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Utilization of Agro-morphology and Near-Infrared Spectroscopy (NIRS) for examining genotypic diversity and seed quality characteristics of various *Brassica juncea* cultivars

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Abstract

The study, conducted on twenty five genotypes of *Brassica juncea* L. in the Agricultural Research Institute (ARI) at Mingora, Swat, Khyber Pakhtunkhwa, between October 2022 and May 2023. The overarching objective was to provide valuable insights crucial for breeding initiatives, cultivation optimization, and enhancing the economic and nutritional values associated with *Brassica juncea*. The mean values analysis exhibited the maximum yield of 3522.2 kg/ha in genotype 108. The outcomes revealed substantial diversity in traits associated with germination, flowering, pod formation, and maturity, as well as notable variations in plant characteristics such as height, population, and 1000-seed weight. In this comprehensive investigation, the utilization of Near-Infrared Spectroscopy (NIRS) served as a powerful and non-invasive tool for examining genotypic diversity and seed quality characteristics within various *Brassica juncea* cultivars. The highest values calculated in a genotype 108 for seed oil percentage (49.99%), protein (36.3%) and oleic acid (57.66%). The maximum age (%) of moisture (8.3%) and least content of glucosinolate (74.66%) was exhibited by 2745. Linolenic acid and erucic acid were observed in highest amounts (18.77% and 24.33%) in 2740 and 114 genotypes, respectively. Genotype-specific analyses highlighted other cultivars with higher linolenic acid percentages were 855 and 106. Statistical analyses, including correlation coefficients and factor loadings, were employed to discern relationships among these traits. These findings bear practical implications, offering guidance for cultivar selection tailored to diverse local environments based on factors such as high yield and short duration. The research briefly delves into the genetic background of *Brassica juncea*, emphasizing its diploid and amphiploid nature, and acknowledges the reference genome assembly for *Brassica juncea*. In conclusion, this study makes a substantial contribution to the agricultural community by providing insights crucial for cultivar selection to improve both economic and nutritional outcomes.

KEYWORDS

Genotypic diversity, NIR Spectroscopy, Agro morphology, Seed quality, Cultivation optimization, *Brassica juncea*.

1.0 INTRODUCTION

Brassica juncea L., commonly known as brown mustard, stands as a versatile member of the *Brassicaceae*, cultivated globally for its manifold applications in vegetables, oilseed, and condiments. The evolutionary saga of *Brassica*, predominantly rooted in the Mediterranean-Middle Eastern region, underscores its substantial genetic diversity, with notable diploid and amphidiploid species contributing to its genetic intricacy [1, 2, 3]. This diversity finds further expression in the genetic variations unearthed in recent genomic studies, highlighting the imperative of comprehending the genetic constitution of *Brassica* *juncea* L. for efficacious breeding programs [4, 5]. Pakistan, with its fertile agricultural terrain, proudly showcases the cultivation of eight *Brassica* species, with rapeseed and mustard collectively constituting the second-largest source of oil after cotton [6]. Nonetheless, the rapeseed production in per-unit area in Indo-Pakistan lags behind developed nations, necessitating strategic enhancements in cultivation practices to satiate the soaring demand for edible oils [7]. The economic significance of the oilseed sector in Pakistan, a sector contributing significantly to employment and curbing edible oil imports, underscores the urgency of amplifying cultivation and augmenting production efficiency [8, 9, 10, and 11]. Canola, a genetically refined variety of rapeseed, holds promise in meeting the escalating demand for edible oils, particularly in regions with favorable agro-climatic conditions like Northern Pakistan [12]. Nevertheless, the accessibility of genetically modified seeds remains a hurdle, requiring an exhaustive understanding of the Canola breeding and genetics values for effective improvement programs. The double challenge of enhancing industrial utility while ensuring food safety in *Brassicaceae* oil seeds is tackled through innovative approaches. The low glucosinolates and erucic acid contents of Canola oil emerge as an alternative favored in the cooking industry. However, elevated erucic acid levels, coupled with glucosinolates, pose health risks, propelling researchers to explore methods to amplify industrial utility while diminishing these substances for food safety. Recent studies have delved into novel techniques to evaluate plant physiology and seed traits. The researchers delved into the utility of NIR(Near Infrared) spectroscopy, a very sound method for evaluating acetolactate synthase (ALS) activity and protein content in rapeseed leaves [13,14]. The immense potential of Near-Infrared Reflectance Spectroscopy (NIRS) in critical small fluctuation in the seed protein amount in plants, revolutionizing promising agriculture by offering unprecedented vision into plant physiology with crop management strategies [15]. In conclusion, the genetic diversity of *Brassica juncea* L. presents both challenges and opportunities in meeting the escalating demand for edible oils in Pakistan and beyond. Innovative approaches, coupled with advanced spectroscopic techniques like NIRS, hold the key to enhancing agricultural productivity and sustainability in *Brassicaceae* oilseed cultivation. This review endeavors to illuminate the significance of genetic variation and advanced spectroscopic techniques in shaping the future of precision agriculture in *Brassica juncea* L.

