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Novel genetic variability in sesame induced via ethyl methane sulfonate

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ABSTRACT

Sesame (*Sesamum indicum* L.) is an ancient oilseed crop with important agronomic, therapeutic, and industrial properties. However, in Morocco, the existing genetic diversity is quite limited. Thus, chemical mutagenesis, using ethyl methane sulfonate (EMS), was applied to induce novel genetic variability. Seeds of two sesame genotypes were treated using two EMS concentrations, 0.5% and 1%. The treated and untreated seeds (control) were planted according to a completely randomized design. Some mutant plants (M_1) were identified and characterized for growth habit, root length, number of carpels per capsule, number of capsules per leaf axil, seed yield per plant, and seed color. Specifically, mutants with a tetra-carpellate capsule, three capsules per leaf axil, determinate growth, diverse seed colors, and a highly developed root system were found to be promising and useful for sesame breeding program aimed at developing productive and high-quality cultivars, particularly for stressful environments.

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Determinate growth; ethyl methane sulfonate; genetic diversity; Moroccan sesame; multi-carpellate capsule; mutation

Introduction

Sesame (*Sesamum indicum* L.) is an ancient oilseed crop that is important from agronomic, therapeutic, and industrial standpoints. It is a mostly self-pollinated diploid plant ($2n = 26$), and its seeds are rich in oil (50–60%), with antioxidant properties (Uzun et al. 2007). It has been cultivated mainly in some Asian and African countries. Almost 70% of the world's production comes from Asia and 26% from Africa (FAOSTAT 2018). In Morocco, average production is around 1800 t per year, with an average yield of <0.8 t/ha from an area of about 2500 ha (FAOSTAT 2018). This yield remains low because of several constraints, such as lack of improved cultivars, drought, lodging, and use of traditional production techniques (PMV

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2008). In addition, sesame still has a few wild characters, including capsule dehiscence, indeterminate plant growth, and asynchronous capsule maturation, leading to low seed yield (Islam et al. 2016). Varietal selection remains the only way to overcome most of these constraints. To do that, a high level of genetic variability should be available. However, a recent study has shown that there was only limited genetic diversity among the Moroccan sesame cultivars (El Harfi et al. 2018). Therefore, it is necessary to expand and broaden the existing genetic base for breeding purposes.

Mutagenesis is a rapid and effective tool for inducing novel and useful genetic variability in sesame, as this plant is predominantly self-pollinated (Saha 2018). Particularly, chemical mutagenesis has been successfully used to obtain new variability in some varieties of cultivated sesame (Begum and Dasgupta 2010). In different countries, novel variants or mutants were induced by treatment with chemical/physical mutagens. Among the obtained mutants, there were those with high yield (Wongyai, Saengkaewsook, and Veerawudh 2001), non-shattering capsule and determinate growth habit (Çağırğan 2006), earliness, synchronous maturity, resistance to diseases, male sterility, and variations in seed coat color (Hoballah 2001), tri-capsule and tetra-carpel capsules (Baydar 2008), and high oil content and modified fatty acid composition (Arslan et al. 2007). The determinate cultivars ensure synchronous maturity of the capsules; thus, minimizing seed loss and reducing the production cost as a result of using mechanized harvesting. Furthermore, the short height of the determinate mutants provides good resistance to lodging and better performance under dense sowing (Çağırğan, Özerden, and Özbaş 2009).

The growth habit in sesame is a very important trait from an agronomic point of view. However, indeterminate growth of sesame plants is the most widespread wild character that limits seed production. Sesame cultivars adapted to modern planting systems, with synchronous maturity and determinate growth are required for mechanized harvesting (Beech and Imrie 2001). Sesame growth habit is controlled by a single recessive gene, which can facilitate selection of cultivars for mechanized harvesting (Uzun and Çağırğan 2009; Zhang et al. 2016, 2018).

