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# ASSESSMENT OF NOVEL EMS-INDUCED GENETIC VARIABILITY IN RAPESEED (*BRASSICA NAPUS* L.) AND SELECTION OF PROMISING MUTANTS

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**Abstract**

Induced mutation may be an effective alternative to get a novel genetic variability that might not be found in natural germplasm. Seeds of *Brassica napus* L. (variety 'INRA-CZH2') were treated with Ethyl Methane Sulphonate (EMS), in 1, 1.2, 1.4 and 1.6% doses for 6, 7 and 14 hours, and were planted to obtain the $M_1$ plants and then the $M_2$ plants. The objective of this study was to evaluate the novel induced variability observed for some important quantitative traits and to select mutants with modified and interesting characteristics. Data were recorded on 10 random plants taken from $M_2$ populations derived from each treatment (dose by duration), along with control plants (wild type), and were evaluated in two contrasted environments. A considerable variability was observed, and EMS treatment had a significant effect on all the traits studied. Compared to control plants, mutants coming from seeds treated with low EMS doses for moderate time, namely 1% EMS (7 hours), flowered and matured earlier and had higher number of pods per plant in both environments. In addition, they were generally more adapted than the check and the other mutants to stressful environments associated with low rainfall, high temperature and late planting at Allal Tazi location. This would explain phenotypic stability and adaptability of these mutants to the environments studied. Besides, late planting at Allal Tazi location was harmful for all the plant materials studied (check and mutants) having exhibited lower performance compared to early planting at Douyet location. Interestingly, this is the first time that a rapeseed mutant combining such desirable characteristics is obtained. This promising mutant exhibiting its stability throughout $M_1$ and $M_2$ generations in both the environments will be valuable and useful for fast development of adapted and agronomically superior rapeseed cultivar.

**Key words:** Cultivar, Earliness, Environment, Mutagenesis, Quantitative traits, Seed yield.

## Introduction

Rapeseed (*Brassica napus* L.) is a very important source of vegetable oil and meal rich in protein, with a substantially increased world production over the last decades (Carré & Pouzet, 2014). In 2016, the overall production was around 69 million tons, ranking third after oil palm and soybean (Anon., 2016). Rapeseed oil is primarily used in human food and biodiesel production, while its meal is used in animal feeding. Rapeseed oil has the lowest level of saturated acid among the other edible oils, with less than 7%, and it has more than 25% of polyunsaturated fatty acids, α-linolenic acid and linoleic acid, which have a beneficial effect on the control of cholesterol levels in human blood (More & Malode, 2016). Also, rapeseed oil is very good for heart health since it is rich in vitamins E, K and plant sterols (McDonald, 2011).

To enhance the sustainable production of any crop, improved and well-adapted varieties should be continuously released. For this purpose, a good genetic variability should be present in the primary gene pool (Shinwari *et al.,* 2013; Shinwari *et al.,* 2014; Kumar *et al.,* 2015; Jan *et al.,* 2017[a, b]). Since there is a limited genetic variability in rapeseed germplasm, various tools should be employed to broaden such variability (Sestili *et al.,* 2010; Khan *et al.,* 2014). Recently, due to the limited genetic variability in nature, conventional cross breeding has been restrictedly used (Sestili *et al.,* 2010). Induced mutation may be an alternative to get a new genetic variability that might not be found in natural germplasm (Szarejko & Forster, 2007). Mutation breeding has the advantage to be potent and effective tool to generate a novel induced genetic variability for several qualitative and quantitative traits in various important crops. In some regions of the world, many researchers did breed and improved some oilseed crops through successful mutagenesis application (Spasibionek, 2006; Ferrie *et al.,* 2008; Velasco *et al.,* 2002; Parry *et al.,*2009; Emrani *et al*., 2015; Hussain *et al.,* 2017). Actually, mutagenesis has been employed to improve a large number of desirable characters like earliness, dwarfness, biotic and abiotic stress resistance, seed yield and oil quality (Schnurbush *et al.,* 2000; Parry *et al.,* 2009; Ali & Shah, 2013; Lee *et al.,* 2018).

Nowadays, the use of some biotechnological tools and techniques for plant breeding purpose, like as mutagenesis, has allowed a sharp increase in global agricultural production. Many mutagen agents, either chemical or physical, are available to create and obtain valuable mutations in crop plants. Each particular mutagen agent acts according to a different and specific mode that determines the nature of alteration in plant genetic background (Meinke *et al.,* 1998). Thus, several rearrangements could happen in DNA fragments and depend upon the mutagen and its dose and the duration of its application, which may result in production of a range of various mutants. Ethyl Methane Sulphonate (EMS) is a chemical mutagen that produces random mutations in plant genome and it is known as one of the available chemical mutagens most commonly used in mutation breeding (Fehr, 1991). It is effective and easy to apply in seeds, and the results obtained can be monitored effortlessly. Usually, there are point mutations in EMS-treated plants, but in some cases, one could also observe a deletion or a loss of a chromosome segment (Okagaki *et al.,* 1991). EMS dose, duration of application and solution temperature are the key factors involved for inducing and obtaining mutations (Alcantara *et al.,* 1996). Different concentrations of EMS mutagen were applied to create a new genetic variability in our rapeseed germplasm. Therefore, this research was carried out to compare the relative effectiveness of different treatment levels of Ethyl Methane Sulphonate (EMS) for inducing novel genetic variability in rapeseed, to evaluate the developed mutants for some important traits, and to isolate and select mutants combining some desirable traits.