2. MATERIALS AND METHODS

The experimental investigation was carried out upon Twenty-five carefully selected *Brassica juncea* genotypes at the Agricultural Research Institute (ARI) in Mingora, Swat, Khyber Pakhtunkhwa, in the interval of October 2022 to May 2023. Employing a Randomized Complete Block Design (RCBD) with three replications, the experiment was established using all agricultural practices, including soil examination.

2.1 Data Collection for Quantitative Characteristics

The quantitative data were collected for characteristics such as germination days, days to 50% flowering, seed per pod, maturity, pods per plant, 1000-seed weight, and gram yield per hectare using the given formula.

The Seeds Yield calculated by using the formula given by [12]:

$$\text{Seeds Yield} = \frac{\text{Seeds Yield X } 10000 \text{ m}^2}{\text{Area } (9 \text{ m}^2)}$$

2.2 Data Collection for Qualitative Characteristics

Qualitative characteristics were observed for growth habit, plant color, leaf margin, leaf color, petal color, and seed color. These characteristics were systematically recorded across all genotypes and replications.

2.3 Biochemical Composition

Biochemical composition analysis included parameters such as protein, oleic acid, oil percentage, linolenic acid, glucosinolates (GSL), moisture, and erucic acid. Near-Infrared Reflectance Spectroscopy (NIRS) at the Nuclear Institute for Food and Agriculture (NIFA) was utilized for non-destructive chemical analysis.

2.4 Statistical Analysis

The statistical analysis of collected data was conducted through ANOVA (Analysis of Variance), a suitable procedure for RCBD (Randomized complete block design). The means were compared by the least significant difference (LSD) test at a significance level of P ≤0.05.

3. RESULTS AND DISCUSSION

Through Microsoft Office Excel worksheets, both qualitative and quantitative traits record of *Brassica juncea* twenty five cultivars were tested. The results are exhibited and explained as follows:

3.1 Agromorphology

3.1.1 Days to 50% Germination

The longest germination period was observed in 2745 and 2759, a maximum of 7 days. The accession numbers, 120, 119, 117, 116, 114, 112, 111, 108, and 107, showed the fastest sprouting, sprouting in just 4 days. Statistical analysis revealed a least significant difference (LSD) of 1.6212 for days to 50% germination, with a significance level of α = 0.05, as shown in Table 1.

3.1.2 Days to 50 % Flowering

Line 2738 showed maximum days (142.67) to have 50 percent (%) flowering, while 108 exhibited minimum days (106) to attain the same age (%), followed by genotypes 114, 116, and 120, which took 108 and 109 days, respectively. The LSD value for 50% flowering was calculated, 3.9055 at the level of significance (α- 0.05) as indicated in Table 1.

