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Identification of High Erucic Acid *Brassica carinata* Genotypes through Multi-Trait Stability Index

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Abstract: *Brassica carinata* is an important and native oilseed crop in Ethiopia. The seed oil from *B.carinata* attracts global attention for its various industrial applications, mainly due to its high erucic acid levels and its superior agronomic traits. Since the demand for high erucic acid from oilseed brassica has been increasing in the world market due to its wider applications in bio-industries, the breeding target of *B. carinata* has recently been focused on enhancing its erucic acid. Several high erucic acid *B. carinata* genotypes have been screened from the pre-breeding activities. Such genotypes, however, need to be tested for their stable performance, for their erucic acid level, and other desirable traits under different environments. The aim of this study was to identify high erucic acid *B. carinata* genotypes with stable performance in multiple desirable traits. Thirty-two *B. carinata* genotypes were grown in a randomized complete block design with three replications at three locations for two years. The genotypes were evaluated for nine desirable traits related to seed oil quality (erucic acid and oil content), seed yield, and other agronomic traits. The results showed that the proportion of genotype by environment interaction (GEI) was clearly observed in erucic acid, which led to a stability and mean performance analysis for selecting the most stable and best-performing genotypes for the desired traits. For such an analysis, we used the multi-trait stability index (MTSI) along with the weighted average of absolute score BLUPs (WAASB). As revealed from the MTSI, five genotypes (G13, G18, G10, G22 and G5) were identified as the most stable in erucic acid, oil content, seed yield, and other agronomic traits. The selected genotypes showed on average 45.7% erucic acid, 3185 kg ha^{-1} seed yield and 45.1% oil content with 4.3%, 25.8% and 6.9% positive selection gain, respectively. The negative selection gain of phenological traits and the plant height of the selected genotypes revealed their early maturity and their lower probability of being affected by lodging. Our findings demonstrated MTSI can be used to select high erucic acid *B. carinata* with a set of desirable traits, which would facilitate breeding efforts in developing novel and high erucic acid *B. carinata* varieties. Our results also showed that MTSI is an effective tool for selecting genotypes across different environments due to its unique ability to select multiple traits simultaneously.

Keywords: *Brassica carinata*; erucic acid; genotype by environment interaction; multi-trait stability index

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1. Introduction

Brassica carinata (carinata), also called Ethiopian mustard, is an amphidiploid (BBCC, 2*n* = 34) oilseed crop that evolved through natural hybridization between the diploid species *B. nigra* (BB, 2*n* = 16) and *B. oleracea* (CC, 2*n* = 18). The crop is well known, and adapted to the highlands of Ethiopia, where its cultivation is believed to date back to the 4th to 5th millennia BC [1]. Currently, carinata is expanding to other countries, including

Canada, India, Australia, Spain and the United States, due to its potential for biofuel and other industrial applications [2]. The most important trait that makes carinata attractive in the world bio-industry market is the presence of high levels of a very long-chain fatty acid, erucic acid (C22:1), in its seed oil [2]. Vegetable oils with high erucic acid (HEA) are potentially used as lubricants, surfactants, fabric and textile softeners, cosmetics, and heat transfer fluids [3,4]. To date, many research initiatives have emerged in carinata for its huge potential as an alternative source of renewable energy, such as biofuel or jet fuel, due to its high erucic acid and other attractive oil profiles [5].

In Ethiopia, carinata oil is traditionally used for cooking or as a spice in the preparation of local food [6]. The carinata oil, however, is not recommended to be used as an edible oil due to the anti-nutritional effects of its higher erucic acid levels, ranging from 39 to 43% [7], which is far beyond the recommended level (<5%) [8,9]. Although research efforts have been made for developing carinata with low erucic acid [10,11], the extent and progress has been limited compared to rapeseed, one of the major oilseed crops in the world used as an edible oil. However, the development of carinata varieties with HEA has become a major breeding goal in recent years, since the current global market demand for oilseed with HEA for bio-industrial feedstock is growing [12].

The evaluation of natural accessions and the available breeding materials would be the first important step for the screening of potential HEA lines in oilseed brassica, including carinata. It is well known that the EA content is affected by both genotype and the environmental conditions where the crop grows, as well as the genotype by environment interactions (GEIs). Many of the previous studies on GEIs in oilseed brassica, including carinata, have focused on quantitative traits, such as seed yield-related traits and oil content [13–20]. One of the crucial reasons for the unstable performance of genotypes in EA is the high impact of GEI under variable environmental stress conditions, such as drought, diseases, and other factors [21]. In order to exploit the GEI effect on EA production in oilseed brassica, systematic studies should be conducted considering all possible environmental variations. This is due to the fact that understanding the effect of GEIs is vital to select relatively stable genotypes for desirable traits, including erucic acid [22]. There are different methods adopted by plant breeders for measuring stability, which in turn depend on how the breeders understood the concept of stability [23].

Selection and stability analyses in multi-environmental trials are usually made by targeting only one trait or univariate, commonly seed yield. Such a univariate selection has a drawback, in that it might ignore other important traits or cause the selection of other undesirable traits. It is thus necessary to use a multivariate selection method which enables the selection of genotypes with a set of desirable traits simultaneously [24]. Apart from the common linear–bilinear model, such as the additive main effects and multiplicative interaction (AMMI), the selection and stability indices suited for the simultaneous selection of the mean performance (MPE) of desirable traits have been developed in a linear mixed model (LMM) [25]. Among the recently released selection and stability indices, the weighted average of absolute scores BLUPs (WAASB), along with the multi-trait stability index (MTSI), are becoming the most popular ones [24,26].