## Material and Methods

**Plant material and EMS mutagenesis:** Plant material used in this study was the rapeseed (*Brassica napus* L.) variety 'INRA-CZH2', from the collection of National Institute for Agricultural Research (INRA) of Morocco (Nabloussi, 2015). Ethyl Methane Sulfonate (EMS) was used as chemical mutagen to induce mutation and novel genetic variability in plants derived from the EMS-treated seeds of this variety. Four EMS concentrations, 1%, 1.2%, 1.4% and 1.6% for 6, 7 and 14 hours were investigated. Nine treatment levels were considered in this study as shown in Table 1.

**Table 1. EMS treatment levels used to induce novel genetic variability in rapeseed.**

| EMS concentration (%) | Application duration (Hour) | Abbreviation treatment |
|---|---|---|
| 1 | 6 | EMS1-6 |
| 1.2 | 6 | EMS1.2-6 |
| 1.4 | 6 | EMS1.4-6 |
| 1.6 | 6 | EMS1.6-6 |
| 1 | 7 | EMS1-7 |
| 1.2 | 7 | EMS1.2-7 |
| 1 | 14 | EMS1-14 |
| 1.2 | 14 | EMS1.2-14 |
| 1.4 | 14 | EMS1.4-14 |

**Field experiment:** Treated and control seeds were planted in November 2014 at the INRA-experimental station of Douyet. This station characterized by a cracking clay soil is located at 10 km from Fez city (34°04' N, 5°07' W), at an elevation of 416 m and with an average rainfall of 425 mm. Climate is of Mediterranean type, with cold and rainy winters, and hot and dry summers. This experimental station is also characterized by a frequent presence of the sirocco wind which could be to some extent harmful for crop growing.

Similarly, seeds collected from individual $M_1$ plants were planted as $M_2$ population in a completely randomized design on 11 November 2015 at the INRA Experimental Station of Douyet and on 20 January 2016 at the INRA-experimental station of Sidi Allal Tazi, as a late planting date. INRA Experimental Station of Sidi Allal Tazi is located at 30 km from Kenitra city (34°31' N, 6° W), at an elevation of 10.5 m and with an average annual rainfall of 550 mm. The soil is limestone clay with higher salinity rate than Douyet experimental station.

**Parameters measured:** Morphological and agronomic parameters were studied in $M_2$ population. Days to 50% flowering and to 50% maturity of each mutant were calculated as the sum of days from emergence date to date when 50% of plants of this mutant had flowered and matured, respectively. Plant height (cm), number of branches per plant and number of pods per plant were determined at the maturity. After harvest, number of seeds per pod was counted in laboratory. Pod length (cm) and diameter (mm) were determined using a caliper, whilst 1000-seed weight (g) was determined by a precision balance.

From each treatment, a total of 10 plants by mutant were considered as a random sample for all observations and measurements done in field and laboratory. Wild type of the variety 'INRA-CZH2' was used as a check.

**Statistical analysis:** The collected data were subjected to an analysis of variance (ANOVA) to test statistically significant differences between treatments, environments (sites) and their interaction levels. Duncan's new multiple range test (DMRT) was applied to compare treatment means. Statistical analyses were conducted with the software package SPSS for Windows (Version 22).

## Results and Discussion

Results of analysis of variance of EMS treatments and environment (sites) for the studied quantitative traits are shown in Table 2. EMS treatments had a significant effect on variation of all traits. Both INRA-experimental stations of Douyet and Sidi Allal Tazi differed significantly for all traits, except number of seeds per pod and pod length. The interaction treatment × environment significantly affected the variation of all traits in $M_2$ population, except plant height and number of branches per plant. Therefore, rapeseed were differentially affected by EMS treatment in terms of induction of novel genetic variations through both environments.

**Climate data analysis:** Figures 1 and 2 show monthly temperatures and rainfall recorded in the experimental stations of Douyet and Sidi Allal Tazi, respectively, during the cropping season 2015/2016.