3.1.3 Days to Maturity

Accessions 2745 and 2740 took the maximum time to mature, requiring 200.33 days. In contrast, accessions 108, 111, 107, 114, and others matured significantly faster, taking around 182-184 days. Specifically, accessions 108 and 111 matured in 182.33 days, accession 114 in 182.67 days, and accessions 107 in 183.00 and 183.67 days showed maturity. The LSD was 1.9267 (α = 0.05), as shown in Table 1**.**

3.1.4 Plant Height (cm)

The observed means range from 84.67 cm to 144 cm. Genotype 854 stood out with the tallest plants, reaching 144 cm, whereas genotype 108 had the shortest plants at 84.67 cm. Statistical analysis showed a least significant difference (LSD) of 2.5350 for plant height at α = 0.05, as shown in Table 1.

3.1.5 Plant Population

Regarding Plant population, accession 2748 presented the highest range in plant population of 45.667, and accession 2734 presented 45.333. Contrary to these, accession 802 had the lowest plant population of 19, followed by genotype 117 with 19.667. The observed LSD value for the parameter was 2.4128 at a significance level (α) of 0.05 (Table 1).

Table 1. Mean values of ANOVA and LSD test for phenotypic traits of

Genotypes	Days to 50% Germination	Days to 50% Flowering	Days to maturity	Plant Height (cm)	Plant Population
106	5	110.67	193	109.33	30.333
107	4	110	183.67	85.33	21.667
108	4	106.33	182.33	84.67	30.333
111	4	129	183	87.33	41
112	4	115.33	184	93	34.333
114	4	108.67	182.67	98.67	31.667
116	4	109	184	85.33	34
117	4	130.33	184.33	114	19.667
119	4	115.67	184.67	90	40.333
120	4	109.67	183	97	30
801	5	111	193	116.67	30.333
802	5	115.33	192.33	132.33	19
854	5	125	192.33	144	24.333
855	6	116	199.67	118.67	29
860	6	131	198.33	104	28.333
2731	6	142.67	197	104.33	33.333
2734	6	128.33	199.67	119	45.333
2739	6	128.33	199	116.67	23.333
2740	6	128	200.33	111	25.333
2743	6	127	199	101.33	29.667
2745	7	118.67	200	106	26
2748	6	116	198	96.33	45.667
2759	7	113	194.33	118.67	31
2764	6	113	194.33	108.67	27
2765	5	123.33	194.33	119.67	30
LSD Value	**1.6212**	**3.9055**	**1.9267**	**2.535**	**2.4128**

LSD= least significant difference

3.1.6 Number of pods plant^{-1}

The most pods per plant (227.67) were found in genotype 801, whilst the fewest pods per plant (50.33), were found in genotype 117. Table 2 shows the least significant difference (LSD) value for the number of pods per plant, which was determined to be 11.495 at a significance level (α) of 0.05.

3.1.7 Number of seeds pod^{-1}

The genotype with the most seeds per pod (19) was 2765, whereas the genotype with the fewest seeds per pod (8) was 854. According to table 2, the number of seeds per pod's least significant difference (LSD) value was determined to be 2.2804 at a significance level (α) of 0.05.

3.1.8 1000 seed weight (gm)

The genotype 854 showed the lowest weight of 1000 seeds at 2.8 gm, while the maximum weight of 1000 seeds was recorded at 4.8 gm for 108. Table 2 shows the least significant difference (LSD) value for the weight of 1000 seeds, which was determined to be 0.1651 at a significance level (α) of 0.05.

3.1.9 Seed Yield (ha^{-1})

The average values range from 3522.2 kg to 1077.1 kg. The highest seed yield, 3522.2 kg/ha, was shown by genotype 108, and the lowest, 1077.1 kg/ha, was shown by genotype 2748. According to table 2 and figure 1, the least significant difference (LSD) value for seed yields in kg/ha was 16.221 at a significance level (α) of 0.05.