In order to enhance EA production in carinata, breeders at Holetta Agricultural Research Center of Ethiopia have focused on developing new carinata breeding lines with HEA. Very recently, a number of advanced breeding lines of carinata were identified and screened for HEA (>40%), along with their better performance in agronomic traits [7]. We hypothesized that those selected genotypes with HEA will not show a stable mean performance due to GEIs that might occur while growing them under various environments. The selected genotypes thus need to be further evaluated in different growing environments for their stability in the desired traits. The objective of this study was to identify HEA genotypes with a stable mean performance for important desirable traits using the multi-trait stability index. This study also demonstrated the potential of MTSI for the selection of the stable and best-performing carinata genotypes grown under different environments.

2. Materials and Methods

2.1. Plant Materials and Field Experiment

In this study, 32 genotypes of carinata (*B. carinata*) were used as plant material (Table S1). These genotypes were selected from advanced breeding lines with erucic acid > 40%, as determined by the fatty acid profile analysis conducted in our previous study [7].

The field trials were conducted in 2021 and 2022 across three locations in Ethiopia, at Holetta (2400 masl, 9°06′ N, 38°50′ E) in the West Shewa zone, Kulumsa (2200 masl, 08°01′ N, 39°16′ E) in the Arsi zone, and Adet (2240 masl, 11°17′ N, 37°28′ E) in the West Gojam zone, where carinata production is predominant (Figure 1). The climatic data of the testing sites, such as precipitation (RF in mm) and temperature (maximum and minimum in °C) on a monthly basis, is presented in Supplementary Table S2. A complete randomized block design was used at each testing location, with three replications. Each plot had a size of 9 m^2, with six rows of 30 cm inter-row spacing and 5 m length. A seed rate of 5 kg/ha was used, based on standard agronomic practices [27]. Phenological data, such as the number of days to reach 50% flowering at the plot blooming stage (DF) and date of maturity (DM), when 95–100% plots reached at a stage of being ready to harvest, were recorded. Seed yield (SYD) and agronomic traits, such as plant height (PH), the number of primary branches (NPB), the number of pods per plant (NPP) and thousand seed weight (TSW), were recorded from the central four rows of the plots.

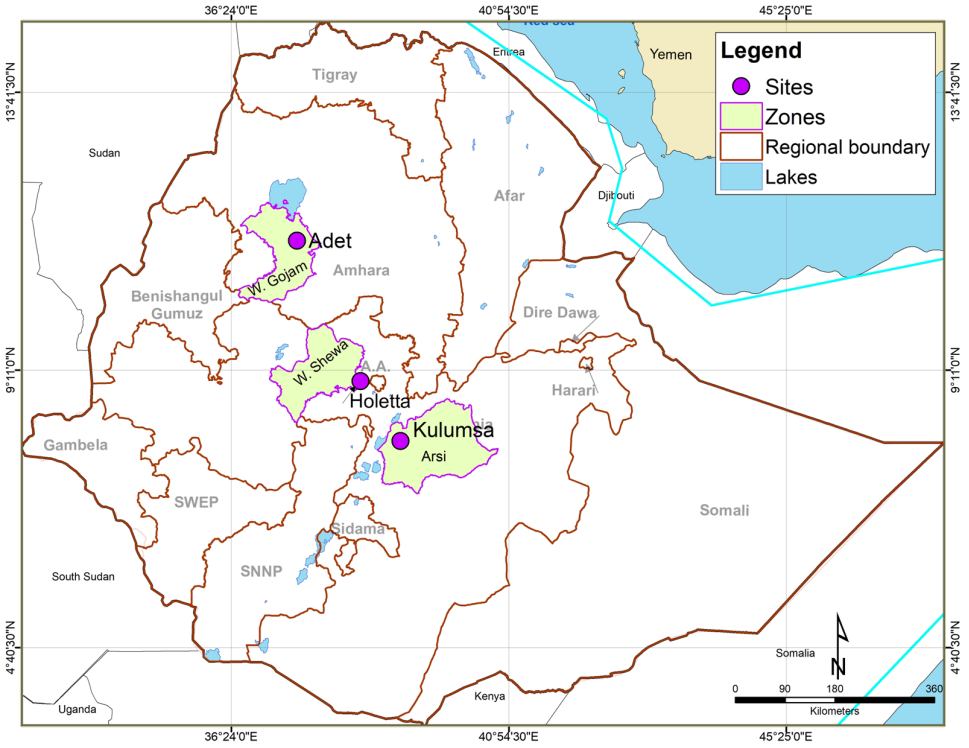

Figure 1. The geographical position of field testing site of 32 carinata genotypes.

2.2. Determination of Oil Content and Erucic Acid

Oil content (OC) was determined by Nuclear Magnetic Resonance Spectroscopy (Newport Analyzer Ltd., Buckinghamshir, UK), following the method described by Madson [28] with minor modifications. Prior to analyzing the experimental samples, the seed oil of carinata with a known oil content was extracted by solvent extraction for the adjustment of the NMR. About 22–24 g of the carinata seeds from each genotype across locations were prepared in triplicates and oven-dried at 130 °C for 2 h. The dried seeds were allowed to cool for 30 min. The instrument was run by adjusting the radio frequency (RF) at a current of 225 µA, and an audio frequency (AF) gain of 400 with a gate width of 1.5 gauss. The

seed samples were placed in NMR sample holder tubes, and oil content was read directly for each triplicate of each sample.

EA was measured using a near-infrared reflectance spectroscopy (NIRS) machine (TANGO FT-NIRS, Bruker, Optics GmbH, Ettlingen, Germany), with 3 g of intact seeds from each sample harvested from each location. The spectra data were converted into the percentages of erucic acid using the calibration equation developed for carinata by Tesfaye et al. [7].

2.3. Statistical Analysis

Data for all the traits were tested and verified for their normality using PROC UNIVARIATE in SAS software version 9.4. We employed the Shapiro test of normality at $p < 0.05$ significance level using the following syntax: (proc univariate data = my_data normal; var my_variable; run). A homogeneity test of variance was also conducted to pool the data for the likelihood test of the joint ANOVA.

