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Morphological, Physiological, and Reproductive Influencing Factors of Yield in Native Iranian Fenugreek Based on Path Analysis

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Abstract

Fenugreek is an important medicinal and nutritionally rich legume with significant potential for cultivation in semi-arid and marginal environments. Twenty-six native Iranian fenugreek populations were evaluated under field conditions through a randomized block scheme with three replications. A comprehensive set of vegetative, reproductive, and biochemical traits; including fresh and dry stem and root weights, leaf dimensions, pod and seed characteristics, and photosynthetic pigments, were recorded to assess their contribution to seed yield. Correlation analysis revealed strong positive associations among vegetative traits, such as stem biomass and leaf area, indicating coordinated plant growth. Reproductive traits, including thousand seed weight, number of pods per plant, and number of seeds per pod, were correlated with seed yield. Also, some negative correlations indicated trade-offs, particularly between root biomass and shoot branching, as well as between pod number and seed number per pod. Path analysis identified number of pods per plant (with coefficient 0.61), number of seeds per pod (0.37), and thousand seed weight (0.93), as the primary direct contributors to seed yield, whereas traits such as pod height, dry stem weight, leaf area, and carotenoid content exerted significant indirect effects through reproductive components. Bootstrap analysis with 2,000 resamples confirmed the stability and reliability of the path coefficients, highlighting the robustness of the model in accounting for multicollinearity among interrelated traits. These findings suggest that integrated selection strategies targeting both reproductive traits and supporting vegetative and physiological attributes can substantially improve seed yield in fenugreek. Genotypes combining vegetative growth, efficient photosynthetic machinery, and superior reproductive performance represent ideal candidates for breeding programs aimed at enhancing productivity.

Keywords: Correlations, Pods per plant, Seeds per pod, Thousand seed weight

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Introduction

As an annual herb belonging to the Fabaceae family, fenugreek (*Trigonella foenum-graecum* L.), has long been cultivated as a valuable medicinal and food plant in many other parts of the world. The leaves and seeds of fenugreek contain a variety of bioactive compounds, including alkaloids, saponins, trigonelline, choline, and several vitamins (Akhtar et al., 2025), which contribute to its therapeutic effects in the treatment of metabolic and digestive disorders and in lowering blood sugar and lipid levels (Syed et al., 2020). In addition to its medicinal importance, fenugreek is nutritionally rich, containing substantial amounts of protein, calcium, iron, phosphorus, and carotenoids (Tewari et al., 2024). The primary center of origin of fenugreek is believed to be the regions of North Africa and the eastern Mediterranean coast; however, historical and biogeographical evidence suggests that it was cultivated in Iran and later spread to various regions of Asia, Europe, and Africa (Seal et al., 2025). Currently, countries in the Indian subcontinent, China, Europe, and North Africa are recognized as major centers of cultivation and distribution of this species (Shahrajabian et al., 2021). The wide geographical range and environmental adaptability of fenugreek have resulted in considerable genetic diversity among its populations and ecotypes.

Because of its symbiotic association with nitrogen-fixing bacteria of the genus Rhizobium, fenugreek can meet part of its nitrogen requirement through biological fixation. Consequently, it serves as a drought-tolerant legume that can be incorporated into crop rotations to enhance soil fertility and contribute to the sustainability of agricultural systems (Mahfouz et al., 2017). Fenugreek is also relatively drought-tolerant, and traits such as root depth and branching help it withstand water limitation. The capacity for nodulation under water stress depends on both plant genotype and rhizobial strain compatibility, making selection of both host and symbiont important for improving performance in marginal environments (Sharma et al., 2021). The native populations of fenugreek exhibit high levels of variability in morphological and agronomic traits, which provides valuable potential for use in breeding programs and for selecting superior genotypes. To evaluate this diversity and identify the traits most closely related to yield performance, multivariate statistical approaches, such as path analysis, are widely applied, as they effectively explain the structural relationships among agronomic traits.

Previous studies on different species and ecotypes of legumes, including alfalfa and fenugreek, have shown that traits such as thousand-seed weight, seed weight per plant, harvest index, number of lateral branches, and chlorophyll content are among the most influential factors affecting seed yield (Camlica and Yaldiz, 2021; Azizi et al., 2025). Given the growing attention to the cultivation of native medicinal plants such as fenugreek, along with the need for efficient use of water and soil resources, identifying high-yielding and well-adapted genotypes and determining key traits contributing to yield improvement are of great importance. In addition, some investigations have reported that traits related to photosynthetic efficiency, pod number per plant, and biomass accumulation are also strongly associated with yield potential under both optimal and stress conditions (Singh et al., 2025; Tilahun et al., 2025). These findings indicate that the coordination between source traits (e.g., chlorophyll concentration, leaf area index) and sink traits (e.g., pod and seed number, seed weight) is fundamental for yield determination in fenugreek and related legumes.

Given the increasing global and regional focus on sustainable agriculture and the growing demand for native medicinal crops, fenugreek is gaining renewed attention as a dual-purpose species valued for both its therapeutic and agronomic significance. Its adaptability to semi-arid and marginal environments, combined with its symbiotic nitrogen fixation capability, makes it an excellent candidate for inclusion in low-input and water-efficient cropping systems (Narayana et al., 2022). The selection of high-yielding genotypes is particularly critical due to increasing water scarcity and soil degradation in traditional cropping regions. Therefore, identifying superior genotypes and elucidating the key morphological and physiological traits that contribute most to yield improvement represent essential steps toward and productive fenugreek cultivars adapted to local environmental constraints.

Despite extensive research on fenugreek's medicinal properties and general agronomic performance, important knowledge gaps remain regarding the relative contribution of morphological and physiological traits to seed yield under diverse environmental conditions. Most previous studies have focused on individual traits or simple correlations with yield, often under a single environment or management regime, which limits the ability to identify key traits with true causal effects on yield. Moreover, comparative evaluations of native fenugreek genotypes using multivariate approaches such as path analysis are scarce, particularly in semi-arid and marginal environments where water availability constrains productivity. The interaction between source-related traits (e.g., chlorophyll content and biomass accumulation) and sink-related traits (e.g., pod number and seed weight) has not been sufficiently quantified to determine their direct and indirect effects on yield formation. To address these gaps, the present study evaluates a diverse set of native fenugreek genotypes and applies multivariate statistical analyses, including path analysis, to disentangle the direct and indirect relationships among yield-related traits. By identifying the most influential morphological and physiological traits contributing to seed yield, this study provides a trait-based framework for selecting high-yielding and well-adapted fenugreek genotypes, thereby supporting breeding efforts and sustainable cultivation in water-limited environments.

