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Research Article

A NOTE ON INDUCED NON-SHATTERING MUTANT IN *NIGELLA SATIVA* L. (BLACK CUMIN)

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ABSTRACT
Six non-shattering mutant plants of *Nigella sativa* L. (Family: Ranunculaceae; common name - black cumin; annual medicinal herb of immense therapeutic uses and potentially possessing spice yielding property of commerce) were screened from field on post harvest at M_2 following different mutagenic treatments (0.25%, 4h EMS; 0.50%, 4h NaN_3; 0.50%, 2h NH_2OH and 50 Gy gamma irradiations). Mutant seeds sown at M_3 and M_4 yielded 3 and 4 non-shattering plants respectively. None of the mutant plants were with detectable phenotypic trait, a major constrain in their identification in field. On post harvest management it was noted that the mutant plants shattered 4.37% seeds in comparison to 22.45% in control. Significance and difficulties of the induced non-shattering mutant have been discussed.
KEYWORDS: Induced mutation, *Nigella sativa,* Non-shattering trait

INTRODUCTION

Shattering of seeds from fully matured pods is a wide spread problem in different plant species[1-8] including *Nigella sativa* L. (Family: Ranunculaceae; common name - black cumin; an annual herb possessing immense therapeutic uses and spice yielding property of commerce – Datta *et al.*[9]). Shattering causes considerable yield losses and therefore a major threat to breeders for crop improvement. The shattering trait is reported to be controlled genetically[6-7].

N. sativa is grown in West Bengal plains as a rabi crop from mid-November (sowing) to mid-March (harvest). The plant species possesses non-synchronous flowering as well as maturity, therefore a compromise (harvesting is done when later formed pods were yet not matured and few flowers in blooming condition) is made during harvest to retain seeds in the earlier formed pods to maximize yield[9]. Therefore, introduction/selection/induction of non-shattering germplasm(s) in black cumin will be the basic raw material for enhancing economic yield. The present communication describes induced (physical and chemical mutagen) non-shattering mutant plant type in the species.

MATERIALS AND METHODS

Screening of the mutant type

In the M_2 (5330 plants scored) mutagenized population (raised following treatments of dry seeds, moisture content-19.04%, of *Nigella sativa* with ethyl methane sulphonate – EMS, sodium azide – NaN_3, hydroxylamine – NH_2OH and gamma irradiations; doses – 0.25%, 0.50% and 1.00% for 2 and 4h durations in case of chemical mutagens and for gamma irradiations – 50 Gy, 100 Gy, 150 Gy and 200 Gy) macromutants were screened from seedling to maturity and harvested separately; while, the rest were kept under field condition and out of which 6 plants in different mutagenic treatments (1 in 0.25%, 4h EMS; 1 in 0.50%, 4h NaN_3; 1 in 0.50%, 2h NH_2OH and 3 in 50 Gy gamma irradiation) were spotted to possess non-shattering trait.

Recovery of the mutant at M_3 and M_4

Seeds of 6 mutant plants were bulked and from which 100 seeds were sown at M_3 and on maturity 3 non-shattering plants were isolated out of 18 plants. Similarly at M_4, 4 non-shattering plants were screened from 26 plants.

Post harvest maintenance

Each mutant and control plants were packed separately with papers carefully so that no seed can fall out. After about a month seed weight of each control plant was taken along with seeds those were shattered and fallen in the paper and subsequently the amount of seed loss due to shattering was calculated. Similar protocol was also followed in mutant plants.

RESULTS AND DISCUSSION

The non-shattering mutant plants assessed over the generations (M_2 to M_4) were shorter in height (34.5 cm ± 1.99 to 40.25 cm ± 0.91; control – 47.0 cm ± 2.78 to 50.39 cm ± 0.94) with reduced number of primary branches (5.54 ± 0.48 to 8.09 ± 0.32; control – 10.11 ± 0.38 to 11.58 ± 0.27) and capsules (7.5 ± 0.81 to 8.6 ± 1.14; control – 8.2 ± 0.74 to 12.1 ± 1.16) per plant than normal plants. Capsule lengths were shorter in the mutant than control (mutant - 0.78 cm ± 0.02 to 0.97 cm ± 0.08; control – 1.09 cm ± 1.02 to 1.15 cm ± 0.08). Control plants were harvested within 110 to 127 days from sowing; while, the mutants were kept in field for prolonged period for full maturity and harvested within 135 to 140 days from sowing. After post-harvest management, the number of retained seeds per capsule (average of first formed 5 capsules) was calculated and it was noted to be 76.03 ± 0.69 in control and 65.33 ± 1.02 in mutant plants. Average seed weight per plant was 1.83 gm ± 0.4 and 2.48 gm ± 0.4 in control and mutant germplasms respectively. Seed loss due to shattering was about 22.45% in control in comparison to 4.37% in mutant. During post harvest few capsules of the mutant showed incision of breaks along the suture. Interesting to note that flower sterility in the non-shattering plant was nil in all cases but in control it ranged from 39.05% to 48.42%. Thus, the significance of the mutant in the species is enormous.

Pascual-Villalobos *et al.*[2] reported EMS induced non-shattering mutant in *Euphorbia lagascae* and on histological investigation it was suggested that a missing mesocarp layer in capsule wall was responsible for the indehiscent trait. Day[3] in sesame found that during capsule senescence mesocarp cells shrank more than the endocarp cells creating tension in the drying capsule wall and such tension forced capsule

opening along the zone of weakness between locules. Further, it was observed that in indehiscent genotypes (idid) cell layers over the median vascular bundle possibly prevented capsule splitting and seed retention. Agrawal *et al*.[5] suggested that shattering occurs due to loss of adhesion between highly active living cells and it is a result of highly coordinated sequence of biochemical events, which leads to cell wall breakdown in one or two rows of cells on either side of the shattering zone. Two hydrolytic enzymes namely, cellulase and polygalactouranase were suggested to be active in the pod-shattering process of *Glycine max*. Langham[10] patented non-dehiscent *Sesamum* variety S 28.

Abd El-Moneim[7] reported that non-shattering character in *Vicia sativa* L. is due to a simple recessive gene; while, Tukamuhabwa *et al*.[6] estimated genetic parameters in soybean and detected the presence of non-allelic interactions of genes affecting pod shattering as well as partial dominance for the trait. In the present investigation, occurrence of 3 mutant plants in M_3 and 4 in M_4 generations suggested that inheritance of non-shattering trait may possibly not be controlled by simple recessive gene.

The non-shattering mutant plants of black cumin isolated at M_2, M_3 and M_4 were without any phenotypic marker trait and therefore a major constraint in their identification before full maturity. In such case, promising non-shattering line(s) could be raised through rigorous selection following application of molecular markers. Prior identification of the non-shattering germplasms under field condition would only enable cytogenetical and biochemical characterization between dehiscent and in-dehiscent genotypes in the species. Boersma *et al*.[8] developed two sequence-specific PCR markers linked to the le gene that reduces pod shattering in narrow-leafed Lupin (*Lupinus augistifolius* L.). As *N.sativa* germplasms are susceptible to out crossing due to open pollination in field planting, the exact genetic make-up of the mutant plants are rather uncertain, although the screened mutants could be important genetic resource for crop improvement.

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