Seed yield in sesame is reportedly associated with a high number of capsules per plant and/or carpels per capsule (Baydar 2008; Diouf et al. 2010; Wei et al. 2015). Qualitatively, compared to bicarpellate and mono-capsule cultivars, tetracarpellate and tri-capsule ones produce increased amount of oil, with a high linoleic acid/oleic acid ratio (Baydar 2008). This suggests that such cultivars should be used for food purposes because of their oil being rich in unsaturated fatty acids (Yoshida and Takagi 1997).

Variants for seed-coat color were also obtained by mutagenesis, and this is very important from agronomic and industrial point of view, given the involvement of seed pigmentation in the metabolism of protein, oil,

antioxidant activity, and resistance to diseases (Zhang et al. 2013). Black sesame seeds contain higher ash and carbohydrate contents compared to white seeds (Kanu 2011), and red sesame seeds are richer in zinc and copper than white seeds (Gebrekidan and Desta 2019). Kermani et al. (2019) found that, compared to genotypes with light-color seed, those with dark seed ones had higher amounts of ellagic, caffeic, and ferulic acids, total phenol content, antioxidant activity, quercetin, and apigenin. Therefore, sesame mutation breeding has enabled inducing many morphological and physiological mutants, useful not only for breeding purposes but also for physiological, genetic, and molecular studies.

In our available sesame germplasm, some of the important traits mentioned above are lacking, mainly tetracarpellate capsule and determinate growth that are, respectively, associated with good seed production, and synchronous maturity and adaptation to modern planting system. Therefore, the objective of this study was to induce novel genetic variability via ethyl methane sulfonate (EMS) in two sesame cultivars (one of Moroccan origin and the other obtained from the USA), to identify potential mutants and to compare them with the parental wild type cultivars.

Materials and methods

Plant material

The plant material used in this study consisted of two sesame genotypes, 'ML13' and 'US06'. ML13 is a Moroccan cultivar collected from the Tadla area in Morocco (El Harfi et al. 2018). It is characterized by light brown seeds, one capsule per leaf axil, bi-carpellate capsules, and indeterminate growth. US06 is a Mexican cultivar 'Ostimuri 89' (accession PI561704), kindly provided by Dr. Bradley Morris of USDA-ARS, Griffin, GA, USA. It has white seeds, three capsules per leaf axil, bi-carpellate capsules, and indeterminate growth.

EMS seed treatment

Ethyl methane sulfonate (EMS) was applied as a chemical mutagen. Healthy and mature seeds of both sesame genotypes (ML13 and US06) were treated with two EMS concentrations, 0.5% and 1%, while the control was treated with distilled water. For each treatment, 200 seeds were used. The seeds were previously soaked in distilled water overnight at room temperature ($24 \pm 1^\circ\text{C}$). A fresh solution of EMS was prepared in distilled water at two concentrations, 0.5% and 1% (v/v). The seeds, anteriorly washed and hydrated in distilled water, were soaked in respective EMS solutions for 5 h at room temperature ($24 \pm 1^\circ\text{C}$). After that, the treated seeds were rinsed with a solution of 3%

sodium thiosulfate for 5 min, then rinsed under running tap water to remove chemical residues, and finally dried on a filter paper. Treated and untreated seeds were placed on Whatman paper in sterile Petri dishes containing distilled water and kept under dark at ambient conditions to induce germination. Distilled water was applied every 2 days to keep the paper moist.

Study site and experiment conditions

After 7 days, the young seedlings obtained were placed in seedling nursery soil blocks filled with peat and kept sufficiently moist, under greenhouse conditions, to ensure their proper growth. At the four-leaf stage, the seedlings were randomly transplanted in a field plot of the INRA-Experimental Station at Ain Taoujdate on 4 April 2019. This station, located 30 km from Meknes city, in the Region of Fes-Meknes (Morocco), at an altitude of 550 m (33°56' N, 5°13' W), is characterized by a warm Mediterranean climate, with a dry summer, cold winter, and an average annual rainfall of 470 mm.