Material and methods

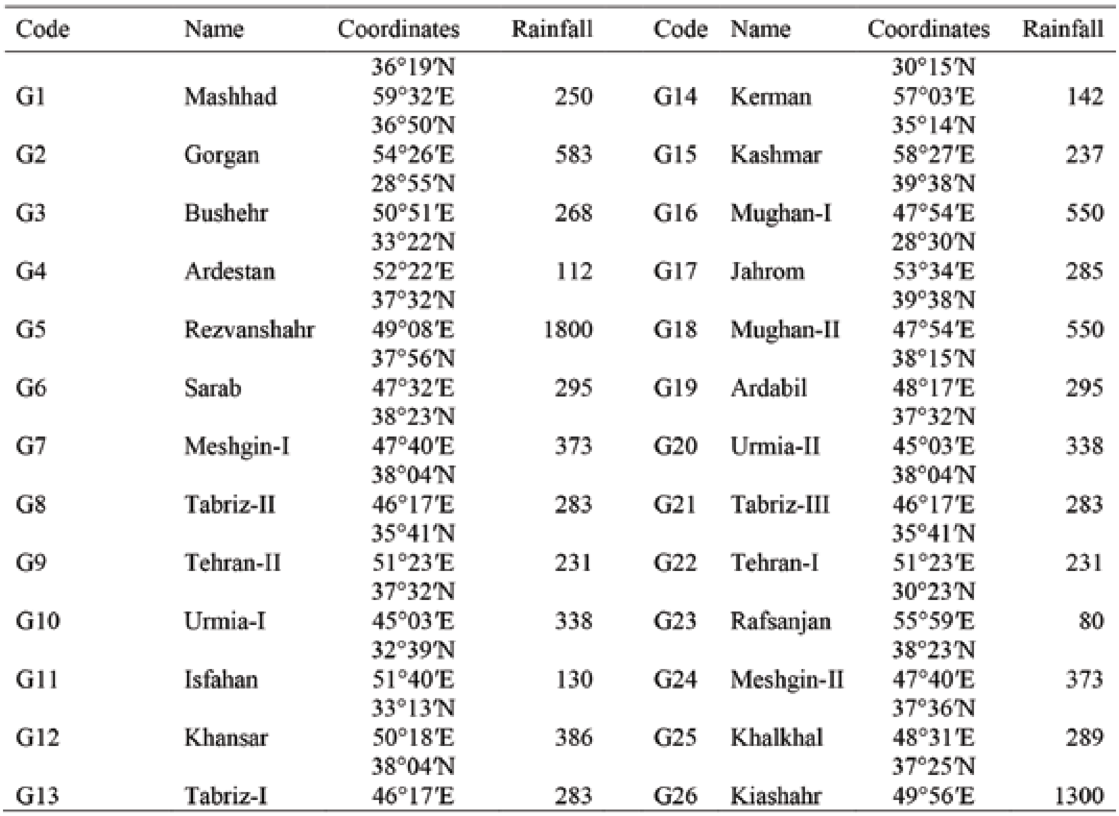
Trial and traits

Twenty-six fenugreek populations were obtained from various areas of Iran (Table 1). A field trial was performed through a randomized block scheme with three replications. Following standard soil preparation (plowing and leveling) in April, plots measuring 50 × 80 cm were established. Uniform irrigation was applied throughout the growing season, and weeds were manually controlled. Fertilization followed local recommendations: 25 kg ha^{-1} N as starter, 60 kg ha^{-1} P, and 25 kg ha^{-1} K. Plots were regularly monitored for pests and diseases, and protective measures were applied as needed. At 50% flowering step, seven sample plants per plot were chosen randomly to measure the morphological and physiological traits, via standard instruments such as calipers, rulers, digital scales, and a leaf area meter (AM-3000, ADC BioScientific Ltd). They were weight of fresh stem (WFS), weight of fresh root (WFR), weight of dry stem (WDS), weight of dry root (WDR), nodes per plant (NP), number of leaves (NL), number of branches (NB), middle leaf length (MML), middle leaf width (MLW), middle leaf area (MLA), lateral leaf length (LLL), lateral leaf width (LLW), lateral leaf area (LLA), number of pods per plant (NPP), height of pods (HP), and number of seeds per pod (NSP). Chlorophyll-a (Chl.a), Chlorophyll-b (Chl.b), and Carotenoid (CAR), were quantified following Arnon's method (Rostami et al., 2022). After harvesting, seed yield (SY), was recorded and the thousand seed weight (TSW), was measured via three random samples.

Data analysis

The dataset was assessed for normality using the Shapiro-Wilk test, and phenotypic correlations among traits were calculated via Pearson's correlation coefficients. These correlations were subsequently partitioned into direct and indirect effects through path analysis. To determine the relative contribution of predictor variables to seed yield while mitigating multicollinearity, stepwise linear regression was conducted using SPSS version 26.0. Although, stepwise regression has been criticized for potential variable selection bias, it was employed in this study as an exploratory tool to identify the most influential yield-related traits from a relatively large set of intercorrelated morphological and physiological variables. Its use was justified by the primary objective of screening candidate predictors and reducing model complexity prior to path analysis, rather than for definitive causal inference. To minimize bias, stepwise regression was combined with correlation and path analyses, allowing cross-validation of selected variables through their direct and indirect effects on seed yield. This integrated analytical approach enhances the robustness of trait selection and supports biologically meaningful interpretation of yield determinants. Predictor variables were ranked based on their influence on yield variation and categorized into first-, second-, and third-order paths. Multicollinearity within each path was evaluated using tolerance; which represents the proportion of variability in a predictor not explained by other predictors, and the variance inflation factor (VIF), the reciprocal of tolerance, reflecting the degree to which a predictor's variance is inflated due to correlations with other variables. Tolerance values below 1.0 or VIF values exceeding 10 were considered indicative of significant multicollinearity. The coefficients of determination for each predictor were derived from the path coefficients, following conventional linear regression procedures. To assess the reliability of the

Table 1. Geographic characteristics of regions for the collected fenugreek (*Trigonella foenum-graecum* L.) genotypes



