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Generational Comparisons in *Brassica campestris*: Implications for Breeding and Crop Improvement

Salsabeel Rauf, Hafiz Basheer Ahmad*, Shazia Saeed, Ahmad Nawaz Gill

Abstract

Plant breeders utilize functional genetic variation within elite germplasm. This research aimed to identify a high-yielding, high-oil-content line with characteristics similar to canola. The experiment was conducted under field conditions, following backcrossing techniques. Four elite lines were used—two from a local source, UAF-11 and Toria, and two imported canola lines, Span and TR-8, of *Brassica campestris*. Statistically significant variation was observed among the parents and their progeny for various traits in all crosses. The F1 cross TR8 × Toria showed the highest values for the number of branches (22.33), seeds per siliqua (25.39), 1000 seed weight (2.82 g), and seed yield per plant (22.02 g). BC1 had the highest oil content (47.35%), F2 exhibited the highest oleic acid content (56.55%), and F1 had linoleic acid (16.47%) with low levels of Erucic acid found in F1 (1.13%), BC1 (1.92%), and F2 (2.73%). A single plant from F3 yielded the highest amount (24.32 g/plant), also displaying double low characteristics: an erucic acid content of 0.77%, a glucosinolate content of 15.77%, and an oil content of 51.65%.

Keywords: Additive variance, Fatty acids, oil contents, mean yield, Erucic acid

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Oilseeds Research Institute, Faisalabad, Pakistan; *Corresponding author e-mail: easahafiz@yahoo.com

Introduction

The worldwide acreage of rapeseed is gradually increasing. Rapeseed is cultivated for oil consumption, feed, and biodiesel production (Marjanović-Jeromela et al., 2007). Oilseed rape is the second-largest oilseed crop, making up 13% of the world's total supply. Commercially, two species, *B. rapa* L. and *B. napus* L., are predominantly cultivated. These species include both spring and winter forms, whose vernalization requirements can be distinguished. Seeds of both species contain more than 40% oil and 35-40% protein in their meal (Raymer, 2002).

Brassica campestris belongs to the famous family Cruciferae, also known as the mustard family. The cultivation of Brassica campestris in South Asia has been used for cooking purposes since approximately 4000 BC (Snowdon, 2007). However, in Europe, it has been cultivated since the 13th century for lighting lamps and as a lubricating agent for engines in the 19th century (Downey & Robbelen, 1989). Its commercial cultivation began in 1942 in Canada and was used as a lubricant in warships (Colton, 1999). Rapeseed (Brassica spp.) is included among first-generation oilseed crops used for fuel, such as soybean (Glycine max) and sunflower (*Helianthus annuus*) (Sindelar et al., 2017). It can replace fossil fuels, which are nonrenewable and environmentally harmful. Brassica oil contains Erucic acid, and erucamide is derived from it, which is used in the plastic industry as a slippery agent. Brassica campestris is a short-duration crop compared to other Brassica species. It is also used as an aphid trap around wheat fields. Farmers prefer to grow it due to its short growing period, high oil content, and good yield. In a comparative analysis, Brassica rapa var. toria demonstrated superior oil quality in various traits compared to *B. nigra* (Kaur et al., 2021). The study of genetic behavior for enhancing yield and yield-contributing traits is crucial in oilseed brassicas. Therefore, current studies were carried out, and generations F1, BC1, BC2, F2, and F3 were developed to produce breeding material for high-yielding, canola-type varieties.

Materials and Methods

The research material consisted of four parental lines of Brassica rapa. Two of these have low erucic acid, while the other two contain high erucic acid but are high-yielding. These four parents were crossed as listed below.

Cross-1

1. F_1 (Span (Canola type) × Toria (Non canola type)
2. BC_1 (F_1 × Span)
3. BC_2 (F_1 × Toria)

The same crossing pattern was followed for each cross separately, i.e.

Cross-2

1. F_1 (TR-8 × Toria)
2. BC_1 (F_1 × TR-8)
3. BC_2 (F_1 × Toria)

Cross-3

1. F_1 (UAF-11 × Span)
2. BC_1 (F_1 × UAF-11)
3. BC_2 (F_1 × Span)

Cross-4′

1. F_1 (UAF-11 × TR-8)
2. BC_1 (F_1 × UAF-11)
3. BC_2 (F_1 × TR-8)

In the following season, F1 plants were grown in the field. The F1 plants were covered to control pollination, and back crosses were made for each specified cross. Seeds were harvested separately and stored for growth the following year.

Development of the generations

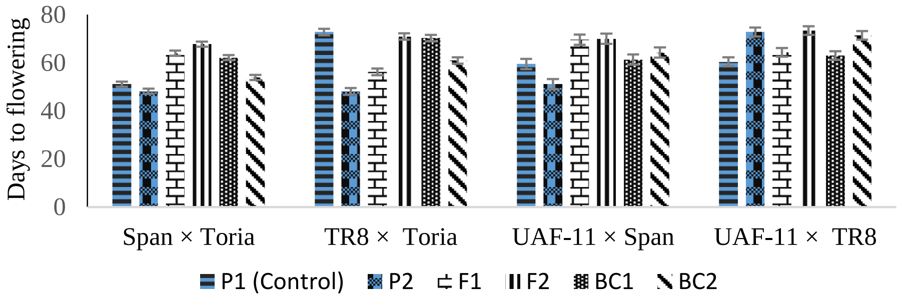
Some F_1 plants were covered during flowering to prevent cross-pollination and produce pure F_2 seeds, which were then sown in the next growing season. Similarly, the F_3 generation for each cross was planted in the following season, and seeds were harvested for subsequent use. Four parent plants along with their generations—F_1, F2, BC1, BC2, and F3—were arranged in a Randomized Complete Block Design with three replications in the field of the Department of Plant Breeding and Genetics at the University of Agriculture Faisalabad (31 ° 26 ′ S, 73 ° 06 ′ E) during 2017-18. A spacing of 15 cm between plants and 45 cm between rows was maintained. The recommended fertilizer doses and three irrigation applications were made. Different rows were designated for different generations. One row was dedicated to each parent and its respective F_1, with ten rows assigned to F_2 and F_3, and three rows to BC_1 and BC_2. Each row measured 500 cm in length. Ten plants were randomly selected and tagged according to their parent and F1 status. From each replication, 100 F2 plants, five F3 plants, and 50 plants each from BC1 and BC 2 were chosen for data collection on various traits (Pandey et al., 2013). Traits measured included days to 50% flowering, days to maturity, plant height (cm), number of branches per plant, siliqua length, seeds per siliqua, 1000 seed weight, seed yield per plant (g), oil content (%), protein content (% in seedcake), oleic acid (%), linolenic acid (%), linoleic acid (%), erucic acid (%), and glucosinolate contents (% in seedcake). The data were statistically analyzed to evaluate genetic variability (Steel, 1997). All biochemical traits were measured using Foss NIRS Systems 6500 near-infrared reflectance spectroscopy (Foss NIRS Systems Inc.). Sample scanning was performed on a monochromator equipped with an auto-sample changer. A standard ring cup filled with 5 g of seed was used for each sample. Reflectance spectra ranging from 400 to 2500 nm (log I/R) were recorded at 2 nm intervals per sample. Calibration and validation procedures were conducted using ISI software, version Ia.I (Infra soft International) (Batten, 1998).

