



DEVELOPMENT OF A NEW OILSEED RAPE VARIETY NIFA SARSON-T20 THROUGH PHYSICAL MUTAGENESIS

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ABSTRACT

Induced mutagenesis has been proven as one of non-conventional approaches to create genetic variability. In line with the objective to develop a high seed and oil yielding oilseed rape cultivar; 4000 healthy seeds of an advanced rapeseed (*Brassica napus* L.) line NR-23 having 8% moisture were irradiated @ 1, 1.2 and 1.4 kGy in 2010 using cobalt-60 source. Single plant selections were executed based on yield and yield contributing traits following pedigree method of selection from M₂-M₄ generations. Homozygous progenies of the pedigree number 011-K-16-3 selected from 1 kGy were bulked during 2013. The mutant with line code 011-K-16-3 was evaluated at station and outstation seed yield trials from 2013-14 to 2017-18. The mutant performed better than checks in Preliminary, Advanced, Zonal and National Uniform Rapeseed Yield Trials (NURYT). It has out yielded Hyola-401 and Faisal Canola by 26% and 23%, respectively in the multi-location adaptation seed yield trials conducted at selected sites in the Khyber Pakhtunkhwa (KP) and Punjab. It has also exceeded the national check Faisal Canola by 5% on location mean basis in the NURYT (2016-17 and 2017-18). It maintained its superiority in oil yields over the check Faisal Canola at station and in NURYTs. It is medium maturing, tall (190-205cm) featured with ramification at middle low, tolerant to alternaria blight, high seed yield (3000-3300 kg ha⁻¹); high oil contents (43-46%) and low erucic acid (< 5%). The Provincial Seed Council approved it with name NIFA Sarson-T20 for growing in the Khyber Pakhtunkhwa (KP).

Keywords: *Brassica napus*, KP, mutant, selection, variety, yield

INTRODUCTION

In Pakistan, edible oil requirement is increasing due to high consumption rate per person and limited indigenous production has resulted in increase in the import share (Mustafa *et al.* 2017). During Financial Year 2021 (July-March); total 3 million tons of edible oil costing over Rs. 574 billion was imported. Indigenous production hovered around 0.374 million tons merely managing 12% against the total availability of edible oil from all sources estimated at 3.291 million tons (Anonymous, 2021). Rapeseed and mustard are second most important source of vegetable oil in Pakistan (Syed and Rahman, 2009) after cotton seed and thus highly adaptable to the local climatic conditions and are well known to the farmers of the country. It covers about 0.23 million hectares including Canola. The national rapeseed normal yield is approx. 1050 kg ha⁻¹ while KP has just 459 kg ha⁻¹ (Anonymous, 2017-18) and the major reason may well be incapacity of available

varieties to perform better under varying macro and micro climatic conditions. Low yield is attributable to many factors but the non-availability of high yielding varieties is the most serious one. It is believed that indigenous production to be focused through developing good varieties to reduce import of edible oil in the country (Ali *et al.*, 2013). To raise the economic health of the oilseed farmers in the country in general and specifically in the KP; there is an urgent need to focus on developing rapeseed varieties having good seed and oil yields potential. In the rapidly changing climatic condition; the replacement of existing crop varieties with better and improved varieties is more vital than ever before. Development of high seed yielding oilseed varieties will increase rapeseed cultivation area and thus provide cushion to agricultural economy through restricting heavy investment on edible oil import.

Genetic alteration through radiation is considered as one of the non-conventional tools for improvement of traits in the plants (Konzak *et al.* 1965 and Anonymous, 1970). The main gain