Code	Name	Coordinates	Rainfall	Code	Name	Coordinates	Rainfall
G1	Mashhad	36°19′N 59°32′E	250	G14	Kerman	30°15′N 57°03′E	142
G2	Gorgan	36°50′N 54°26′E	583	G15	Kashmar	35°14′N 58°27′E	237
G3	Bushehr	28°55′N 50°51′E	268	G16	Mughan-I	39°38′N 47°54′E	550
G4	Ardestan	33°22′N 52°22′E	112	G17	Jahrom	28°30′N 53°34′E	285
G5	Rezvanshahr	37°32′N 49°08′E	1800	G18	Mughan-II	39°38′N 47°54′E	550
G6	Sarab	37°56′N 47°32′E	295	G19	Ardabil	38°15′N 48°17′E	295
G7	Meshgin-I	38°23′N 47°40′E	373	G20	Urmia-II	37°32′N 45°03′E	338
G8	Tabriz-II	38°04′N 46°17′E	283	G21	Tabriz-III	38°04′N 46°17′E	283
G9	Tehran-II	35°41′N 51°23′E	231	G22	Tehran-I	35°41′N 51°23′E	231
G10	Urmia-I	37°32′N 45°03′E	338	G23	Rafsanjan	30°23′N 55°59′E	80
G11	Isfahan	32°39′N 51°40′E	130	G24	Meshgin-II	38°23′N 47°40′E	373
G12	Khansar	33°13′N 50°18′E	386	G25	Khalkhal	37°36′N 48°31′E	289
G13	Tabriz-I	38°04′N 46°17′E	283	G26	Kiashahr	37°25′N 49°56′E	1300

estimated path coefficients, standard errors were obtained through bootstrap analysis. Since breeders often require not only point estimates but also measures of variability and confidence intervals for true parameter values, resampling methods such as bootstrapping are particularly useful. In this study, the mean direct effects obtained from 2,000 bootstrap samples closely corresponded to the observed direct effects of the predictor variables.

Results and discussion

Correlation analysis (Table 2), indicated strong integration among stem, leaf, and root traits, reflecting coordinated vegetative growth in fenugreek. Positive associations between stem biomass (WFS and WDS), root weight, and leaf dimensions suggest that overall plant vigor is governed by common growth and resource-allocation processes. Rather than functioning independently, these organs develop in concert, allowing plants with greater structural biomass to support expanded photosynthetic surfaces and below-ground resource acquisition. The strong correlations between leaf size components (length, width, and area) further indicate that leaf expansion follows a coordinated developmental pattern. Larger leaves likely enhance light interception and carbon assimilation, thereby supporting greater dry matter accumulation and reproductive development. The positive association of dry stem weight with pod height and thousand-seed weight suggests that structural biomass acts as a critical source reservoir, facilitating assimilate translocation to developing seeds. Similar relationships between dry matter production and yield components have been reported in fenugreek and other legumes (Gurjar et al., 2016). Branch number showed positive relationships with leaf and pod numbers, highlighting its role in determining sink capacity. Increased branching expands the photosynthetic canopy and provides additional sites for pod formation, ultimately contributing to yield potential. This agrees with earlier findings that greater leaf area and branch number are key determinants of seed yield in fenugreek (Parmar et al., 2021). These results emphasize that yield formation in fenugreek is primarily driven by integrated vegetative vigor, where stem biomass, leaf development, and branching collectively enhance both source strength and sink capacity. Consequently, selection strategies targeting these interconnected traits may be more effective than focusing on single yield components in isolation.

Reproductive traits also demonstrated important associations, whereas seed yield (SY), was associated with dry stem weight (WDS), and thousand seed weight (TSW) (Table 2), thus plants with more vegetative growth potential tended to produce larger seeds and higher overall yield. This relationship reflects a strong source–sink linkage, in which greater stem biomass enhances assimilate storage and transport capacity, enabling more efficient partitioning of photoassimilates toward developing seeds. Consequently, plants with higher structural reserves and improved sink strength produce heavier seeds and achieve greater overall yield. Pod height (HP), was associated positively with the number of pods per plant (NPP), suggesting that taller pods may support higher pod numbers. This is in agreement with studies by Shakthi et al. (2020), who reported positive relations between yield and its attributing characters, including number of seeds per pod and 1000-seed weight. Additionally, chlorophyll pigments, Chl a and Chl b, were very strongly correlated (Table 2), reflecting coordinated photosynthetic pigment accumulation. This finding is consistent with the variability observed in chlorophyll content among fenugreek genotypes, as reported by Azizi et al. (2025), who found significant variation in chlorophyll content across different genotypes. Interestingly, some negative correlations revealed potential trade-offs in resource allocation. Dry root weight (WDR) was negatively correlated with nodes per plant (NP) and lateral leaf area (LLA), suggesting that increased root biomass may limit branching and leaf expansion, possibly reflecting resource allocation constraints. Similarly, the negative correlation between number of pods per plant (NPP), and number of seeds per pod (NSP) indicates a trade-off between pod number and seed size or number per pod, which is a classic reproductive allocation pattern in plants. Similar trade-offs have been reported in other studies, such as the one by Shakthi et al. (2020), who observed significant negative correlations between certain vegetative and reproductive traits. The observed correlations highlight that vegetative growth, leaf development, and reproductive performance are highly interdependent, while certain traits, particularly root biomass and pod-seed relationships, reflect trade-offs that may influence ideotype selection. These findings can guide breeding programs by identifying key traits such as stem and leaf biomass that indirectly contribute to higher seed yield, as well as highlighting potential constraints due to negative associations between vegetative and reproductive allocation. Meena et al. (2021), reported the highest positive correlations among reproductive and yield components, like seed yield with pods per plant, seeds per pod, and seed yield per plot. The reproductive traits of fenugreek such as seeds per pod often load onto components separate from vegetative growth traits, indicating partly distinct genetic control (Roba and Mohammed, 2024). To examine the contribution of various traits to seed yield (SY), a regression model was fitted while accounting for multicollinearity (Table 3). Initially, all traits were included as first-order predictors, with SY as the target variable, and about half of traits exhibited high multicollinearity, so for addressing this issue, standardized coefficients were estimated using a stepwise regression strategy, which permitted identification of the most influential traits while minimizing multicollinearity effects.

