Desirable Macromutants Induced by Chemical Mutagens in Sesame (Sesamum indicum L.)

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Received April 23, 2004; accepted May 14, 2004

Summary Eleven desirable macromutants namely, viridis (seedling colour as marker, increased seed protein content), broad leaf (high capsule number/plant), thick leaf and diffused branching (enhanced number of capsules with high amount of seed protein and fatty oil contents), early flowering (synchronous maturity, enhanced fatty oil content), white flower (marker trait), globular fruit (enhanced seeds/capsule with high oil content in seed), non-shattering capsule (intensed pigmentation on flowers as marker trait apart from the unique trait it possessed for breeders and farmers), dark reddish brown seed-coat I and II (high oil content) and bold seeded (high protein content) have been reported in sesame (Sesamum indicum L. var B-67) which were the outcome of induced chemical mutagenesis. Meiosis in control and mutants was nearly normal (13 bivalents at MI and 13/13 separation of chromosomes at AI mostly) with high pollen fertility (75.15 to 100.0%). All mutant traits were recessive to normal and excepting viridis (digenic) showed monogenic inheritance pattern.

Key words Sesamum indicum, Desirable macromutants, Chemical mutagenesis.

Experimentally induced mutation provides an important source of developing and creating genetic variations in crop plants for improvement. In sesame (Sesamum indicum L.; family: Pedaliaceae), an important oilseed crop with rich protein source and therapeutic use, induced mutagenesis has been performed mostly with physical (Nair 1961, Kobayashi 1964, 1965, 1973, Nayer 1969, Nair and Nair 1978, Ankinudu et al. 1968, Govindarasu et al. 1997, 1998, Sorour et al. 1999) and rarely with chemical (Kamala and Sasikala 1985, Lee et al. 1986, Mary and Jayabalan 1995, Kang et al. 1996) mutagens, however, reports on desirable macromutations are rather meagre (Kobayshi 1964, 1965, Ankinudu et al. 1968, Kamala and Sasikala 1985, Sorour et al. 1999). With a view to raise superior plant types of interest, a research programme on induced chemical mutagenesis has been undertaken in sesame (Sengupta and Datta 2002, 2003a) and this communication is a report on some desirable macromutations induced.

Material and methods

Out of 21 macromutants (Sengupta and Datta 2003b) induced in sesame (Sesamum indicum L. var. B-67) following treatments of dry seeds (moisture content: 6.446%) with different doses (0.25, 0.5, and 1.0% for 2, 4 and 6 h durations at 21±1°C at pH 6.8) of 7 chemical mutagens, namely ethylmethane sulfonate (EMS), diethylsulphate (dES), nitrous acid (HNO₂), hydroxylamine (NH₂OH), dimethyl sulfoxide (DMSO), sodium azide (NaN₃) and hydrogen peroxide (H₂O₂), 11 were considered desirable on the basis of qualitative and quantitative assessment. Frequency of desirable macromutants was estimated at M₂ (6137 plants screened) as per 100 plants (Gaul 1964). The colour of seedlings, flowers and seeds (of identical maturity) in mutants and control were laid with reference to Horticultural Colour Chart I and II (1968) and Munsell Soil Colour Chart (1975). Chlorophyll content was assessed quantitatively (leaf tissues) in control and viridis plant types fol-
lowing the method of Arnon (1949).

**Meiotic analysis**

Meiotic analysis in control and in 11 mutant plant types was performed as suggested earlier by Sengupta and Datta 2003b. Photomicrographs were taken from temporary preparations.

**Inheritance of traits**

Reciprocal crosses were made between normal (N) and mutant (M) plant types (excepting seed mutants) and subsequently F1 and F2 plants were raised. The F2 plants were used for estimating the segregating ratio for different mutant traits by using $\chi^2$ test analysis, however, segregation pattern of seed mutants (dark reddish brown seed-coat I and II and bold seeded) was ascertained from M2 seeds of the mutants sown in M3 generation.

**Quantitative analysis**

Quantitative traits were assessed from true breeding M4 macromutants and selfed control lines. A total of 60 plants from each plant type have been analyzed. Test of significance (student t-test) was computed between sample means of control and mutants to estimate significant variations and only those have been documented in the text. Seed protein was extracted following Osborne (1962) and estimated (5 replicas for each plant type) quantitatively using the method of Lowry et al. (1951). Extraction (3 replicas) of fatty oil was done in a soxhlet apparatus in petroleum ether (boiling point 60–80°C).

