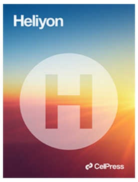
Heliyon 8 (2022) e12649

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon



Research article

Inheritance of dwarfism and narrow lobed-leaf in two rapeseed (*Brassica napus* L.) mutant lines

Souhail Channaoui [a,b,1], Hamid Mazouz [b], Mustapha Labhilili [c], Mohamed El Fechtali [a], Abdelghani Nabloussi [a,*]

[a] *Plant Breeding and Plant Genetic Resources Conservation Research Unit, Regional Agricultural Research Center of Meknes, National Institute of Agricultural Research, PO. Box 415, Rabat 10090, Morocco*

[b] *Laboratory of Plant Biotechnology and Molecular Biology, Department of Biology, Faculty of Science, University Moulay Ismail, PO. Box 11201 Zitoune Meknes 50100, Morocco*

[c] *Plant Biotechnology Research Unit, Regional Agricultural Research Center of Meknes, National Institute of Agricultural Research, PO. Box 415, Rabat 10090, Morocco*

ARTICLE INFO

Keywords:
Dwarfism
Lobed-leaf size
Mutant lines
Rapeseed
Simple inheritance

ABSTRACT

There is a need for dwarf and narrow lobed-leaves rapeseed cultivars to reduce transpiration under drought prone areas. A dwarf mutant line ‘H2M-1’ and a mutant with reduced lobed-leaf ‘H2M-2’ were developed. To exploit these mutated traits properly in an effective breeding program, one should understand their mode of inheritance. There are conflicting findings for plant dwarfism and limited studies for leaf size in mutant genetic backgrounds. Therefore, the objective of this study was to investigate the inheritance of dwarfism and narrow lobed-leaf mutated traits. Plants of the wild-type variety ‘INRA-CZH2’ were reciprocally crossed with plants of the line ‘H2M-1’ and plants of the line ‘H2M-2’. A genetic study was conducted by analyzing segregation of mutated traits in F_1, F_2 and BC_1F_1 generations. The results revealed that two recessive genes with dominant epistasis action controlled the heredity of plant height in the dwarf line, whereas only a single recessive gene is involved in determining reduced lobed-leaf in the line H2M-2. Thus, there is a possibility to easily and quickly transfer these characters into rapeseed breeding germplasm or varieties towards the development of suitable cultivars for areas marked by increasing drought stress.

1. Introduction

Rapeseed (*Brassica napus* L.) is one of the three most important oil seed crops in the world, known for its high seed oil content and its interesting fatty acids composition that make it suitable for both food and industrial uses. In 2018, it ranks as the second oilseed crop after soybean at the global level, with an overall production of about 75 million tons [1]. Its production is mainly destined for edible oil, animal feed, and biodiesel [2, 3, 4]. As a result of the rapid and continuous global population growth, there is an increased demand for edible oil, which requires a sustainable improvement of crop yield and quality through plant breeding, particularly in the actual context of climate change. In Morocco, rapeseed was introduced as a promising oilseed crop that could improve vegetable oil production. Thus, to enhance this production, there is a need to develop and release productive and adapted cultivars for diverse environmental conditions.

The development of new rapeseed lines with improved yield requires information regarding the gene actions involved in expression of plant phenological, morphological and yield traits [5, 6, 7]. Measurement of genetic variation and mode of inheritance of quantitative and qualitative traits are of prime importance in designing breeding program efficiently and effectively [8]. Heritability of any trait depends upon genetic properties of breeding materials and environmental conditions in which these materials are experimented.

In different countries, mutation breeding has been adopted and used to induce novel genetic variability and select interesting and desirable economic traits [9, 10, 11, 12, 13, 14, 15]. Among the economic traits improved by mutation breeding, one could cite earliness, dwarfness,

---

* Corresponding author.
E-mail address: abdelghani.nabloussi@inra.ma (A. Nabloussi).

[1] Present affiliation: Oasis System Research Unit, Regional Center of Agricultural Research of Errachidia, National Institute of Agricultural Research, PO. Box 415, Rabat 10090, Morocco.

https://doi.org/10.1016/j.heliyon.2022.e12649
Received 15 May 2022; Received in revised form 5 July 2022; Accepted 20 December 2022

resistance or tolerance to abiotic or biotic stresses, seed yield and oil quality [16, 17, 18, 19, 20]. Dwarf mutant lines have been exploited in plant breeding programs to improve resistance to lodging and increase plant density. The development and use of dwarf cultivars were key factors in the success of the 'Green Revolution', particularly in wheat and rice [21, 22]. In many species, dwarf mutants have been identified and selected; however, in rapeseed, a few dwarf germplasms are available [23, 24, 25, 26, 27, 28]. Plant height of natural dwarf rapeseed may be controlled by quantitative trait loci that are affected by environmental conditions, making it difficult to transfer this desirable trait from one genotype to another. Furthermore, the actual height reduction in natural germplasm is quite limited [29]. Therefore, artificial mutagenesis techniques were used to obtain mutants with new sources of major dwarfing genes in rapeseed. Nevertheless, controversial findings were reported with regard to the inheritance of dwarfism in the developed rapeseed mutant lines. Previous studies have shown that one major gene either with additive [23, 25] or dominance action [30], two major recessive genes [26, 30], two additive genes [24], a pair of additive-dominant major genes [27] or up to three pairs of recessive genes were involved in the genetic control of dwarfism in rapeseed mutants.

