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Selection and phenotypic stability of M4 mutants of pearl millet (*Pennisetum glaucum* (L.) R. Br.) derived from gamma rays induced mutagenesis in Niger

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Abstract

Gamma irradiation mutagenesis is an approach that offers a wide range of possibilities for varietal selection. In the simultaneous induction of multiple mutations to modify several plant traits. The aim of the present study was to select seed lots for four mutants of the M4 generation. An experiment was carried out in an experimental plot to purify drought-tolerant M4 genotypes based on morphological characteristics. Potential mutants MI 02/82, MI 13/63, MI 12/72, and MI 10/54 were tested in a randomized complete block design. The parameters measured were: the number of tillers, stem height, number of internodes, number and length of ears, stem and spike diameters, and cycle duration. A dendrogram was first generated to identify the homogenous subgroups. Then an analysis of variance ANOVA was conducted between individuals of the same subgroups to evaluate the variance. On morphological parameters, results showed that MI 13/63 and MI 10/54 genotypes have a homogeneous population from M4 onwards. MI 12/72 can be classified into two different subgroups according to ear length. MI 02/82, on the other hand, showed a high degree of variability at M4. These results will contribute to the selected new varieties adapted to the needs of rural producers in order to improve the productivity of pearl millet in Niger.

Keywords: *Pearl millet* - Gamma rays - M4 mutants - Crop breeding - Morphological diversity

Introduction

Millet [*Pennisetum glaucum* (L.) R. Br.] is the sixth most important cereal in the world, cultivated on thirty million hectares mainly in the arid and semi-arid zones of Asia and Africa (Satyavathi et al., 2021). Millet culture is facing the effects of climate change, crossing losses of up to 20% in grain and 5% in biomass (Benoit & Mandéla, 2015). The vegetative, reproductive and physiological characteristics of millet make it tolerant to low soil fertility, high soil pH, drought, high temperatures and salinity (Varshney et al., 2017). Selection by mutagenesis is a flexible and practical approach, that can be applied crops with precise objectives and appropriate selection methods (Oladosu et al., 2016). It has become an interesting approach for plant improvement and its advantage lies in its ability to improve one or more specific traits of the preferred variety (Hase et al., 2020). Gamma rays have the advantage of being highly effective in generating a high frequency of mutations that can lead to loss of gene function (Kazama et al., 2017).

Therefore gamma rays have become the most frequently used mutagen agent in plant breeding (Beyaz & Yildiz, 2017; Hase et al., 2020). However, in millet breeding, few studies have been conducted on the rate and effectiveness of physical mutagens on the characteristics of inbred millet lines due to natural genetic variation (Serba et al., 2017). The effects of gamma ray mutagenesis on the morphological parameters of millet are multiple and manifest stem height and diameter of stems, leaf shrinkage, seed size, earliness or cycle length and ears length (Ambli & Mullainathan, 2015). These mutations are characterized by a high heritability of characters in M2 (Abdalla et al., 2016) and on the number of nodes, stem and ear diameter in the M3 generation (Ambli, 2018). However, selection in the M3 generation of millet mutants is less effective because of the high genetic variability (Maryono et al., 2020).

As part of the genetic improvement program for water stress tolerance, the millet variety HKP (Haini Kirey Precoce) was irradiated with 250 Gray gamma rays. From a morphological term, HKP has an average tillering capacity, with stems 190 to 200 cm long, and ears of intermediate length. It has the particularity of being an earlier local variety, with a sowing-maturing cycle of 75 to 90 days (MA/Niger, 2021). The aim of this study is to investigate

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the morphological variability of M4 genotypes for the effective selection of four water-stress tolerant millet mutants.

Methods

The material consists of populations of four drought-tolerant M4 genotypes MI 02/82, MI 13/63, MI 12/72 and MI 10/54 of millet derived from a mutagenesis process using gamma irradiation at the *Laboratoire de Biotechnologie et Amélioration des Plantes (LABAP) de l'Institut des Radio Isotopes (IRI) de l'Université Abdou Moumouni (UAM) de Niamey*. The device is a randomized complete block (Figure 1). All measures were taken to ensure controlled pollination. The following parameters were measured: heading date (HD), number of tillers (NT) per plant, stem diameter (SD) and ear diameter (ED), plant height (PLH) and ear length (EL), number of ears (NE) and number of nodes (NN).