We obtained 165 possible mutant plants, along with 20 plants from each of the check cultivars, ML13 and US06. Plants were harvested on 28 September 2019. During the experiment period, the minimum and maximum temperatures were 21°C (on 15 April) and 42°C (on 17 August). The sesame plants tested were subjected to natural climatic conditions. Weeding was carried out as needed. Drip irrigation was used throughout the plant cycle to meet the crop's water requirements. The first irrigation was applied just before plants were transplanted.

Analysis

The M_1 plants and the control plants were individually characterized, analyzed, and compared with each other on the basis of morphological and agronomic characters, i.e., growth habit, root system, number of carpels per capsule, number of capsules per leaf axil, seed yield per plant and seed color. Five plants from each control cultivar (wild type) were randomly taken to be analyzed and compared with mutants. The differences between mutants and control plants were examined via Student's t-test for the following traits: root length and yield components (number of capsules per plant, number of seeds per capsule, weight of 1000 seeds, and yield per plant). The check (wild type) population average was compared to specific value of a mutant. Student's t-test was performed using SPSS software for Windows (Version 22).

Results and discussion

Growth habit

A total of 11 sesame mutants with determinate growth habit were observed and isolated from the M_1 population. These mutants, accounting for 6.7% of the population, were ML1-17, ML1-32, and ML1-42 (related to the check cultivar ML13), and US1-1, US1-3, US1-5, US1-8, US2-10, US2-12, US2-14 and US2-18 (derived from the check cultivar US06). [Figure 1](#) represents a mutant characterized by determinate growth in comparison with the wild type with indeterminate growth.

In our study, we used EMS mutagenesis to induce determinate growth in sesame, whereas in some previous studies gamma rays were used (Ashri 1981; Çağırğan 2006). However, these gamma rays-induced mutants with determinate growth have shown low seed yield and late maturity, even when they were early-flowering (Ashri 1994; Uzun and Çağırğan 2006; Çağırğan, Özerden, and Özbaş 2009). Our investigation revealed a novel mutant exhibiting earlier flowering and maturity than the parental wild type.

Root system

We compared the length of the root system in four sesame mutants (ML2-1, ML75-3, US75-1, and US2-3) and their parents, ML13 and US06. Large morphological differences were observed in the roots of the mutants obtained ([Figure 2](#)). These variations were statistically significant according to the Student's t-test ([Table 1](#)). In fact, the mutants ML2-1 and ML75-3, derived

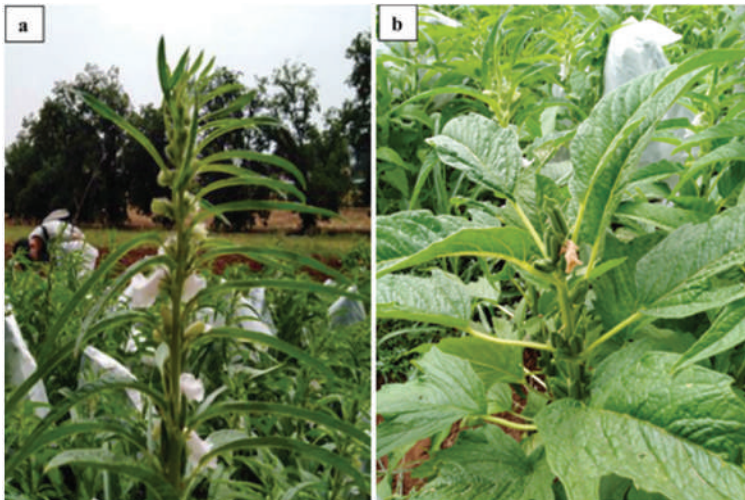


Figure 1. Growth habit in sesame plants: (a) indeterminate growth of the parent US06, (b) determinate growth of the mutant US1-5.



Figure 2. Root system of some sesame mutants, in comparison with the check cultivars, at seedling (A 1, B 1) and adult plant stages (A 2, B 2).