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of mutation breeding is the higher probability of improving one to few characters without altering the rest of the genotype. During present times, induced mutations have been widely used for breeding annual oilseed crops (Ferrie *et al.*, 2008; Emrani *et al.*, 2015 and Hussain *et al.*, 2017). Mutagenesis has successfully been used for improving traits like earliness, dwarfness, biotic and abiotic stress resistances or tolerance, seed yield and oil quality (Parry *et al.*, 2009; Ali and Shah, 2013 and Lee *et al.*, 2018). Plant breeders select mutants with modified genetic architecture of plant in respect of plant height, number of pods per plant, number of grain per pod, grain weight, grain yield, oil content and disease resistance (Javed *et al.*, 2000; Mahla *et al.*, 1972 and 1990; Robbelen, 1990; Rahman, 1996; Shah *et al.*, 1990; Shah *et al.*, 1998 and Shah *et al.*, 1999). Mutants with reduced plant height coupled with high yield potential have been developed from populations of rapeseed and mustard, respectively (Das and Rahman, 1988 and Shah *et al.*, 1990) while Das *et al.* (1999) developed early maturing and high yielding rapeseed mutant varieties. Low glucosinolate containing germplasm of *Brassica campestris* (rapeseed) was developed through spontaneous mutants, intra- and inter-specific hybridization (Potts *et al.*, 1999). One hundred and sixty three mutant cultivars of annual oilseed crops have been officially released for cultivation in 26 countries (Bhatia *et al.*, 1999). Abasin-95 and NIFA-Raya were the first ever mutant cultivars of rapeseed and mustard group respectively, released in Pakistan possessing early maturity, wider adaptability against respective checks (Syed *et al.*, 2009). Gamma radiation reported to have influenced the biochemical profile of the oilseed rape by inducing different spectrum of variation in the fatty acid profile (Ali *et al.*, 2013).

The above lines conclude that induced mutagenesis can be an alternative or supporting tools for the qualitative and quantitative improvement of oilseed brassicas.

The oilseed breeding programme at Nuclear Institute for Food and Agriculture (NIFA), Peshawar is contributing in the research and development sector of the country through developing high seed and oil yielding; early maturing with better quality rapeseed varieties. The objectives are approached through the techniques of induced mutations and hybridization alone or in combination. The induction of this variety in the agricultural

production system will certainly improve per hectare yield at framers' fields.

MATERIALS AND METHODS

The project activities were commenced during 2010-11 at Nuclear Institute for Food and Agriculture, Peshawar and Summer Agricultural Research Station (SARS), Kaghan, Khyber Pakhtunkhwa, Pakistan. The experimental material consisted of an advanced rapeseed line (NR-23) having good quality but low seed yield. The mutation breeding procedure outlined by International Atomic Energy Agency (IAEA, 1997) was adopted to irradiate the seed material and developing M₁-M₃ generations. About 4000 uniform seeds of NR-23, with about 8% moisture were irradiated at 1, 1.2 and 1.4 kGy (⁶⁰Co gamma source) during 2010. Pursuing shuttle breeding (getting two generations in a year), irradiated material was planted at Summer Agricultural Research Station (SARS), Kaghan in isolation as M₁ generation during 2010 (May to September). The same year (October to April, 2010-11), desirable mutants were assorted in M₂ at NIFA, Peshawar. 011-K-16-3 was selected in M₃. Based on high seed yield and other yield contributing traits of selected mutant; homozygous progenies of the pedigree number 011-K-16-3 were bulked. The mutant line was comprehensively assessed for yield in different replicated trials at NIFA, Peshawar, Zonal Yield Trial (ZYT) in Khyber Pakhtunkhwa and selected locations in the Punjab. It was evaluated for two years in National Uniform Rapeseed (NURYT, 2017-18 and 2018-19) throughout Pakistan. The soil types over different locations were clay loam to sandy loam. Two to three irrigations were applied on as and when required basis generally on the critical growth stages of the crop. The experiments were planted under rain-fed condition at six locations viz., Barani Agricultural Research Institute (BARI), Chakwal, Arid Zone Research Station (AZRI), Bhakkar, Barani Agricultural Research Station (BARI), Fateh Jhang, Barani Agricultural Research Station (BARI), Kohat, Agricultural Research Institute (ARI), Swat and Agricultural Research Institute (ARI), Quetta. Experiments at all sites were laid out in Randomized Complete Block Design (RCBD) with three replications, having six rows, 5m long and 30cm apart. The yield trials data were analyzed using computer based statistical package (MSTAT-C, 1991). The total oil contents, fatty acid profile and total seed glucosinolates of various mutants were analyzed through Near Infrared Reflectance Spectroscopy

(NIRS-Foss 6500 and Perten DA 7250) systems at NIFA, Peshawar and Ayub Agriculture Research Institute (AARI), Faisalabad.

