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EVALUATION AND SELECTION OF RAPESEED (*Brassica napus* L.) MUTANT LINES FOR YIELD PERFORMANCE USING AUGMENTED DESIGN

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ABSTRACT

The present study was undertaken to evaluate rapeseed mutants for yield and yield components at an early stage of entry selection in a non-replicated trial. Sixty five mutant entries were selected from gamma irradiated population of 1.0, 1.2 and 1.4 K Gys. and planted in augmented incomplete block design in four blocks. The data on days to 50% flowering, seed yield (kg ha^{-1}), 1000 seed weight (g) and oil yield (kg ha^{-1}) were recorded and analyzed to workout variance and contrast analyses. Significant differences were revealed for all the traits among the mutants and with the control except 1000-seed weight. Three mutant lines were significantly superior both in seed yield and oil yield. Ten mutant lines took significantly less number of days to 50% flowering. The study confirmed the effeciency of augmented design in the evaluation and selection of superior genotypes in early generation with better yield potential.

Key words: Rapeseed, Gamma Rays, Non-Replicated, Augmented Design.

INTRODUCTION

Rape (*Brassica napus* L.) is the world's third most important source of vegetable oil after palm and soybean (Beckman, 2005). The development of canola quality rapeseed (Downy and Rakow, 1987) has made rapeseed one of the major plant oil sources at the global level with constant tendency to increase its share in production of oilseeds (Bartkowiak-Broda *et al*., 2005).

In Pakistan, it is grown over an area of about 0.23 million ha but its production per unit area is merely 803 kg ha^{-1}(Anonymous, 2012). Pakistan is facing chronic edible oil shortage. Domestic production of edible oil from all traditional and non-traditional oilseeds is only sufficient to meet about one fourth of local demand, remaining requirement is met through heavy imports (Anonymous, 2012). There are many factors responsible for low yield, but the most important one is the non availability of high yielding varieties. It is, therefore, imperative to develop improved varieties of oilseed Brassica to bridge the gap between local production and import of edible oil in the country (Khatri *et al*., 2005).

Literature shows that more than 2700 mutant varieties of different crops with improved agronomic traits have been developed and released to the farmers for general cultivation all over the world (Maluszynski *et al.*, 2000; Shu, 2009). Mutagenesis technique has also been successfully employed in rapeseed and mustard by the plant breeders (Naz and Islam, 1979; Javed *et al*., 2000) to alter the genetic architecture of plant and isolate the possible mutants with desired economic plant characters.

In plant selection programme, breeders usually start with a large number of test entries which comes either from crossing, induced mutation or introduction from foreign sources. The number of the lines can range even up to hundreds. To conduct a field experiment for such a large population is extremely difficult for number of reasons like, environmental heterogeneity in the field can not be easily taken in to account. To complicate the matter further, material available for each test line is often limited, some being sufficient for only one replication. Thus, design for variety trial involving for large numbers of test lines, for example, lattice, lattice square and quasi factorial designs, all of which require replications, cannot be used; design such as chain blocks (Youden and Connor, 1953; Mandel, 1954), which require that a substantial number of test lines have atleast two replications can not be applied. To circumvent the difficulties arising from non-replicated experiments, Federer (1956, 1961 and 1991), Federer and Raghavarao (1975) introduced the class of augmented designs to replace the systematically spaced arrangement for screening new genotypes in plant breeding research investigation (Mudeheri and Obiero, 2006). The present study was thus undertaken to evaluate developed mutants for yield and its components at an early stage of selection in a non-replicated trial. This will help in short listing promising mutants for yield assessment in the replicated trials at station and across different environments in the country to evolve high yielding brassica oilseeds.