Table 1. A comparison of M_1 mutants with their wild type for root length, measured in seedling and adult plant stages, via the Student's t-test.

| Genotypes | Root length (cm) | |
|---------------------|----------------------|-------------------|
| | Young seedling stage | Adult plant stage |
| ML75-3 | 2.60 | 54.80 |
| ML13 (Check) | 1.40 | 37.00 |
| t-Value | 4.48 | 18.99 |
| p-Value | <0.05 | <0.001 |
| ML2-1 | 3.80 | 68.30 |
| ML13 (Check) | 1.40 | 37.00 |
| t-Value | 8.96 | 33.39 |
| p-Value | <0.001 | <0.001 |
| US75-1 | 1.30 | 47.00 |
| US06 (Check) | 2.10 | 66.20 |
| t-Value | 1.99 | 14.83 |
| p-Value | 0.12 | <0.001 |
| US2-3 | 0.90 | 22.00 |
| US06 (Check) | 2.10 | 66.20 |
| t-Value | 2.99 | 34.14 |
| p-Value | <0.05 | <0.001 |

from the check cultivar ML13, exhibited a significantly more developed root system ($p < 0.001$), with a more pronounced root length than that of the check parent ML13, both at the seedling and adult plant stages (Figure 2A1, A2 and Table 1). In contrast, the mutants US75-1 US2-3 had significantly shorter roots compared to their check parent US06 at the adult plant stage (Figure 2B2; Table 1). However, at the seedling stage, US75-1 was comparable to the check, whereas US2-3 exhibited significantly shorter roots (Figure 2B1 and Table 1).

To the best of our knowledge, this is the first report of sesame mutants with strong root system. A well-developed root system is indicative of high yield and drought resistance. A significant correlation has been reported between a well-developed root system and high seed yield in sesame (Su et al. 2019), as well as in other crops, such as barley (Svačina, Středa, and Chloupek 2014), maize (Wang et al. 2015) and rice (Liu et al. 2018). In peanut, Li et al. (2014) found that genotypes with a well-developed root system were highly tolerant to drought. Therefore, the novel genotypes, with a stronger root system compared to parental cultivar in our study, should be useful for breeding for high productivity and drought resistance or tolerance in the context of climate change.

Capsules per leaf axil and carpel per capsule

One of the mutants was found to have multicarpellate capsules (Figure 3) and another with multiple capsules per leaf axil (Figure 4), which represent good and valuable agronomic characteristics in sesame. In contrast to its check parent US06 with bicarpellate capsules, the mutant US2-7 had tetra-carpellate capsules. Also, this mutant had 93 capsules/plant, which is statistically equal ($t = 1.95$, $p > 0.12$) to the check cultivar US06 with 95.7 capsules/plant (Table 2). The mutant US2-7 produced 88.1 seeds/capsule, which was significantly ($t = 12.64$, $p < 0.001$) higher than that of the check cultivar US06 (72.30 seeds/capsule). With respect to 1000-seed weight, there was no significant difference ($t = 2.24$, $p = 0.09$) between the mutant US2-7 and the wild type parent US06. The yield per plant was significantly ($t = 5.46$, $p < 0.01$) higher in the mutant US2-7 (32.77 g) compared to the check parent US06 (27.77 g).

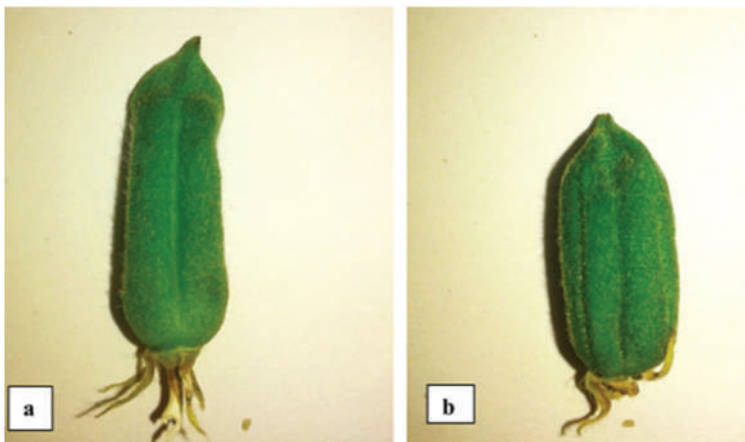


Figure 3. Number of carpels per capsule, (a) bi-carpellate capsule in the check cultivar US06 and (b) tetra-carpellate capsule in the mutant US2-7.