Regarding plant leaves, organs that have a crucial role in transpiration, photosynthesis and nutrition, show large variation in their size, shape and position, which could affect the plant life [31, 32]. Canopy evapotranspiration, sunlight and pesticides impact, pest preference, crop yield and oil quality are all influenced by the leaf characters [33]. There is large leaf shape diversity in *Brassica* species, including lobed leaves that are reported to be more suitable for high-density planting than entire or serrated leaves [34, 35]. Some genetic studies on leaf shape and lobed-leaf character in particular, have been carried out [36, 37, 38, 39]. However, and to the best of our knowledge, there is little information on inheritance and genetic control of leaf size [40].

Recently, we have developed various rapeseed mutants, including a dwarf line (H2M-1) and a narrow lobed-leaf line (H2M-2), through chemical and physical mutagens, ethyl methane sulphonate (EMS) and gamma rays [15]. Based on previous literature, there are conflicting findings for plant dwarfism and too limited studies for leaf size, especially in mutant genetic backgrounds, which necessitate the present study. Therefore, the objective of this research was to study the inheritance of dwarfism and narrow lobed-leaf traits observed, respectively, in the mutant lines H2M-1 and H2M-2.

2. Materials and methods

2.1. Plant material

The plant material used in this study consisted of two promising advanced M_3 mutant lines of rapeseed (*B. napus* L.) ‘H2M-1’ and ‘H2M-2’ that were selected on the basis of their particular morphological characteristics compared to the wild-type variety ‘INRA-CZH2’ [15]. The mutated traits in these two lines were recorded in two consecutive generations, i.e. M_2 and M_3 plants and were maintained over various and contrasted environments. This suggested that these mutants were stable and, hence, could be used and exploited properly in the study of inheritance of these mutated traits.

2.2. Genetic study

Plants of the wild-type variety INRA-CZH2 (P_1) were reciprocally crossed in 2015/2016 with the mutant plants H2M-1 and H2M-2 (P_2) to obtain the F_1 generation. In the parent as female, flower buds of female parent were emasculated and pollinated by fresh pollen from mature flowers of the male parent. Then, pollinated flower buds were isolated from any other source of pollen by using grease proof paper bags. In 2016/2017, F_1 plants from reciprocal crosses were self-pollinated to obtain F_2 seeds and also backcrossed to both parents to get BC_1F_1 ($F_1 {}^\times P_1$ and $F_1 {}^\times P_2$) seeds. Original reciprocal crosses were repeated to obtain F_1 seed under the same environment as the F_2 and BC_1F_1 seeds. The resulting seeds of P_1, P_2, F_1, F_{1r}, F_2, F_{2r}, BC_1F_1 were planted in a completely randomized design with three replicates, at the experimental station of Sidi Allal Tazi (ATZ) in 2017/2018. Generations P_1, P_2, F_1 and BC_1F_1 were represented each by two rows of 5 m long spaced by 60 cm, while F_2 generation seeds were planted in four rows of 5 m long spaced by 60 cm. Conventional and uniform cultural practices were followed [15] to avoid field variation and environmental effects.

2.3. Parameters studied

On the established populations, particular characteristics distinguishing mutants from the control wild-type were studied, namely plant height for H2M-1 (92.8 ± 4.64 cm) and leaf shape for H2M-2 (lobed-leaf with the reduced shape) (Figure 1 b, d). The wild-type presented an average plant height of 161 ± 8.40 cm and normal leaf shape (lobed broad leaves) (Figure 1 a, c). For P_1, P_2 and F_1 populations, ten genetically homogeneous plants per population were randomly used for all observations and measurements, whilst in F_2 and BC_1F_1 populations, the number of plants per population was much higher, but variable due to availability of such plants as indicated in Tables 1 and 2.

2.4. Statistical analysis

The independent *t*-test was used to compare the reciprocal F_1 means in order to evaluate the maternal effect, and the reciprocal F_2 means to evaluate the cytoplasmatic effect. The maternal effect on the expression of the mutated trait is shown by the significant difference between the reciprocal F_1 means. Similarly, the cytoplasmic effect is proved by the significant difference between the reciprocal F_2 means. In addition, the dominance relation between alleles of the same locus was evaluated by comparing F_1 means and mid-parent mean values. Significant difference between both values indicates a dominance of wild-type trait over the mutated one or mutated trait over the wild-type one. Regarding the Chi-square (χ^2) test, it is used to evaluate the goodness of fit of observed data to the proposed segregation ratios in F_2 and BC_1F_1 generations. The Chi-square (χ^2) is calculated using the following formula:

$$\chi^2 = \sum_{i}^{n} \frac{(O_i - E_i)^2}{E_i}$$

where Oi is the observed value for phenotype i, Ei is the expected value for phenotype i, and n is the number of phenotypes (individuals). To decide whether the difference between the observations and the expectations ($O_i - E_i$ in the equation) is large enough to be statistically significant, the chi-square value is compared to a critical value. The critical chi-square value is found by using a chi-square value table or statistical software. A non-significant difference suggests that the distribution of the observed data in F_2 and BC_1F_1 generations segregation fits to the proposed or expected segregation ratios.