MATERIALS AND METHODS

The rapeseed (*Brassica napus* L.) mutant lines of $M_{2:4}$ generation were developed for improvement in yield at Nuclear Institute for Food and Agriculture (NIFA), Peshawar. The mutant lines comprised of 65 entries selected from gamma irradiated population of 1.0, 1.2 and 1.4 K Gys. The commercial variety Durr-e-NIFA was included as check in the experiment. . The experiment was conducted at experimental fields of NIFA during 2009-10 in an augmented incomplete block design. The experimental design consisted of four randomized blocks in each of which commercial check Durr-e-NIFA was repeated three times. In first three blocks 17 mutant lines and in fourth 14 lines were planted. The check variety was grown in each block, but each mutant line to be evaluated was planted only once in the experiment. The position of check and the mutant lines in each block were fully randomized and each entry planted on a 6 m^2 size plots. Nitrogen and phosphorous fertilizers were applied @ 32 and 23 kg per acre, respectively. Three irrigations were applied on as and when needed basis at different stages of plant growth.

The data on days to 50% flowering, seed yield (kg ha^{-1}), 1000 seed weight (g) and oil yield (kg ha^{-1}) were recorded and subjected to statistical analysis to workout variance and contrast analyses of test and control treatments, and critical differences for performing treatment comparisons in augmented design as suggested by Rathore *et al*. (2004) following the approach of Federer (1956).

RESULTS AND DISCUSSION

Results for four quantitative traits showed significant differences among all the test treatments in terms of all traits measured except 1000-seed weight while block mean square was non-significant for day to 50% flowering only (Table 1). The coefficient of determination (R^2) may also be read as square of the correlation coefficient revealed high correlation between the unadjusted and adjusted means for all the traits. The small values for root mean square error (RMSE) as compared to observed variation in the measurements, and coefficient of variation (CV) indicated that the observations had no wide spread data relative to the size of the means.

The contrast analysis among controls, tests and test versus control revealed stirring results (Table 2). The control Durr-e-NIFA exhibited non-significant differences for all the traits under study that revealed the genetic uniformity of the material. The effects of the checks could be considered fixed in plant breeding, as they are generally standard released varieties (Santos *et al.,* 2002). This also signified the least soil heterogeneity of the experimental plot which otherwise might be a great limiting factor in drawing final conclusion. Test against control treatments (Table 2) showed highly significant differences ($p \leq 0.01$) depicting the variability in the genotypes and fair scope of short listing better performing mutants.

Early flowering provides suffcient time for seed filling which could result better seed yield. The block effects were significant for the trait (Table 1) that might be attributable to two main reasons i) morphological trait observed on visual basis not numerically measured or recorded the plants and ii) inderminate flowering in brassica species. All the test treatments attained 50% flowering from 51 (OA-12) to 141 (OA-38) days compared to the control treatment which took 95.66 (sum means over blocks) days. It is evident from the results (Table 3) that ten test treatments viz., OA-5 to OA-13 and OA-1 consumed significantly less days to 50% flowering at $p \leq 0.01$ and 0.05, respectively. Thurling and Depittayanan (1992) found M_2 plants carrying the mutant gene flowered as early as 59 days before the parental line. These major gene mutations could be rapidly exploited in the development of agronomically superior cultivars for short-season, lower rainfall environments. Flowering time is not only of scientific interest, it is also important in agriculture, because its modification may enable the geographical range of the *Brassica* crop to be extended (Rae *et al*., 1993).

All the test treatments (65) exhibited non-significant differences (Table 1) for 1000-seed weight. However, nine test treatments in aggregate gained 1000-seed weight either at par (OA-17, 24, 39 and 53) or more than (OA-16, 21, 50, 51 and 60) the control treatment that achieved 3.60 g (sum means over blocks). The increased 1000-seed weight ranged from 0.2 – 0.4 g (Table 3). Some yield components significantly affect the seed yield either directly or indirectly through other components. Seed weight is an important component of seed yield and depends on environmental conditions; although genetic variation in the seed weight existed but would have negative effects on other yield components (Diepenbrock, 2000). The test treatments that achieved good seed yield and fairly high 1000-seed weight as mentioned above may be worth promoting for next year trial. Aytac and Kinaci (2009) observed negative interrelationship between seed yield and 1000-seed weight while Engqvist and Becker (1993) suggested improvement in seed yield and oil and protein contents at the same time. Therefore, it is revealed that 1000-seed weight alone might not be the selection base of the present study.