Results

Data regarding economic attributes were recorded and then subjected to biometric analysis to determine genetic variation among traits. In current research, parents and their related generations exhibited different behaviors in all four crosses studied. The magnitude and direction of all statistical parameters were found to be different for all four crosses and their subsequent generations. Results revealed the presence of significant variation in breeding material used and developed after hybridization and backcrossing.

The average performance of morpho-phenological traits of different generations, along with their parents, is presented in Figs. 1 to 15. In Span × Toria and TR8 × Toria, P_2 (Toria) showed the shortest days to 50% flowering (48.09 days). The range was 48.09 days to 67.75 days. F_2 showed the longest days to 50% flowering (67.75 days) in Span × Toria. In TR8 × Toria, the range of days to 50% flowering starts from 48.09 days to 72.78 days. P_1

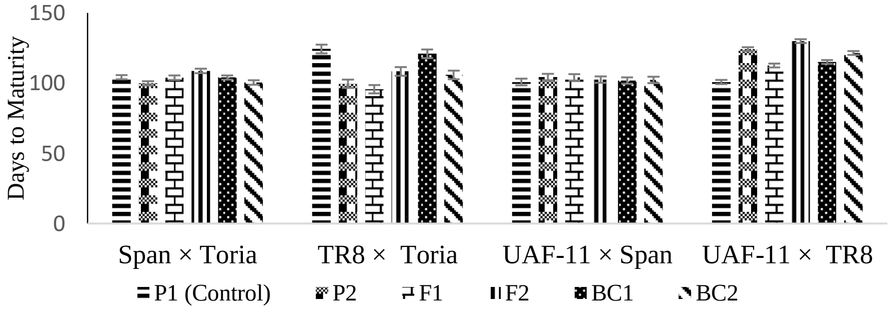
(TR8) in the TR8 × Toria cross showed the longest days to 50% flowering. Among the parents and generations of cross UAF-11 × Span, parent-2 (span) had the shortest days to 50% flowering (51.13 days), followed by P_1 (UAF-11) having 59.47 days. The longest days to flowering (69.98 days) were observed in F_2. The range of 50% flowering was from 51.13 days to 69.98 days. In UAF-11 × TR8, the shortest days to 50% flowering were shown by parent-1 (UAF-11), 60.37 days, followed by BC1 (62.96 days), and the range was 60.37 to 73.40 days (Fig. 1).



I.Cd1= 1.06, Cd2 =1.51, II.Cd1= 1.36, Cd2 =1.94, III. Cd1= 1.06, Cd2 =3.03, IV. Cd1= 1.83, Cd2 =2.60

Figure 1. Days to 50% flowering of six generations in four crosses

In Span × Toria, days to maturity range from 99.87 days for Toria to 108.73 days for F2. P2 (Toria) showed shorter days to mature than P1 (Span). In TR8 × Toria, P2 (Toria) had the shortest days to maturity (99.87 days), while P1 (Span) had the longest (124.33 days). P1 (UAF-11) in the UAF-11 × Span cross showed the shortest days to maturity (100.77 days), followed by BC2 (102.30 days), with the longest being F2 and P2 (104.02 days). In UAF-11 × TR8, the range was from 100.77 days for UAF-11 to 129.94 days for F2 (Fig. .2).

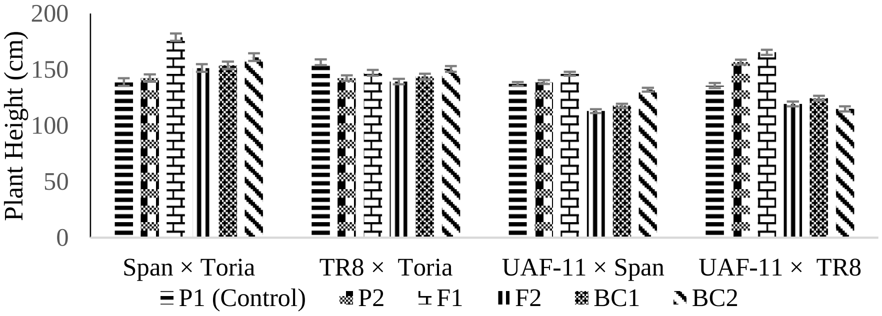


I.Cd1= 1.36, Cd2 =1.94, II.Cd1= 3.04, Cd2 =4.32, III.Cd1= 2.30, Cd2 =3.28, IV.Cd1= 1.43, Cd2 =2.04