**Results and discussion**

**Desirable plant types**

The types (Figs. 2–4, 6, 8) of desirable macromutants are: *viridis* (seedling colour–scheelea green–860/3, control–leek green–00858; chlorophyll content in mg/gm of tissue: chlorophyll a, chlorophyll b and total chlorophyll, 0.0106, 0.1682 and 0.1788 respectively as compared to 0.1194, 0.2904 and 0.4096 in control; maximum occurrence in 1.0%, 2 h DMSO: 20.0% and overall mutation frequency: 0.88%), *thick leaf* (only noted in 1.0%, 4 h dES treatment: 2.78%; over the mutagen population: 0.02%), *broad leaf* (length, breadth and area: 19.68 cm±0.5, 18.95 cm±0.6 and 181.5 sq.cm±0.6 respectively as compared to 13.65 cm±0.2, 14.02 cm±0.3 and 106.0 sq.cm.±0.2 in control (Fig.1); maximum in EMS: 0.5% 2h, 20.0%, 0.39% over the population), *diffused branching* (angle of divergence of primary branches in relation to main axis 29.35° in comparison to 20.75° in control; highest frequency: 0.25%, 2 h EMS: 6.67% and overall frequency: 0.21%), *early flowering* (10–15 d earlier than control plants and the mutant plants showed synchronous maturity; maximum frequency 4.55% in 0.5%, 4 h H2O2, 0.44% over mutagen treatments), *white flower* (flower colour: completely white $8/2$ in relation to *phlox purple*: 632 to 632/3 in flaps and white colour of corolla tubes in control, recovered only from 1.0% 2 h NaN3 treatment: 3.77%; overall frequency: 0.02%), *globular fruit* (6–8 loculed globular shaped fruit (Fig. 5a), as compared to 4 loculed oblong fruit in control (Fig. 5b); spotted only from 0.5%, 2h NH2OH: 2.82%, 0.07% frequency in overall population), *non-shattering capsule* (intense pigmentation on flower which facilitated their identification from segregating population: corolla tube–*phlox purple*–632/3 and flap–*phlox purple*–632/1; matured plants can be kept in field without being shattering of capsules and only after one month from harvest during the process of drying the capsules showed sign of breaks along the suture resulting in 1.0 to 2.0% seed loss whereas in other plant types it was 20.18 to 38.89%; highest frequency in 1.0%, 2 h HNO2: 6.25% and frequency over the population: 0.07 %), *dark reddish brown seed-coat* I (seed-coat colour–$3/4$, control–dark red–$3/6$; spotted only from 0.25%, 2 h NH2OH: 0.48% and overall frequency: 0.02%), *dark reddish brown seed-coat* II (seed-
coat colour - 3/4 and concomitantly associated with diffused branching trait; isolated only from 1.0%, 6 h EMS: 0.5% and estimated frequency in overall population: 0.02%) and bold seeded (maximum frequency in 0.25%, 6 h HNO₂: 2.5%, 0.03% over the population).

Meiotic analysis
Meiotic studies performed in control (106 PMCs analyzed) and mutant plant types (44–158 cells scored) revealed 2n=26 chromosomes (Figs. 9, 10). Average chromosome association noted in control at MI has been 12.91 II+0.19 I. Viridis, diffused branching, early flowering, white flower, dark reddish brown seed-coat II and bold seeded mutants formed 13 II only in their meiocytes. Univalents were observed in PMCs of broad leaf (0.14/cell), thick leaf (0.19/cell), globular fruit (0.52/cell), non-shattering capsule (0.08/cell) and dark reddish brown seed-coat I (0.09/cell) mutant plant types; while a single meiocyte with one quadrivalent was noted in thick leaf mutant. Mostly AI cells (76.15–95.25%) were balanced (13/13 separation of chromosomes) resulting in high pollen fertility (75.15–92.40%) among the plant types.