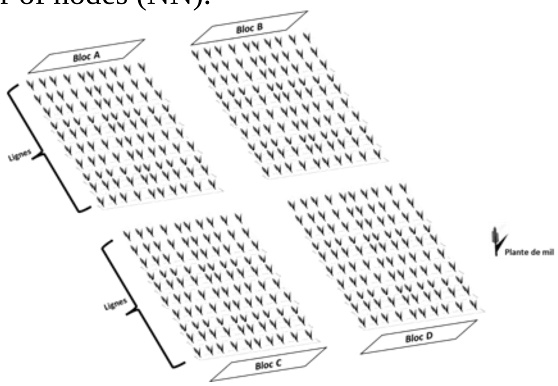

Figure 1: Schematic diagram of the experimental set-up. Each block (A, B, C and D) is dedicated to a single genotype family

Data analysis

A hierarchical classification was carried out to generate dendrograms in the populations of each of the four genotypes, using R software. Correlations between measured parameters were highlighted by bottom-up classification. Branches at the precise height of 2 dendrogram units were used as the discrimination threshold. The averages of individuals from the different class were analyzed by ANOVA (at the 5% threshold) using GenStat software, to highlight their homogeneity.

Results

Analysis of genotype diversity based on dendrograms shows a higher height for MI 02/82 and MI 13/63 (10 and 7 units) than for MI 12/72 and MI 10/54 (6 and 5 units) (Figure 2). At the precise height of 2 units on the dendrogram scale in Figure 2a, MI 02/82 genotypes hold more branches (13 branches) compared with only 6, 5 and 4 branches for MI 13/63, MI 12/72 and

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MI 10/54 respectively (Figure 2b, c and d). Through the subfamilies, MI 13/63 and MI 10/54 genotypes have 10.7 and 8.5 nodes respectively for stem heights of 182.8 and 157 cm. Whereas MI 12/72 has an average of 8.8 nodes for a stem height of 150 cm (Tables S2 and S3).

The results of the analysis of variance allowed us to transform the branches of the MI 13/63, MI 12/72 and MI 10/54 genotypes into different classes, based on the high similarity between individuals. This allows us to distinguish 13 class for MI 02/82, 6 class for MI 13/63, 5 and 4 class, respectively for MI 12/72 and MI 10/54.

These results indicate that MI 02/82 classes were statistically different for the parameters studied (Table S1). Contrary to MI 10/54 genotypes, which are statically identical for all the parameters studied. The MI 13/63 and MI 12/72 genotypes were different in number of tillers (Table S2) and ear length (Table S3), respectively. For MI 13/63 genotype, the difference between subgroups was in the number of tillers, which varied from 9.5 to 19.5 tillers (Figure 3). Whereas in MI 12/72, ear length varies with an average of 36 to 52 cm between classes (Figure 4).