Figure 2. Days to maturity for six generations in four crosses

Toria, which was P2 in the Span × Toria cross, had the shortest height of 142.45 cm, and the longest was found in F_1 (179 cm). 139.29 cm plant height was seen by F_2 in cross TR8

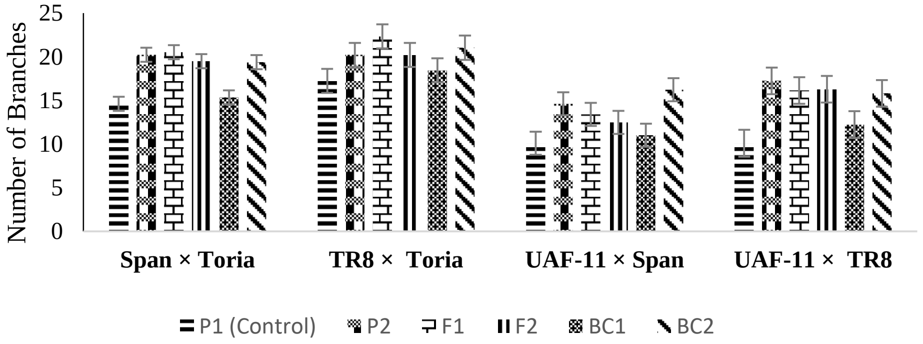
× Toria and longest by P_1 (156.67 cm). The shortest plant was found in F_2 (113.01 cm), and the tallest was in F_1 generation (146.27 cm) in UAF-11 × Span. In cross UAF-11 × TR8 (119.30 cm) height was shown by F_2 and tallest by F_1 (165.47 cm) **(**Fig. 3).



I.Cd1= 3.33, Cd2 =4.73, II.Cd1= 2.55, Cd2 =3.63, III.Cd1= 1.68, Cd2 =2.38, IV.Cd1= 2.24, Cd2 =3.18

Figure 3. Plant height for six generations in four crosses

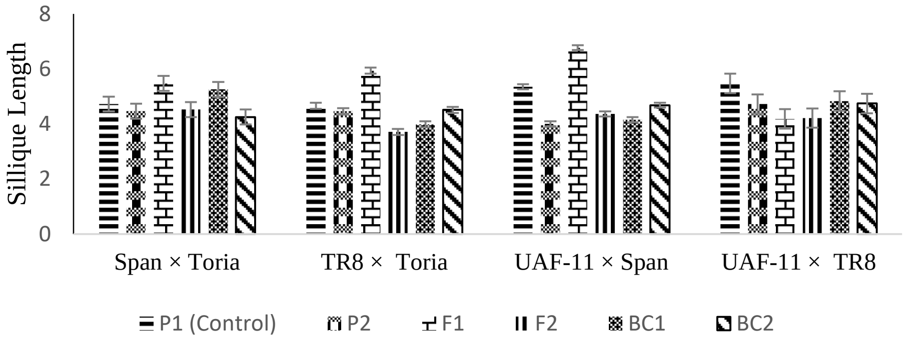
The highest number of branches was observed in P2 (Span) (14.6) of the cross UAF-11 × Span, followed by F1 (13.38). In the UAF-11 × TR8 cross, P2 (TR-8) exhibited the most branches (17.23), while BC1 had the fewest (12.21). The parent Toria in the crosses Span × Toria and TR8 × Toria showed the highest number of branches (20.21) (Fig. 4).



I.Cd1= 0.81, Cd2 =4.73, II.Cd1= 1.38, Cd2 =1.96, III.Cd1= 1.32, Cd2 =1.89, IV.Cd1= 1.53, Cd2 =2.17

Figure 4. Number of branches for six generations in four crosses

In cross Span × Toria, the parent Span had a siliqua length of 4.72 cm, followed by BC1 at 5.25 cm. In TR8 × Toria, siliqua length ranged from 3.71 cm to 5.93 cm. The longest siliqua among the parents was observed in P1 (UAF-11) in UAF-11 × Span (5.35 cm), and among the offspring, it was observed in F1 (6.77 cm). This was the longest siliqua observed in all generations and parents of the four crosses studied. In UAF-11 × TR8, P1 (UAF-11) had the longest siliqua length at 5.47 cm, followed by BC1 (4.83 cm) (Fig. 5).



I.Cd1= 0.27, Cd2 =0.38, II.Cd1= 0.11, Cd2 =0.16, III.Cd1= 0.09, Cd2 =0.12, IV.Cd1= 0.35, Cd2 =0.49

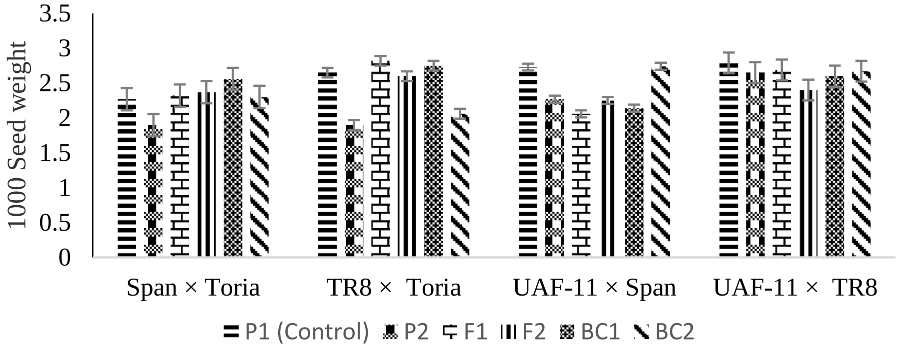
Figure 5. Siliquae length for six generations in four crosses

Fig. 6 shows that the range of the number of seeds per siliqua varies from 10.04 to 25.39 across four crosses. Among the F1 generations, TR8 × Toria had the highest number of seeds per siliqua (25.39), followed by UAF-11 × TR8 (22.86). P1 (UAF-11) in the crosses UAF-11 × Span and UAF-11 × TR8 remained the top parent for the number of seeds per siliqua (21.25, 22.54). The lowest number of seeds per siliqua was observed in P2 (Span) in UAF-11 × Span (10.94) and P2 (TR8) in UAF-11 × TR8 (10.04).