Inheritance of mutant trait
The F₁’s raised from reciprocal crossings were all normal and the pattern of F₂ segregation noted in broad leaf (mutant as pollen parent: N=65, M=17, χ²=0.79 for 3 : 1, p>0.30, mutant as stigma parent: N=44, M=10, χ²=1.21 for 3 : 1, p>0.20), thick leaf (mutant as pollen parent: N=38, M=10, χ²=0.51 for 3 : 1, p>0.30, mutant as stigma parent: N=20, M=06, χ²=0.05 for 3 : 1, p>0.70), diffused branching (mutant as pollen parent: N=72, M=16, χ²=2.18 for 3 : 1, p>0.10, mutant as stigma parent: N=26, M=04, χ²=2.18 for 3 : 1, p>0.10), early flowering (mutant as pollen parent: N=115, M=30, χ²=1.44 for 3 : 1, p>0.20, mutant as stigma parent: N=80, M=25, χ²=0.08 for 3 : 1, p>0.70), white flower (mutant as pollen parent: N=17, M=04, χ²=0.40 for 3 : 1, p>0.50, mutant as stigma parent: N=21, M=05, χ²=0.46 for 3 : 1, p>0.30), globular fruit (mutant as pollen parent: N=25, M=09, χ²=0.04 for 3 : 1, p>0.70, mutant as stigma parent: N=70, M=21, χ²=0.18 for 3 : 1, p>0.50) and non-shattering capsule (mutant as pollen parent: N=33, M=05, χ²=2.84 for 3 : 1, p>0.05, mutant as stigma parent: N=66, M=24, χ²=0.13 for 3 : 1, p>0.30) mutants revealed that mutant traits were recessive and was under the control of one gene locus excepting viridis which showed digenic mode of inheritance (mutant as pollen parent: N=78, M=09, χ²=2.49 for 15 : 1, p>0.10, mutant as stigma parent: N=102, M=10, χ²=1.37 for 3 : 1, p>0.20). Segregation pattern of dark reddish brown seed-coat I and II and bold seeded mutant was noted to be 1:1 [bold seeded: N=10 (Fig. 7a), M=8 (Fig. 7b), χ²=0.49 for 1 DF, p value >0.50] and 3:1 (dark reddish brown seed-coat I: N=18, M=4, χ²=0.545–for 1 DF, p value >0.50; dark reddish brown seed-coat II: N=29, M=10, χ²=0.034 for 1 DF, p value >0.80). Thus, cytogenetical analysis of the macromutants suggested that they have possibly arisen as the consequence of gene mutation.

Quantitative analysis
Analysis of quantitative parameters has indicated that viridis was dwarf (range: 22.0 to 47.0 cm, mean: 37.8 cm±1.9, control range: 63.0 to 95.0 cm, mean: 77.7 cm±1.9, t=4.10, p>0.001) with increased seed protein content (28.5/100 gm of tissue±0.03; control 24.07/100 gm of tissue±0.4, t=4.50, p>0.001); while, broad leaf (plant height: range: 89.0 to 98.0 cm, mean: 93.5 cm±2.0, t=1.99, p>0.05) and thick leaf (plant height: range: 64.0 to 113.0 cm, mean: 91.9 cm±4.1, t=2.01, p>0.05) mutants were larger plants with significantly higher number of capsules on main axis (broad leaf: range: 2 to 39 units, mean: 26.3±7.8; control range: 7 to 27 units, mean: 17.5±1.1, t=2.54, p>0.05; thick leaf: range: 16 to 46 units, mean: 27.0±3.3, t=2.20, p>0.05) and on total plant (broad leaf: range: 23 to 102 units, mean: 55.2±8.1, t=2.12, p>0.05). Further, thick leaf mutant possessed high amount of seed protein (33.6/100 gm of tissue±0.4,
$t=4.10$, $p>0.001$) and fatty oil (56.7%, control 46.7%) contents like diffused branching mutant (seed protein: 26.3±0.5 units, $t=1.92$, $p>0.05$; fatty oil 61.5%). Diffused branching trait may not be suitable for increasing plant population per unit area but high number of capsules present in them (range: 29 to 114 units, mean: 62.6±6.3, $t=2.11$, $p>0.05$) may compensate the loss. Possessing high amount of oil (60%) in seeds, the globular fruit mutant plants were with high number of seeds/capsules (range: 15 to 73 units, mean: 47.3±2.5; control range: 19 to 62 units, mean: 47.3±2.5; control range: 19 to 62 units, mean: 

Figs. 1–10. 1) Control plant type. 2–8) Mutant traits. 2) Thick leaf. 3) Diffused branching. 4) Globular fruit. 5) Globular shaped fruits (a) along with oblong shaped capsules (b) in control. 6) Non-shattering capsule (matured capsules →). 7) Bold seeded mutant trait (b) with normal seeds (a). 8) Broad leaf. 9–10) MI configurations ($2n=26$). 9) 12 II+2 I. 10) 13 II.
40.9±0.8, t=2.40, p>0.05). Dark reddish brown seed-coat I (30.5±0.40, t=3.42, p>0.001) and bold seeded (29.1±0.40, t=3.33, p>0.001) had high protein content; while, oil content enhanced over control in seed-coat colour mutants (60.0%) and in early flowering (56.7%) plants types.

Thus, broad leaf, thick leaf, globular fruit and diffused branching mutants may be directly used as superior plant types; while, white flower, viridis and seed-coat colour mutants may serve as genetic markers in sesame breeding not withstanding the utmost importance of early flowering and non-shattering capsule traits to breeders and farmers.

Acknowledgement

Financial assistance from CSIR, India is gratefully acknowledged.

References