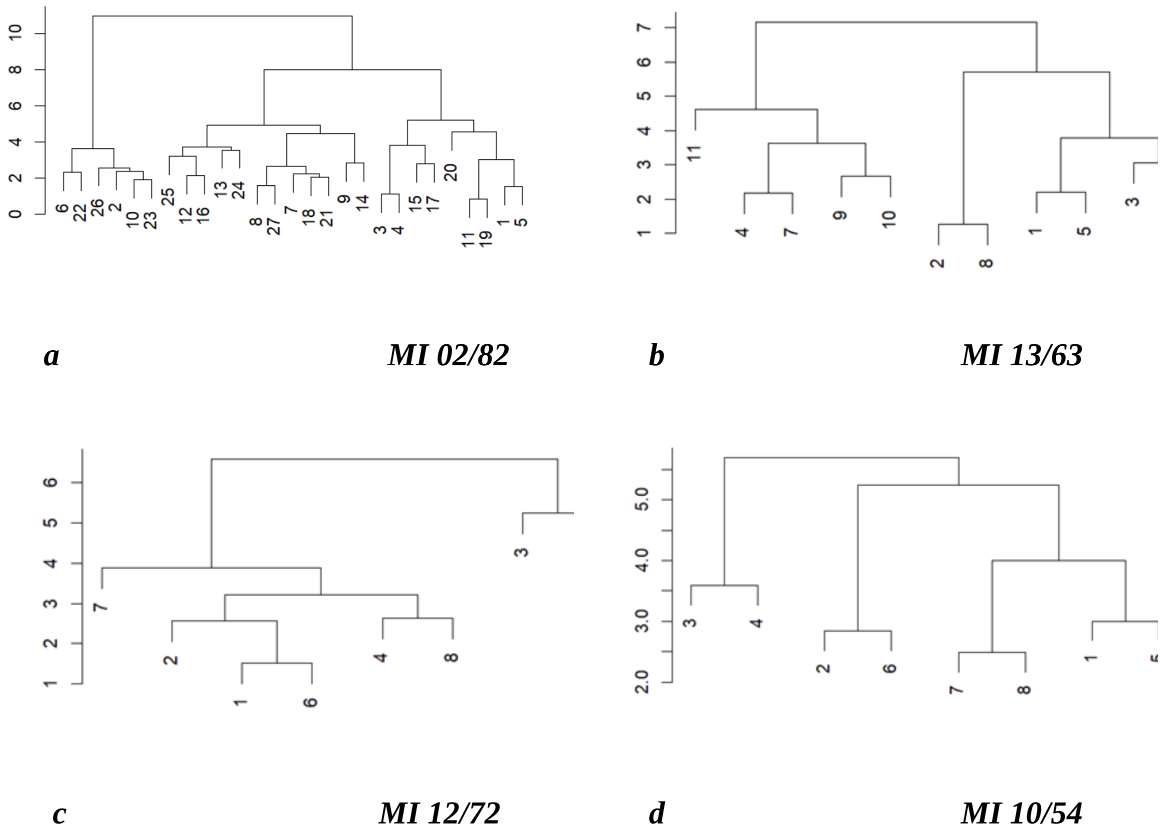

Figure 2: Hierarchical classification of genotypes. *The letters a, b, c and d, designate the MI 02/82, MI 13/63, MI 12/72 and MI 10/54 dendrograms respectively*

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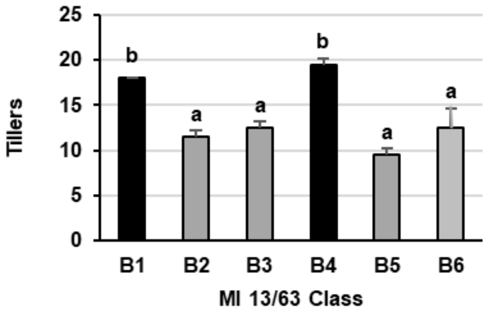

Figure 3: MI 13/63 number of tiller (NT) classification (*p <0,001*). *The letters a and b indicate significantly different classes for the number of tillers Parameter.*

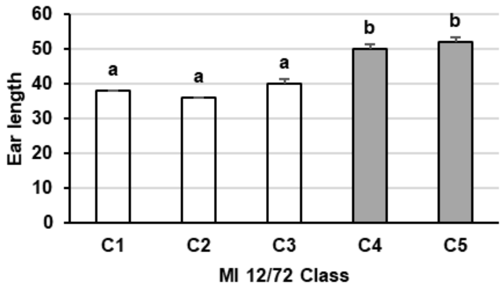

Figure 4: MI 12/72 ear length classification (EL)
(*p = 0,001*). *The letters a and b indicate significantly different classes for the ear length parameter*