I.Cd1 = 0.21, Cd2 = 0.30; II.Cd1 = 0.32, Cd2 = 0.46; III.Cd1 = 0.61, Cd2 = 0.87; IV.Cd1 = 0.52, Cd2 = 0.74.

Figure 6. Number of seeds per siliqua for six generations in four crosses

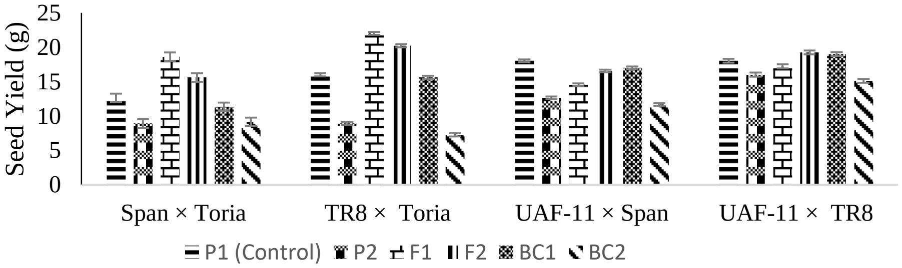
Overall, four crosses were studied. P1 UAF-11 found the highest 1000 seed weight among parents. 2.79 g in cross UAF-11 × TR8, and among generation F1 (2.82 g) of TR8 × Toria, followed by BC_2 (2.74 g) of cross UAF-11 × TR8. The range was found to be from 1.90 g to 2.82 g (Fig. 7).



I.Cd1= 0.16, Cd2 =0.23, II.Cd1= 0.07, Cd2 =0.09, III.Cd1= 0.05, Cd2 =0.07, IV.Cd1= 0.15, Cd2 =0.22

Figure 7. 1000 seed weight for six generations in four crosses

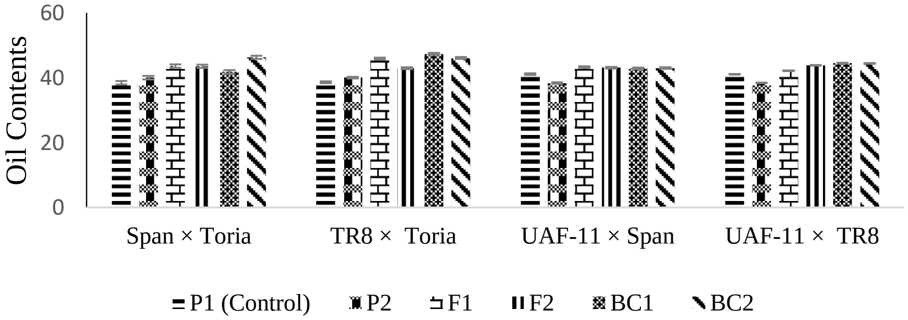
In cross Span × Toria, F_1 showed the best yielding generation (18.61 g), followed by F_2 (15.61 g). F_1 of TR8 × Toria cross showed the highest yield (22.02 g). TR8 × Toria showed the lowest yield by BC2 (7.25 g) and the highest by F_1 (20.02 g). P_1 (UAF-11) showed the highest yielder parent (18.03 g) among parents in all four crosses, followed by TR-8 (16.01 g). In the cross UAF-11 × Span, among generations, BC1 showed the highest yield per plant (17.03 g). The yield range was found to be from 12.65 g to 18.03 g in UAF-11 × Span. In cross UAF-11 × TR8, F_2 stood at the highest rank for seed yield per plant by holding 19.25 g yield, followed by BC_1 (19.01 g) (Fig.8).



I.Cd1= 0.62, Cd2 =0.89, II.Cd1= 0.23, Cd2 =0.33, III.Cd1= 0.18, Cd2 =0.25, IV.Cd1= 0.29, Cd2 =0.42

Figure 8. Seed yield for six generations in four crosses

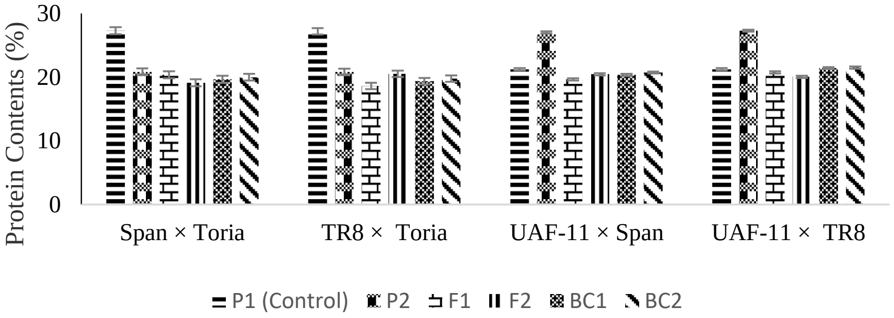
In Span × Toria among parents, P2 (Toria) showed 40.01% oil contents, while among generations, BC2 showed the highest amount of oil contents (46.27%). In TR8 × Toria, BC_1 exhibited the highest oil content (47.35%). It is the highest amount of oil content obtained from the four crosses studied, followed by BC_1 (46.06%). Among the parents, UAF-11 showed high oil contents (41.12%), and among generations, F_1 showed 43.23% and F_2 (43.09%) of oil contents in UAF-11 × Span. BC_1 and BC_2 have approximately the same amount of oil content and are slightly lower than F_1 and F_2. In UAF-11 × TR8, UAF-11 showed (41.04%) oil contents, and among generations BC1, the highest oil contents (44.49%) followed by BC_2 (44.42%) (Fig.9).