Table 2. Mean Values of ANOVA, LSD test for Quantitative traits

Genotypes	Pods/Plant	Seeds/Pod	1000Seeds weight	SeedsYield kg/hectare
106	134	15	4.7	1610.2
107	130.33	9	3.8	2155.2
108	136.67	16.667	4.8	3522.2
111	104.67	13.667	4.1	3050.2
112	149.33	12.667	4	2855.2
114	171	12.667	4	2210.2
115	164.67	16	4.2	2655.1
117	50.33	13.333	4.4	1985.3
119	92.67	17	3.8	2844.4
120	98.67	15.333	4.1	2933.3
801	227.67	13	4.2	2122.2
802	160	10	3.9	2644.4
854	95.67	8.333	2.8	2522.2
855	140.67	14.333	3.2	1844.4
860	196	15.333	4.1	1144.4
2731	140.33	15.667	4.4	1933.3
2734	111.67	10.333	3.1	1322.2
2739	120.67	13	3.6	2044.4
2740	156	10.333	4.1	1455.5
2743	128.67	12.667	3.7	3112.2
2745	54.33	9.333	3.5	1721.2
2748	78.33	14	4.3	1077.1
2759	115	13.333	4.4	1583.7
2764	139.67	17.333	4.1	1985.3
2765	111.67	19	3.9	2021.2
LSD Value	**11.495**	**2.2804**	**0.1651**	**16.221**

LSD (least significant difference)

3.2 NIR Spectroscopy

3.2.1 Seeds oil percentage (%)

Significant variations across the genotypes were found by the statistical analysis of the oil content (Fig. 3, table 3). Oil percentages for genotypes 801, 2740, and 2764 were 45.997, 45.971, and 45.373, respectively, while genotype 108 had the highest proportion at 49.99%. The genotype 2731, on the other hand, showed the lowest oil proportion (33.35%).

3.2.2 Protein proportion of seeds (%)

There are notable variations, according to the protein content statistical study. At 36.3%, genotype 108 had the highest protein content (Table 3, figure 2, 3).

3.2.3 Seed glucosinolates (mole/gm)

Significant variations between genotypes are revealed by the statistical analysis of glucosinolates shown in figure 3. Glucosinolate levels were highest in genotype 116 (107.6 units) and lowest in genotype 2745 (74.66 units) (Table 3, figure 2 & 3).

3.2.4 Seed moisture percentage (%)

According to Table 3, genotype 855 had the lowest moisture level (7.4%), while 2745 had the greatest moisture content (8.3%).

Table 3. Comparative Biochemical Analysis of *Brassica juncea* Genotypes

Genotype	Seed Oil (%)	Seed Protein (%)	Seed Glucosinolates (Mole/Gm)	Seed Moisture (%)	Seed Oleic Acid (%)	Seed Linolenic Acid (%)	Seed Erucic Acid (%)
106	38.14	21.8	95.36	7.967	44.777	11.00	32.333
107	39.33	24.9	94.00	7.200	45.333	9.83	37.000
108	49.99	36.3	93.66	7.933	57.667	9.00	35.667
111	38.20	24.2	90.33	7.800	40.777	12.33	30.667
112	36.55	23.7	99.71	5.900	55.000	9.66	32.333
114	38.65	22.4	100.3	7.400	44.000	8.33	24.333
116	38.22	23.7	107.6	7.567	46.000	9.33	39.000
117	38.99	21.8	97.66	7.433	40.667	9.77	35.000
119	37.77	23.6	89.97	7.967	46.000	8.56	30.333
120	38.24	22.1	95.07	7.333	45.667	8.66	39.66
801	45.99	20.8	95.35	7.777	45.667	8.20	38.000
802	44.40	22.7	93.46	7.667	45.333	8.10	34.333
854	38.55	25.9	103.6	7.667	47.667	7.667	36.667
855	38.40	27.1	104.0	7.400	43.000	11.00	37.000
860	37.40	25.8	102.9	5.667	44.667	8.30	32.667
2731	33.35	19.5	96.23	7.667	43.000	8.66	39.667
2734	39.27	26.2	86.56	7.777	41.000	9.33	32.333
2739	44.41	31.1	86.46	7.867	46.000	8.56	39.500
2740	45.97	31.9	79.77	7.500	37.333	18.777	43.200
2743	40.81	27.6	82.33	8.100	40.667	10.77	31.667
2745	41.27	27.5	74.66	8.333	41.667	10.00	31.667
2748	37.44	23.2	89.00	8.300	39.333	10.33	28.333
2759	39.77	26.8	88.67	8.000	41.000	10.00	38.000
2764	45.37	31.4	88.77	7.277	39.333	9.66	38.000
2765	39.88	25.9	86.00	7.000	44.000	10.000	39.000