2.3.1. Variance Component Analysis

Each trait was analyzed using the linear mixed model (LMM), where GEI was assumed to be random, while the effects of the environment and block-within environment were assumed to be fixed [26]. The LMM was performed by the function *gamem_met*() of the R package metan version 4.2.0 [29], which is explained by the following model [30]:

$$\mathbf{y} = \mathbf{X\beta} + \mathbf{Zu} + \boldsymbol{\varepsilon}$$

where $\mathbf{y}$ is an n $[=\sum_{i=1}^{e} (gb)] \times 1$ vector of response variable (e.g., EA), $\mathbf{y} = [y_{111}, y_{112}, \ldots, y_{geb}]'$; $\boldsymbol{\beta}$ is an (eb) $\times$ 1 vector of fixed effects, $\boldsymbol{\beta} = [\mu + \Upsilon_{11}, \Upsilon_{12}, \ldots, \Upsilon_{eb}]'$; $\mathbf{u}$ is an m[=g + ge] $\times$ 1 vector of random effects, $\mathbf{u} = [\alpha 1, \alpha 2, \ldots, \alpha g, (\alpha\tau)11, (\alpha\tau)12, \ldots, (\alpha\tau)ge]'$; $\mathbf{X}$ is an n $\times$ (eb) design matrix of 0s and 1s relating $\mathbf{y}$ to $\boldsymbol{\beta}$, $\mathbf{Z}$ is an n $\times$ m design matrix of 0s and 1s relating $\mathbf{y}$ to $\mathbf{u}$, and $\boldsymbol{\varepsilon}$ is an n $\times$ 1 vector of random errors $\boldsymbol{\varepsilon} = [y_{111}, y_{112}, \ldots, yg_{eb}]'$. The variance components were obtained by restricted maximum likelihood using the expectation maximization algorithm, as described by Dempster et al. [31]. The likelihood ratio test was used to test the significance of random effects by a two-tailed x^2 test with one degree of freedom. Heritability, in a broad sense on a genotype-mean basis ($\mathbf{h^2_{gm}}$), was computed as follows:

$$\mathbf{h^2_{gm}} = \boldsymbol{\sigma^2_g} / [\boldsymbol{\sigma^2_g} + \frac{\boldsymbol{\sigma^2_i}}{\mathbf{e}} + \frac{\boldsymbol{\sigma^2_e}}{(\mathbf{eb})}]$$

where $\boldsymbol{\sigma^2_g}$, $\boldsymbol{\sigma^2_i}$, and $\boldsymbol{\sigma^2_e}$ are the variances related to genotypes, GEI and residuals, respectively; **e** and b are the number of environments and blocks per environment, respectively. The analysis was performed using the function *gamem_met*() of the R package metan version 4.2.0 [29].

2.3.2. Genotypic Stability Index

The genotypic stability index of each genotype was estimated by a weighted average of absolute scores BLUPs (WAASB), from the singular decomposition of the matrix of BLUPs for the GEI effects generated by an LMM [26]:

$$\mathbf{WAASB}_i = \sum_{k=1}^{n} |\mathbf{IPCA}_{ik} \times \mathbf{EP}_x| \Big/ \sum_{k=1}^{p} \mathbf{EP}_k$$

where $\mathbf{WAASB}_i$ is the weighted average of absolute scores of the *i*th genotype (or environment); $\mathbf{IPCA}_{ik}$ is the score of the *i*th genotype (or environment) in the *k*th IPCA, and $\mathbf{EP}_x$ is the amount of the variance explained by *k*th IPCA. The most stable genotype will be the one with the lowest WAASB value, which is also the one that deviates least from the average performance across environments.

2.3.3. Simultaneous Selection for Performance and Stability

The simultaneous selection for mean performance (MPE) and stability were determined by the following equation, as described by Olivoto et al. [26].

$$\mathbf{WAASBY}_i = \frac{(\mathbf{rY_i} + \theta_\mathbf{Y}) + (\mathbf{rW_i} + \theta_\mathbf{S})}{\theta_\mathbf{Y} + \theta_\mathbf{S}}$$

where $\mathbf{WAASBY}_i$ is the simultaneous selection index for the *i*th genotype that weights between MPE and stability (WAASB); $\theta_\mathbf{Y}$ and $\theta_\mathbf{S}$ are the weights for the response trait (**Y**) and the WAASB, respectively. The weight for mean performance ($\theta_\mathbf{Y}$) was assumed to be 50 to 65%, and $\theta_\mathbf{S}$ is supposed to be $100 - \theta_\mathbf{Y}$. $\mathbf{rY_i}$ and $\mathbf{rW_i}$ are the rescaled values (0–100) for the response traits (**Y**) and WAASB, respectively. The selection goal for each trait was determined as increase or decrease based on the target of breeding (i.e., best-performing HEA genotype with better oil content, seed yield, early maturing and other desirable agronomic traits). To this end, the traits such as EA, OC, SYD and yield component variables (NPB, NPP and TSW) were considered as desired traits that need to be increased. Meanwhile, DF, DM and PH were determined as traits that need to be decreased to capture early maturing and lodging resistant genotypes.

The WAASBY index was also used for the analysis of Pearson's correlation and factor analysis, as demonstrated by Olivoto et al. [26].