I.Cd1= 0.53, Cd2 =0.76, II.Cd1= 0.29, Cd2 =0.42, III.Cd1= 0.24, Cd2 =0.35, IV.Cd1= 0.09, Cd2 =0.12

Figure 9. Oil contents for six generations in four crosses

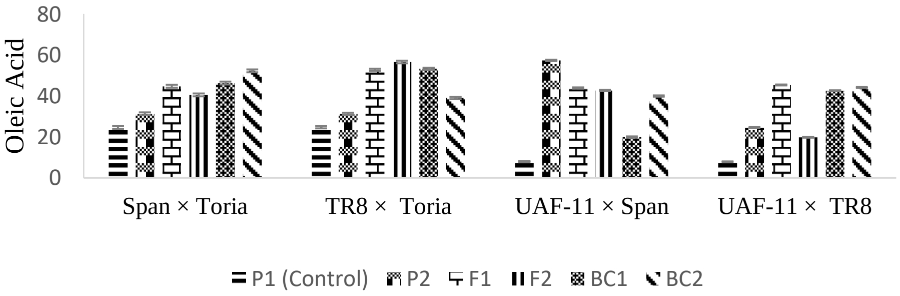
P_2 (TR-8) showed a high protein content (27.27%), and among generations, BC2 showed the highest protein content at 21.49%. In Span × Toria and TR8 × Toria generations, protein contents could not exceed their parents. Among the parents of UAF-11 × Span, P2 (Span) showed a higher protein content (26.98%) than P1, and their offspring showed nearly the same amount of protein content (20%), which was less than either of the parents (Fig. 10).



I.Cd1= 0.54, Cd2 =0.77, II.Cd1= 0.50, Cd2 =0.71, III.Cd1= 0.17, Cd2 =0.24, IV.Cd1= 0.17, Cd2 =0.22

Figure 10. Protein contents for six generations in four crosses

The highest amount of oleic acid among the generations was found in F2 (56.55%) for TR8 × Toria, followed by BC2 (52.28%) in Span × Toria. In Span × Toria and TR8 × Toria, both parents had medium oleic acid levels, and their respective generations showed the highest oleic acid content: 56.55% in F2, 53.19% in BC1, and 52.70% in F1 of TR8 × Toria. In UAF-11 × Span, P1 (UAF-11) exhibited a very low oleic acid level (7.72%), while parent-2 (Span) showed the highest content (57.36%), with their generations ranging from 19.93% to 43.87% oleic acid. In UAF-11 × TR8, one parent had the lowest level (7.72%), whereas P2 (TR8) had a medium level (24.43%), and the generations ranged from 19.71% in F2 to 45.33% in F1 (Fig.11).

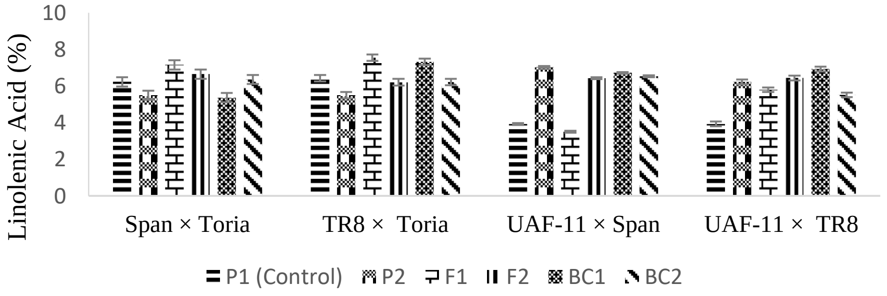


I.Cd1= 0.69, Cd2 =0.99, II.Cd1= 0.56, Cd2 =0.79, III.Cd1= 0.31, Cd2 =0.44, IV.Cd1= 0.23, Cd2 =0.33

Fig. .11 Oleic acid for six generations in four crosses

In cross Span × Toria, F1 showed the highest amount of linolenic acid (7.16%), followed by F2 (6.65%). Cross TR8 × Toria had the highest amount in F1 (7.55%), followed by BC1 (7.32%). The amount of linolenic acid was lowest in P1 (UAF-11) at 3.93%, while P2 (Span) showed the highest amount at 7.04%, observed in the cross UAF-11 × Span.

Their subsequent generations showed 6.72% in BC1 and 6.54% in BC2, with F1 having the lowest amount. This low amount is desirable. The same pattern was observed in the cross UAF-11 × TR8 (Fig.12).

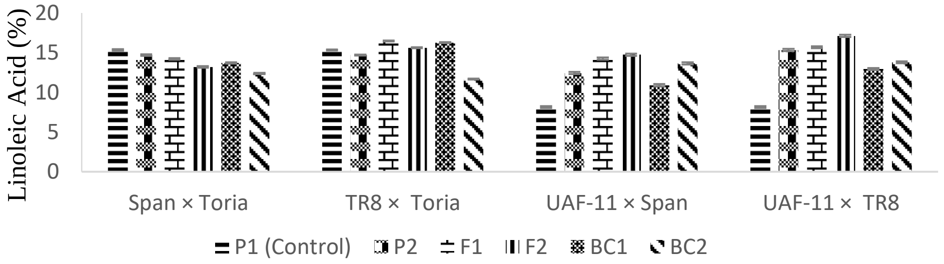


I.Cd1= 0.25, Cd2 =0.35, II.Cd1= 0.18, Cd2 =0.25, III.Cd1= 0.05, Cd2 =0.07, IV.Cd1= 0.13, Cd2 =0.18

Figure 12. Linolenic acid for six generations in four crosses

In Span × Toria, both parents had approximately the same amount of linoleic acid, but their generations could not surpass their parents. In TR8 × Toria, F1 showed the highest amount of linoleic acid (16.17), followed by BC1 (16.27) and F2 (15.03). Among the parents, P2 (Span) exhibited a relatively higher amount of linoleic acid (12.44) than P1 (UAF-11), which had (8.15) from the cross UAF-11 × Span. Among the generations of UAF-11 × Span, F2 showed the highest amount (14.75), followed by F1 (14.26). The F2 value indicated maximum segregation toward a higher amount, as it exceeds the better parent.