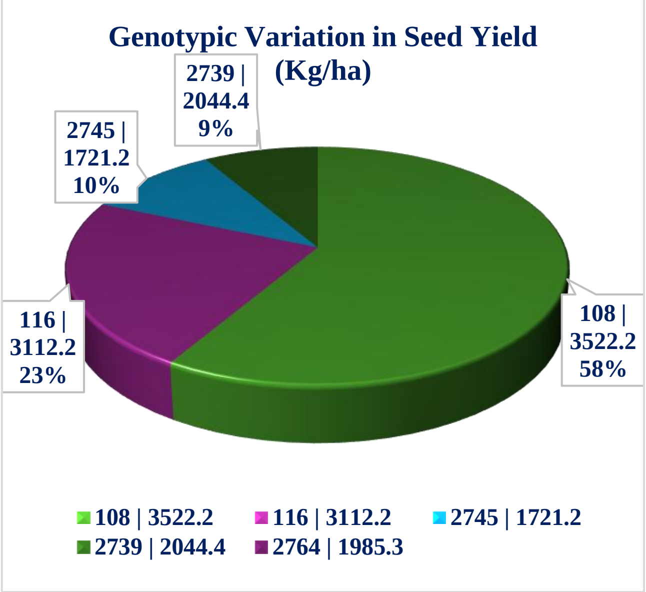

Figure 1. Genotypic variation in seed yield (Kg/ha) among different genotypes.