In breeding field crops, the ultimate goal is to improve the yield as compared to contemporary released varieties inclusive of other edges in various traits. The block effects were non significant for the trait that refelcted the least soil variation in the experimental area while highly significant differences were obtained $p \leq$

0.01 among all test treatments (Table 1). The data revealed that seventeen test treatments achieved seed yield either higher or at par with the control treatment that produced 2403.54 kg ha^{-1} (sum means over blocks) Table 3. Two test treatments, OA-17 and OA-25 produced SY as 3569 and 3486 kg ha^{-1}, respectively and outyielded the control treatment at CD1 while another two test treatments OA-42 (3154 kg ha^{-1}) and OA-11 (3071 kg ha^{-1}) surpassed the control at CD2. The improvement in polygeneic characters through induced mutations might be due to genetic changes in certain other related but simply inherited characters which could have possitive effect on seed yield and oil content (Micke *et al*., 1990). Shah and Rehman (2009) had evolved high yielding and superior quality mutant varieties of rapeseed 'Abasin-95' and mustard 'NIFA-Raya' from mutagenized population of Canadian varieties Tower and DLJ-3, respectively.

All the studied mutant lines varied highly significantly for oil yield (Table 1) at $p \leq 0.01$. Sixteen mutants yielded either at par or exceeded in oil yield than the control treatment that produced 1178.63 kg ha^{-1} (sum means over blocks) Table 3. However, test treatments OA-17, OA-42 and OA-25 harbored 1727.39, 1655.85 and 1627.96 kg ha^{-1} oil yield and outclassed the control treatment (1178.63 kg ha^{-1}) when compared at CD1 and CD2. This revealed that selection for high seed yield may also improve oil yield from certain level. Seed oil content, besides seed yield, is one of the highly demanding criteria in developing rapeseed cultivars (Engqvist and Becker, 1993). Thus, it is essential to improve seed yield and oil content at the same time (Aytac and Kinaci 2009). The present results corroborate the findings of Shah and Rehman (2009) who reported improved oil contents in newly developed mutant (43-47%) compared to its parent variety (41-43%).

The present research was conducted to asses 65 mutants at early stage in a non-replicated trial with augmented randomized incomplete design for their yield potential and to sort out the promising ones for future evaluation in replicated trial at breeding site and other locations.

The data revealed that mutant lines OA-17, 25, and 42 are the best in performing test treatments in respect of all traits under study except OA-17 that consumed significantly more number of days to flowering compared to control. Earliness in respect of flowering may achieve early maturity, however, test treatment that took longer to flower, may be considered for zones where the cropping pattern is flexible to accommodate slightly long duration variety. 1000-seed weight in the range of 3.00 to 3.60 g may be useful for getting higher seed yield and oil yield. It is suggested that all the mutant lines that performed significantly better for yield or at par to control, may be advanced for further extensive field evaluation.

Table 1. Basic statistics for quantitative traits of brassica mutants 2009-10.

Traits	Grand mean	Mean squares		R^{2} [c]	CV% [d]	RMSE [e]
		Block	*Treatment*			
Days to flower	105.43	163.20^{*} [a]	282.23^{*}	0.99	5.32	5.60
1000 seed weight	3.129	0.12^{ns} [b]	0.23^{ns}	0.96	10.27	0.32
Seed yield	2138.59	10094.29^{ns}	226138.09^{**}	0.98	8.57	183.38
Oil yield	1052.42	3054.33^{ns}	54943.65^{**}	0.99	7.76	81.75

[a] *, ** significant at $p \leq 0.05$ & 0.01, respectively; [b] non-significant; [c] Correlation coefficient; [d] coefficient of variation; [e] root mean square error.

Table 2. Contrast analysis (mean squares) of different sources of test and control treatments in brassica mutants 2009-10.

Source	Degree of Freedom	Days to flower	1000 seed weight (g)	Seed yield	Oil yield
Among controls	2	13.69^{ns} [a]	0.13^{ns}	50088.77^{ns}	8633.25^{ns}
Among tests	64	273.17^{**} [b]	0.17^{ns}	219326.79^{**}	53719.56^{**}
Test vs control	1	1624.89^{**}	4.60^{**}	1005483.43^{**}	224848.80^{**}

[a] non significant; [b] significance at $p \leq .01$

Table 3. Mean values for observed traits of 65 rapeseed mutant lines with commercial check variety Durr-e-NIFA for augmented design.