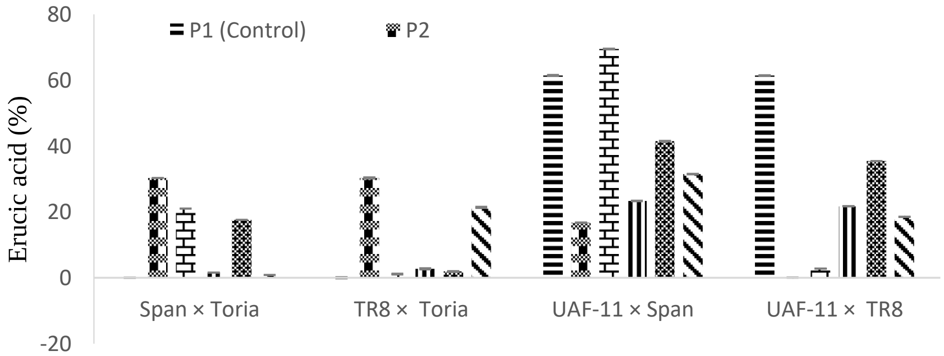
A high amount of linoleic acid is desirable. BC2 also showed a high level of this trait, indicating that P2 (Span) contributed more to BC2's better performance. Analysis of variance results revealed significant variability in this trait. Several researchers, including Khan et al. (2003), Rahman et al. (2009), and Abideen et al. (2013), have reported variations in this trait. In UAF-11 × TR8, similar results were observed as in UAF-11 × Span. P2 (TR8) had a higher linoleic acid content (15.35) compared to P1 (UAF-11) (8.15). Consequently, F2 had the highest amount (17.12), followed by F1 (15.69). BC2 exhibited a slightly higher amount than BC1 (Fig. 13).



I.Cd1= 0.09, Cd2 =0.14, II.Cd1= 0.06, Cd2 =0.08, III.Cd1= 0.14, Cd2 =0.19, IV.Cd1= 0.13, Cd2 =0.18

Figure 13. Linoleic acid for six generations in four crosses

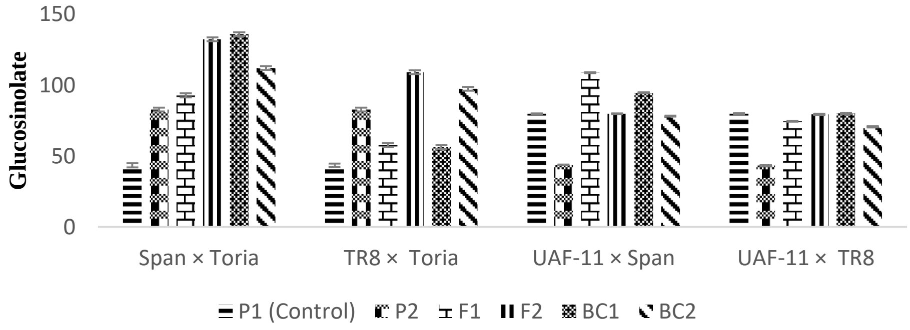
In Span × Toria and TR8 × Toria, Toria was a common parent and had a higher amount of erucic acid (30.33) compared to P1 (Span) in crosses of Span × Toria and TR8 × Toria. In Span × Toria, the lowest erucic acid content was observed in BC2 (0.92%), followed by F2 (1.52%). This cross produced very valuable results because breeders need plants with low erucic acid content. The lower-value parent (Span) contributed more than P1. Excellent results were obtained from the cross between TR8 and Toria. P1 (TR8) had a low amount of erucic acid (0.02), and its progeny showed low erucic acid levels. The lowest amount was found in F1 (1.13), followed by BC1 (1.92), and F2 (2.73). BC2 results indicated that P2 (Toria) contributed more in this specific generation, while BC1 showed that P1 (TR8) contributed more. The highest erucic acid content was found in UAF-11 (61.47), which was a common parent in the first two crosses. This high-erucic acid parent was crossed with a low-erucic acid parent (Span). However, none of the generations showed low erucic acid levels in UAF-11 × Span, suggesting that P1 (UAF-11) contributed more than the other parent. The lowest erucic acid value was observed in the F2 generation (23.35), followed by BC2 (31.48). The situation was slightly different in UAF-11 × TR8. The F1 showed the lowest erucic acid amount (2.74) among the generations, but it increased again in BC1 (35.45), while BC2 showed a medium level of erucic acid (18.55). These results reveal not only variability in the breeding material but also the presence of low erucic acid levels, which could be utilized to develop low-erucic acid varieties. The findings of Iqbal et al. (2003), Rahman et al. (2009), and Abideen et al. (2013) support these results (Fig.14).



I.Cd1= 3.36 E-14, Cd2 =4.78 E-14, II.Cd1= 0.17, Cd2 =0.24, III.Cd1= 0.11, Cd2 =0.16, IV.Cd1= 0.05, Cd2 =0.07

Figure 14. Erucic acid (%) for six generations in four crosses

In Span × Toria and TR8 × Toria, the parent (Toria) showed a higher amount of glucosinolate content (82.68) than the other parents, and among generations, its range was found to be 92.72 to 135.92. TR8 × Toria had a better situation regarding glucosinolate contents, but could not achieve as low an amount as recommended. The range was found to be 56.31 to 109.06. UAF-11 showed a high amount of glucosinolate (79.81) among the parents of UAF-11 × Span and UAF-11 × TR8. F_1 showed the highest amount (108.74), and the lowest was shown by BC_2 (78.13) in UAF-11 × Span. In UAF-11 × TR8, P_2 (TR8) had a low amount (43.37) as compared to parents, while in generations, it was found in the range of (70.57) in BC_2 to (8.57) in BC_1. Results revealed that P_1 (UAF-11) made the most significant contribution among the others (Fig. 15).



