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Variability in M_2 Generations and Characteristics of Advanced Mutant Lines of Rapeseed (*Brassica napus* L.)

W.M. Thagana[1*], C.M. Ndirangu[2], E.O. Omolo[2] and T.C. Riungu[1]

[1]Kenyatta University, P.O. Box 43844-00100, Nairobi, Kenya
[2]Egerton University, P.O. Box 538, Njoro
[*]Cell: 0722697576, [*]E-mail: wtmuriithi@gmail.com

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ABSTRACT Commercial varieties of rapeseed grown in Kenya have various production constraints. Mutation breeding has been used globally to develop varieties with desirable traits. The objectives of the studies were to identify the best level of irradiation for creating variability in M_2 and to characterise selected M_3 and M_6 mutant lines. M_2 generations of three varieties of rapeseed which had been irradiated at various levels were planted in blocks. M_2 plants were selected at random and data taken on plant height and pods per plant for each level of irradiation. M_3 lines selected based on earliness were planted and data was taken on various characters. Observations were also made on M_6 lines. There were significant (P<0.01) differences between the levels of irradiation in the number of pods per plant and significant interaction (P<0.01) between varieties and levels of irradiation for pods per plant. Association among the parents and M_6 mutant lines revealed by the PCA indicated that the M_6 mutant lines had higher genetic diversity. Genotypes that were distinctly different from the parents were observed at M_3. Mutation breeding was effective in creating variability and in the development of new plant genotypes that may be of agronomic value.

INTRODUCTION

Rapeseed (*Brassica napus* L.) is an important oilseed crop in Kenya (Oggema et al. 1988). Rapeseed which belongs to the Brassicaceae family is suitable for crop rotation in wheat and barley producing areas (Senior and Bavage 2003). Rapeseed can be grown in many areas in Kenya including all wheat and malting barley-growing areas (National Plant Breeding Research Centre [NPBRC] 2000). About 14 varieties of rapeseed have been released in Kenya (NPBRC 2000). Currently, commercial varieties are introductions from other countries (Kenya/Canada, Wheat and Oilseed Research Project [KCWORP] 1983). Those introductions have various production constraints which include shattering, lodging, low oil content, late maturity, tall plant height and susceptibility to diseases which lead to low rapeseed yield of 1.5MT/ha in Kenya compared with an average of 3 MT/ha in Europe (KCWORP 1983; FAO 1999). Mutation breeding has been used to develop mutant varieties with desirable traits such as high viability, seedling growth, lodging resistance, stress tolerance, earliness, high yield, high oil content and oil quality (IAEA 2000; Malek et al. 2012). The mutant varieties have global impact on food production and quality enhancement (Ahloowalia et al. 2004). M_2 and M_3 lines selected for earliness from three varieties of rapeseed, Karat, Regent and Topaz which had been irradiated at 600 Gy, 800 Gy and 1000 Gy were used for this study. The three varieties had been reported to be distinct from each other and also recommended for mutation breeding (Thagana et al. 2005a).

Objectives

(i) To identify the best level of irradiation for creating variability in M_2
(ii) To characterise selected M_3 and M_6 lines.

MATERIAL AND METHODS

Site Description

Njoro in Nakuru County is located at an altitude of 2143 masl and experiences cool temperatures with a mean annual temperature of 16 C, temperature range of 9-24 C. It experiences annual rainfall of 943 mm.

Experimental Description and Field Management

M_2 generations of three rapeseed varieties, Karat, Regent and Topaz which had been irradiated at 0 Gy, 600 Gy, 800 Gy and 1000 Gy were planted in blocks 18 metres long and 28 metres wide with Oljord tractor in the long rains of 2004. The spacing between rows was 18 cm and recommended agronomic practises were used in the management of the trial. At maturity 25 plants from each treatment were selected at random and data was taken on plant height and pods per plant. In the 2004 short rains, M_3 seeds derived from M_2 plants selected based on early maturity were planted in single rows. The parental varieties Karat, Regent and Topas were also planted in single rows as controls. The spacing between rows was 30 cm.

Data Analysis

Data was recorded on plant height, pods per plant, time of days to first flower and days to 50% flowering. In 2006, observations were made on M_6 lines planted in single rows. The data was analysed using SAS general linear model (GLM) and means separated using Duncan's Multiple Range Test (DMRT). The data was also analysed using Principle component analyses (PCA) (SAS Institute Inc.[SASII]).

RESULTS AND DISCUSSION

There were differences in number of pods per plant resulting from different levels of irradiation The interaction between varieties and levels of irradiation were significantly different (P<0.01) for plant height (Table 1) and pods per plant (Table 2). Studies on M_1 generations had also revealed significant differences (P<.01) among levels of irradiation but did not reveal significant differences (P>0.05) among genotypes (Thagana et al. 2005_b). Irradiation at 800 Gy resulted in the highest number (73) of pods per plant and was significantly different (P>0.05) from the non-irradiated control (54) (Table 2).

Table 1: Height (cm).of three varieties resulting from different levels of radiation

Level of irradiation	*Karat*	*Regent*	*Topaz*	*Overall mean*
0	88.2 b	81.5 b	92.8 a	87.5 a
600	92.1 ab	90.0 ab	80.7 b	87.6 a
800	98.8 a	83.1 b	95.0 a	92.3 a
1000	89.0 b	93.1 a	87.6 ab	89.9 a
Overall mean	92.0 x	86.9 x	89.0 x	89.3

Means followed by same letter (a,b,c) in the same column are not significant different at 5 % level of significance using DMRT. Means followed by same letter (x) in the same row are not significant different at 5 % level of significance using DMRT.

