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EVALUATION OF ELITE GERMPLASM OF MOTH BEAN (*Vigna aconitifolia* Jacq.) USING PATH COEFFICIENT ANALYSIS AND D2 ANALYSIS

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ABSTRACT

Moth bean [*Vigna aconitifolia* (Jacq.)] is an important leguminous crop known for its drought resistance and nutritional value. This study evaluates Path coefficient analysis and D^2 analysis among 20 elite moth bean genotypes. Conducted at ITM University, Gwalior, the experiment followed a randomized block design (RBD) with three replications. Eleven quantitative traits, including seed yield, harvest index and protein content, were analyzed. Path analysis revealed that more than 93% of variation in seed yield was explained by component traits, indicating the adequacy of the studied parameters. Harvest index exhibited the highest positive direct effect (0.56614⁸; 0.74298^P) and strong correlation ($r = 0.9578^8$; 0.9378^P) with yield, followed by number of pods per plant (direct effect: 0.13352⁸; 0.26238^P; $r = 0.8536^8). Pod length showed moderate direct effects (0.40571⁸), while seed weight had negative effects (−0.19349⁸), indicating yield dependence on seed number rather than size. Days to maturity also showed negative association ($r = -0.3745^8$), favoring early genotypes. Multivariate analysis classified 20 moth bean genotypes into four distinct clusters, indicating substantial genetic divergence among the tested material. Cluster III exhibited the highest mean seed yield per plant (6.30 g) and harvest index (36.17%), while Cluster IV showed earliness (41.33 days to 50% flowering) and highest protein content (24.45%), demonstrating their superiority for yield and nutritional improvement, respectively. The maximum inter-cluster distance between Cluster III and Cluster IV ($D^2 = 3527.49$) highlights their potential as highly diverse parents for hybridization, enabling heterosis exploitation and development of transgressive segregants. Yield-contributing traits, particularly seeds per pod (44.89%) and harvest index (17.80%), accounted for the maximum contribution toward total genetic divergence, emphasizing their importance for selection and breeding progress.$

Keywords : Moth bean, Path coefficient analysis, Multivariate analysis, Mahalanobis D^2 statistics

Introduction

Moth bean (*Vigna aconitifolia* (Jacq.) Maréchal) belongs to the family Fabaceae (sub-family Papilionoideae) and is a self-pollinated diploid species ($2n = 22$) (Kohakade *et al.*, 2017). The plant has a prostrate, creeping or mat-like growth habit: its trailing stems form a dense canopy that covers the soil surface, which helps conserve soil moisture and protects the soil from erosion-traits that make it especially well-suited for cultivation under arid and semi-arid, dryland conditions (Kumar *et al.*, 2025).

Moth bean is highly tolerant to drought and heat stress, and can grow under low-input and rainfed

conditions (Kanishka *et al.*, 2023). This resilience makes it one of the more dependable pulses among “minor” legumes under marginal and harsh environments (Kanishka *et al.*, 2023).

Nutritionally, moth bean seeds are rich in protein (reported ~ 23–26% on dry-weight basis), along with carbohydrates, minerals and vitamins-making it a valuable source of plant-based protein especially for resource-poor and low-income populations (Bhadkaria *et al.*, 2022);(Kumar *et al.*, 2025).The pods, sprouts and seeds are consumed in various forms (dal, sprouted seeds, green pods, etc.), which contributes to nutritional and food security in rural and marginal farming communities (Kumar *et al.*, 2025).

Globally, as of 2020, the total area under moth bean cultivation was estimated at approximately 0.43 million hectares, and world production was around 413,406 metric tons (Ganapathy *et al.*, 2025). India is recognized as the world's largest producer of moth bean, contributing roughly 90% of global production (Ganapathy *et al.*, 2025).

At the national level, during the 2020–2021 period, the total area under moth bean cultivation in India was reported as ~ 0.41 million hectares, with a production of about 361,200 metric tons (Ganapathy *et al.*, 2025). Major moth bean–producing states in India include Rajasthan, Maharashtra, Gujarat, Madhya Pradesh and Uttar Pradesh (Kanishka *et al.*, 2023); (Kumar *et al.*, 2025).