3. Results

3.1. Plant height: cross between the wild type INRA-CZH2 and the dwarf mutant line H2M-1

Plants of INRA-CZH2 had an average plant height of 161 ± 8.40 cm (mean ± SD) and ranged from 146 to 178 cm, whereas those of H2M-1 had a mean value of 92.80 ± 4.64 cm and a variation from 80 to 100 cm (Figure 2). Plant height in F_1 population from the cross INRA-CZH2 × H2M-1 averaged 157.92 ± 10.10 cm (Figure 2), significantly higher than the mid-parent value ($t = 3.629$, $p < 0.01$), which indicates that the wild-type trait (high plant) is dominant over the mutated trait (dwarf plant). In other words, the obtained mutation is controlled by a recessive gene(s). Also, F_1 plants in the reciprocal cross had a mean height of 156.92 ± 9.12 cm, which is not statistically different from the former ($t = 0.801$, $p > 0.05$), indicating

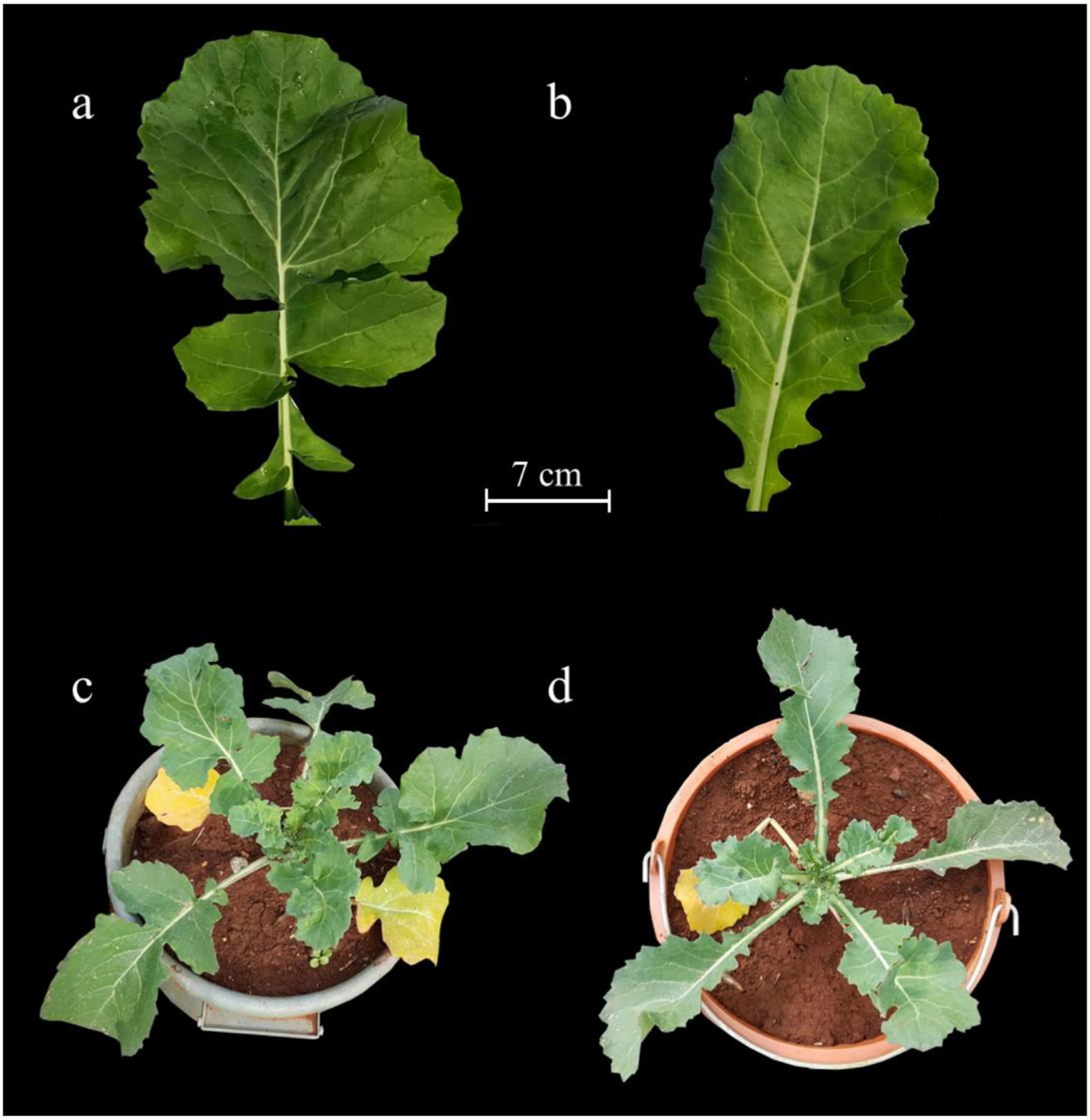


Figure 1. Leaves of wild-type INRA-CZH2 (**a**), and narrow lobed-leaf mutant H2M-2 (**b**). Plants of INRA-CZH2 (**c**), and H2M-2 (**d**) at eight-leaves stage.