2.3.4. Multi-Trait Stability Index and Genotype Selection

The stability analysis of multiple traits, considering the mean performance of variables, was carried out using the multi-trait stability index (MTSI), as described by Olivoto et al. [24].

$$\mathbf{MTSI}_i = \left[\sum_{\mathbf{j=1}}^{\mathbf{f}} (\mathbf{F_{ij}} - \mathbf{F_j})^\mathbf{2}\right]^{0.5}$$

where the $\mathbf{MTSI}_i$ is the multi-trait stability index for the *i*th genotype, $\mathbf{F_{ij}}$ is the jth score of the *i*th genotype, and $\mathbf{F_j}$ is the jth score of ideotype. MTSI was computed by the function *mtsi*() of the R package metan [29]. The genotype with the lowest MTSI is then closer to the ideotype, and therefore shows a high MPE for all analyzed variables.

2.3.5. Determination of Selection Differential

The selection differential of the genotypes subjected to the WAASY index was determined based on the assumption of 15% selection intensity. The selection differential can be expressed by a percentage (SD%), with reference to the mean performance of selected genotypes (Xs) and original population (Xo) as follows:

$$\mathbf{SD\%} = \frac{(\mathbf{Xs} - \mathbf{Xo})}{\mathbf{Xo}} \times \mathbf{100}$$

3. Results

3.1. Mean Performance and Likelihood Test

The mean performance of the genotypes for the nine traits across the six environments is presented in Table 1. The EA content of the carinata genotypes ranged from 35.5 to 49.8% with an overall mean of 43.1%. The grand mean of SYD and OC were 2490.6 kg ha^{-1} and 41.6%, respectively, and there were genotypes that scored a maximum productivity of 3986 kg ha^{-1} and oil content of 50.7%. The overall mean for maturity was 150 days, while 50% flowering was 71 days. The genotypes that matured first and last as compared to the rest of the genotypes had 127 and 166 days to maturity, respectively. The overall height of the tested genotypes ranged from 97 to 197 cm, with a grand mean of 151 cm. The likelihood test showed that the effect of genotype and GEI were highly significant for all traits at $p < 0.001$ (Table 1).

Table 1. The mean performance and likelihood ratio test results of 32 carinata genotypes grown at three locations (Holetta, Kulumsa and Adet) for two years (2021 and 2022).

Traits	Mean ± SD	Max.	Min	LRTg	LRTgxe
DF	70.88 ± 7.43	96	53	111.33 ***	579.31 ***
DM	149.95 ± 5.52	166	129	112.09 ***	263.76 ***
PH	150.54 ± 19.14	194	97	41.18 ***	1191.90 ***
NPB	8.3 ± 1.69	13	5	153.87 ***	34.03 ***
NPP	246.74 ± 44.17	370	110	50.44 ***	1058.15 ***
TSW	4.13 ± 0.54	6.0	3.0	168.72 ***	93.94 ***
SYD	2490.63 ± 580.52	3986.35	1403.47	126.6 ***	1254.8 ***
OC	41.63 ± 3.69	50.65	30.9	53.74 ***	732.59 ***
EA	43.12 ± 2.62	49.78	35.51	28.39 ***	807.51 ***

*** Significant at $p < 0.001$; Abbreviation used: DF, date of 50% flowering. DM, date of maturity. PH, plant height in cm. NPB, number of primary branch. NPP, number of pods per plant. TSW, thousand seed weight. SYD, seed yield in kg ha^{-1}. OC, oil content in %. EC, erucic acid in %. LTRg and LTRgxe, Likelihood ratio tests for genotype and GEI, respectively.

3.2. Variance Component

The proportion of phenotypic variation explained by genotype (GEN), environment, and their interaction (GEI), are shown in Figure 2. The effect of genotypic variance from the phenotypic variance component was found to be higher for SYD, TSW, NPB, DF and DM (Figure 2), and such traits also revealed a high heritability (Table 2). The proportion of GEI to phenotypic variance was dominant for the main target trait, EA, and its extent was also larger for PH, OC, and NPP (Figure 2). This implies the need to examine genotypic stability for selecting HEA genotypes with better and stable mean performance.

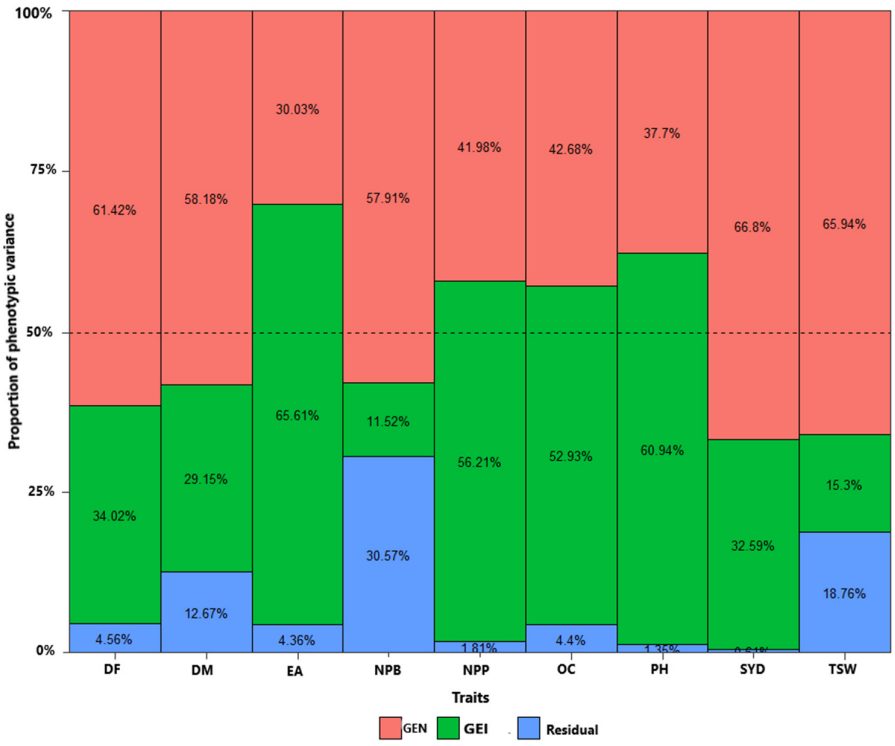

Figure 2. Proportion of phenotypic variance for nine traits of *B. carinata* genotypes grown in the six environments. DF, date of 50% flowering. DM, date of maturity. PH, plant height in cm. NPB, number of primary branches. NPP, number of pods per plant. TSW, thousand seed weight. SYD, seed yield in kg ha^{-1}. OC, oil content in %. EA, erucic acid in %. GEN, genotypic variance. GEI, genotypic by environment interaction, Residual refers to the environmental variance.