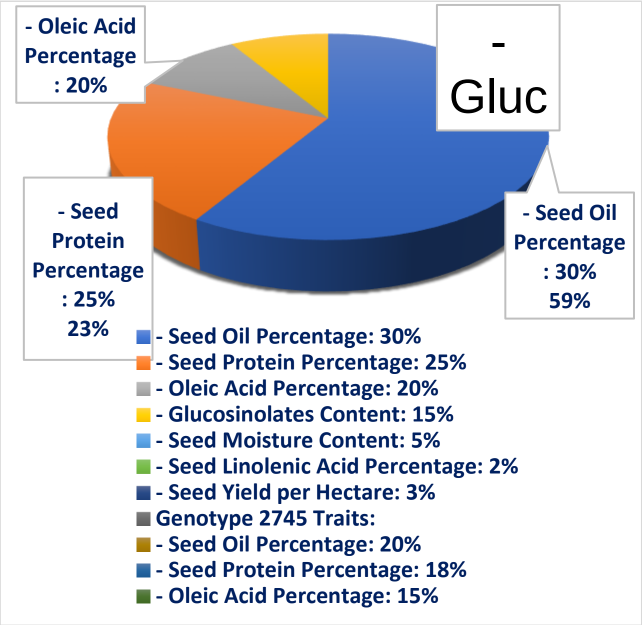

Figure 2. Chemical composition representation of Genotypes 108, 2745 and 854

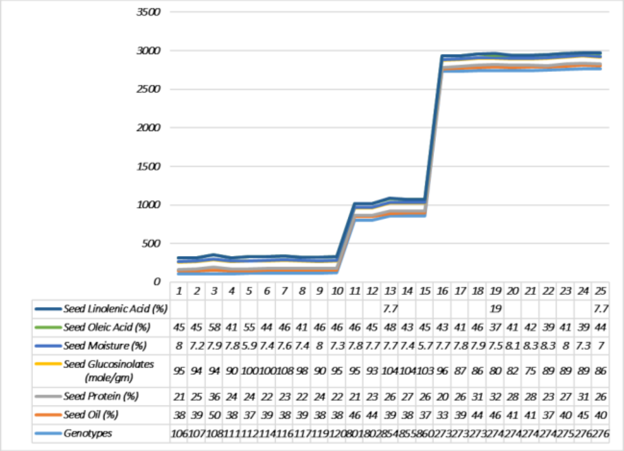

Figure 3. Multifactorial Analysis of *Brassica Juncea* Genotypes: Elevating Seed Quality and Yield through Trait Valuation

3.3 Correlation Coefficient

Days to Flowering (DTF) and Days to Germination (DTG) have a positive connection ($r = 0.55$, $p < 0.05$). Likewise, there was a significant positive association between Plant Population and Seed Yield (GY) ($r = 0.784$, $p < 0.01$). There is a significant inverse relationship between oil content and glucosinolates ($r = -0.631$, $p < 0.01$). There was a favorable connection between erucic acid and seed yield ($r = 0.349$, $p < 0.05$). Additionally, there is a high positive link between oil and protein concentration ($r = 0.849$, $p < 0.01$). Table 4 shows a substantial positive connection ($r = 0.865$, $p < 0.01$) between Oil Content and Days to Flowering.

Table 4. Comprehensive Multivariate Analysis of *Brassica juncea* Genotypes: Traits, Relationships, and Yield Optimization

	Genotype	Rep	DTG	DTF	DPF	Mat	PH	SPP	Sw	PPP	PP	GY	EA	GL	Oil	Prot	Mos	OL	LIN
Genotype	1																		
Rep	6.18E+00	1																	
DTG	0.326	0.55	1																
DTF	-0.124	0.035	0.174	1															
DPF	-0.378	-0.033	0.612	0.171	1														
Mat	-0.157	0.062	0.504	0.211	0.087	1													
PH	0.357	0.016	0.331	0.125	0.281	0.063	1												
SPP	0.221	0.033	0.231	0.059	-0.539	0.173	0.055	1											
Sw	0.153	0.439	0.646	0.442	0.019	0.272	0.236	0.077	1										
PPP	-0.139	0.052	0.366	0.033	0.831	0.138	0.253	0.139	0.232	1									
Plant population	0.135	0.046	0.784	0.557	0.031	0.114	0.305	0.026	0.076	0.077	1								
yeild in gram	-0.476	0.156	0.821	0.225	-0.205	0.165	0.151	0.347	0.298	0.043	0.196	1							
Erucic acid	0.289	0.349	0.355	0.173	-0.235	0.245	0.144	0.379	0.165	0.144	0.074	0.249	1						
Glucosinolates	0.634	-0.552	0.096	0.183	-0.011	0.034	0.058	0.012	0.171	0.067	0.631	0.106	0.638	1					
Oil	0.048	0.865	0.195	0.069	0.499	0.334	0.297	0.149	0.217	0.165	0.149	0.068	0.863	0.135	1				
Prot	-0.185	0.177	0.849	0.107	-0.075	0.048	0.245	0.024	0.125	0.124	0.325	0.178	0.021	0.173	0.178	1			
Mos	0.622	0.161	0.313	0.319	0.317	0.135	0.065	0.146	0.096	0.086	0.164	0.052	0.148	0.574	0.222	0.334	1		
OL	-0.521	-0.791	0.226	0.179	0.241	0.145	0.087	0.051	0.072	0.176	0.182	0.125	0.117	0.493	0.117	0.235	0.432	1	
LIN	0.187	0.021	0.148	0.019	-0.219	0.083	0.367	0.164	0.151	0.424	0.234	0.459	0.337	0.389	0.065	0.099	0.262	0.513	1