Table 1. Plant height distribution and chi-squared test for the segregation ratio in the F_1, F_2 and BC_1F_1 resulting from reciprocal crosses between the wild-type line INRA-CZH2 and the mutant H2M-1.

Population	Number of plants	Segregation observed ratio			Expected theoretical ratio	χ^2	p
		Plant height (cm)					
		80–100	104–142	146–178			
INRA-CZH2	10	0	0	10	0 : 0: 1	0	1
H2M-1	10	10	0	0	1 : 0: 0	0	1
INRA-CZH2 × H2M-1							
F_1	10	0	0	10	0 : 0: 1	0	1
F_2	244	16	51	177	1 : 3: 12	0.839	0.36
BC_1F_1 (F_1× INRA-CZH2)	25	0	5	20	0 : 0: 1	1.00	0.32
BC_1F_1 (F_1 × H2M-1)	20	4	5	11	1 : 1: 2	1.2	0.27
H2M-1 × INRA-CZH2							
F_1	10	0	0	10	0 : 0: 1	0	1
F_2	200	18	35	147	1 : 3: 12	3.15	0.08

Table 2. Leaf shape distribution and chi-squared test for the segregation ratio in F_1, F_2 and BC_1F_1 resulting from reciprocal crosses between the wild-type line INRA-CZH2 and the mutant H2M-2.

Population	Number of plants	Segregation observed ratio		Expected theoretical ratio	χ^2	p
		Leaf shape				
		Normal	Reduced			
INRA-CZH2	10	10	0	1 : 0	0	1
H2M-1	10	0	10	0 : 1	0	1
INRA-CZH2 × H2M-1						
F_1	10	10	0	1 : 0	0	1
F_2	702	532	170	3 : 1	0.23	0.63
BC_1F_1 ($F_1\times$ INRA-CZH2)	60	42	0	1 : 0	0	1
BC_1F_1 ($F_1 \times$ H2M-1)	14	18	14	1 : 1	0.5	0.48
H2M-1 × INRA-CZH2						
F_1	10	10	0	1 : 0	0	1
F_2	434	315	119	3 : 1	1.36	0.24

an absence of maternal effects on this trait. Similarly, no cytoplasmic effect was observed as the plant height average in F_2 plants from the cross INRA-CZH2 × H2M-1 (146.47 cm, ranging from 82 to 178 cm) and that of F_2 plants in the reciprocal cross (146.14 cm, varying from 83 to 175 cm) (Figure 2), are not significantly different ($t = 0.253$, $p > 0.05$).

In the pooled F_2 population, plants were divided into three discrete classes for plant height. The first class consists of plants with a height similar to the parent H2M-1, ranging from 80 to 100 cm; the second comprises plants of intermediate height with a variation from 104 to 142 cm; and the third class is made of plants with a height between 146 and 178 cm similar to the parent INRA-CZH2 (Figure 2). Also, in each of the two reciprocal F_2 populations, plants were distributed in three classes. In fact, the number of individuals in the F_2 population resulting from the cross INRA-CZH2 × H2M-1 and found in the first class [80–100 cm] represents about 1/16 of the total individuals, while the number of plants in the other classes [104–142 cm] and [146–178 cm] constitutes, respectively, 3/16 and 12/16 of the total (Table 1). Similarly, the individuals in the reciprocal F_2 population follow the same distribution, with proportions of about 1/16 for the first class [80–100 cm], 3/16 for [104–142 cm], and 12/16 for the [146–178 cm] class (Table 1).

Based on these observations, it is suggested that two recessive genes with dominant epistasis control the inheritance of the plant height in the mutant line H2M-1. The expected F_2 ratios, based on this hypothesis, would be 12:3:1. The Chi-square test (χ^2) of the fit of observed ratios to theoretical ones is significant for both the reciprocal F_2 populations (F_2: $\chi^2 = 0.839$, $p = 0.36$; F_{2r}: $\chi^2 = 3.15$, $p = 0.08$) and also for aggregated population ($\chi^2 = 1.742$, $p = 0.19$).

The involvement of two recessive genes with dominant epistasis in the control of dwarfism is also confirmed by the analysis of plant height from BC_1F_1 ($F_1 \times P_2$), for which a tri-modal distribution was obtained with distinct classes: 80–100 cm, 101–140 cm and 141–180 cm (Table 1), and where segregation followed 2:1:1 ratios ($\chi^2 = 1.2$, $p = 0.27$). This is also strongly supported by examining plants from the BC_1F_1 ($F_1\times P_1$). In fact, 20 plants out of the 25 analyzed fell in the parental class (INRA-CZH2), and five plants had an intermediate height between 125 and 140 cm (Table 1). However, this observed segregation fitted statistically with the expected ratios 0:0:1 ($\chi^2 = 1.00$, $p = 0.32$), thus confirming the recessive digenic inheritance. The observation of a few plants (five plants) with intermediate height in this population (Table 1) might be due to an environmental effect and/or experimental error.