Path analysis indicated that the stepwise model described almost all of SY variation, with NSP, NPP, and TSW identified as significant predictors and acceptable multicollinearity levels (Table 4). Identification of these traits as the main contributing components in seed yield of fenugreek, is verified in many field and vegetable crops like *Medicago sativa* (Sengul, 2006), *Glycine max* (Czopek et al., 2023), and *Nigella sativa* (Fikre et al., 2023). In the subsequent regression steps (Table 4), the number of pods per plant (NPP) was primarily driven by lateral leaf area (LLA) and carotenoid content (CAR), whereas dry root weight (WDR) exerted a negative effect, together explaining 63% of the variation. This pattern suggests that enhanced photosynthetic surface and pigment concentration increase reproductive sink formation, while excessive belowground biomass may divert assimilates away from pod development. Similarly, the number of seeds per pod (NSP) was positively associated with lateral leaf length (LLL) but negatively affected by LLA, indicating that leaf elongation rather than leaf expansion may be more efficient in supporting seed set, accounting for 54% of the variation. Thousand-seed weight (TSW) was positively influenced by dry stem weight (WDS) and negatively by leaf number (NL), explaining 47% of its variation. This implies that greater structural biomass supports assimilate storage and translocation to seeds, whereas excessive leaf proliferation may increase intra-plant competition for resources. Comparable relationships between leaf area, root biomass, and pod development have been reported in *Phaseolus vulgaris* and *Hibiscus sabdariffa*, reinforcing the biological relevance of these associations (Alemu et al., 2017; Fallahi et al., 2017). Further regression analysis revealed that variation in LLA was largely explained by leaf width traits, reflecting coordinated lateral leaf expansion, while WDR was positively associated with middle leaf length but negatively with pod number, supporting a trade-off between root biomass accumulation and reproductive output. Carotenoid content was strongly de-

Table 2. Pairwise correlation coefficients among morphological traits of fenugreek genotypes

	WFR	WDS	WDR	NP	NL	NB	MML	MLW	MLA	LLL	LLW	LLA	NPP	HP	NSP	TSW	Chl.a	Chl.b	CAR	SY
WFS	0.44	0.71	0.20	0.11	0.43	0.37	0.62	0.46	0.44	0.41	0.27	0.35	0.35	0.27	-0.03	0.22	0.49	0.52	0.52	0.40
WFR		0.04	0.23	-0.01	0.14	0.22	0.39	0.20	0.22	0.37	0.17	0.24	0.08	-0.19	-0.20	0.05	0.27	0.25	0.26	0.01
WDS			0.35	-0.05	0.32	0.26	0.57	0.54	0.52	0.42	0.23	0.43	0.20	0.53	0.04	0.55	0.37	0.43	0.40	0.65
WDR				-0.40	0.05	0.18	0.48	0.01	0.41	0.45	0.02	0.01	-0.39	-0.01	-0.11	0.47	0.33	0.39	0.34	0.15
NP					-0.20	0.18	-0.04	0.29	0.07	-0.08	0.27	0.35	0.56	0.39	0.23	-0.13	0.15	0.10	0.13	0.30
NL						0.52	0.23	0.26	0.08	0.17	0.39	0.25	0.36	0.09	0.08	-0.15	0.32	0.24	0.35	0.11
NB							0.41	0.48	0.53	0.57	0.63	0.54	0.38	0.23	0.04	-0.01	0.31	0.34	0.33	0.23
MML								0.51	0.70	0.50	0.22	0.41	0.11	0.04	-0.07	0.24	0.45	0.53	0.48	0.26
MLW									0.65	0.59	0.70	0.89	0.57	0.40	0.19	0.08	0.15	0.18	0.17	0.50
MLA										0.59	0.32	0.60	0.28	0.29	0.09	0.24	0.24	0.38	0.28	0.43
LLL											0.54	0.67	0.22	0.03	-0.17	0.14	0.18	0.22	0.17	0.22
LLW												0.78	0.52	0.42	0.12	-0.11	0.11	0.12	0.14	0.27
LLA													0.59	0.41	0.12	-0.02	0.01	0.01	0.02	0.40
NPP														0.34	0.44	-0.43	0.26	0.18	0.27	0.38
HP															0.36	0.39	0.21	0.21	0.23	0.71
NSP																-0.13	0.00	-0.12	0.00	0.52
TSW																	0.25	0.35	0.26	0.62
Chl.a																		0.94	0.99	0.37
Chl.b																			0.96	0.37
CAR																				0.39

Significant correlation coefficients at 24 degrees of freedom (DF) were 0.39 and 0.50 at the 0.05 and 0.01 probability levels, respectively. Traits were weight of fresh stem (WFS), weight of fresh root (WFR), weight of dry stem (WDS), weight of dry root (WDR), nodes per plant (NP), number of leaves (NL), number of branches (NB), middle leaf length (MML), middle leaf width (MLW), middle leaf area (MLA), lateral leaf length (LLL), lateral leaf width (LLW), lateral leaf area (LLA), number of pods per plant (NPP), height of pods (HP), number of seeds per pod (NSP), chlorophyll-a (Chl.a), chlorophyll-b (Chl.b), carotenoid (CAR), seed yield (SY), and the thousand seed weight (TSW).