Block	Entry	Days to Flowering	1000 Seed Weight (g)	Seed Yield (kg ha^{-1})	Oil Yield (kg ha^{-1})
1	OA-1	77	2.6	1826	832.65
1	OA-2	84	2	2324	1062.07
1	OA-3	94	2.6	1660	790.16

1	OA-4	92	2.4	1909	922.04
1	OA-5	60	3	1370	637.05
1	OA-6	64	2.4	1411	673.04
1	OA-7	64	2.6	1494	721.6
1	OA-8	62	2.4	1743	841.86
1	OA-9	57	2.2	1328	652.04
1	OA-10	57	2.6	1162	554.27
1	OA-11	62	3.2	3071$^{*\ a}$	1421.87
1	OA-12	51	2.4	1245	578.92
1	OA-13	60	2.6	1743	819.21
1	OA-14	84	2.4	1909	887.68
1	OA-15	82	2.4	2075	1008.45
1	OA-16	96	3.8	2407	1169.8
1	OA-17	127$^{**\ a}$	3.6	3569**	1727.39**
2	OA-18	94	3.4	1909	958.31
2	OA-19	127**	2.8	2490	1230.06
2	OA-20	110	2.8	2739	1402.36
2	OA-21	86	3.8	2200	1091.2
2	OA-22	128**	3.4	1743	817.47
2	OA-23	128**	2.8	1992	976.08
2	OA-24	102	3.6	2075	1045.8
2	OA-25	109	3	3486**	1627.96**
2	OA-26	131**	3.4	2200	1067
2	OA-27	111	3.4	2573	1271.06
2	OA-28	111	3.4	2656	1266.91
2	OA-29	125**	3	2490	1192.71
2	OA-30	134**	2.6	2241	1095.84
2	OA-31	134**	3	1909	895.32
2	OA-32	127**	3	2407	1112.03
2	OA-33	128**	3.4	2656	1296.12
2	OA-34	102	3.4	2075	1039.6
3	OA-35	121**	2.6	1660	796.8
3	OA-36	128**	3	2075	1047.87
3	OA-37	127**	2.8	1826	892.91
3	OA-38	141**	2.8	1577	769.57
3	OA-39	100	3.6	1992	1023.88
3	OA-40	112*	3.2	2075	1027.12
3	OA-41	113*	2.8	2158	1126.47
3	OA-42	108	3.2	3154*	1655.85**
3	OA-43	127**	2.8	2075	1091.45
3	OA-44	105	3	2490	1267.41
3	OA-45	123**	3.2	2490	1222.59
3	OA-46	111	3	2407	1203.5
3	OA-47	132**	2.6	1909	916.32
3	OA-48	132**	3.2	1992	972.09
3	OA-49	111	3.4	2158	1059.57
3	OA-50	122**	3.8	2158	1029.36
3	OA-51	111	4	2490	1277.37
4	OA-52	127**	3.2	1909	965.95
4	OA-53	102	3.6	1494	785.84
4	OA-54	109	3.4	2158	1102.73
4	OA-55	132**	3	1577	796.38
4	OA-56	130**	3	2158	1055.26
4	OA-57	141**	2.4	1992	997.99
4	OA-58	133**	3.2	1909	986.95
4	OA-59	131**	3.2	2324	1217.77

4	OA-60	109	3.8	2407	1198.68
4	OA-61	123 **	3.2	913	465.63
4	OA-62	127 **	3.2	2075	1066.55
4	OA-63	111	3.2	2158	1130.79
4	OA-64	91	3.4	1909	1015.58
4	OA-65	128**	2.4	2075	1041.65
Durr-e-NIFA (Mean performance)		95.66	3.6	2403.54	1178.63
Critical Difference (1)		23.2	1.45	832.61	371.17
Critical Difference (2)		15.68	0.96	549.6	245.01
SEd		6.63	0.39	224.6	100.12

[a] *, ** significance at p ≤ .05 & .01, respectively

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