Table 2. Heritability, genetic parameters and variance components for nine traits in 32 carinata genotypes.

Genetic Parameters	Traits								
	DF	DM	PH	NPB	NPP	TSW	SYD	OC	EA
PV	52.43	17.58	318.9	2.09	1347	0.26	30,289	10.90	6.85
Heritability	0.61	0.58	0.38	0.58	0.42	0.66	0.67	0.43	0.30
$GEIr^2$	0.34	0.29	0.61	0.12	0.56	0.15	0.33	0.53	0.66
h^2mg	0.91	0.91	0.79	0.94	0.82	0.95	0.92	0.83	0.73
AS	0.96	0.96	0.89	0.97	0.90	0.97	0.96	0.90	0.85
rge	0.88	0.68	0.98	0.27	0.97	0.45	0.98	0.92	0.94
cvg	8.01	2.13	7.28	13.24	9.64	10.02	18.06	5.18	3.32
cvr	2.18	0.99	1.38	9.62	2.00	5.35	1.72	1.66	1.27
cv ratio	3.67	2.14	5.28	1.38	4.82	1.88	10.48	3.12	2.63

Note: DF, date of 50% flowering. DM, date of maturity. PH, plant height in cm. NPB, number of primary branch. NPP, number of pods per plant. TSW, thousand seed weight. SYD, seed yield in kg ha^{-1}. OC, oil content in %. EA, erucic acid in %. PV, phenotypic variance. $GEIr^2$ GEI coefficient of determination. h^2mg, heritability of genotypic mean. AS, accuracy of selection. rge, correlation among genotypic value across environment. cvg, genotypic coefficient of variation. cvr, residual coefficient of variation. cv, coefficient of variation.

The genetic parameters of the nine traits of carinata are presented in Table 2. The accuracy of the genotype selection of the nine traits ranged from 0.85 for EA to 0.97 for NPB or TSW. The coefficient of determination for the GEI effect ($GEIr^2$) was high for EA, PH, NPP and OC, implying that GEI played a major role on the phenotypic component. All the traits showed high heritability on genetic mean bases ($h^2mg > 0.60$).

3.3. Correlation and Factor Analysis

Pearson's correlation matrix revealed a high degree of positive correlation among SYD, NPP, NPB and TSW, which can be grouped as a set of common factors with more close relations to each other, i.e., a change in one trait will have a positive effect on the others (Figure 3). EA showed a weak positive association with all traits except DF, for which a weak negative correlation was exhibited.

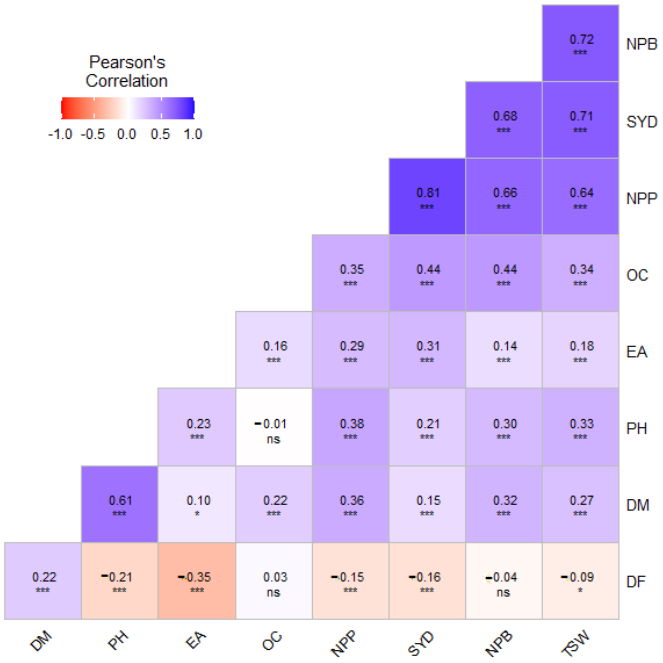

Figure 3. Pearson's correlation coefficient among the WAASBY index for nine traits of carinata tested at six environments. DF, date of flowering. DM, date of maturity. PH, plant height in cm. NPB, number of primary branch. NPP, number of pods per plant. TSW, thousand seed weight. SYD, seed yield in kg ha^{-1}. OC, oil content in % and EC, erucic acid in %. *, *** represents significance at $p = 0.05$, and 0.001, respectively and "ns" refers to non-significance.

According to the factor analysis by the WAASBY index, three principal factors were maintained (eigenvalue > 1), with the accumulated variance of 75.69% (Table 3). The eigenvalues explained variances, and their accumulated values are presented in Supplementary Table S3. The nine traits analyzed with WAASBY were grouped into three factors as follows: SYD, NPB, NPP, TSW and OC were grouped as the first factor (FA1), DM and PH were grouped as the second factor (FA2), and EA and DF were grouped as the third factor (FA3) (Table 3).

Table 3. Eigenvalues, explained variance, factorial loadings after varimax rotation, and communalities in the factor analysis.