Table 2: The number of pods per plant. of three varieties resulting from different levels of radiation

Level of irradiation	*Karat*	*Regent*	*Topaz*	*Overall mean*
0	58.3 b	51.2 b	51.6 a	53.7 c
600	55.0 b	73.9 a	51.2 a	60.0 bc
800	96.2 a	52.4 b	70.1 a	72.9 a
1000	67.2 b	78.4 a	65.6 a	70.5 ab
Overall mean	69.2 x	64.0 x	59.6 x	64.3

Means followed by same letter (a,b,c) in the same column are not significant different at 5 % level of significance using DMRT. Means followed by same letter (x) in the same row are not significant different at 5 % level of significance using DMRT.

Since there was significant interaction between varieties and levels of irradiation in both height and pods per plant, the optimum irradiation level was different for different varieties. Irradiation at 800 Gy resulted in plants that were tall and also had the greatest variability in height for Karat (98.8±17.5) and Topas (95±16.1). In addition, irradiation at 800 Gy resulted in plants that had many pods per plant and also had the greatest variability in pods per plant for Karat (96.2±53.6) and Topas (70.1±37.7). For variety Regent, irradiation at 1000 Gy resulted in tall plants that had the highest variability in height (93.1±15.1). Irradiation at 1000 Gy also resulted in the highest pods per plant and highest variability (78.4±43.9). The best irradiation for inducing variability was 800 Gy for varieties Karat and Topas but for Regent it was 1000 Gy. The seed coat of Regent may be thicker and hence requires more powerful gamma rays to penetrate. The correlation between plant height and number of pods per plant was positive (R, 0.51) and highly significant (P<0.01). Tall plants had more pods per plant and since pods per plant are an important component of yield, tall plants may be expected to yield highly (Thurling 1974). Similarly, genetic variability has recently been observed among mutant lines for important quality traits such as levels of erucic acid and glucosinolates that were positively correlated (Bashir et al. 2013). Principle component analyses (PCA) for M_3 lines, revealed that the means were 67.9, 93.8, 61.3 and 72.3 for plant height (cm), pods per plant, days to first flower and days to 50% flowering, respectively. Number of pods per plant was positively and highly correlated to plant height and negatively correlated to days to first flower and days to 50% flowering. The number of days to first flower was also positively and highly correlated to days to 50% flowering. The principle components were 1.68, 1.55, 0.41 and 0.36 for the first, second, third and forth components, respectively (Table 3).

Table 3: Eigen values of the Correlation Matrix

	Eigen value	*Difference*	*Proportion*	*Cumulative*
Principle 1	1.6802	0.1254	0.4200	0.4200
Principle 2	1.5548	1.1492	0.3887	0.8087
Principle 3	0.4056	0.0461	0.1014	0.9101
Principle 4	0.3595		0.0899	1.0000

The principle components 1 and 2 were the most important and accounted for 81% of the total variation. The first principle component accounted for 42 % of the total variation, whereas the second principle component accounted for 39 % of the variation. Days to first flower and days to 50% flowering contributed highly to the first principle component whereas height and pods per plant highly contributed to the second principle component (Table 4).

Table 4: Eigen vectors for the principle components

	Principle 1	*Principle 2*	*Principle 3*	*Principle 4*
Height	-0.2702	0.6607	-0.6956	-0.0815
Pods per plant	-0.3933	0.5882	0.7023	0.0784
First flower	0.6208	0.3317	-0.0094	0.7103
50% flowering	0.6220	0.3279	0.1513	-0.6948

Association among the parents and M_3 mutant lines revealed by the PCA indicated that the M_3 mutant lines had higher genetic diversity and were located far away from the parents (SASII, 1998). Genetic diversity is important for the selection of parents for crossing in a rapeseed hybrid development programme (Ali et al. 1995). Mutant lines advanced to M_6 comprised of homozygous genotypes that were distinctly different from the parents and comprised of early maturing lines, vigorous plants, dwarf plants and a male sterility plant with high female fertility (Fig. 1).

Fig. 1. Male sterile plant selected in M_6

The male sterile plant produced flower buds that were apetalous, devoid of anthers and pollen with protruding stigma compared with the normal plants that had normal petals. On pollinating the stigma with pollen derived from non-irradiated mother plants of variety regent vigorous pods filled with big seeds were produced hence the male sterile plant had high female fertility. Mutation was therefore effective in creating new genotypes. Rapeseed varieties have been developed from mutant lines after further selection (Barret et al. 1996). Male sterile lines with high fertility may be used to develop commercial hybrids using the Cosegregation method (Ilarslan et al. 1999)

CONCLUSION

Irradiation induced changes in plant height and pods per plant and created variability. Irradiation at 800 Gy was the optimum for varieties Karat and Topas whereas 1000Gy was the optimum for variety Regent. The PCA revealed that the M_3 lines had higher genetic diversity compared with the parents. Mutation was therefore effective in producing new homozygous genotypes that may be of agronomic value.

RECOMMENDATION

Further studies are required to classify the male sterility system of the mutant lines. Seeds for the studies are available from the author

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