I.Cd1= 1.39, Cd2 =1.98, II.Cd1= 1.31, Cd2 =1.86, III.Cd1= 0.28, Cd2 =0.39, IV.Cd1= 0.38, Cd2 =0.55

Figure 15. Glucosinolate for six generations in four crosses

Comparison of current mean results with reported values

The plant height of Brassica campestris is relatively short compared to other species, and UAF-11 falls under the dwarf variety category among the *B. campestris* group. It matures earlier and is resistant to lodging. Europe has a very long season from sowing brassica to harvesting, due to snow, so tall plants with long maturation periods were found there. In Pakistan, the growing season of brassica is comparatively shorter than that of European countries due to high temperatures in April and May, and brassica completes its lifecycle before the hard season. Therefore, short or dwarf plants are the best suited to our environment. The higher the height of a plant, the longer it will take to mature. Shorter plants will mature earlier than taller plants. Table 1 shows the range of current results of plants from 91 to 175 cm. It had an excellent range, encompassing dwarf, semi-dwarf, and tall plants, in line with the reported range. Very tall plants were not present in the genetic material developed, and selection for dwarf and semi-dwarf plants was relatively easy due to the high variability present in an extensive range of plants.

Good variability was found in the number of branches, which ranged from 8 to 24. The plants developed had neither too many scant branches nor too many thick branches. Internationally reported numbers of branches in Brassica ranged from 4 to 32. A diverse range of plants was available for use in the following program. Siliqua length is one of the essential morphological traits in Brassica. The range of variability in the present research was found between 4 cm and 7 cm.

Table 1. Comparison of current mean results of morpho-phenological traits with reported values

	Character	**Current Results**	**Reported range results**	**References**
Mo rp	PH (cm)	91-175	124-142	(Aytaç and Kınacı 2009)

			120 - 135	(Aytaç and Kınacı 2009)
			180 - 250	(Lodhi et al. 2016)
			179 - 214	(Yadava et al. 2011)
			179 - 240	(Yadav et al. 2021)
	BRA	10.09 - 22.23	5.6 - 6.9	(Aytaç and Kınacı 2009)
			5.2 - 6.9	(Aytaç and Kınacı 2009)
			10.1 - 32.1	(Lodhi et al. 2016)
			12.4 - 20.6	(Yadava et al. 2011)
			4.3-11.2	(Kumar et al. 2013)
	SL (cm)	3.71 - 6.94	6.78-7.98	(Aytaç and Kınacı 2009)
			6.67 - 7.62	(Aytaç and Kınacı 2009)
			3.4 - 6.1	(Lodhi et al. 2016)
			3.23 - 4.71	(Yadava et al. 2011)
			4.66-6.17	(Kumar et al. 2013)
			4.08 - 6.22	(Yadav et al. 2021)
	DOF (days)	48 - 73	53 -44	(Lodhi et al. 2016)
			46 - 62	(Yadava et al. 2011)
			44 - 61	(Yadav et al. 2021)
			64 -72	(Bahadur et al. 2021)
	DOM (days)	96 - 130	146 - 141	(Lodhi et al. 2016)
			133 - 148	(Yadava et al. 2011)
			122 - 139	(Yadav et al. 2021)
			102 - 112	(Bahadur et al. 2021)

PH stands for plant height, BRA for total number of branches, SL for siliquae length, DOF for days to 50%flowering, and DOM for days to maturity.

The number of seeds per siliqua showed the best range, i.e., 8 to 24, with an upper limit slightly greater than the reported values of 9-22. The 1000-seed weight had a current range from 1.001 g to 4.001 g. However, researchers reported its range from 1.84 g to 6.7 g. Sometimes, when the number of seeds increased per siliqua, its size was reduced, ultimately decreasing the 1000-seed weight. The present research material also showed a good range of seed yield per plant, i.e., 9.0 g to 22.0 g for *B. campestris*. *B. campestris* has a lower yield potential than B. napus and B. juncea; however, it has a significant advantage because it matures earlier than both *B. napus* and *B. juncea*. That could fit in a growing pattern easily, and growers can also cultivate wheat well in time (Table 2).

Table 2. Comparison of current mean results of yield-related traits with reported values

	Character	**Current results**	**Reported range results**	**References**
Yield-related traits	SS	8 - 24	11 - 22	(Lodhi et al. 2016)
			11 – 16	(Yadava et al. 2011)
			9-15	(Kumar et al. 2013)
			13- 17	(Yadav et al. 2021)
	TSW (g)	1.001-4.001	3.83 - 5.05	(Aytaç and Kınacı 2009)
			3.67 - 4.40	(Aytaç and Kınacı 2009)
			3.5 - 6.7	(Lodhi et al. 2016)
			3.32-5.79	(Yadava et al. 2011)
			1.84-3.79	(Kumar et al. 2013)
			2.18- 5.66	(Yadav et al. 2021)
	SY (g)	8.91-22.0	277-389.5 kg/ha	(Aytaç and Kınacı 2009)
			202.3 - 312.3 kg/ha	(Aytaç and Kınacı 2009)
			14- 40	(Lodhi et al. 2016)
			15–33	(Yadava et al. 2011)
			7 – 20	(Kumar et al. 2013)
			09 – 18	(Yadav et al., 2021)
			5 – 13	(Bahadur et al. 2021)

SS stands for the number of seeds per silique, TSW for 1000-seed weight, and SY for seed yield per plant.