Although detailed recent data for moth bean production specific to Madhya Pradesh (MP) are limited in the publicly available sources, Madhya Pradesh is consistently listed among the important moth bean–growing states in India (Kanishka *et al.*, 2023); (Kumar *et al.*, 2025). In certain reviews, MP–along with Maharashtra and Rajasthan is identified as a key state where moth bean cultivation is significant under rainfed conditions (Kanishka *et al.*, 2023).

Given its drought tolerance, low water and input requirements, and high nutritional value, moth bean holds considerable promise for improving food and nutritional security particularly in arid and semi-arid agro-ecologies and resource-poor regions. Its ability to thrive in marginal lands with minimal inputs makes it a suitable crop for dryland agriculture and subsistence farming systems (8,18). (Kanishka *et al.*, 2023).

Despite these advantages, moth bean remains underutilized compared with major legumes, partly due to its low yields under traditional management, scant research, and limited adoption of improved agronomy or breeding approaches (Ganapathy *et al.*, 2025); (Kumar *et al.*, 2025). With changing climate patterns, increasing incidence of drought, and growing need for sustainable agriculture, moth bean deserves renewed research focus, breeding efforts, and policy support to unlock its full potential as a climate-resilient, protein-rich pulse.

The present study was undertaken to analyze the relationship among yield and its component traits in moth bean using path coefficient analysis. The aim was to partition correlation into direct and indirect effects to identify key traits influencing seed yield. Additionally, genetic divergence among genotypes was assessed using Mahalanobis D^2 statistics. The study also focused on grouping genotypes into distinct clusters for effective selection. Furthermore, it aimed

to identify diverse and superior parents for hybridization. Ultimately, the objective was to improve yield potential through informed selection and breeding strategies.

Materials and Methods

The experimental material for the study consisted of 20 genotypes of Moth Bean obtained from the National Bureau of Plant Genetic Resources, Pusa, New Delhi. The experiment was laid out in RBD with three replications at Crop Research Centre-I, School of Agriculture, ITM University, Gwalior. By adopting recommended spacing between rows and plants respectively. Defined agronomic practices were followed to raise good and healthy crop stand. No irrigation was given to *kharif* experiments as rains were sufficient before and after sowing of the experiments during the season. *Kharif* experiments were sown on July 31, 2022.

Observations were recorded on five randomly selected plants for 11 characters *viz.* days to 50% flowering, days to maturity, plant spread at harvest (cm), No. of branches per plant, No. of pods per plant, pod length (cm), No. of seeds/pod, 100 seed weight(g), protein content (%), harvest Index%, seed yield /plant (g).

The statistical software used for the analysis was R Studio (Popat *et al.*, 2020) and Agri analyze (Popat *et al.*, 2024). Path coefficient analysis was carried out to partition the correlation coefficients into direct and indirect effects, following the method of Dewey and Lu (1959). Mahalanobis D^2 statistics for estimation of genetic divergence among genotypes and subsequent clustering were computed using the procedure outlined by Mahalanobis (1936) and further elaborated by Rao (1952)

Result and Discussion

Genotypic Path analysis and Phenotypic Path analysis (Fig:1, Fig:2)

The combined path analyses (genotypic and phenotypic) demonstrated that over 93% of variation in seed yield per plant was accounted for by yield-component traits, reflecting that most major determinants of yield were included in the study. This underscores the adequacy of the trait set and reinforces the reliability of the results.

Harvest Index (%)

Harvest Index consistently emerged as the key determinant of yield under both genotypic (direct effect = 0.56614; $r = 0.9578$) and phenotypic (direct effect = 0.74298; $r = 0.9378$) analyses. These high direct effects

and strong correlations indicate that efficient partitioning of total biomass into economic seed yield is the principal driving force for productivity. Given that earlier studies by Sahoo *et al.* (2018) also identified harvest index as major yield contributor, our results further validate HI as the most stable and reliable selection criterion in moth bean.