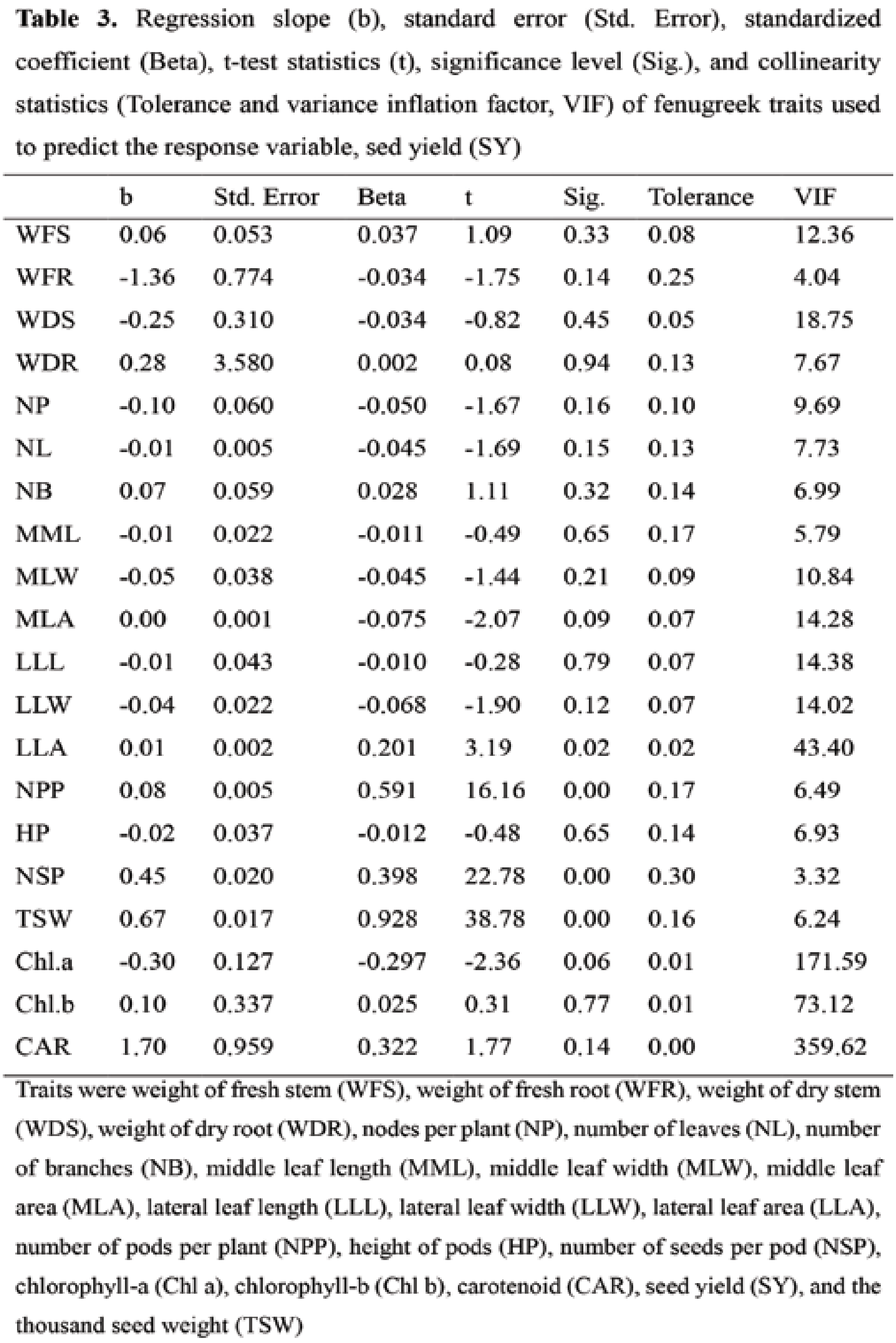


Table 3. Regression slope (b), standard error (Std. Error), standardized coefficient (Beta), t-test statistics (t), significance level (Sig.), and collinearity statistics (Tolerance and variance inflation factor, VIF) of fenugreek traits used to predict the response variable, sed yield (SY)

	b	Std. Error	Beta	t	Sig.	Tolerance	VIF
WFS	0.06	0.053	0.037	1.09	0.33	0.08	12.36
WFR	-1.36	0.774	-0.034	-1.75	0.14	0.25	4.04
WDS	-0.25	0.310	-0.034	-0.82	0.45	0.05	18.75
WDR	0.28	3.580	0.002	0.08	0.94	0.13	7.67
NP	-0.10	0.060	-0.050	-1.67	0.16	0.10	9.69
NL	-0.01	0.005	-0.045	-1.69	0.15	0.13	7.73
NB	0.07	0.059	0.028	1.11	0.32	0.14	6.99
MML	-0.01	0.022	-0.011	-0.49	0.65	0.17	5.79
MLW	-0.05	0.038	-0.045	-1.44	0.21	0.09	10.84
MLA	0.00	0.001	-0.075	-2.07	0.09	0.07	14.28
LLL	-0.01	0.043	-0.010	-0.28	0.79	0.07	14.38
LLW	-0.04	0.022	-0.068	-1.90	0.12	0.07	14.02
LLA	0.01	0.002	0.201	3.19	0.02	0.02	43.40
NPP	0.08	0.005	0.591	16.16	0.00	0.17	6.49
HP	-0.02	0.037	-0.012	-0.48	0.65	0.14	6.93
NSP	0.45	0.020	0.398	22.78	0.00	0.30	3.32
TSW	0.67	0.017	0.928	38.78	0.00	0.16	6.24
Chl.a	-0.30	0.127	-0.297	-2.36	0.06	0.01	171.59
Chl.b	0.10	0.337	0.025	0.31	0.77	0.01	73.12
CAR	1.70	0.959	0.322	1.77	0.14	0.00	359.62

Traits were weight of fresh stem (WFS), weight of fresh root (WFR), weight of dry stem (WDS), weight of dry root (WDR), nodes per plant (NP), number of leaves (NL), number of branches (NB), middle leaf length (MML), middle leaf width (MLW), middle leaf area (MLA), lateral leaf length (LLL), lateral leaf width (LLW), lateral leaf area (LLA), number of pods per plant (NPP), height of pods (HP), number of seeds per pod (NSP), chlorophyll-a (Chl a), chlorophyll-b (Chl b), carotenoid (CAR), seed yield (SY), and the thousand seed weight (TSW)

termined by chlorophyll a and b, consistent with the coordinated regulation of photosynthetic pigments and their sensitivity to developmental and environmental factors, as previously reported in fenugreek (Kadam et al., 2017). Lateral leaf length was mainly controlled by leaf width components, whereas dry stem weight was associated with fresh stem weight and pod number but negatively influenced by pod height. In addition, leaf number was directly related to branch number, highlighting the architectural control of canopy development. These resulted indicate that seed yield formation in fenugreek

Table 4. Coefficients of determination (CD), standardized coefficients (SC), and collinearity diagnostics (Tolerance and variance inflation factor, VIF) for fenugreek genotypes in the stepwise regression model applied to predict the target traits

Target	CD	Predictor	SC	Tol.	VIF	Target	CD	Predictor	SC	Tol.	VIF
SY	0.99	NPP	0.61	0.67	1.49	WDR	0.37	MML	0.46	1.00	1.00
		NSP	0.37	0.81	1.24			NP	-0.38	1.00	1.00
		TSW	0.93	0.81	1.23						
						CAR	0.95	Chl a	0.72	0.12	8.19
NPP	0.63	LLA	0.58	1.00	1.00			Chl b	0.29	0.12	8.19
		WDR	-0.55	0.88	1.13						
		CAR	0.44	0.88	1.13	LLL	0.53	MLA	0.53	0.87	1.15
								LLW	0.52	0.78	1.28
NSP	0.54	LLL	-0.45	0.55	1.83			HP	-0.35	0.79	1.26
		LLA	0.42	0.55	1.83						
						WDS	0.66	WFS	0.62	0.93	1.08
TSW	0.47	WDS	0.66	0.90	1.11			HP	0.48	0.79	1.26
		NL	-0.36	0.90	1.11			NP	-0.30	0.85	1.18
LLA	0.83	MLW	0.68	0.51	1.97	NL	0.52	NB	0.52	1.00	1.00
		LLW	0.30	0.51	1.97						