Oil content showed a wide range of variability, from 37.0% to 51.65%, whereas the reported oil content ranged from 35.37% to 48%. Although high protein content in seedcake is desirable for the poultry feed industry, the observed variation was from 17.89% to 27.99%, compared to the reported range of 18% to 24%. This could attract poultry feed company owners and ultimately benefit the government. High levels of oleic acid are desirable for human health, and the developed material contained a high amount of oleic acid, ranging from 7.03% to 61.53%, while researchers reported levels from 9% to 54%. Low levels of linolenic acid are also preferred, with variation in this study from 3% to 8%, compared to the reported range of 6% to 13%, making selection for low linolenic acid feasible. A significant variation was observed for erucic acid, ranging from 0.009% to 70.86%. Low erucic acid is desirable for human consumption, while high amounts are proper for industrial purposes. The current materials exhibited both low and high ranges, making them suitable for direct use or in breeding programs and industrial applications. Canola-type characteristics were not previously identified in B. campestris in Pakistan. The breeding material developed in this research exhibits canola-type traits in B. campestris. Developing double low varieties involves reducing the content of erucic acid and glucosinolate. The recommended glucosinolate level is less than 30 µmoles per free oil meal. The variability range for glucosinolate was from 7.42% to 139%, providing a good opportunity to reduce glucosinolate levels across a wide range. In contrast, other researchers reported ranges from 32% to 55% (Table 3).

Table 3. Comparison of current mean results of biochemical (quality) traits with reported values

	Character	**Current results**	**Reported range results**	**References**
Biochemical (Quality) traits	Oil content (%)	37.3-51.65	38.32-42.78	(Aytaç and Kınacı 2009)
			37.03 - 41.92	(Aytaç and Kınacı 2009)
			36.8 -43.2	(Lodhi et al. 2016)
			44 – 48	(Azam et al. 2013)
			35.37- 39.41	(Kumar et al. 2013)
			36.06 - 41.71	(Yadav et al., 2021)
			41 – 45	(Bahadur et al. 2021)
	Protein content (%)	17.89-27.99	20.83-22.70	(Aytaç and Kınacı 2009)
			18.27 - 20.17	(Aytaç and Kınacı 2009)
			23-24	(Azam et al. 2013)
	Oleic acid (%)	7.03-61.53	51-54	(Azam et al. 2013)
			9.01-14.36	(Kumar et al. 2013)
	Linolenic acid (%)	3.02-8.42	9-10	(Azam et al. 2013)
			6.49-12.92	(Kumar et al. 2013)
	Linoleic acid (%)	8.01-21.48	14.39-20.14	(Kumar et al. 2013)
	Erucic acid (%)	0.009-70.86	32-42	(Azam et al. 2013)
			36.19-54.85	(Kumar et al. 2013)

	Gluco-sinolate (%)	7.42-138.92	50-66	(Azam et al. 2013)

Selection of plants with double low characteristics continued in the F3 generation.

Table 4 presents data on yield and biochemical traits of F3 plants from four crosses under study. Most of the plants listed in this table not only yielded better results but also exhibited good biochemical characteristics beneficial for human consumption. The range of seed yield of these plants was from 741 kg/ acre to 1441 kg/ acre across four crosses. Eleven plants showed erucic acid < 1% which was an improvement. Six plants had the highest percentage of oil content, i.e., 49.64% to 51.65%. Two plants had glucosinolates within the required limits. The highest amount of oleic acid (56.33%) was observed in the F3 plant of the cross TR8 × Toria. Plant number 2 of F_3 from the cross TR8 × Toria yielded the highest, at 1441 kg/acre, and also contained erucic acid at 0.77% and glucosinolate at 15.77%, with an oil content of 51.65%. Consequently, these plants may be used either directly or indirectly as in a breeding program for the development of high-yielding as well as double-low varieties.

Table 4. Seed yield and biochemical traits of F_3 plants of four crosses

Cross	**Sr. No**	**Yield**			**Oil content (%)**	**Oleic Acid (%)**	**Erucic Acid (%)**	**Gluco-sinolate**
		g / plant	Kg / ac	mond/ acre				
Span ×Toria	P-1	12.51	741.33	18.53	47.05	36.97	**0.76**	97.73
	P-2	14.23	843.26	21.08	**50.28**	33.42	26.05	74.45
	P-3	12.56	744.30	18.61	**45.73**	**49.04**	**0.44**	86.21
	P-4	13.45	797.04	19.93	44.65	39.49	**0.53**	**7.42**
	P-5	15.81	936.89	23.42	**50.05**	34.01	16.8	83.95
	P-6	13.33	789.93	19.75	**49.49**	47.47	**1.17**	91.42
TR8× Toria	P-1	20.08	1189.93	29.75	**49.88**	28.05	37.47	88.77
	P-2	**24.32**	**1441.19**	**36.03**	**51.65**	**44.29**	**0.77**	**15.77**

	P-3	19.00	1125.93	28.15	**49.64**	26.85	39.6	58.58
	P-4	21.09	1249.78	31.24	47.43	**55.20**	**1.17**	71.26
	P-5	20.56	1218.37	30.46	48.84	**56.33**	1.93	85.35
UAF-11× Span	P-1	13.11	776.89	19.42	38.46	43.62	**0.7**	94.1
	P-2	16.59	983.11	24.58	46.99	27.83	30.48	99.08
	P-3	14.21	842.07	21.05	36.3	33.99	**0.21**	120.72
	P-4	16.75	992.59	24.81	43.39	38.44	**0.78**	143.77
	P-5	15.23	902.52	22.56	41.03	43.57	**0.66**	107.48
	P-6	16.75	992.59	24.81	43.06	**48.97**	2.07	100.27
	P-7	15.98	946.96	23.67	41.47	36.28	**0.45**	82.13
UAF-11× TR8	P-1	16.25	962.96	24.07	37.72	**53.28**	**0.76**	97.08
	P-2	18.41	1090.96	27.27	43.9	37.68	**0.59**	102.33
	P-3	17.55	1040.00	26.00	39.73	31.66	25.02	92.57
	P-4	16.39	971.26	24.28	44.43	25.9	35.35	96.18
	P-5	17.21	1019.85	25.50	47.06	35.69	23.54	84.82

Conclusions

The cross TR8 × Toria had low Erucic acid contents along with good seed yield which may be further subjected to pure line selection. Trangressive segregants from his cross may be used for the development of canola version cultivars in future breeding programs.

Competing Interests

The authors declare that the research was conducted without any commercial or financial relationships that could be perceived as a potential conflict of interest.

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