Biologically, a high harvest index implies that the genotype effectively channels assimilates (photosynthates) into pod/seed formation rather than vegetative growth a desirable trait under resource-limited, arid/semi-arid conditions typical for moth bean cultivation (Kanishka *et al.*, 2023)

Number of pods per plant

Number of pods per plant stood out as the most influential structural yield component with strong direct effects (0.13352 genotypic; 0.26238 phenotypic) and high positive correlations ($r = 0.8536^{\text{g}}$; 0.8373^{p}). The sizeable positive indirect effects via harvest index especially at the phenotypic level (indirect via HI = 0.60449) — underscore that pod load is the principal “sink” determinant in yield formation. This aligns with earlier findings Rajora *et al.* (2012) and Sahoo *et al.* (2018) and multiple variability-analysis studies, which consistently show pods per plant as the most reliable yield component in moth bean germplasm. From a breeding perspective, focusing on genotypes with high pod-number per plant is likely to yield the greatest improvements in seed yield per plant.

Pod Length (cm) and Number of Seeds per pod

Pod Length had positive direct effects (0.40571^{g} ; 0.09297^{p}) though its genotypic ($r = 0.1862$) and phenotypic ($r = 0.1765$) correlations were modest. Number of Seeds per Pod likewise had moderate contributions (direct effect phenotypic = 0.00912; genotypic direct effect was negative in your data, but in some studies number of seeds/pod contributed positively). Combined with positive indirect effects via harvest index and pods per plant, both traits show supportive but secondary influence on final seed yield. These findings resonate with earlier works Rajora *et al.* (2012) reported pod length as the only trait (among several) with positive significant association with yield under arid conditions, while Sahoo Sahoo *et al.* (2019) included pod length and seeds per pod among useful supportive traits.

Seed Weight (g)

Contrary to some expectations, 100-seed weight (or seed weight) showed negative direct effects at both levels (-0.19349^{g} ; -0.04488^{p}) and negligible or slightly negative correlations ($r = -0.0192^{\text{g}}$; -0.0141^{p}). This

suggests that in the present germplasm, heavier seeds do not contribute to higher yield; rather, yield depends more on seed number (pods $\times$ seeds per pod) than on individual seed size. Variability studies Chaudhary *et al.* (2021) also highlight that seed weight offers limited yield gain potential compared to pods per plant and harvest index in moth bean. Biologically, in a resource-limited or drought-prone environment, producing more seeds (even if lighter) may be advantageous for yield stability rather than fewer heavy seeds, because seed set and number compensates for individual weight loss.

Plant Spread and Number of Branches per plant

Plant Spread showed negative direct effects (-0.19906^{g} ; -0.1068^{p}) while correlation with yield was minimal ($r = 0.0194^{\text{g}}$; 0.007^{p}). Similarly, number of branches per plant had negative genotypic correlation ($r = -0.4697^{\text{g}}$) even though the direct effect was slightly positive (0.00127^{g}). Negative indirect effects (via pods per plant, harvest index, seed weight) further undermined their net contribution. These results suggest that excessive vegetative growth (branching, spread) may be detrimental to yield, likely because biomass is partitioned into structural growth rather than reproductive sinks. Several variability analyses Soni and Modi (2023) report high heritability for branching and other vegetative traits, but those are often not associated with yield, highlighting the importance of reproductive allocation over vegetative proliferation.

Days to 50 % flowering and Days to Maturity

Both phenology traits showed negative correlation and negative direct effects on seed yield (e.g., days to maturity $r = -0.3745^{\text{g}}$; -0.3609^{p} ; direct effects -0.44243^{g} ; -0.12681^{p}). This suggests that earlier flowering and maturity favor higher yield in the studied environment. Earlier studies by Rajora *et al.* (2012) demonstrated similar patterns, where delayed maturity was negatively associated with yield under hot arid conditions. Given that moth bean is largely grown in arid/semi-arid zones, early maturing genotypes may escape terminal drought or heat stress during pod filling — thus ensuring better seed set and yield stability. The review by Kanishka *et al.* (2023) supports this, noting the importance of adaptive traits (early maturity, drought tolerance) for moth bean cultivation under climate-stress environments.

Protein Content (%)

Protein content showed moderate positive genotypic correlation ($r = 0.4041^{\text{g}}$) but a negative direct phenotypic effect (-0.04945^{p}). Its positive indirect effects (via harvest index, pods per plant) suggest that improving yield and seed quality (protein) simultaneously might be possible, but direct selection

solely for protein content may not guarantee yield increase. Given the nutritional importance of moth bean as highlighted in recent reviews (e.g., moth bean as a drought-tolerant nutritional legume) protein content remains desirable, especially if selection targets both yield and quality. Because in variability studies, protein content often shows lower heritability compared to major yield-component traits (Chaudhary *et al.*, 2021) improvement may require careful multi-trait selection or crossing to bring together high yield and high protein genotypes.