Traits were weight of fresh stem (WFS), weight of fresh root (WFR), weight of dry stem (WDS), weight of dry root (WDR), nodes per plant (NP), number of leaves (NL), number of branches (NB), middle leaf length (MML), middle leaf width (MLW), middle leaf area (MLA), lateral leaf length (LLL), lateral leaf width (LLW), lateral leaf area (LLA), number of pods per plant (NPP), height of pods (HP), number of seeds per pod (NSP), chlorophyll-a (Chl a), chlorophyll-b (Chl b), carotenoid (CAR), seed yield (SY), and the thousand seed weight (TSW).

is governed by a balance between photosynthetic capacity, biomass partitioning, and sink development, with lateral leaf traits, stem biomass, and pigment content playing central roles. The consistency of these relationships with path analysis outcomes supports their use as biologically meaningful selection criteria in fenugreek breeding programs.

Path analysis indicated indirect contributions to response variables, whereas in SY, the indirect impact of NPP via NSP was low, and via TSW was moderately negative (Table 5). The indirect impact of NSP via NPP was relatively low, and via TSW was negatively low. Also, the indirect impacts of TSW via NPP and NSP, were negatively low, thus the importance of direct impacts of NSP, NPP, and TSW traits on SY were more than the indirect impacts (Table 5). Evaluation of the indirect impacts of LLA, WDR, and CAR on NPP, as well as indirect impacts of MLA, LLW, and HP on LLL, and indirect impacts of WFS, HP, NP on WDS were less pronounced than the direct effects (Table 5). Path analysis (Table 5) revealed that most indirect effects among traits were weak to moderate, indicating that yield formation in fenugreek is driven primarily by a few key direct relationships, with secondary modulation through interconnected vegetative and physiological traits. For example, the reciprocal indirect effects between lateral leaf length (LLL) and lateral leaf area (LLA) on seeds per pod (NSP) were small and opposite in direction, suggesting that leaf size

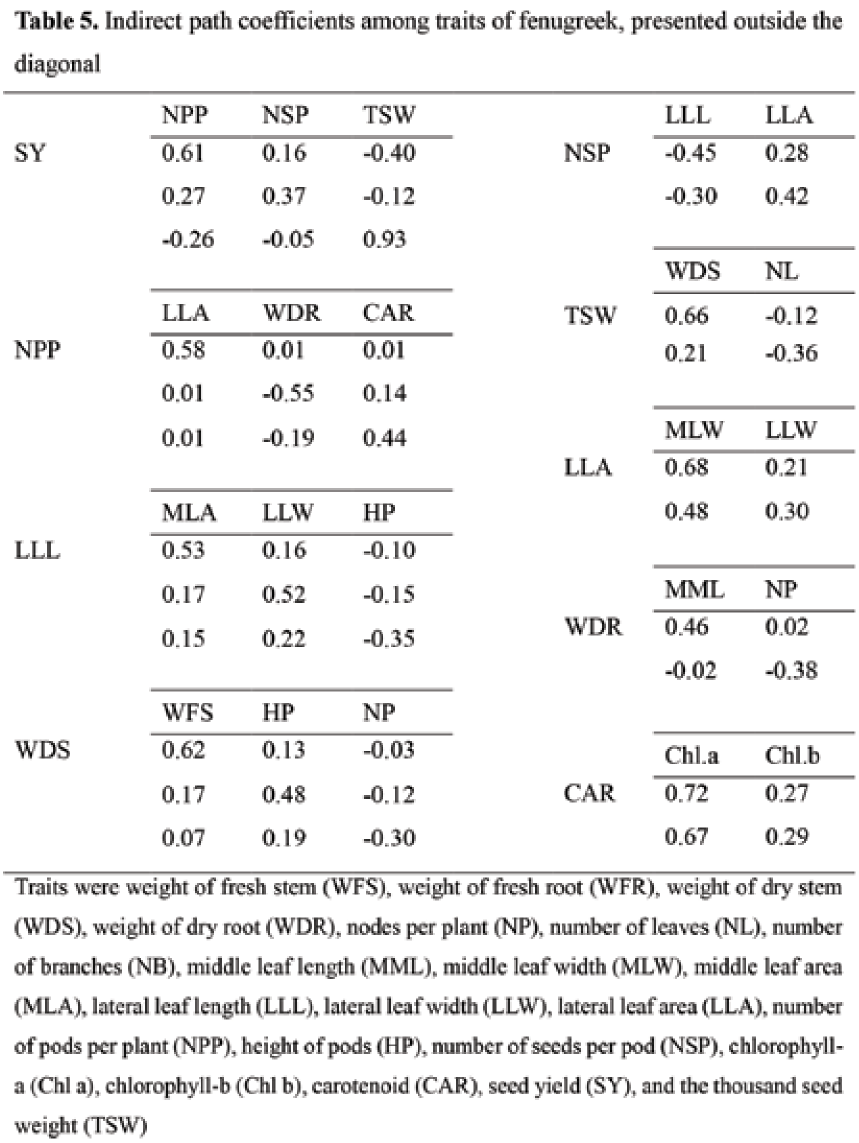


Table 5. Indirect path coefficients among traits of fenugreek, presented outside the diagonal