During cropping cycle of $M_2$ population grown in Douyet, minimum temperature was 2°C recorded on November, while maximum temperature was 44.8°C registered on July (Fig. 1). Moreover, Figure 1 shows a strong monthly rainfall variation. After planting, and until January, there was no precipitation, so that two irrigations were carried out to ensure a good germination and seedling emergence. Cumulative rainfall was around 218 mm to which the quantities of 20 and 25 mm brought by both irrigations were added. The most rainy month was March with about 52 mm (Fig. 1). On the other hand, at INRA experimental station of Sidi Allal Tazi, minimum (0.7°C) and maximum (46.2°C) temperatures recorded in February and July, respectively (Fig. 2). Also, Figure 2 shows a clear monthly rainfall variation. In late April, there was no precipitation, so that irrigation were carried out to have unstressed flowering conditions. Cumulative rainfall was around 213.9 mm to which were the quantity of 30 mm brought by irrigation was added. Like as Douyet, the most rainy month in Sidi Allal Tazi was March, with about 45.4 mm. In both experimental stations, the overall water supply (cumulative rainfall and irrigations) remained lower than average rainfall, indicating that experiment was conducted under relatively dry conditions.

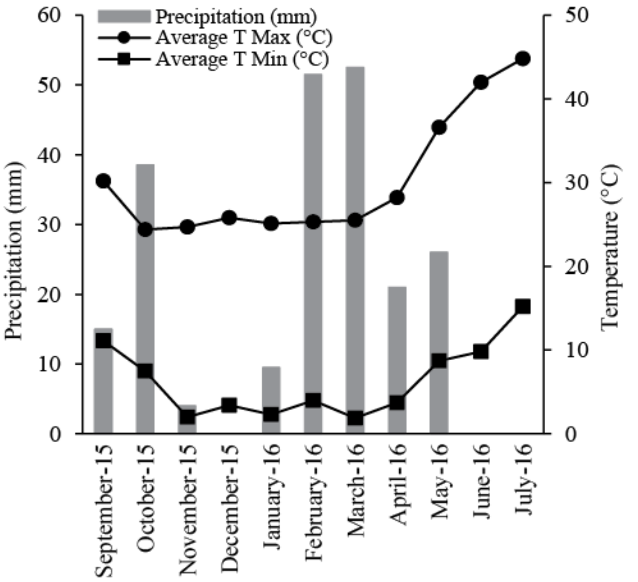


Fig. 1. Average maximum and minimum monthly temperatures and rainfall recorded in the experimental station of Douyet during 2015/2016.

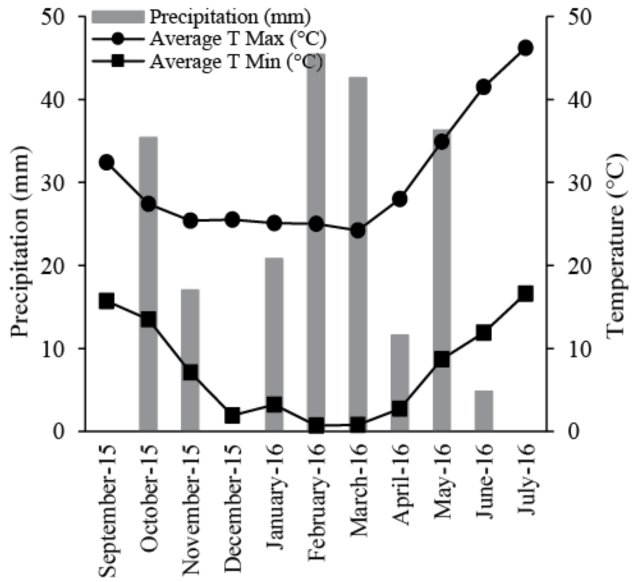


Fig. 2. Average maximum and minimum monthly temperatures and rainfall recorded in the experimental station of Sidi Allal Tazi during 2015/2016.