Traits	FA1	FA2	FA3	Communality	Uniqueness
DF	−0.086	−0.412	**−0.688**	0.650	0.350
DM	0.077	**−0.921**	0.083	0.861	0.139
PH	0.091	**−0.724**	−0.388	0.684	0.316
NPB	**0.925**	−0.132	0.178	0.904	0.096
NPP	**0.633**	−0.508	−0.208	0.702	0.298
TSW	**0.912**	0.095	−0.096	0.850	0.150
SYD	**0.738**	−0.305	−0.319	0.740	0.260
OC	**0.557**	0.165	−0.555	0.645	0.355
EA	0.141	−0.046	**−0.868**	0.775	0.255
Eigenvalues	3.694	1.821	1.297		
Variance	41.04	20.23	14.41		
Accumulated, %	41.04	61.27	75.69		

DF, date of 50% flowering. DM, date of maturity. PH, plant height in cm. NPB, number of primary branch. NPP, number of pods per plant. TSW, thousand seed weight. SYD, seed yield in kg ha^{-1}. OC, oil content in %. EA, erucic acid in % and FA, the factor retained. Bold values show the traits grouped within each factor.

3.4. Multi-Trait Stability Index and Genotype Selection

According to the MTSI index, with the assumption of 15% selection intensity, the following five genotypes were selected as the most stable among the 32 genotypes for the mean performance of the desired traits: G13 (MTSI = 1.64), G18 (MTSI = 2.33), G10 (MTSI = 2.62), G22 (MTSI = 2.72), and G5 (MTSI = 3.17) (Figure 4), Supplementary Table S4. The MTSI value 3.17, where G5 was found, is taken as the cut point, as shown in the red circle in Figure 4. The genotype G8 was closer to this circle, indicating its potential in expressing the stability for the desired traits next to the above mentioned top five genotypes. In future studies, it would be interesting to investigate the performance of those genotypes that are closest to the cut point. The mean performance of each genotype, including the selected genotypes for targeted traits, is presented in Supplementary Table S5.

The selection made using MTSI analysis in Figure 4 has also enabled us to estimate the selection differential (SD) of the WAASBY index, as shown in Table 4. The mean performance of the selected genotypes (Xs) was higher than the mean performance of the original population (Xo) for all traits. For instance, the mean performance of EA for the selected genotypes (Xs) was 45.7%, which was greater than the original population by 2.56% (Table 4). We observed smaller mean values of DF, DM and PH in selected genotypes (Xs) than the original population (Xo). In this case, smaller mean values imply a better mean performance, since the selection target was a decrease in these traits, to capture early maturing genotypes with moderate height and lodging resistance. The mean performance of each selected genotype for the nine traits is presented in Supplementary Table S5, where G18 showed the highest EA (47.13%) with SYD (3214 kg ha^{-1}), followed by G13 (46.78%) with maximum SYD (3409 kg ha^{-1}). Considering the mean performance of the genotypes for EA in response to environmental conditions, G13 produced the highest EA (49.3%) at Holetta in 2021, followed by G5 (48.8%) in the same location in 2022 and G18 (48.5%) at Adet in 2022 (Table S6). The overall mean performance of selected genotypes for EA at each environment indicated that the maximum EA (47.3%) was recorded at Holetta in 2022 (Table S6).

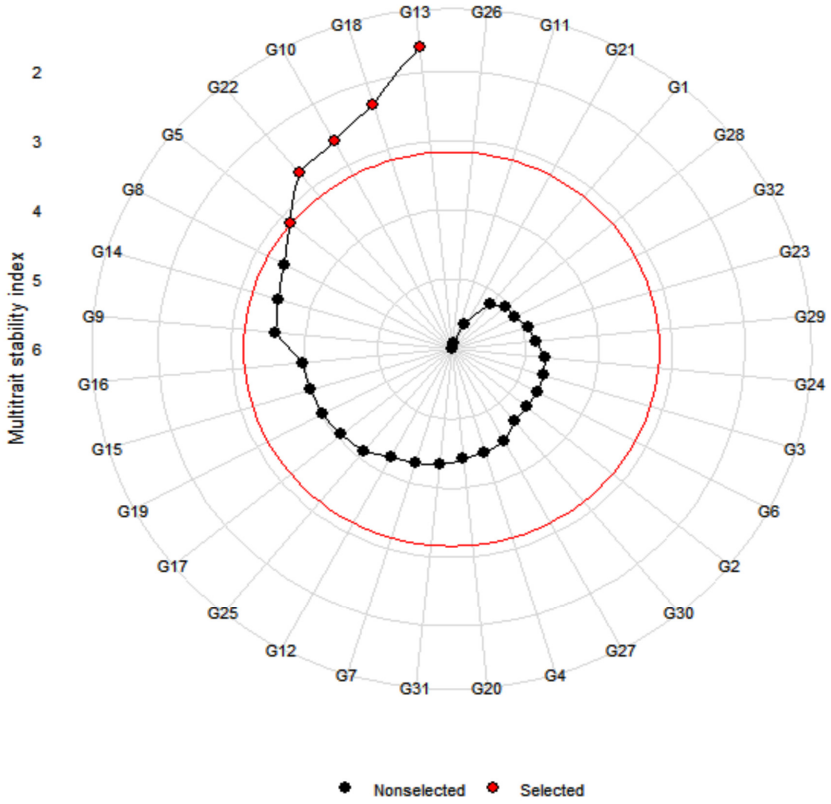

Figure 4. The multi-trait stability indexes of the 32 genotypes involved in the field trials. The selected stable genotypes located on the red circle or beyond, with red dots, considering 15% selection intensity.

Table 4. Selection differential of the WAASBY index for the nine traits of carinata.