3.2. *Leaf shape: cross between the wild type INRA-CZH2 and the reduced lobed-leaf mutant line H2M-2*

F_1 plants from the cross between INRA-CZH2 and H2M-2 were all of normal leaf-shape (Table 2). This shows that the mutated reduced lobed-leaf was determined by recessive gene(s). The same results were found for the reciprocal cross H2M-2 × INRA-CZH2 (Table 2), indicating the absence of maternal effects. In addition, plants from both the reciprocal F_2 populations were segregated into plants with normal leaf-shape and plants with reduced lobed-leaf (Table 2). The respective proportions observed fit perfectly with the expected ratio of 3:1 ($\chi^2 = 0.23$, $p = 0.63$; $\chi^2 = 1.36$, $p = 0.24$), which indicates that a single gene is involved in determining leaf shape, with normal leaf shape dominant. The reciprocal crosses gave the same results (Table 2), indicating the lack of involvement of cytoplasmic factors in leaf shape determination. Similarly, the pooled F_2 population segregated according to the same ratio of 3:1 ($\chi^2 = 0.118$, $p = 0.73$). The backcross populations did confirm the simple inheritance of the muted trait, due to a single recessive gene. In fact, the BC_1F_1 ($F_1\times$ INRA-CZH2) was entirely composed of plants with normal leaf shape, whereas the individuals of the BC_1F_1 ($F_1 \times$ H2M-1) were divided into a class of normal lobed-leaf shape and a class of reduced lobed-leaf shape, with proportions of 18 and 14 individuals, according to the ratios 1:1 ($\chi^2 = 0.5$, $p = 0.48$) (Table 2).

4. Discussion

This study showed that both mutated traits investigated were under simple genetic control where either one or two genes are involved.

The novel dwarf mutant, H2M-1, recently developed, showed a significant phenotypic variation in plant height, compared to the wild-type INRA-CZH2. The tri-modal distribution of plants in the segregated populations (F_2, BC_1F_1) indicated that two recessive genes with dominant epistasis might control the dwarfism trait. This is in agreement with the findings of Zeng et al. [26] having reported that a pair of recessive genes was involved in the height reduction in the mutant bnaC.dwf, and those of Xiang et al. [28] having discovered that dwarfism was controlled by two or a few genes with major effects that can be completely recessive or partially dominant. On the other hand [27], discovered a semi-dwarf mutant (10D130) in rapeseed and inheritance analysis on F_1 and F_2 populations revealed that the dwarfism was under the control of a pair of additive-dominant major genes, besides additive-dominant-epistatic polygenes. However, in previous studies on other mutants, monogenic inheritance was reported, with a major dominant locus in mutant NJ7982 [25] or a major gene with additive effects in mutant Bzh [23] and in mutant NDF-1 [24]. In addition [41], described a dwarf mutant bnd2 in rapeseed using EMS mutagenesis and inheritance analysis on F_2 population revealed that the dwarf phenotype of bnd2 was controlled by a single recessive gene. Following bulked segregant analysis (BSA) by resequencing [41], reported that BND2 to be located in the 13.77–18.08 Mb interval of chromosome A08, with a length of 4.31 Mb [41]. found that the gene was narrowed to a 140-Kb interval ranging from 15.62 Mb to 15.76 Mb, after using a fine mapping with single nucleotide polymorphism (SNP) and insertion/deletion (InDel) markers. According to