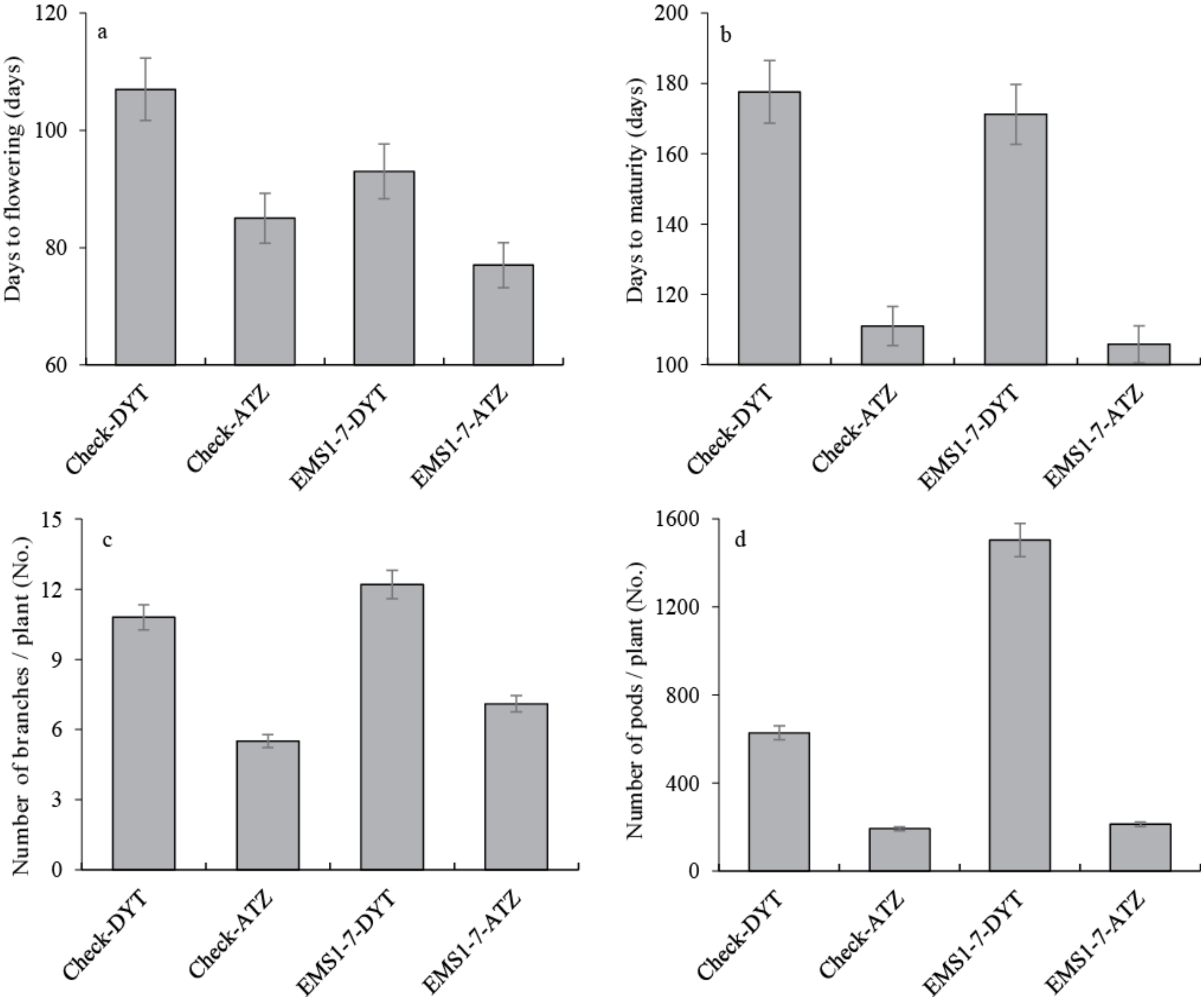


Fig. 3. Genetic gain in agro-morphological parameters of $M_2$ mutant population derived from 1% EMS during 7 hours (EMS1-7) and check variety 'INRA-CZH2'of rapeseed evaluated in two different environments, Douyet (DYT) and Sidi Allal Tazi (ATZ). **a**: Days to 50% flowering (days), **b**: Days to 50% maturity (days), **c**: Number of branches per plant (No.), **d**: Number of pods per plant (No.).

**Table 2. Analysis of variance (mean squares) of EMS treatments for quantitative traits evaluated in $M_2$ populations of *Brassica napus* L.**

| Source of variation | Degree of Freedom | Days to flowering (days) | Days to maturity (days) | Plant height (cm) | Number of branches / plant (No.) | Number of pods / plant (No.) | Number of seeds / pod (No.) | Pod length (cm) | Pod diameter (mm) | 1000-seed weight (g) |
|---|---|---|---|---|---|---|---|---|---|---|
| Treatment | 8 | 575.68*** | 865.86*** | 1422.59** | 17.12* | 1046116.05*** | 184.51*** | 5.19*** | 0.66*** | 1.52** |
| Site | 1 | 19236.59** | 163598.40*** | 94067.52*** | 715.82*** | 11538729.71*** | 75.51 | 0.24 | 15.01*** | 15.81*** |
| Treatment *Site | 8 | 89.37*** | 268.28*** | 505.867 | 8.12 | 814714.73*** | 26.54* | 1.04** | 0.27* | 1.33** |

*, **, *** Significant at 0.05, 0.01 and 0.001 levels, respectively.

**Table 3. Effect of different EMS treatment levels on quantitative traits in $M_2$ rapeseed populations evaluated in two different environments.**