Clustering of the genetic material using Tocher's method and Mahalanobis D² distance

Variance and Covariance Matrix

The variance–covariance matrix presented in Table 1 indicates the presence of measurable phenotypic and genotypic variability among the 20 moth bean genotypes. The higher variance observed for traits like protein content and harvest index suggests wide variability and a stronger influence of genetic factors on these characters. Positive covariance values among key yield components such as pods per plant, seeds per pod, and seed yield per plant indicate favorable trait associations important for selection strategies. The observed covariance trends imply that simultaneous improvement of yield and yield components may be feasible through direct selection. A similar influence of yield-associated covariance was reported earlier in moth bean genetic studies (Chaudhary *et al.*, 2021).

Cluster Composition and Grouping

Based on Mahalanobis D² statistics and Tocher's method, the 20 genotypes were grouped into four clusters, highlighting substantial genetic divergence in the studied material. Cluster II was the largest, comprising 13 genotypes, followed by Cluster I with 4 genotypes, Cluster III with 2 genotypes, and Cluster IV containing a single genotype, indicating its extreme uniqueness. The unequal distribution of genotypes across clusters suggests that sufficient genetic diversity exists and that non-random grouping has occurred, which is desirable for selecting superior parents in hybridization programs (Rao, 1952) ;(Murty and Arunachalam, 1966).

Cluster Mean Performance

Cluster means revealed remarkable differences for both yield-contributing and quality traits. Cluster III demonstrated the highest seed yield per plant (6.30 g) and harvest index (36.17%), suggesting its superiority for productivity enhancement. Cluster IV, although monogenotypic, exhibited earliness (41.33 days to 50%

flowering) and highest protein content (24.45%), indicating its value for improving nutritional traits and earliness. Cluster II showed moderate but stable performance across traits, implying wider adaptability and potential as a general donor parent. These mean variations clearly indicate substantial divergence among clusters and scope for character-specific selection. These findings align with past reports where clusters with high mean values for yield were prioritized in moth bean improvement (Nisar *et al.*, 2007).

Contribution of Traits to Total Divergence

Trait contribution analysis revealed that number of seeds per pod contributed the highest proportion (44.89%) toward total divergence, followed by harvest index (17.80%), and number of pods per plant (9.63%). Yield-related traits collectively contributed more than 70% of the genetic divergence, demonstrating their major role in discriminating among the evaluated genotypes. Hence, selection strategies should prioritize yield components for effective genetic improvement. Similar dominance of yield traits in genetic divergence has been observed previously in legumes (Singh *et al.*, 2014).

Intra- and Inter-Cluster Distance

D² values revealed that the maximum inter-cluster divergence occurred between Cluster III and Cluster IV (D² = 3527.49), followed by Cluster I and Cluster IV (D² = 3188.55), indicating that the genotypes grouped in these clusters are highly divergent and would serve as promising parents for hybridization to exploit heterosis and transgressive segregation. Similar emphasis on selecting distantly located parents to maximize recombination potential has been reported earlier in moth bean and other legumes (Chaudhary *et al.*, 2021).

The lowest inter-cluster distance was recorded between Cluster I and Cluster II, reflecting their close genetic relationship and relatively limited scope for generating wider variability through crossing. Cluster IV exhibited zero intra-cluster distance due to its monogenotypic nature, highlighting its extreme uniqueness and usefulness as a rare genetic donor in crop improvement programs.

High D² divergence among clusters, as observed in this study, validates the availability of substantial genetic diversity within the germplasm, which may be effectively exploited in breeding programs aimed at developing high-yielding and nutritionally superior cultivars (Singh *et al.*, 2014); (Rao, 1952); (Murty and Arunachalam, 1966).