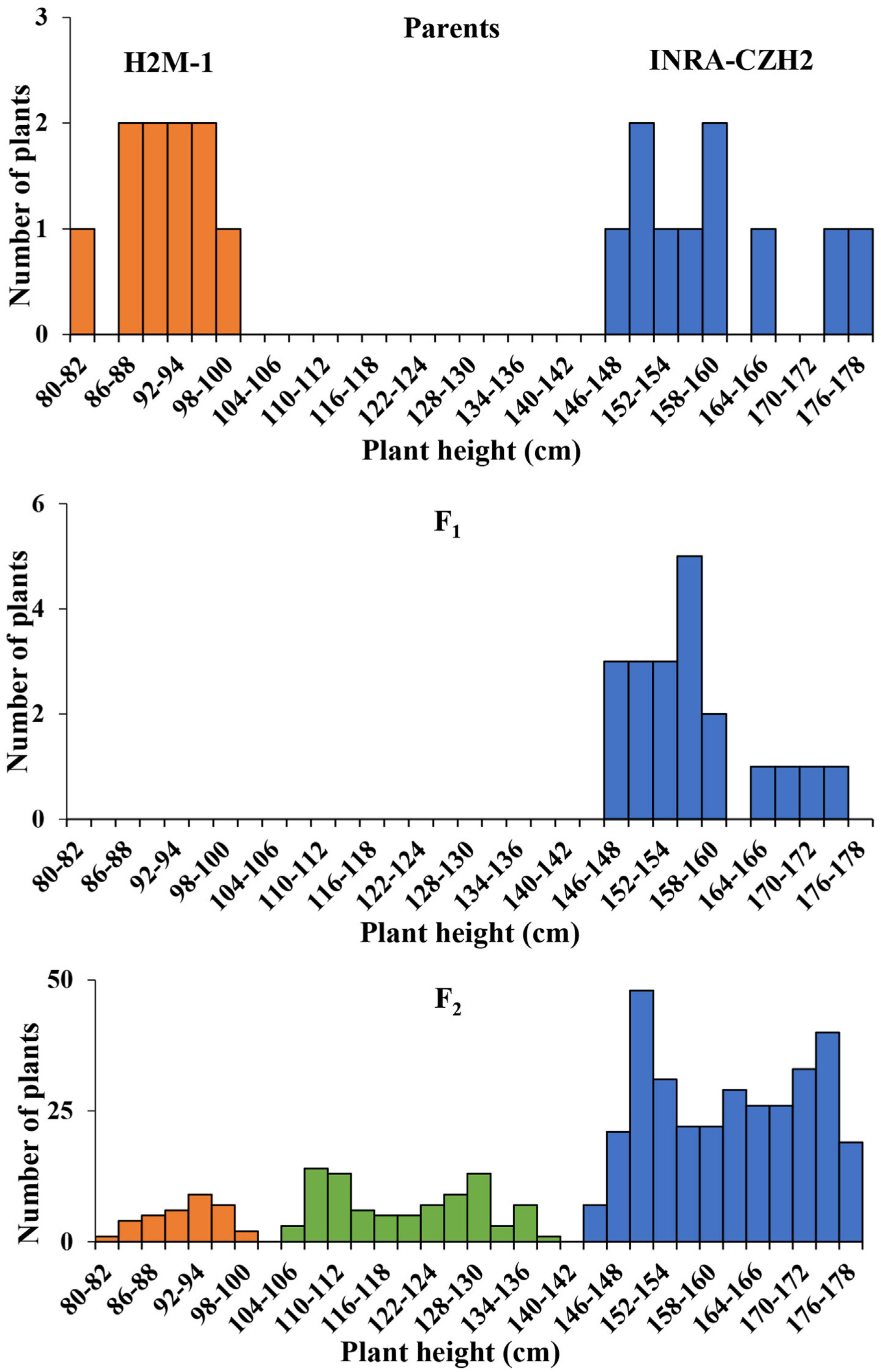


Figure 2. Distribution of plants of the parent lines INRA-CZH2 and H2M-1 and their F_1 and F_2 generations for plant height (cm).

reference genome annotation, there were 27 genes in the interval, of which BnaA08g20960D had an SNP type variation in the intron between the mutant and its parent, which may be the candidate gene corresponding to BND2 [41].

The differences observed in the inheritance of dwarfism in rapeseed mutants might be due to the genetic background of the mutants studied, the kind and nature of mutagenesis used to obtain such mutants, the number and functions of the mutated genes, and the complex molecular mechanisms controlling dwarf traits in plants [30].

In the present study, a rapeseed mutant, H2M-2, with narrow and reduced lobed-leaf was developed. Analysis of the frequency distribution for leaf shape in F_1, F_2 and BC_1F_1 revealed that a single recessive gene is involved in the determination of this mutated trait, with the absence of any maternal or cytoplasmic effect. Contrarily [40], identified two QTLs for lamina width, five QTLs for lamina length and two QTLs for total leaf length, indicating that leaf size in rapeseed is under polygenic control. Nevertheless, it is important to notice that this quantitative inheritance is related to the variation naturally found among diverse genetic materials. In contrast, the monogenic inheritance reported in our study is linked to the sharp mutation that occurred in H2M-2. This is, so far, the first report of monogenic inheritance of reduced leaf size associated with shallower lobes in the H2M-2 mutant line.

The obtained results did confirm that EMS-mutagenesis technique leads to a punctual mutation in the genome, for which the mutated traits are often controlled by one or a few genes. The simple inheritance of dwarfism and reduced-lobed leaves will allow transferring these attributes easily into advanced genetic stocks, germplasms or cultivars. However, no molecular analysis was integrated in this study to confirm and support the obtained results. In-depth knowledge of the molecular changes and mechanisms associated with the induced mutations will facilitate an effective genomic breeding strategy. A further investigation using TILLING molecular method is planned to identify the point mutations in target genes or sequences of the genome coding parts that could be at the origin of the observed and characterized mutations.

5. Conclusion

In this work, we studied the inheritance of the mutated traits in a new dwarf mutant H2M-1 and a new narrow lobed-leaf mutant H2M-2 isolated using EMS mutagenesis. The results showed that two recessive genes with dominant epistasis control the inheritance of dwarfism, while a single gene is involved in the determination of leaf shape, with a dominance of the normal shape of wild-type. These findings should be confirmed and supported by genomic and molecular studies. The obtained and characterized mutant lines H2M-1 and H2M-5 can be used as valuable germplasms in rapeseed breeding program.

Declarations

Author contribution statement

Souhail Channaoui: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Hamid Mazouz, Pr.: Conceived and designed the experiments; Analyzed and interpreted the data.

Mustapha Labhilili, Dr.: Contributed reagents, materials, analysis tools or data.

Mohamed El Fechtali: Performed the experiments.

Abdelghani Nabloussi, Ph.D: Conceived and designed the experiments; Wrote the paper.

Funding statement

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Data availability statement

Data will be made available on request.

Declaration of interest's statement

The authors declare no competing interests.

Additional information

No additional information is available for this paper.