| EMS treatment levels | Days to flowering (days) | Days to maturity (days) | Plant height (cm) | Number of branches / plant (No.) | Number of pods/plant (No.) | Number of seeds/pod (No.) | Pod length (cm) | Pod diameter (mm) | 1000-seed weight (g) |
|---|---|---|---|---|---|---|---|---|---|
| Control | $95.80^{c}$ | $144.30^{cd}$ | $133.65^{a}$ | $8.15^{abc}$ | $409.7^{b}$ | $26.56^{a}$ | $6.35^{ab}$ | $4.03^{a}$ | $2.12^{bc}$ |
| 1% of 6hrs | $87.15^{de}$ | $129.90^{e}$ | $127.55^{ab}$ | $8.05^{abc}$ | $309.9^{b}$ | $25.31^{ab}$ | $6.35^{ab}$ | $3.69^{b}$ | $2.28^{bc}$ |
| 1% of 7hrs | $85.00^{e}$ | $138.50^{d}$ | $123.85^{ab}$ | $9.65^{ab}$ | $858.5^{a}$ | $25.64^{a}$ | $6.46^{ab}$ | $3.85^{ab}$ | $2.39^{b}$ |
| 1% of 14hrs | $106.33^{a}$ | $164.33^{a}$ | $116.78^{ab}$ | $7.22^{bc}$ | $364.8^{b}$ | $20.28^{de}$ | $5.60^{cd}$ | $3.33^{c}$ | $1.29^{d}$ |
| 1.2% of 6hrs | $96.95^{bc}$ | $146.30^{bc}$ | $112.55^{b}$ | $6.30^{c}$ | $276.9^{b}$ | $22.27^{cd}$ | $5.66^{cd}$ | $3.71^{b}$ | $1.85^{c}$ |
| 1.2% of 7hrs | $89.85^{d}$ | $143.95^{cd}$ | $121.75^{ab}$ | $8.10^{abc}$ | $830.6^{a}$ | $27.05^{a}$ | $6.63^{a}$ | $3.79^{ab}$ | $2.27^{bc}$ |
| 1.2% of 14hrs | $100.45^{b}$ | $151.90^{b}$ | $111.00^{b}$ | $8.55^{abc}$ | $279.7^{b}$ | $19.55^{e}$ | $5.22^{de}$ | $3.65^{b}$ | $1.88^{bc}$ |
| 1.4% of 6hrs | $97.35^{bc}$ | $146.20^{bc}$ | $131.90^{a}$ | $8.85^{ab}$ | $454.0^{b}$ | $23.11^{bc}$ | $6.01^{bc}$ | $3.86^{ab}$ | $2.27^{bc}$ |
| 1.4% of 14hrs | $97.10^{bc}$ | $165.10^{a}$ | $120.10^{ab}$ | $10.10^{a}$ | $381.0^{b}$ | $18.72^{e}$ | $4.95^{e}$ | $3.57^{bc}$ | $3.00^{a}$ |
| 1.6% of 6hrs | $98.90^{bc}$ | $150.15^{bc}$ | $126.35^{ab}$ | $8.90^{ab}$ | $202.7^{b}$ | $21.07^{cde}$ | $5.69^{c}$ | $3.77^{ab}$ | $2.29^{bc}$ |

Values with different alphabetical superscripts are significantly different ($p \leq 0.05$) according to DMRT

**Table 4. Effect of different EMS treatment levels on quantitative traits in $M_2$ rapeseed populations evaluated in the experimental stations of Douyet and Sidi Allal Tazi.**

| EMS treatment levels | Days to flowering (days) | | Days to maturity (days) | | Plant height (cm) | | Number of branches / plant (No.) | | 1000-seed weight (g) | |
|---|---|---|---|---|---|---|---|---|---|---|
| Sites | Douyet | Sidi Allal Tazi | Douyet | Sidi Allal Tazi | Douyet | Sidi Allal Tazi | Douyet | Sidi Allal Tazi | Douyet | Sidi Allal Tazi |
| Control | $107^{bc}$ | $84.6^{ab}$ | $177.6^{ab}$ | $111^{bcd}$ | $155.5^{a}$ | $111.8^{a}$ | $10.8^{ab}$ | $5.5^{ab}$ | $2.59^{bc}$ | $1.66^{abc}$ |
| 1% of 6hrs | $98.5^{d}$ | $75.8^{c}$ | $152.7^{c}$ | $107.1^{cd}$ | $145.4^{ab}$ | $109.7^{a}$ | $9.6^{ab}$ | $6.5^{ab}$ | $2.71^{b}$ | $1.86^{abc}$ |
| 1% of 7hrs | $92.70^{e}$ | $77.4^{c}$ | $171.2^{b}$ | $105.8^{d}$ | $154.8^{a}$ | $92.9^{ab}$ | $12.2^{a}$ | $7.1^{ab}$ | $2.34^{bc}$ | $2.45^{a}$ |
| 1% of 14hrs | $115^{a}$ | $89^{a}$ | $186^{a}$ | $121^{a}$ | $127.67^{b}$ | $95^{ab}$ | $8.17^{b}$ | $5.33^{ab}$ | $1.36^{d}$ | $1.17^{c}$ |
| 1.2% of 6hrs | $108.6^{b}$ | $85.3^{ab}$ | $177^{ab}$ | $115.6^{ab}$ | $136.5^{ab}$ | $88.6^{ab}$ | $8.7^{ab}$ | $3.9^{b}$ | $2.08^{c}$ | $1.62^{abc}$ |
| 1.2% of 7hrs | $97.7^{de}$ | $82^{bc}$ | $177.7^{ab}$ | $110.2^{bcd}$ | $152^{a}$ | $91.5^{ab}$ | $10.9^{ab}$ | $5.3^{ab}$ | $2.26^{bc}$ | $2.28^{ab}$ |
| 1.2% of 14hrs | $112.5^{ab}$ | $88.4^{ab}$ | $183.8^{a}$ | $120^{a}$ | $136^{ab}$ | $86^{ab}$ | $9.8^{ab}$ | $7.3^{a}$ | $2.41^{bc}$ | $1.36^{bc}$ |
| 1.4% of 6hrs | $110.4^{ab}$ | $84.3^{ab}$ | $181.6^{ab}$ | $110.8^{bcd}$ | $150.8^{a}$ | $113^{a}$ | $11^{ab}$ | $6.7^{ab}$ | $2.39^{bc}$ | $2.15^{ab}$ |
| 1.4% of 14hrs | $102.71^{cd}$ | $84^{ab}$ | $187^{c}$ | $114^{abc}$ | $140.14^{ab}$ | $73.33^{b}$ | $12.14^{a}$ | $5.33^{ab}$ | $3.63^{a}$ | $1.55^{abc}$ |
| 1.6% of 6hrs | $113.1^{ab}$ | $84.7^{ab}$ | $185.2^{a}$ | $115.1^{ab}$ | $154^{a}$ | $98.7^{ab}$ | $10.2^{ab}$ | $7.6^{a}$ | $2.65^{bc}$ | $1.95^{abc}$ |