RESULTS AND DISCUSSION

Station yield trials

Stable and high yielding nine rapeseed mutants/recombinants were evaluated in the Preliminary Yield Trial along with a commercial check, Hyola-401 at NIFA-Peshawar, during 2013-14. The mutant 011-K-16-3 exhibited superior seed yield (3815 kg ha⁻¹) and out classed the check Hyola 401 (2920 kg ha⁻¹) by 16%. Eight high seed yielding and better performing rapeseed mutants were evaluated in the Advanced Seed Yield (AYT) trial compared with a commercial check, Faisal Canola at NIFA-Peshawar, during 2014-15. The rapeseed mutants 011-K-16-3 displayed high seed yield viz., (3561 kg ha⁻¹) against control Faisal Canola (2789 kg ha⁻¹) and out classed it by 21% (Table 1).

Table 1. Yield performance of 011-K-16-3 in station trials at NIFA, Peshawar

Year	Name of trial	Seed Yield (kg per ha.)			LSD at 5%
		011-K-16-3	Hyola-401	Faisal Canola	
2013-14	Preliminary yield trial	3815	2920	-	767
2014-15	Advanced yield trial	3561	-	2789	767
Average		3688	2920	2789	-

Zonal yield trials (ZYT)

The performance of 011-K-16-3 mutant was assessed in comparison with commercial check Faisal Canola at 04 and 05 locations in the KP and Punjab during 2015-16 and 2017-18, respectively. The data revealed that rapeseed mutants 011-K-16-3 produced 2210 and 2074 kg ha⁻¹ average seed yield during the respective years as against commercial check Faisal Canola that exhibited average seed yield of 1696 and 1534 kg ha⁻¹ during two respective years. The stable performance 011K-16-3 can be

judged for high mean seed yield from the two years of evaluation for seed yield at selected locations remained over 2000 kg ha⁻¹ (Table 2).

Table 2. Yield performance of 011-K-16-3 in zonal yield trials

Year	Varieties	Seed Yield (kg per ha.)					Genotypic means
		NIFA, Peshawar	BARS, Kohat	AZRC, DI Khan	BARI, Chakwal	RARI Bahawalpur	
2015-16	011-K-16-3	3056	1554	2360	1870	-	2210
	Faisal Canola	3028	1033	980	1744	-	1696
	Lsd (5%)	397.7	675	434	266	-	-
2017-18	011-K-16-3	2972	762	3094	251	3290	2074
	Hyola-401	2111	519	3219	243	1577	1534
	LSD (5%)	972	738	806	59	275	-

National uniform rapeseed yield trial (NURYT)

011-K-16-3 was assessed for the first year compulsory trial in National Uniform Rapeseed Yield Trial (NURYT-2016-17) inclusive of eighteen candidate lines contributed by other oilseed breeders of the country and commercial check Faisal Canola. The rapeseed mutant (011-K-16-3) exhibited 4.71% higher seed yield (1772 kg ha⁻¹) than commercial check Faisal Canola (1688 kg ha⁻¹) on overall mean basis. It showed the worth by displaying 3 to 21% high seed yield over seven of fourteen locations on mean value (Table 3). For the second year obligatory adaptability testing in the NURYT 2017-18; 011-K-16-3 produced high seed yield at seven locations out of fourteen locations among 22 different hybrids and open pollinated varieties (OPVs) and achieved 5.13% higher seed yield (1695 kg ha⁻¹) than a control Faisal Canola (1608 kg ha⁻¹) (Table 4). The average % increase of 011-K-16-3 over the check remained to nearly 5% (Table 5).

Table 3. Yield performance of 011-K-16-3 in national uniform rapeseed yield trials 2016-17

Entry Name	NARC, Islamabad	BARI, Chakwal	ORI, Faisalabad	RARI, Bahawalpur	ORS, Khan pur	Pioneer, Sahiwal	AZRC, D.I. Khan	BARS, Kohat	ARI, Tamab	NIFA, Peshawar	ARI, Swat	ARI, Tandojam	NIA, Tandojam	ARI, Quetta	Entry Mean
011-K-16-3	3009	516	1639	1871	917	1371	1684	2272	1389	2700	1022	1620	557	693	1772
Faisal Canola (c)	2155	568	2044	1871	833	1217	2718	1652	1694	1633	1053	1695	508	692	1688
Location mean	2545	546	1570	1463	931	1393	2340	1954	1453	2512	1141	1478	478	607	-
Lsd (5%)	221	73.28	233.3	157	364	248	124.5	233	279	436	NS	317	101	54.5	-