Tableau 4: Classification of MI 10/54 genotypes into subfamilies

Parameters	*Classes*					
	D1	*D2*	*D3*	*D4*	*Means*	*Probability*
HD	65,5 ±2,1	56,5 ±2,1	52,0 ±2,8	61,5 ±9,2	**59,00**	0,283
NN	9,5 ±0,7	7,0 ±0,0	9,0 ±0,0	8,5 ±0,7	**8,50**	0,072
NT	18,5 ±2,1	15,0 ±0,0	13,0 ±4,2	13,0 ±0,0	**14,88**	0,179
NE	9,5 ±4,9	5,0 ±2,8	8,0 ±1,4	6,5 ±0,7	**7,20**	0,558
SD	1,3 ±0,3	0,8 ±0,0	1,1 ±0,1	1,5 ±0,0	**1,170**	0,068
PLH	168,5 ±16,3	140,0 ±28,3	158,5 ±2,1	161,0±5,7	**157,00**	0,419
EL	36,5 ±7,8	36,5 ±3,5	38,5 ±0,7	57,0 ±9,9	**42,1**	0,052
ED	1,5 ±0,1	1,7 ±0,0	1,8 ±0,2	2,0 ±0,2	**1,762**	0,187

(p = 0,001). *The letters a and b indicate significantly different classes for the ear length parameter*

HD*: Heading date,* ***NN*** *: Number of nodes,* ***NT*** *: Number of tillers,* ***NE*** *: Number of ears,* ***SD*** *: Stem diameter,* ***PLH*** *: Plant height,* ***EL*** *: Ear length,* ***ED*** *: Ear diameter.*

Discussion

Mutagenesis by gamma irradiation induces genetic variability in millet, making it possible to obtain mutants with outstanding agronomic performance (Addai & Yahaya, 2018). Selection significantly reduces genotypic and phenotypic variance of the M4 generation of gamma irradiated sesame mutants (ARISTYA et al., 2017). In millet, the number of effective

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stems per plant, panicle length and panicle diameter have high heritability and genetic advance (PA et al., 2023). In this study, analysis by hierarchical classification shows morphological diversity that varies to genotype. All four genotype families were performed individually. MI 02/82 shows more variability at generation M4 than genotypes MI 13/63, MI 12/72 (Figure 2). MI 10/54 genotype represents the most homogeneous of all the parameters studied in the M4 generation (Table 4). Morphological variability in gamma-irradiated genotypes varies with dose and variety (Saibari et al., 2023). Because irradiation mutation randomly affects morphological characteristics (Abdullah et al., 2018). All four genotype families performed individually. This confirms the existence of intraspecific variability in crop radio-sensitivity reported by Hazra et al., (2022).

The MI 13/63 genotype family could be considered as morphologically homogeneous group. However, the number of tillers contributes significantly to the agronomic traits of millet. (Naoura et al., 2020), but has a low heritability (Yahaya, 2015). In rice M4 mutant, irradiation did not affect the number of tillers (Bella, 2021). On the contrary, the average length of the ears is a selection index for millet (Patil et al., 2018). Thus, it is possible to distinguish two subgroups of MI 12/72 (short ears and medium ears) with a significant difference in ear length. Based on the classification of Singh et al., (2016), the MI 13/63 and MI 10/54 genotypes have a low number of nodes and medium stem height (Tables S2 and 4). While MI 12/72 has short stems. This indicates that the MI 13/63 and MI 10/54 genotypes are similar in stem height to HKP, while MI 12/72 is shorter and this reduction in stem height could be induced by gamma irradiation. Similar effects were observed in about M4 rice mutants (Hanifah et al., 2020).

Conclusions

In this study, we sought to assess the homogeneity of millet mutants in the M4 generation, with a perspective on morphological purification. The results showed that morphological purification is variable between mutants. Genotypes MI 13/63 and MI 10/54 are homogeneous, MI 12/72 has an intermediate homogeneity and MI 02/82 shows variability in all the parameters studied. In conclusion, morphological homogeneity can be obtained on the M4 generation in millet mutagenesis by gamma irradiation. But it will be necessary to conduct this study on subsequent generations to determine the specific stage for the purification of each mutant.

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