Values with different alphabetical superscripts are significantly different ($p \leq 0.05$) according to DMRT

**Days to 50% flowering and to 50% maturity:** Effect of different EMS treatment levels on days to flowering and to maturity are shown in Table 3. EMS1-6 and EMS1-7 were found to be more effective for inducing earliness in flowering and maturity, compared to the check and the other EMS treatment levels. In fact, mutant population derived from EMS1-7 had the lowest average number of days to flowering, 85, and mutant population coming from EMS1-6 exhibited the lowest average number of days to maturity, 130, compared to 96 and 144 days, respectively, in the check variety (Table 3). These results showed that mutants derived from low EMS concentrations and short application duration exhibited earlier flowering and maturity than the original variety. Further experiments using the same EMS treatment in the same conditions are needed to confirm such results. In the present study, mutant population coming from EMS1-6 and EMS1-7 needed short time duration to flower (99 and 93 days, respectively) and to mature (153 and 171 days, respectively) at Douyet, compared to 107 and 178 days, respectively in the check variety (Fig. 3a,b). In Sidi Allal Tazi, days to flowering of mutant population coming from EMS1-6 and EMS1-7 were 76 and 78 days, respectively, whilst days to maturity were 107 and 106, respectively, compared to 85 and 111 days, in the control variety respectively. Mutant population derived from EMS1-7 produced flowers earlier than the check and the other EMS treatment levels regardless planting date and locations (Table 4). This would explain phenotypic stability and adaptability of this mutant over the environments studied. In addition, this mutant was generally more adapted than check and other mutants to stressful environments associated with low rainfall, high temperature and contrasted sowing dates. Early flowering provides sufficient time for seed filling, often under conditions with less drought and heat stresses than late flowering, which could result in better seed yield. In our study, experimental conditions in both environments were characterized by high maximum temperatures during March-April period, coinciding with flowering and seed filling (Figs. 1, 2). Therefore, one could expect that early flowering mutant would be more productive in terms of seed yield and seed oil content under such conditions. Modification of *Brassica* species flowering time is very relevant in agriculture since it may allow extending the geographical range of these crops (Rae *et al.,* 1999). Induction of maturity earliness is one of the traits most frequently used in mutation breeding programs in *Brassica* crops (Kharkwal *et al.,* 2004), and many early mutants have been developed (Rahman *et al.,* 1992; Das *et al.,* 1999; Barve et *al.,* 2009; Malek & Monshi, 2009). In the present study, 14 and 6 days earliness in maturity was induced by EMS1-6 and EMS1-7, respectively, in $M_2$ mutants. This indicated there was a genetic gain in terms of maturity earliness of 9.73 and 4.17%, respectively, in those mutants, compared to wild type (Table 3). In *Brassica juncea*, characterized by much longer crop cycle than *B. napus*, Barve *et al.,* (2009) found one mutant derived from seeds treated with 0.02% EMS during 3 hours, with 47 days earliness in maturity compared to control, which represented a cycle reduction of 33.57%. Also, application of EMS1-6 and EMS1-7 induced earliness in flowering in $M_2$ mutants, with a reduction in days to flowering of 9.38% and 11.45%, respectively, compared to wild type (Table 3). In a previous study, Thurling & Depittayanan (1992) found one $M_3$ mutant derived from seeds treated with 0.75% EMS during 12 hours that flowered 20 days earlier than the parental line. Also, Emrani *et al.,* (2012) had found 6 days earliness in flowering in $M_3$ lines induced by gamma ray treatment (1200 Gy) of 'Sarigol' variety seeds.