Source: National Coordinator, PARC, Islamabad, 2016-17

Table 4. Yield performance of 011-K-16-3 in national uniform rapeseed yield trials 2017-18

Entry Name	NARC, Islamabad	BARI, Chakwal	ORI, Faisalabad	RARI, Bahawalpur	ORS, Khan pur	Pioneer, Sahiwal	AZRC, DIKhan	BARS, Fateh. Jang	AZRI, Bhakkar	ARI, Tarnab	NIFA, Peshawar	ARI, Swat	ARI, Tandojam	ARI, Quetta	Entry Mean
011-K-16-3	3203	489	2374	3228	1019	2522	2188	631	2074	773	2539	489	1376	824	1695
Faisal Canola (C)	2543	679	1991	2489	1259	2156	2446	470	2111	1700	2044	698	1234	687	1608
Location Mean	2735	634	2319	2551	1068	2706	2284	594	2098	998	2322	839	1192	794	-
LSD (0.05)	207	146	457	75.5	368.4	268	206.6	34.7	455.6	92.3	908	NS	101	72.8	-

Source: National Coordinator, PARC, Islamabad, 2017-18

Reaction to disease and insect

Twenty rapeseed entries were evaluated for insect pest attack and disease infestation under field conditions of NARC during 2016-17 and 2017-18. Overall no insect pest attack or disease incidence was observed on these rapeseed entries. However, aphid attack on these rapeseed entries was reported from AZRC, D. I. Khan (NURYT 2016-17). The aphid attack ranged from 15 to 23% (Table 6).

Table 5. Performance of candidate variety (011-K-16-3) against national check

Locations: 14			
2016-2017		2017-2018	
Entry	Seed yield (kg/ha)	Entry	Seed yield (kg/ha)
011-K-16-3	1772	011-K-16-3	1695
Faisal Canola	1688	Faisal Canola	1608
% Inc. over check	4.71	% Inc. over check	5.14
Avg. % Increase over check			5.13 4.95

Calculated from NURYT Report 2016-17 & 2017-18, PARC, Islamabad

Table 6. Insect/Pest attack (%) at AZRC, DI Khan 2016-17

Ent. #	Entry Name	Aphid attack %
1	HBO-555	18
2	AZRI Rapeseed	16
3	RBN-13018	17
4	HSO-1	18
5	KN-265	17
6	MUN-1	16
7	HBO-63	17
8	Kingola	19
9	011-K-16-3	17
10	CRH-374	16
11	CRH-101/16	18
12	Rapeseed Cold Tolerant	15
13	11CBN-006	16
14	RBN-13028	19
15	Faisal Canola (C)	18
16	MUN-3	19
17	KN-277	23
18	CRH-119	18
19	Hyola-401(C)	17
20	CRH-319	19

Source: NURYT Report 2016-17, PARC, Islamabad

Twenty two rapeseed entries were evaluated under natural field conditions at NARC and Pioneer, Sahiwal during 2017-18. The disease severity was rated on 0-5 scale (Mayee and Datar, 1986). Overall no disease was observed on these entries at NARC. However incidence of alternaria blight was recorded. NIFA Sarson-T20 (011-K-16-3) was found resistant to alternaria blight (Table 7).

Table 7. Evaluation of rapeseed entries in NURYT (2017-18) against alternaria blight diseases from 2 locations

Sr. No.	Entry Name	NARC, Islamabad	Pioneer, Sahiwal
1	HC-021C	0	1
2	Kingola	0	1
3	ChakwalSarson	0	1
4	CHS-2	0	1
5	WS-520	0	1
6	CHS-9	0	1
7	KN-294	0	1
8	RR-41-4	0	1
9	Hyola-401 (C)	0	1
10	MUN-2	0	1
11	KN-279	0	1
12	011-K-16-3	0	1
13	RBN-13029	0	1
14	14CBN-001	0	1
15	MUN-4	0	1
16	HC-022B	0	1
17	MUN-3	0	2
18	MUN-1	0	2
19	RBN-13028	0	2
20	14CBN-009	0	2
21	CHS-16	0	2
22	Faisal Canola (C)	0	2

Source: NURYT Report 2016-17, PARC, Islamabad

0= highly resistant, No symptoms 1= resistant, 1% diseased area. 2= moderately resistant = 2-10% diseased plant area 3= moderately susceptible, 11-25% diseased plant area. 4= susceptible, 26-50% diseased plant area, 5= highly susceptible above 50% diseased.