	NPP	NSP	TSW
SY	0.61	0.16	-0.40
	0.27	0.37	-0.12
	-0.26	-0.05	0.93

	LLA	WDR	CAR
NPP	0.58	0.01	0.01
	0.01	-0.55	0.14
	0.01	-0.19	0.44

	MLA	LLW	HP
LLL	0.53	0.16	-0.10
	0.17	0.52	-0.15
	0.15	0.22	-0.35

	WFS	HP	NP
WDS	0.62	0.13	-0.03
	0.17	0.48	-0.12
	0.07	0.19	-0.30

	LLL	LLA
NSP	-0.45	0.28
	-0.30	0.42

	WDS	NL
TSW	0.66	-0.12
	0.21	-0.36

	MLW	LLW
LLA	0.68	0.21
	0.48	0.30

	MML	NP
WDR	0.46	0.02
	-0.02	-0.38

	Chl.a	Chl.b
CAR	0.72	0.27
	0.67	0.29

Traits were weight of fresh stem (WFS), weight of fresh root (WFR), weight of dry stem (WDS), weight of dry root (WDR), nodes per plant (NP), number of leaves (NL), number of branches (NB), middle leaf length (MML), middle leaf width (MLW), middle leaf area (MLA), lateral leaf length (LLL), lateral leaf width (LLW), lateral leaf area (LLA), number of pods per plant (NPP), height of pods (HP), number of seeds per pod (NSP), chlorophyll-a (Chl a), chlorophyll-b (Chl b), carotenoid (CAR), seed yield (SY), and the thousand seed weight (TSW)

components influence seed set in a nuanced manner rather than through strong cascading effects. This supports the idea that seed formation is more sensitive to specific aspects of leaf morphology than to overall leaf expansion alone. Similarly, the indirect effects of dry stem weight (WDS) and leaf number (NL) on thousand-seed weight (TSW) were weak and compensatory, reflecting a trade-off between structural biomass and canopy size in regulating assimilate allocation to seeds. Moderate indirect effects observed between leaf width traits (MLW and LLW) on lateral leaf area indicate coordinated leaf development, but their influence on yield components remains largely indirect. Biochemical traits showed a clearer hierarchical structure: carotenoid content (CAR) was strongly regulated by chlorophyll pigments, particularly chlorophyll b, confirming the tight physiological coupling among photosynthetic pigments. In contrast, indirect effects involving root biomass (WDR) and pod number (NP) were weak, reinforcing the notion that excessive allocation to roots may have limited influence on reproductive traits beyond direct effects. While indirect pathways contribute to trait integration, seed yield in fenugreek is largely governed by strong direct effects of reproductive components such as pods per plant, seeds per pod, and thousand-seed weight, consistent with earlier findings (Meena et al., 2021). However, unlike more linear yield models reported previously (Singh et al., 2019), the present study demonstrates a multilayered trait network, in which vegetative architecture and biochemical efficiency subtly shape yield components through indirect interactions. This highlights the importance of balanced selection strategies that integrate reproductive, morphological, and physiological traits to optimize fenugreek performance across diverse environments.

Bootstrap analysis with 2,000 resamples confirmed the stability of path coefficients, with low standard errors and minimal biases for all direct effects (Table 6), demonstrating the robustness of the methodology even among highly interrelated traits. The use of stepwise regression model minimized multicollinearity by maintaining relative independence among predictors at each modeling stage. Structuring predictors into primary, secondary, and tertiary, categories have been successfully applied in previous studies on crops such as *Carthamus tinctorius* (Shekari et al., 2025), and *Nigella sativa* (Sabaghnia et al., 2025), supporting the effectiveness of this strategy for analyzing complex trait interactions in fenugreek.

Path analysis further identified the most efficient paths influencing SY in fenugreek, whereas the paths HP → WDS → TSW → SY, and Chl.b → CAR → NPP → SY, were the most effective, each associated with high seed yield performance (Fig. 1). These results suggest that increasing NPP, TSW, WDS, HP, Chl.b and CAR can enhance fenugreek seed yield. The above-mentioned paths were followed by NSP → LLA → LLW → SY, in the next step, so considering leaf properties and NSP can be useful in fenugreek breeding programs. Finally, paths Chl a → CAR → NPP → SY, and LLW → LLA → NPP → SY, were the other efficient paths in seed yield performance of fenugreek. The efficient trait paths identified in current research extend the findings of Sharma and Sastry (2008), who reported number of pods per plant, number of seeds per pod, and seed yield per plot as the main direct contributors to yield. In contrast, the current analysis indicated a more integrated and hierarchical network, where vegetative vigor (HP, WDS) and photosynthetic capacity (Chl a, Chl b, CAR) indirectly enhance yield through improvements in pod number, seed size, and weight. This broader perspective indicates that yield performance is influenced not only by direct reproductive efficiency but also by other factors controlling resource capability, assimilate partitioning, and pigment-driven photosynthesis (Buckley, 2021; Liang et al., 2023). Therefore, selection for genotypes combining strong vegetative growth, efficient photosynthetic machinery, and superior reproductive traits may provide a more robust breeding strategy for increasing seed yield potential, particularly under environments, whereas physiological efficiency and biomass partitioning play key roles.

Understanding the relationships among traits is essential for improving seed yield in fenugreek. The current research demonstrated that fenugreek has a significant di-

Table 6. Bootstrapped path coefficients for target traits and predictor traits (X) in fenugreek genotypes according to the stepwise regression model

Target	Predictor	Mean	Bias	Std. Error	Sig.	Lower	Upper
SY	NPP	0.613	0.000	0.02	0.00	0.58	0.64
	NSP	0.373	-0.004	0.02	0.00	0.33	0.40
	TSW	0.927	0.003	0.02	0.00	0.90	0.96
NPP	LLA	0.580	-0.013	0.16	0.00	0.26	0.89
	WDR	-0.551	-0.008	0.14	0.00	-0.88	-0.30
	CAR	0.444	0.010	0.11	0.00	0.26	0.69
NSP	LLL	-0.446	0.003	0.04	0.04	-1.17	0.21
	LLA	0.417	-0.046	0.04	0.03	-0.44	1.03
TSW	WDS	0.665	-0.002	0.13	0.00	0.41	0.89
	NL	-0.363	-0.004	0.16	0.03	-0.69	-0.05
LLA	MLW	0.680	-0.133	0.02	0.00	0.08	0.87
	LLW	0.303	0.198	0.03	0.04	0.12	1.18
WDR	MML	0.462	0.015	0.01	0.00	0.21	0.81
	NP	-0.380	-0.012	0.02	0.03	-0.78	-0.12
CAR	Chl.a	0.719	-0.001	0.06	0.00	0.60	0.84
	Chl.b	0.289	0.003	0.06	0.00	0.18	0.40
LLL	MLA	0.528	-0.002	0.02	0.01	0.18	0.87
	LLW	0.518	0.010	0.01	0.00	0.22	0.80
	HP	-0.346	-0.022	0.02	0.11	-0.81	0.00
WDS	WFS	0.619	0.021	0.18	0.01	0.31	1.09
	HP	0.476	-0.035	0.18	0.02	0.02	0.73
	NP	-0.303	-0.003	0.15	0.06	-0.60	-0.02
NL	NB	0.521	0.001	0.18	0.04	0.15	0.86