**Plant height:** Table 3 shows the effect of different EMS treatment levels on plant height. There was a decrease trend in average plant height with all EMS treatments. For EMS1.2-6 and EMS1.2-14, plant height was reduced significantly to 112.55 and 111 cm, respectively, as compared to control (133.65 cm). Significant variation was observed in this parameter for both locations. At Douyet, the check produced the tallest plants, with an average of 155.5 cm, while mutant developed from EMS1-14 had the shortest ones, with a mean value of 127.67cm (Table 4). At Sidi Allal Tazi, EMS1.4-6 mutants produced the tallest plants, with an average of 113 cm, while EMS1.4-14 mutants produced the shortest plants, with a mean value of 73.33 cm (Table 4). As a result of long-duration EMS application, short statured plants were obtained (Table 3). Dwarfism in mutant plants can be explained by decline of mitotic activity of meristematic tissues and reduction in moisture content in seeds (Khalil *et al.,* 1986). Kumar & Yadav (2010) reported that plant height was found to be significantly reduced by high doses of mutagenic treatment, but in some cases, plants respond positively to lower mutagen doses and recorded a slight increase in their height. Improvement in seed yield may be associated to reduction in plant height (More & Malode 2016), and one could remember that use of dwarfing genes was a key factor in the success of green revolution (Khush, 2001). Dwarfing genes may improve seed yield through reduced lodging and increasing harvest index. Many mutation breeders isolated dwarf mutants in rapeseed and mustard using physical and chemical mutagenesis (Chauhan & Kumar, 1986; Das & Rahman, 1988; Zanewich *et al.,* 1991; Rai & Singh, 1993; Shah *et al.,* 1990; Javed *et al.,* 2003).

**Number of branches per plant:** Significant variation of plant branching was observed as an aftereffect of different EMS treatment levels (Table 3). The highest average number of branches per plant was 10.10, noticed for EMS1.4-14, followed by 9.65 for EMS1-7. The control had a mean value of 8.15. At Douyet, EMS treatments EMS1-7 and EMS1.4-14 enabled to produce mutants with most elevated number of branches per plant (12.2 and 12.14, respectively), whereas EMS1-14 led to mutants with lowest branches (8.17).The control had a mean value of 10.8 (Table 4). At Sidi Allal Tazi, mutants coming from EMS1.6-6 produced highest number of branches per plant (7.6), while mutants derived from EMS1.2-6 showed lowest branches (3.9). The control had an average of 5.5 (Table 4). Similarly, in a previous research, having used EMS mutagenesis, there was an increase in number of branches in a *Brassica napus* mutant (More & Malode, 2016). Our findings showed that application of EMS1.4-14 led to an increase in number of branches per plant by 24%, compared with the control. More & Malode (2016) had found mutants derived from seeds presoaked in water for 18 hours and treated with 0.03 and 0.06% EMS for 6 hours, exhibited an increase of 60%, compared with the check. Plant branching is one of the most relevant factors that contribute to high productivity in rapeseed. In our study, the lowest average value ever obtained was 6.30 branches per plant for the treatment EMS1.2-6, which corresponded to a decrease of about 23%, compared with the control. More & Malode (2016) had found a reduction of 17% in this trait, for a rapeseed mutant obtained by seed presoaked in water during 12 hours and treated by 0.08% EMS during 6 hours. Reduction in number of branches through mutagenesis was also reported by Waghmare & Mehra (2000) in grasspea and Patil & Wakode (2011) in soybean.

**Number of pods per plant:** EMS treatment affected significantly number of pods per plant (Table 3). By applying a treatment of EMS1-7 and EMS1.2-7, a substantial rise in number of pods per plant was observed. In fact, this parameter was 859 and 831 pods/plant, in respective mutants, which is more than twice, compared to that of the control (410 pods/plant). In each experimental location, this trait varied significantly. At Douyet, mutants derived from EMS1-7 and EMS1.2-7 produced much higher number of pods per plant, respectively 1505 and 1458 respectively compared to the check having an average of 628 pods per plant. At Sidi Allal Tazi, highest number of pods per plant (213) was observed in EMS1-7mutant, which was significantly higher than that of control (192 pods per plant). Therefore, compared to the check, number of pods per plant increased significantly in EMS1-7 mutants grown in both environments (Fig. 3d). This could be due to stability of such mutation. Late planting at Sidi Allal Tazi resulted in lower number of pods per plant compared to early planting at Douyet (Fig. 3). Actually, early planting often allows better vegetative growth and development, which is traduced by higher plant height and branching, and consequently more flowers and fruits (pods) than late planting. In dry land agriculture, moisture availability plays a crucial role in crop performance. Thus, late sowing of rapeseed could result in drought and heat stresses during flowering and seed filling, which would worsen pods formation and development. Number of pods per plant, like other quantitative traits, is influenced by genotype and environmental conditions (Sana *et al.*, 2003). Other authors had also reported an increase in pods per plant in oleifurous *Brassica* after mutagen treatments (Naz & Islam, 1979; Chauhan & Kumar, 1986; Shah *et al.,* 1990; Siddiqui *et al.,* 2009). However, higher levels of mutagen EMS treatment could affect negatively number of pods per plant (Table 3). Treatment with EMS1-7 induced simultaneously higher number of pods per plant and higher number of primary branches per plant, compared to control (Table 3). Mutants exhibiting more branches and pods than the check varieties had been already found in oilseed *Brassica* (Naz & Islam, 1979; Chauhan & Kumar, 1986; Shah *et al.,* 1990; Javed *et al.,* 2003; Malek & Monshi, 2009). It is well known that number of pods per plant is an important component of seed yield, as there is a strong and positive correlation between both agronomic traits (Sultan *et al.,* 2012; Zada *et al.,* 2013; Nabloussi, 2015; Jan *et al.,* 2018). Therefore, mutants having increased number of pods should be a very promising germpalsm for seed yield improvement.