Factor	Traits	Xo	Xs	SD	SD (%)
FA 1	NPB	8.230	8.9	0.60	7.26
FA 1	NPP	246.7	281.7	35	14.18
FA 1	TSW	4.13	4.41	0.28	6.77
FA 1	SYD	2491	3185	694.2	27.87
FA 1	OC	41.63	45.10	3.48	8.36
FA 2	DM	150	147.2	−2.73	−1.82
FA 2	PH	150.5	141.1	−9.44	−6.27
FA 3	DF	70.88	65.86	−5.03	−7.09
FA 3	EA	43.12	45.68	2.56	5.94

DF, date of 50% flowering. DM, date of maturity. PH, plant height in cm. NPB, number of primary branch. NPP, number of pods per plant. TSW, thousand seed weight. SYD, seed yield in kg ha^{-1}. OC, oil content in %. EA, Erucic acid in %. Xo, mean for WAASBY index of the original population. Xs, mean for WAASBY index of the selected genotypes (G13, G18, G10, G22 and G5) and SD, selection differential.

The selection differential of the WAASBY index was positive for the traits targeted to increase, whereas it was negative for the traits targeted to decrease. The higher SD values were obtained for SYD (27.87%) and NPP (14.18%), which were found in the common factor group, FA1 (Table 4). The SD of EA and OC were found to be 5.9% and 8.36%, respectively, which were found in FA3 and FA1 factor groups, respectively (Table 4).

The selection gain (SG) achieved for all traits is presented in Table 5. The traits expected to increase had positive selection gains, i.e., SYD (25.77%), NPP (11.57%), OC (6.89%), NPB (6.83%), TSW (6.42%) and EA (4.33%); while the traits targeted to decrease showed negative selection gains, i.e., DF (−6.47%), PH (−4.93%) and DM (−1.66%). The heritability of all traits with reference to the selected genotypes was higher than 70%, indicating success in selecting superior genotypes for all the evaluated traits (Table 5).

Table 5. Heritability and selection gain (%) for the mean performance for the nine traits of carinata.

Factor	Traits	h^2	SG	SG%	Sense	Goal
FA 1	NPB	0.94	0.57	6.83	Increase	100
FA 1	NPP	0.82	28.56	11.57	Increase	100
FA 1	TSW	0.95	0.27	6.42	Increase	100
FA 1	SYD	0.92	641.7	25.77	Increase	100
FA 1	OC	0.83	2.87	6.89	Increase	100
FA 2	DM	0.91	−2.494	−1.66	Decrease	100
FA 2	PH	0.79	−7.43	−4.93	Decrease	100
FA 3	DF	0.91	−4.58	−6.47	Decrease	100
FA 3	EA	0.73	1.87	4.33	Increase	100

DF, date of 50% flowering. DM, date of maturity. PH, plant height in cm; NPB, number of primary branch. NPP, number of pods per plant. TSW, thousands seed weight. SYD, seed yield in kg gha^{-1}. OC, oil content in %. EA, erucic acid in %. SG, selection gain and h^2, heritability.

3.5. The Strengths and Weakness View of the Selected Genotypes

The radar plot (Figure 5) reveals the strengths and weaknesses of the selected genotypes. The factor's contribution towards MTSI is ranked from the most contributing factor (close to the plot center) to the least contributing factor (close to the plot edge). The selected genotypes showed weakness related to the factor FA1 that holds the traits NPB, NPP, TSW, SYD and OC, whereas these genotypes, except G5, showed strengths related to FA2 with a desirable negative gain of DM and PH. Regarding FA3, the selected genotypes, except G10, perform well for the EA and DF traits. The smaller proportions, explained by a factor that is placed closer to the external edge, indicate that the trait within that factor is more similar to the ideotype.

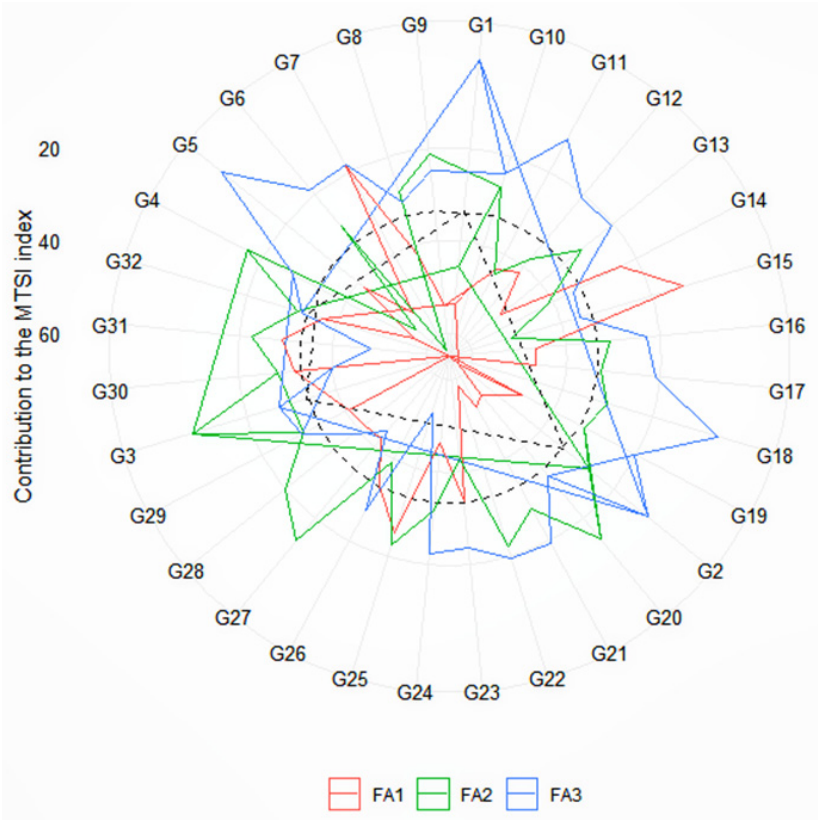

Figure 5. A view of the strengths and weakness of selected carinata genotypes. The graph depicted the proportion of each factor (FA) on the computed multi-trait stability index (MTSI). The smaller the proportion explained by a factor (closer to the external edge), the closer the traits within that factor are to the ideotype. The dashed line shows the theoretical value if all the factors had contributed equally.