Traits were weight of fresh stem (WFS), weight of fresh root (WFR), weight of dry stem (WDS), weight of dry root (WDR), nodes per plant (NP), number of leaves (NL), number of branches (NB), middle leaf length (MML), middle leaf width (MLW), middle leaf area (MLA), lateral leaf length (LLL), lateral leaf width (LLW), lateral leaf area (LLA), number of pods per plant (NPP), height of pods (HP), number of seeds per pod (NSP), chlorophyll-a (Chl.a), chlorophyll-b (Chl.b), carotenoid (CAR), seed yield (SY), and the thousand seed weight (TSW) versity in morphological, biochemical, and yield-related traits that can be used in breeding programs. Correlation analysis revealed that plants with more vigorous stems tended to accumulate biomass in multiple organs, supporting the notion that vegetative vigor is closely related to reproductive potential. Similar trends have been reported in *Brassica napus* and *Triticum aestivum*, where biomass-related traits were positively correlated with components of seed yield (Zhang and Flottmann, 2016; Shamuyarira et al., 2023). Leaf traits, including median and lateral leaf dimensions, were highly correlated with each other, suggesting that selection for larger leaves may indirectly increase photosynthetic capacity and overall biomass accumulation. These results highlight the functional integration of source traits, such as leaf area and pigment content, with sink traits, such as pod and seed production, which is critical for optimizing yield. Seed yield performance was related with stem dry weight, thousand seed weight, and pod height, suggesting that stronger vegetative growth supports higher reproductive output. Photosynthetic pigments, were highly correlated, indicating coordinated accumulation of pigments. Given that photosynthetic efficiency directly affects the availability of

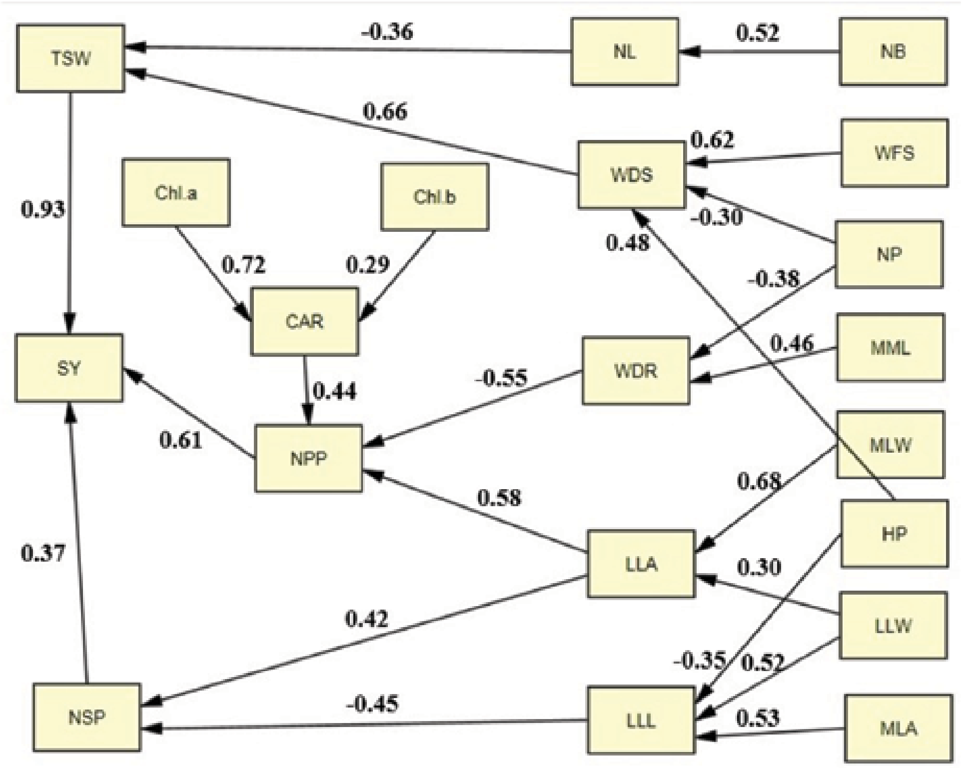


Fig. 1. Traits were weight of fresh stem (WFS), weight of fresh root (WFR), weight of dry stem (WDS), weight of dry root (WDR), nodes per plant (NP), number of leaves (NL), number of branches (NB), middle leaf length (MML), middle leaf width (MLW), middle leaf area (MLA), lateral leaf length (LLL), lateral leaf width (LLW), lateral leaf area (LLA), number of pods per plant (NPP), height of pods (HP), number of seeds per pod (NSP), chlorophyll-a (Chl.a), chlorophyll-b (Chl.b), carotenoid (CAR), seed yield (SY), and the thousand seed weight (TSW).

photosynthetic materials for reproductive development (Yaldiz and Camlica, 2022; Qiao et al., 2024), these findings emphasize the importance of considering physiological traits in addition to morphological characteristics to improve yield.

Some traits showed negative correlations, indicating potential exchange in resource allocation, whereas root dry weight was negatively correlated with node per plant and lateral leaf area, suggesting that increased root investment may limit branching or leaf expansion. Similarly, the negative correlation between pods per plant and seeds per pod suggests a reproductive exchange between pod number and size or seeds number. Such exchanges are common in legumes and reflect inherent physiological constraints, where resource allocation must balance vegetative growth, root development, and reproductive output. Understanding these trade-offs is important for ideal breeding, as it indicates that selection for one trait may affect other traits and potentially limit yield if not carefully balanced. Path analysis provided further insights into the hierarchical and dependent relationships between traits. Stepwise regression minimized multicollinearity and identified pods per plant, seeds per pod, and thousand seed weight, as the most effective predictors of grain yield. These traits collectively accounted for the majority of yield variation, supporting their use as key selection criteria in breeding programs. Path analysis revealed that vegetative and physiological traits indirectly contribute to yield by influencing reproductive components.

Conclusions

For effective fenugreek improvement, breeding programs should adopt a multi-trait selection strategy that integrates both reproductive and vegetative attributes. Prioritizing genotypes with high pods per plant, seeds per pod, and thousand-seed weight can directly enhance yield, while traits such as stem biomass, leaf area, and photosynthetic efficiency can indirectly support assimilate production and partitioning. Maintaining balanced root and shoot development is essential to avoid trade-offs between vegetative growth and reproductive output. This approach provides a practical framework for developing high-yielding, resilient fenugreek cultivars adapted to semi-arid and marginal environments, enabling more efficient and sustainable crop production.

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