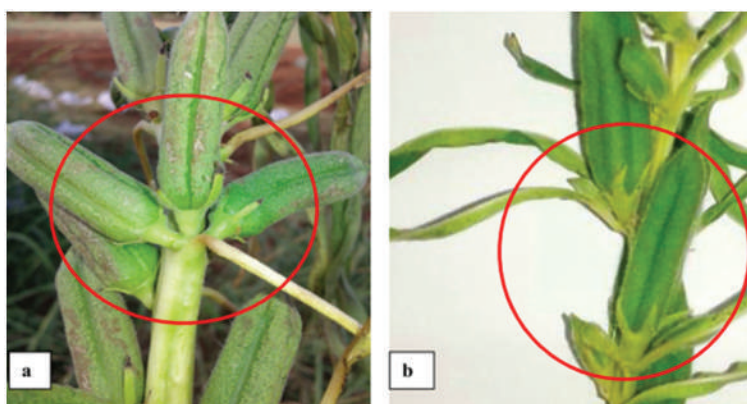


Figure 4. Number of capsules per leaf axil in sesame, (a) three capsules per axil in the mutant ML2-68 and (b) a single capsule in the wild type variety ML13.

Table 2. A comparison of M_1 mutants with their wild type for yield components via the Student's t-test.

| Genotypes | Number of capsules per plant | Number of seeds per capsule | 1000-seed weight (g) | Yield per plant (g) |
|----------------|------------------------------|-----------------------------|----------------------|---------------------|
| US2-7 | 93.00 | 88.10 | 4.00 | 32.77 |
| US06 | 95.70 | 72.30 | 4.10 | 27.77 |
| (Check) | | | | |
| t-Value | 1.95 | 12.64 | 2.24 | 5.46 |
| p-Value | 0.12 | <0.001 | 0.09 | <0.01 |
| ML2-68 | 82.00 | 57.90 | 3.02 | 14.33 |
| ML13 | 68.30 | 60.70 | 3.85 | 14.79 |
| (Check) | | | | |
| t-Value | 8.08 | 2.16 | 1.33 | 0.38 |
| p-Value | <0.01 | 0.1 | 0.25 | 0.72 |

Mutant ML2-68 produced three capsules per leaf axil, whereas the check cultivar ML13 had only one capsule per leaf axil (Figure 4). In addition, this mutant produced 82 capsules/plant, which was significantly ($t = 8.08$, $p < 0.01$) higher than its parent ML13's 68.3 capsules/plant. However, there was no significant difference between the mutant ML2-68 and the check cultivar ML13 with respect to the number of seeds per capsule, 1000-seed weight, and yield per plant (Table 2).

In two novel genetic materials, i.e., US2-7 and ML2-68, there was a significant increase in number of seeds per plant and in number of capsules per plant, which are important components of seed yield in sesame. The use of these mutants as a novel germplasm in our breeding program will open up the possibility of rapidly developing cultivars with higher seed yield than the existing ones. Our findings are in accordance with those of Baydar (2008) and Diouf et al. (2010), who reported that the increase in seed yield in sesame required the development of cultivars with a high number of capsules per

plant and/or carpels per capsule. Also, Wei et al. (2015) found that sesame cultivars with three capsules per leaf axil produced higher seed yield than standard cultivars with a single capsule.

