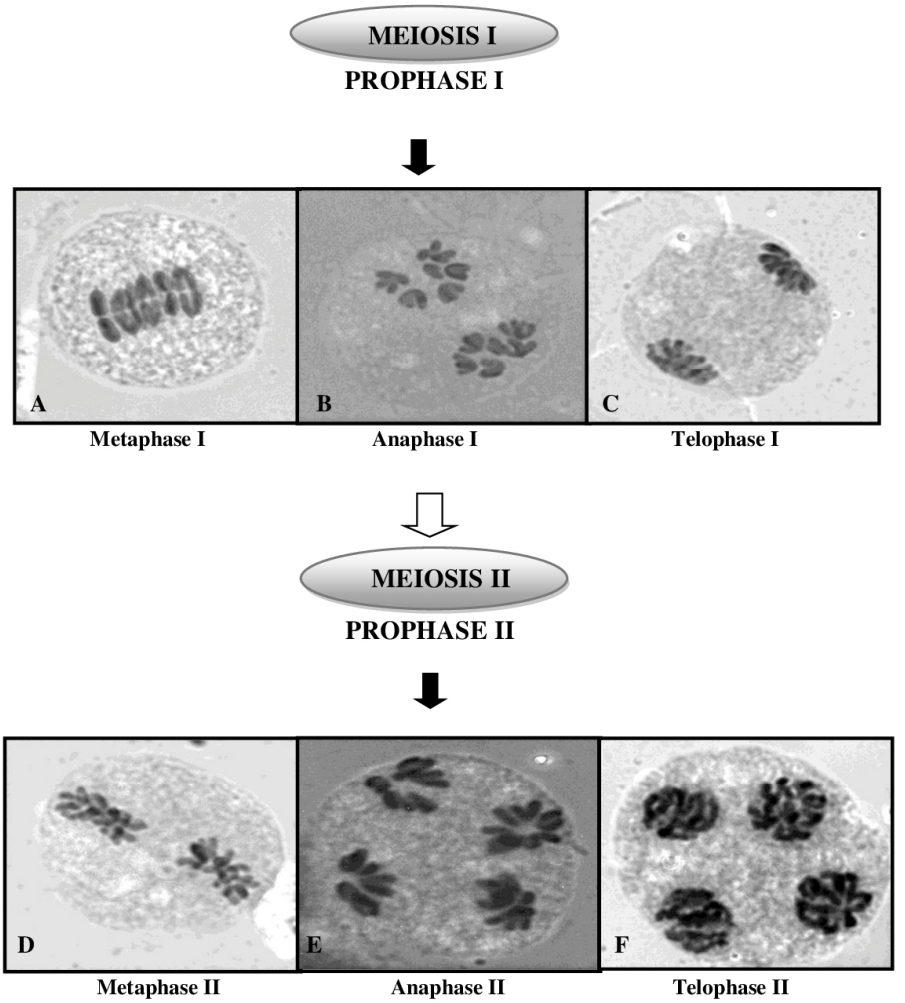


Figure 2: Illustration of Normal Cell Cycle Observed in Control and Early Mutant

DISCUSSION

Chemical and physical mutagens have been in use since time for the induction of mutants in plants. Induced mutants in plants on account of mutagenesis have been studied by various workers[12-16]. Limited work has been done on Kalonji for evaluation of useful mutants as a result of chemical mutagenesis. The present investigation proved fruitful for the induction of viable early maturing mutants on screening the M_2 generation which clearly indicated the potential of Caffeine in *Nigella*. Stimulatory effect of caffeine on different qualitative and quantitative traits has also been suggested by Khursheed *et al.*[17] in sunflower. In present work also Caffeine has shown its ameliorative action for modulating useful drug plant with meiotic stability. Such mutant type with normal meiosis is quite similar with that of early flowering mutant of Datta and Biswas[18]. Mutant evaluated due to stimulatory effect of caffeine showed increased height with higher number of branches. The probable cause is the loss of apical dominance which results in lateral distribution of growth hormone and hence the increased branching. Mutant with increased height and branches was also obtained by Gnanamurthy and Dhanavel[19] in Cowpea due to mutagenic effect of EMS. Mutant isolated in our case could be highly useful because early maturity supports the plant to escape late season drought[20]. It bears the entire traits superior, especially the yield is higher than the control plant. Total yield per plant was directly related with number of capsules per plant because whenever the number of capsules per plant increased, total number of seeds increased and hence the yield also increased. With an agreement of our result early maturing kalonji with higher yield has also been evaluated by Rabbani *et al.*[21]. Moreover, the mutant is hexapetalous having six petals instead of five (in control plant) which is the index of increased number of seeds and better yield. The similar peculiar character was also noticed by Kumar and Gupta[22] in *Nigella* by mutagenic efficiency of gamma rays. Besides yield, big seed size positively raised the quantity of important chemical constituents which are prized for pharmacological domain and enhanced level of

nutritive oil. Usually induced mutants exhibit different types of chromosomal lesions due to toxic effect of mutagens but very few of the literature recorded normal chromosomal nature despite of mutagenic effect of mutagens. In such cases the mutagens exhibit the stimulatory effect on plant morphology without disturbing the cell cycle as found earlier in mutants of *Nigella*[18]. Similar is the case of our results for normal meiosis exhibited in mutant plant.

Thus, mutant isolated in the present study is highly significant having practical utility for plant breeders as it is early matured with improved qualitative traits coupled with increased height, number of branches, yield and other yield attributing traits. Similar findings have also been observed by Basu *et al*.[23]. The mutant is also meiotically stable without any DNA damage which can be directly linked with greater pollen fertility. Datta and Biswas[18] reported 96.89% pollen fertility in early mutant of *Nigella.* Therefore this mutant type is safe, could be used as spice of commerce, could be recommended for pharmaceutical and pharmacological products and for safe utilization of cumin based medicaments.

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