Conclusion

The present study revealed significant genetic variability and divergence among moth bean genotypes, indicating strong potential for genetic improvement. Path coefficient analysis showed that most of the variation in seed yield was explained by the studied traits. Harvest index emerged as the most important trait, exhibiting high positive direct effects and strong association with seed yield, making it a reliable selection criterion. Number of pods per plant also showed substantial positive influence, confirming its role as a key yield component. Pod length and number of seeds per pod contributed moderately, while seed weight, plant spread, and number of branches per plant showed negative or negligible effects, indicating

that yield is more dependent on reproductive efficiency than vegetative growth. Early flowering and maturity were negatively associated with yield, suggesting the advantage of early genotypes under stress conditions. D² analysis grouped genotypes into four clusters, with maximum divergence between Cluster III and IV, suggesting their suitability for hybridization. Cluster III showed superior yield performance, while Cluster IV possessed traits for earliness and higher protein content.

Overall, selection based on harvest index, pods per plant, and early maturity, along with the use of diverse parents, can effectively enhance yield and adaptability in moth bean.

Table 1: Variance & Covariance matrix

Traits	DT5F	DTM	PS	NOBPP	NOPPP	PL	NOSPP	SW	PC	HI	SYP
DT5F	2.02	0.31	0.30	-0.011	-0.12	-0.01	0.007	0.011	-0.491	0.049	-0.03
DTM	0.316285702	3.13	0.55	0.06	0.33	-0.03	0.02	0.028	-1.576	0.095	-0.0029
PS	0.30	0.552	1.870	0.064	-0.1943	0.036	-0.002	-0.02		0.259	-0.055
NOBPP	-0.0116	0.060	0.0643	0.0403	-0.0296	0.012	-0.0006	-0.003	-0.047	0.043	-0.005
NOPPP	-0.129	0.334	-0.194	-0.029	1.96	0.042	0.015	0.008	0.119	0.127	0.056
PL	-0.0167	-0.035	0.036	0.0123	0.042	0.024	0.0017	-0.0015	-0.127	0.051	0.0024
NOSPP	0.0079	0.020	-0.002	-0.00063	0.01538	0.001	0.01	0.001	-0.118	0.006	0.018
SW	0.01159	0.028	-0.025	-0.003	0.0087	-0.00151	0.0011	0.006	-0.021	-0.0088	0.0022
PC	-0.491	-1.576	-1.542	-0.0478	0.11958	-0.1274	-0.118	-0.021	9.465	-1.0128	-0.098
HI	0.049	0.095	0.259	0.0434	0.1277	0.051	0.006	-0.0088	-1.012	0.581	0.0079
SYP	-0.033	-0.002	-0.055	-0.005	0.056	0.0024	0.018	0.0022	-0.098	0.00797	0.0497

Table 2: Germplasm Clustering Groups

Cluster	Total Genotypes	Genetic Material
cluster 1	4	T1 IC370471, T18 IC415134, T4 IC415124, T5 IC311416
cluster 2	13	T10 IC370508, T12 IC311434, T13 IC311439, T14 IC415116, T15 IC415127, T16 IC415133, T17 IC329051, T19 IC415136, T2 IC415103, T20 IC415139, T3 IC415104, T7 IC311447, T9 IC370476
cluster 3	2	T11 IC311398, T6 IC311427
cluster 4	1	T8 IC370469

Table 3: Mean of Traits

Trait	cluster 1	cluster 2	cluster 3	cluster 4
Days to 50% Flowering	48.53	46.85	48.63	41.33
Days to Maturity	105.14	96.54	92.53	92.31
Plant Spread (cm)	23.06	25.25	28.52	26.56
No. of Branches per plant	2.97	3.50	2.83	3.67
No. of Pods per Plant	44.52	50.19	62.88	65.44
Pod Length (cm)	3.13	3.74	2.83	3.26
No. Of Seeds per pod	3.33	4.98	3.69	5.89
Seed weight (g)	1.87	2.20	2.61	2.63
Protein content (%)	19.64	21.17	22.51	24.45
Harvest Index (%)	22.19	24.02	36.17	22.34
Seed Yield plant (g)	4.09	4.69	6.30	4.47

Table 4: Contribution of Traits

Traits	Singh_statistic	Proportion	Cumulative_proportion
No. of Seeds per pod	971664.9692	44.8978	44.8978
Harvest Index (%)	385249.76	17.8013	62.6991
No. of Pods per Plant	208376.5906	9.6285	72.3276
Seed Yield plant (g)	162880.5053	7.5262	79.8538
Days to Maturity	134009.0781	6.1922	86.046
Seed weight (g)	119513.8066	5.5224	91.5684
Plant Spread (cm)	54697.01494	2.5274	94.0958
No. of Branches per plant	46933.62382	2.1687	96.2644
Days to 50 % Flowering	35988.82225	1.6629	97.9274
Pod Length (cm)	33448.74117	1.5456	99.473
Protein content (%)	11406.17261	0.527	100