The exhaustive investigation into *Brassica juncea* L. genotypes has uncovered substantial variations in germination traits, flowering dynamics, pod formation, days to maturity, plant morphology, and yield components. These findings underscore the pivotal role of genetic diversity and its profound impact on crop management decisions. Our results align seamlessly with prior studies [16], emphatically emphasizing the genetic factors' crucial influence on various agronomic traits. Days to 50% germination, a pivotal phase in crop establishment, exhibited remarkable variations among *Brassica juncea* genotypes, accentuating the inherent genetic diversity and advocating for meticulous genotype selection to optimize overall crop performance. This resonates with [16] emphasis on genetic diversity in germination traits, reaffirming its relevance within the context of *Brassica juncea*. Flowering time stands as a critical factor shaping crop adaptation strategies, and our study identified significant variations in days to 50% flowering. The observed genetic disparities in flowering time underscore the imperative of a nuanced understanding of these traits for effective crop management decisions. Our findings harmonize with previous research [17,18,19], robustly highlighting the enduring significance of genetic diversity in the traits related to flowering. Days to 50% pod formation in *Brassica juncea* genotypes offered invaluable insights into crop development and potential yield outcomes. The observed variations underscore the pivotal role of genetic diversity in inducing the duration of pod formation [19, 20, 21]. Optimal pod formation is indispensable for overall yield, emphasizing the need to select genotypes with favorable pod characteristics is crucial. Divergent days to maturity among different genotypes underscored significant disparities, underscoring the importance of comprehending and managing maturity timelines for effective crop planning and harvest management [22, 23]. The number of pods per plant and the number of seeds per pod exhibited significant variations among various genotypes, offering valuable insights into potential seed yield outcomes. These findings echo studies emphasizing the consistent pattern of significant variability in these traits. The factors affecting yield, such as increased pod density, early planting date, and environmental conditions, underscore the intricate interplay of genetic and external factors. These discoveries improve our knowledge of the factors that influence *Brassica juncea* seed output, which helps to guide breeding plans and farming methods. Genetic diversity linked to important seed quality metrics has been revealed through the use of Near Infrared Spectroscopy (NIRS) in the study of *Brassica juncea* genotypes. The effectiveness of NIRS in providing accurate quantitative assessments of oil content, protein content, glucosinolates content, moisture content, and fatty acid profiles underscores its potential for guiding breeding programs and cultivation practices [24, 25]. These findings align emphatically with previous studies by others, emphasizing NIRS's pivotal role in optimizing economic returns for farmers through the identification of cultivars with superior traits. These findings contribute to a holistic perspective on the genetic diversity in *Brassica juncea*, offering valuable insights for targeted breeding and cultivation practices.

Erucic Acid and Seed Yield ($r = 0.349$, $p < 0.05$), Oil Content and Protein Content ($r = 0.849$, $p < 0.01$), Seed Yield (GY) and Plant Population ($r = 0.784$, $p < 0.01$), Days to Germination (DTG) and Days to Flowering (DTF) all show positive correlations ($r = 0.55$, $p < 0.05$). Intriguing research questions are raised by *Brassica juncea's* inverse link between oil accumulation and developmental time period.

2.0 CONCLUSION

NIRS plays a vital role in advancing crop research and sustainable farming practices, as demonstrated by our findings to prioritize Genotype 2740 for agricultural endeavors due to its exceptional linolenic acid content (18.777%), offering potential health benefits and increased profitability through seed oil production. The Genotypes 855 and 106 as secondary options, with slightly lower linolenic acid content (11.00%), yet still valuable for cultivation and breeding programs, contributing to the diverse genetic portfolio of *Brassica juncea* for optimized agricultural outcomes.

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CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

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