References

[1] FAOSTAT, Food and Agriculture Organization of the United Nations (FAO), 2020. Available from, http://www.fao.org/faostat/fr/#data/QC/visualize. (Accessed 30 April 2020).

[2] R.K. Downey, G.F. Rakow, In: principles of cultivar development, in: W. Fehr (Ed.), MacMillan Publishing Co., New York, 1987, pp. 437–486.

[3] B.E. McDonald, Oil properties of importance in human nutrition, in: D.S. Kimber, D.I. McGregor (Eds.), *Brassica* Oilseeds–Production and Utilization, CABI, Wallingford, 1995, pp. 291–299.

[4] R. Snowdon, W. Luhs, W. Friedt, Oilseed rape, in: C. Kole (Ed.), Genome Mapping and Molecular Breeding in Plants, Springer, Berlin Heidelberg, 2007, pp. 55–114.

[5] S.U. Khan, J. Yangmiao, S. Liu, K. Zhang, M.H.U. Khan, Y. Zhai, Y. Zhou, Genome-wide association studies in the genetic dissection of ovule number, seed number, and seed weight in *Brassica napus* L, Ind. Crop. Prod. 142 (2019) 111877.

[6] Y. Zhai, K. Yu, S. Cai, L. Hu, O. Amoo, L. Xu, Y. Zhou, Targeted mutagenesis of BnTT8 homologs controls yellow seed coat development for effective oil production in *Brassica napus* L, Plant Biotechnol. J 18 (2020) 1153–1168.

[7] M.H. Khan, L. Hu, M. Zhu, Y. Zhai, S.U. Khan, S. Ahmar, Y. Zhou, Targeted mutagenesis of EOD3 gene in *Brassica napus* L. regulates seed production, J. Cell. Physiol. 236 (2021) 1996–2007.

[8] S.U. Khan, S. Saeed, M.H.U. Khan, C. Fan, S. Ahmar, O. Arriagada, F. Mora-Poblete, Advances and challenges for QTL analysis and GWAS in the plant-breeding of high-yielding: a focus on rapeseed, Biomolecules 11 (2021) 1516.

[9] S. Spasibionek, New mutants of winter rapeseed (*Brassica napus* L.) with changed fatty acid composition, Plant Breed. 125 (2006) 259–267.

[10] A.M.R. Ferrie, D.C. Taylor, S.L. MacKenzie, G. Rakow, J.P. Raney, W.A. Keller, Microspore mutagenesis of *Brassica* species for fatty acid modifications: a preliminary evaluation, Plant Breed. 127 (2008) 501–506.

[11] L. Velasco, J.M. Fernandez-Martinez, A.D. Haro, Inheritance of reduced linolenic acid content in the Ethiopian mustard mutant N2-4961, Plant Breed. 121 (2002) 263–265.

[12] S.N. Emrani, A. Arzani, G. Saeidi, M. Abtahi, M. Banifatemeh, M.B. Parsa, M.H. Fotokian, Evaluation of induced genetic variability in agronomic traits by gamma irradiation in canola (*Brassica napus* L.), Pakistan J. Bot. 44 (2012) 1281–1288, 2012.

[13] S. Hussain, W.M. Khan, M.S. Khan, N. Akhtar, N. Umar, S. Ali, S.S. Shah, Mutagenic effect of sodium azide (NaN_3) on M_2 generation of *Brassica napus* L. (variety Dunkled), Pure Appl. Biol. 6 (2017) 226–236.

[14] S. Channaoui, M. Labhilili, H. Mazouz, M. El Fechtali, A. Nabloussi, Assessment of novel EMS-induced genetic variability in rapeseed (*Brassica napus* L.) and selection of promising mutants, Pakistan J. Bot. 51 (2019) 1629–1636.

[15] S. Channaoui, M. Labhilili, M. Mouhib, H. Mazouz, M. El Fechtali, A. Nabloussi, Development and evaluation of diverse promising rapeseed (*Brassica napus* L.) mutants using physical and chemical mutagens, OCL 26 (2019) 35.

[16] T. Schnurbusch, C. Mollers, H.C. Becker, A mutant of Brassica napus with increased palmitic acid content, Plant Breed. 119 (2000) 141–144.

[17] M.A. Parry, P.J. Madgwick, C. Bayon, K. Tearall, A. Hernandez-Lopez, M. Baudo, M. Labhilili, Mutation discovery for crop improvement, J. Exp. Bot. 60 (2009) 2817–2825.

[18] H.M.A. Ali, S.A. Shah, Evaluation and selection of rapeseed (*Brassica napus* L.) mutant lines for yield performance using augmented design, J. Anim. Plant Sci. 23 (2013) 1125–1130.

[19] Y.H. Lee, W. Park, K.S. Kim, Y.S. Jang, J.E. Lee, Y.L. Cha, K. Lee, EMS-induced mutation of an endoplasmic reticulum oleate desaturase gene (FAD2-2) results in elevated oleic acid content in rapeseed (*Brassica napus* L.), Euphytica 214 (2018) 28.

[20] S. Ahmar, Y. Zhai, H. Huang, K. Yu, M.H.U. Khan, M. Shahid, Y. Zhou, Development of mutants with varying flowering times by targeted editing of multiple SVP gene copies in *Brassica napus* L, Crops J 10 (2022) 67–74.