4. Discussion

In plant breeding, multi-environmental trials with a set of genotypes are tested over different sites and years, which is a major approach for enabling the selection of genotypes with more stable performance across multiple environments [32]. In this study, we evaluated nine traits of 32 carinata genotypes at three locations in two consecutive years. The combined analysis of the nine traits indicated a wide range of responses for the target traits. Such variations could be genotypic, environmental, or an interaction between them (GEI). The main causes of variation between genotypes in their stability is the wide occurrence of GEI due to the differential response of genotypes to various environmental factors [22]. Our results from the likelihood ratio test of the joint ANOVA showed that the GEI effects for all analyzed traits were highly significant ($p < 0.001$).

Seed yield is a complex trait which is often influenced by GEI. In this study, the major proportion of phenotypic variability for seed yield was from genotypic variance, indicating a high heritability for the seed yield of carinata. This is not in agreement with some other studies on carinata, where the seed yield was highly influenced by the environment and GEI [33]. This discrepancy might be due to the difference in climate conditions or genotypes used for different studies. The extent and direction of the correlations among studied traits would assist the plant breeders in identifying the traits that can be improved simultaneously [34]. In our study, the Pearson's correlation showed the existence of a stronger association between SYD and yield component traits (NPP, NPB and TSW), implying that selection based on yield component traits will increase the seed yield of carinata.

EA is one of the main important quality traits in carinata that make the crop suitable for various industrial applications [2,3]. Although the current carinata cultivars grown in Ethiopia are considered as being high in erucic acid, with its content ranging from 39.17 to 42.98% [7], the current industrial market demands are above this range. Additionally, the relative amount of EA in carinata would vary across different environments, due to the effect of GIE [18,19].Thus, investigating the genetic stability is vital where the GEI effect is dominant. In this study, the proportion of GEI from phenotypic variance was prominent for EA, which supports the need to test the stability of genotype performance [35].

The use of MTSI for stability analysis is advantageous in selecting potential genotypes with a combination of desirable traits [24]. Accordingly, our study focused on the identification of HEA carinata genotypes with stable mean performance for seed quality traits (EA and OC), seed yield, and other agronomic traits. As far as we know, this study is the first report on stability analysis for carinata using the multi-trait stability index. We were able to select five HAE genotypes (G13, G18, G10, G22 and G5) with stable performance for desirable traits. These genotypes produced EA in the range of 43.73 to 47.13%, with a mean performance of 45.68%. Thus, the selected genotypes can be better candidates for the further breeding of carinata for industrial applications as compared to the current released varieties [7,36]. The mean value of phenological traits (i.e., DF and DM) indicated that the selected genotypes were found to be early maturing, which enables the timely harvesting of the crop for next crop cultivation. The selection of early mature genotypes might cause a yield penalty, as reported by Kumar et al. [33]. Interestingly, the seed yield of the selected genotypes, however, was found to be superior compared to the rest of the genotypes. With reference to the environmental response, the selected genotypes showed relatively better and stable mean performance in EA at Holetta. This might be due to the impact of the continuous selection or pre-breeding activities of carinata conducted at Holetta, which in turn might bring about further adaption in those genotypes to that location.

Other important parameters that can be obtained from MTSI index are selection differential (SD) and selection gain (SG). We found that our selected genotypes showed better mean performance in comparison to the overall performance of the 32 genotypes. Similarly, the selected genotypes revealed the desired selection gain for the mean performance of all traits. The success of the selection gain was also supported by the presence of high heritability, which was >70%.

The contribution of each factor towards the MTSI index is explained by a graphical tool (Figure 5), to identify the strengths and weaknesses of the genotypes in terms of traits or sets of traits within that factor. With the exception of G10, the selected genotypes showed strength related to the factor FA3 that holds EA and DF. Similarly, the selected genotypes, except G5, perform well for DM and PH, which are found under FA2. Thus, G13, G18 and G22 have simultaneously showed strength for EA, which was targeted for an increase, as well as for DF, DM and PH, that were targeted for a decrease. Thus, these genotypes could be considered as better-performing, early-maturing, and HEA genotypes.

5. Conclusions

This study demonstrated the selection of HEA carinata genotypes with stable mean performance for a set of desirable traits using MTSI. The use of genotypic stability WAASB, along with MTSI for multi-environment trials, enables us to select the stable and best-performing genotypes across environments for a set of desirable traits. Using these methods, we selected five carinata genotypes (G13, G18, G10, G22 and G5) as stable and best performing, among the 32 genotypes included in the study. The selected genotypes showed HEA above the average value (>43.12%), along with a better oil content, seed yield and other agronomic traits. These genotypes can be a better sources of EA for industrial application. The selected genotypes were found to mature early, on average within 147 days, and can be used as early maturing cultivars in production to avoid the risk of limited rainfall and to enable double cropping for quick economic return. Furthermore, the selected genotypes can be used as source of genetic material for future carinata breeding, aiming at developing HEA for industrial applications.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agriculture14071100/s1, Table S1: *Brassica carinata* genotypes used for field trials conducted at three locations (Holetta, Kulumsa and Adet) for two years (2021 and 2022); Table S2: Precipitation (mm) and temperature °C (maximum and minimum) on a monthly basis for testing sites (Holetta, Kulumsa and Adet); Table S3: Principal components (PC) of the correlation matrix with the WAASBY values of the nine traits of *B. carinata*; Table S4: Multi-trait stability index (MTSI) of the first top 10 *B. carinata* genotypes; Table S5: Mean performance of 32 *B. carinata* genotypes for nine traits tested under six environments; Table S6. Mean performance of selected genotypes for erucic acid (%) under six different environments.

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