Table 5: Intra Inter Cluster Distance

Cluster Number	cluster.1	cluster.2	cluster.3	cluster.4
cluster 1	569.6262381	1428.64294	1674.715735	3188.547267
cluster 2	1428.64294	677.6194699	2288.339401	1021.756116
cluster 3	1674.715735	2288.339401	925.1817493	3527.485861
cluster 4	3188.547267	1021.756116	3527.485861	0

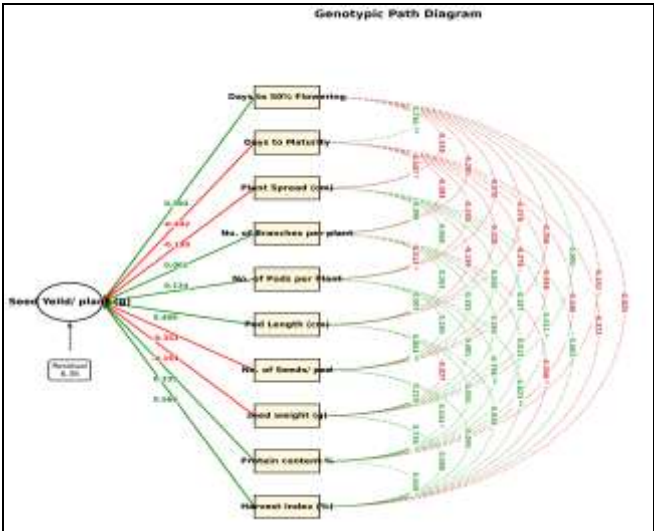


Fig. 1: Genotypic Path Diagram

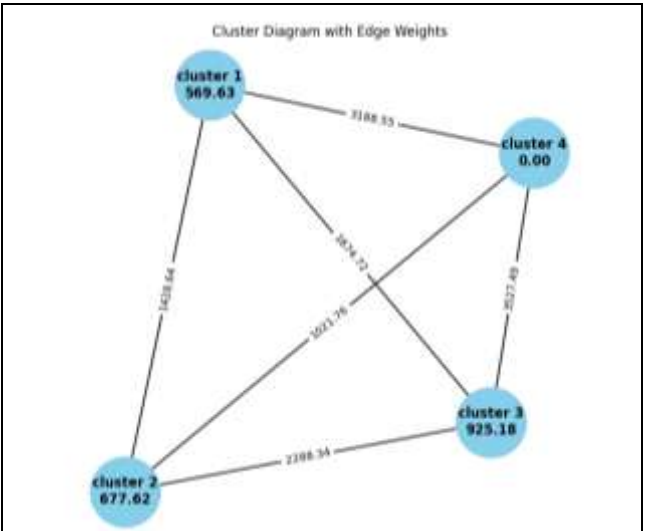


Fig. 3: Cluster diagram

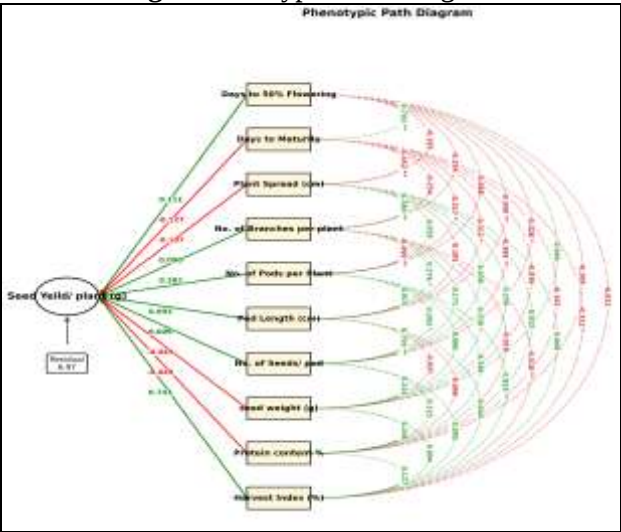


Fig. 2: Phenotypic Path Diagram

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