**Number of seeds per pod:** A considerable variation was noted in number of seeds per pod for different EMS treatment levels (Table 3). The highest mean value seeds/pod (27) was recorded for EMS1.2-7. However, this was statistically equal to the average of the control (26.6 seeds/pod) (Table 3). Also, both environments tested in this research were comparable for this trait. In inconsistent way, More & Malode (2016) had isolated mutants with more seeds per pod than the check. On the other hand, our data showed that higher levels of EMS treatment affected negatively and drastically this trait. In fact, EMS1.2-14 and EMS1.4-14 reduced number of seeds/pod to 19.6 and 18.7, respectively (Table 3).

**Pod length and diameter:** Both parameters varied significantly according to different EMS levels and duration of its application (Table3). The longest pod, 6.63 cm, was obtained for EMS1.2-7, which was significantly higher than the control average (6.35 cm). However, and like number of seeds per pod, both experimental locations were comparable for pod length. Improvement in pod length had also been reported in *Brassica juncea* through gamma rays mutagenesis (Nayer & George, 1970; Kumar & Das, 1973). On the other hand, high levels of EMS treatment caused a decrease in pod length, and the lowest value 4.95 cm, was obtained for EMS1.4-14 (Table 3). Regarding pod diameter, it was negatively affected by all EMS treatment levels, and the highest average value (4.03 mm) was recorded in the control (Table 3).

**1000-seed weight:** Significant differences were observed among EMS treatment levels for 1000-seed weight (Table 3). EMS1.4-14 enabled to get a mutant with highest 1000 seed weight, which was 3.00 g significantly higher than the control (2.12 g). At Douyet, the highest 1000-seed weight was recorded for EMS1.4-14 (3.63 g), whilst the lowest was observed for EMS1-14 (1.36 g). The control had a mean value of 2.59 g (Table 4). At Sidi Allal Tazi, EMS1-7 mutant produced the highest 1000-seed weight (2.45 g) and EMS1-14 mutant had the lowest one (1.17 g) (Table 4). Overall, mutants and control produced bigger seeds in early planting than late one. Previously, mutants in oilseed *Brassica* having an improved seed size were obtained using gamma rays (Chauhan & Kumar, 1986; Shah *et al.,* 1990). In our study, one could observe that overall 1000-seed weight, regardless the EMS treatment levels, was lower than standard value which was about 3.50-4.00 g (Nabloussi, 2015). This was likely due to unfavorable environmental conditions under which the experiment was conducted, particularly characterized by drought and heat stress in both experimental environments (Figs. 1, 2). In fact, seed weight is an important seed yield component that is strongly affected by environmental conditions (Diepenbrock, 2000).

## Conclusion

The present study showed that EMS mutagenesis was found to be potent and effective for inducing novel variability in some important agronomic traits in rapeseed. Different EMS treatments enabled to develop various mutants with modified and interesting characteristics. Interestingly, modifications and particular characteristics of these mutants were recorded at the level of $M_1$ and $M_2$ plants, and were maintained over both environments of this study. This suggested that these mutants were stable and, thus, they could be used and exploited efficiently in rapeseed breeding program. Among all EMS treatments used in this investigation, EMS1-7 was found to be the most effective for obtaining earliness in flowering and maturity and for increasing number of pods per plant, the most important component of seed yield. Furthermore, that early and highly productive mutant, derived from that EMS treatment was also characterized by higher branching shorter plants, compared with control. Thus, this might suggest that low EMS doses during moderate time can be fruitfully applied to obtain interesting mutants that could be used as a germplasm to develop new rapeseed varieties combining desirable agronomic, phenological and morphological traits such as high seed yield, early flowering and maturity, high branching and reduced plant height. However, this experiment must be carried out again at least twice or three times to confirm this hypothesis. Besides, and to best of our knowledge, this is the first report of a rapeseed mutant exhibiting simultaneously various traits that are very interesting for this crop breeding. Obtaining such a stable mutant may enable to develop efficiently and quickly new rapeseed cultivar with diverse desirable characteristics.

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