Table 8. Mean Values of Quality Parameters of 011-K-16-3 done at NIRS (Foss 6500) & Perten (DA7250)

Entry vs Control	Oil (%)	Protein (%)	GSL (umol/g)	Moisture (%)	18:01(%) ω-9	18:03 (%) ω-3	22:01 (%)
011-K-16-3	43	26.38	50	5.7	58.5	10.68	2
Faisal Canola	42	27.33	70	5.4	60.3	10.75	27.20

Source: Oilseed Brassica Lab., NIFA, Peshawar& AARI, Faisalabad

Agronomic and botanical description

The salient agronomic and botanical description was recorded according to descriptor established by Federal Seed Certification and Registration Department, Islamabad. Growth habit of the variety is intermediate with 190-205 cm plant height having determinate plant type. Its leaf is green, open and large in size and petiole base is deeply lobbed. Flower colour is yellow, petal length and width is long and broad respectively. It takes about 60-70days for opening. Pod attitude is divaricated, shape is intermediate and shattering is low. Numbers of grains per pod are 23-26. Its 1000 seed weight is 3.9-4.4g. Seed is bold and colour is black having 43-46% edible oil.

Variety approval

The KP Provincial Seed Council in its 40th meeting held on April 07, 2021 at Cereal Crop Research Institute (CCRI) approved the mutant 011-K-16-3 as NIFA Sarson-T20 for general cultivation in the irrigated and rain-fed areas of Khyber Pakhtunkhwa (KP).

It was previously reported that various mutagenic including gamma radiation were efficient treatments for mutagenesis (Siddiqui *et al.*, 2009; Emrani *et al.*, 2012; More and Malode, 2016). The findings of above researchers confirmed that genetic variability in rapeseed quantitative traits was increased by using these mutagenic treatments.

In the current study, the mutant performed better than checks in Preliminary, Advanced, Zonal and National Uniform Rapeseed Yield Trials (NURYT). Channaoui *et al.* (2019) reported that plant height and higher number of pods per plant was increased in seeds irradiation with gamma rays-1300 Gy.

In our study agronomical and botanical parameter were also recorded and found that growth habit of the variety was intermediate ranged 190-205 cm plant height having determinate plant type. Its leaf was green, open

and large in size and petiole base was deeply lobbed. Flower colour was yellow, petal length and width were long and broad respectively. Pod attitude was divaricated, shape was intermediate and shattering was low. Numbers of grains per pod was recorded as 23-26. Its 1000 seed weight was found as 3.9-4.4g. Seed was bold and colour was black having 43-46% edible oil. Previously, increased of 1000-weight was observed in mutant plants derived from seeds treated by gamma rays-1300 Gy (Channaoui *et al.*, 2019, Siddiqui *et al.*, 2009). Mutants in oilseed *Brassica* with improved seed size were achieved by gamma rays treatments (Chauhan and Kumar 1986; Diepenbrock, 2000).

CONCLUSION

Plant breeding for development of new crop varieties is continuous process and due to the rapid changes in the climate; the life span of varieties has been reduced in term of performance. Therefore, the development and release of field crop varieties are needed to be on fast track than ever before. On one side newly developed crop varieties are often cited as a possible adaptation to changing climate but on the other hand their performance in respect of qualitative or quantitative traits are assumed to be better than the existing varieties. NIFA Sarson-T20 in term of its performance throughout the developmental cycle has shown worth over respective commercial check (s) and approved by Provincial Seed Council, KP, for general cultivation in the province. It is hoped that the adoption of this variety by the farmer will certainly improve per acre yield.

AUTHOR'S CONTRIBUTION

H. M. Ahmed: 80%

K. Ahmed: 20%

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