Pigmentation of seed coat

EMS-mutagenesis induced variations in the seed-coat pigmentation of some of the mutants. A pale black color was observed in the integument of mutant US2-6; seeds of the wild cultivar US06 are white (Figure 5a,b). On the other hand, a dark brown coloration of the seed coat was obtained in mutant ML2-12; the seeds of the check cultivar ML13 have a light brown pigmentation (Figure 5c,d).

Through mutation breeding, we could get novel seed variants that were missing in our germplasm before. Variation in sesame seed coat is related to the chemical and biochemical composition of the seed. Furthermore, pigmentation of sesame seed coat is involved in the metabolism of protein, oil, phenolic acids, flavonoids, antioxidant activity, and resistance to diseases (Zhang et al. 2013; Kermani et al. 2019). The seeds of the mutants and their wild types should be characterized for various chemical and biochemical parameters to determine the modifications that occurred in these seeds as a result of mutation. The mutants obtained, i.e., US2-6 and ML2-12, would

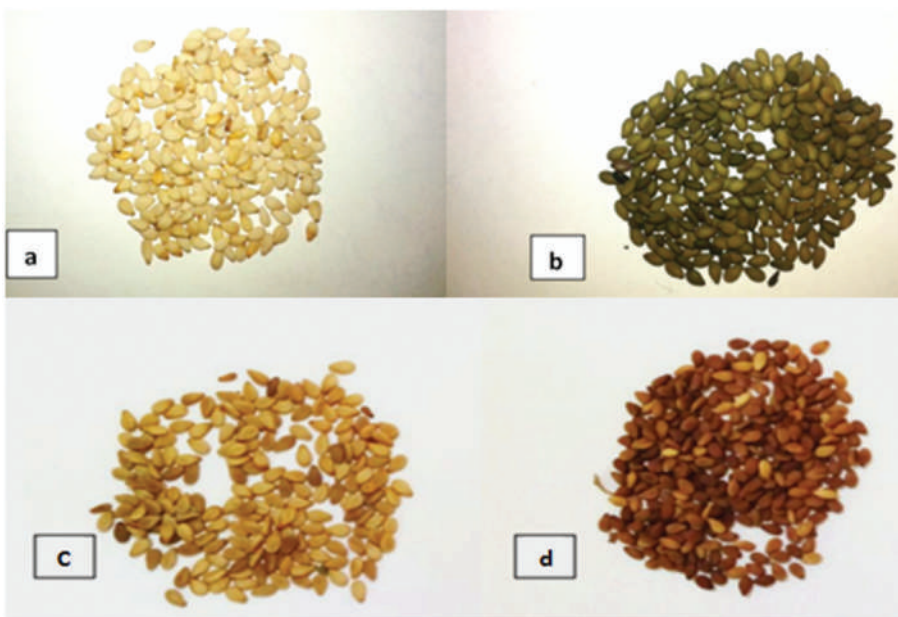


Figure 5. Pigmentation of seed coat: (a) White seeds of the wild cultivar US06, (b) pale black seeds of the mutant US2-6, (c) light brown seeds of the wild cultivar ML13, (d) and dark brown seeds of the mutant ML2- 12.

be useful for improving some physiological, nutritional, industrial, and medicinal properties in Moroccan sesame.

By using EMS mutagenesis breeding, the existing genetic variability in Moroccan sesame germplasm has been expanded and broadened, and, as a result, new important and interesting characters were induced. This should accelerate and facilitate the development of sesame cultivars with many improved traits.

Conclusions

Chemical mutagenesis by EMS produced viable and interesting sesame mutants, both in Moroccan and Mexican materials, for some morphological and agronomic traits. The most interesting mutants that have been identified were those having one or more of the following characters: a determinate growth habit, highly developed root system, different seed colors, three capsules per leaf axil, and tetra-carpellate capsule. All these mutants could serve as useful germplasm for the sesame breeding program in Morocco. However, these mutants should be evaluated in different environments to obtain the M₂ generation and to confirm the stability of mutated traits.

Disclosure statement

No potential conflict of interest was reported by the authors.

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