[21] G.S. Khush, Green revolution: the way forward, Nat. Rev. Genet. 2 (2001) 815–822.

[22] P. Hedden, The genes of the green revolution, Trends Genet. 19 (2003) 5–9.

[23] N. Foisset, R. Delourme, P. Barret, M. Renard, Molecular tagging of the dwarf BREIZH (Bzh) gene in *Brassica napus*, Theor. Appl. Genet. 91 (1995) 756–761.

[24] M.L. Wang, Y. Zhao, F. Chen, X.C. Yin, Inheritance and potentials of a mutated dwarfing gene ndf1 in *Brassica napus*, Plant Breed. 123 (2004) 449–453.

[25] H. Li, Y. Wang, X. Li, Y. Gao, Z. Wang, Y. Zhao, M. Wang, A GA-insensitive dwarf mutant of *Brassica napus* L. correlated with mutation in pyrimidine box in the promoter of GID1, Mol. Biol. Rep. 38 (2011) 191–197.

[26] X. Zeng, L. Zhu, Y. Chen, L. Qi, Y. Pu, J. Wen, T. Fu, Identification, fine mapping and characterisation of a dwarf mutant (bnaC. dwf) in *Brassica napus*, Theor. Appl. Genet. 122 (2011) 421–428.

[27] Q. Zhou, J. Li, C. Cui, H. Bu, T. Ying, Y. Yan, Z. Zhang, Genetic analysis of plant type in semi-dwarf new line (10D130) of rapeseed, Acta Agron. Sin. 39 (2013) 207–215.

[28] Y. Xiang, C. Tong, S. Yu, T. Zhang, J. Zhao, S. Lei, S. Liu, Genetic segregation analysis of a rapeseed dwarf mutant, Pakistan J. Bot. 48 (2016) 1629–1635.

[29] H.L. Liu, J. Tang, A review on the plant ideotype research in oilseed rape, J. Crops 4 (1990) 2–5.

[30] Y. Wang, J. He, L. Yang, Y. Wang, W. Chen, S. Wan, R. Guan, Fine mapping of a major locus controlling plant height using a high-density single-nucleotide polymorphism map in *Brassica napus*, Theor. Appl. Genet. 129 (2016) 1479–1491.

[31] H. Tsukaya, Mechanism of leaf-shape determination, Int. J. Dev. Biol. 57 (2006) 477–496.

[32] K. Nikovics, T. Blein, A. Peaucelle, T. Ishida, H. Morin, M. Aida, P. Laufs, The balance between the MIR164A and CUC2 genes controls leaf margin serration in Arabidopsis, Plant Cell 18 (2006) 2929–2945.

[33] Q.H. Zhu, J. Zhang, D. Liu, W. Stiller, D. Liu, Z. Zhang, I. Wilson, Integrated mapping and characterization of the gene underlying the okra leaf trait in *Gossypium hirsutum* L, J. Exp. Bot. 67 (2016) 763–774.

[34] H.M. Pu, S.Z. Fu, C.K. Qi, J.F. Zhang, Y.M. Wu, J.Q. Gao, X.J. Chen, Inheritance of divided leaf trait of rapeseed (*Brassica napus*) and application in hybrid breeding, Chin. J. Oil Crop Sci. 23 (2001) 60–62.

[35] Y.Q. Tu, J. Sun, X.L. Dai, J. Tang, W.F. Tu, H.Q. Shao, Character and genetic analysis of lobed-leaf traits in *Brassica napus*, Chin. J. Oil Crop Sci. 35 (2013) 93–96.

[36] D.J.K. Geltink, Inheritance of leafshape in turnip (*Brassica rapa* L. partim) and rape (*Brassica napus* L.), Euphytica 32 (1983) 361–365.

[37] X. Ni, J. Huang, B. Ali, W. Zhou, J. Zhao, Genetic analysis and fine mapping of the LOBED-LEAF 1 (BnLL1) gene in rapeseed (*Brassica napus* L.), Euphytica 204 (2015) 29–38.

[38] L. Hu, H. Zhang, Q. Yang, Q. Meng, S. Han, C.C. Nwafor, Y. Zhou, Promoter variations in a homeobox gene, BnA10. LMI1, determine lobed leaves in rapeseed (*Brassica napus* L.), Theor. Appl. Genet. 131 (2018) 2699–2708.

[39] M. Yang, C. Huang, M. Wang, H. Fan, S. Wan, Y. Wang, R. Guan, Fine mapping of an up-curling leaf locus (BnUC1) in *Brassica napus*, BMC Plant Biol. 19 (2019) 324.

[40] H. Jian, B. Yang, A. Zhang, L. Zhang, X. Xu, J. Li, L. Liu, Screening of candidate leaf morphology genes by integration of QTL mapping and RNA sequencing technologies in oilseed rape (*Brassica napus* L.), PLoS One 12 (2017).

[41] X. Li, F. Xiang, W. Zhang, J. Yan, X. Li, M. Zhong, X. Zhao, Characterization and fine mapping of a new dwarf mutant in *Brassica napus*, BMC Plant Biol. 21 (2021) 1–12.