Original Research Article

Assessment of Genetic Diversity and Direct and Indirect Effects of Traits on Seed Yield in Sunflower (*Helianthus annuus* L.)

ABSTRACT

Present investigation was carried out with an objective to study the genetic divergence and character association among 45 sunflower inbred lines. Findings revealed significant differences among inbred lines for the studied traits. The inbred lines were grouped into six clusters using Tocher's method. Cluster I is the largest cluster with 34 inbred lines. Maximum intra cluster distance of 15.17 is associated with cluster I whereas maximum inter cluster distance of 79.75 is found between cluster II and cluster V. Cluster VI recorded maximum means for six traits. Path analysis revealed that seed yield (g/plant) and oil yield (kg ha^{-1}) exhibited high and positive direct effects on seed yield (kg ha^{-1}) whereas days to maturity, plant height, volumetric weight and oil content exhibited negative direct effects on seed yield (kg ha^{-1}) at both phenotypic and genotypic levels. All the traits exhibited positive indirect effects on seed yield (kg ha^{-1}) via seed yield (g/plant) and oil yield. The study contributes to estimate amount of genetic diversity present among inbred lines and also nature of association between yield and its component traits. These insights can guide the breeding programmes in selecting diverse inbred lines and promising traits for hybrid development and to improve the seed yield.

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KEY WORDS: Sunflower, genetic diversity, D^2 analysis and path coefficient analysis

INTRODUCTION

Sunflower belongs to family Compositae (*Asteraceae*) and is diploid with chromosome number 2n = 34. It is one of the four most widely grown and consumed oil crops in the world (Li *et al.,* 2024). Presently, it is cultivated in an area of 20.00 million hectares globally with production of 30.00 million tonnes and productivity of 1,500 kg ha^{-1} (Kumar *et al*., 2024). Sunflower seed oil is widely acclaimed in several countries compared to other vegetable oils owing to its easy availability and several health benefits including less serum cholesterol, low-density lipoprotein levels, antioxidants, regulating blood pressure, anti-inflammatory, skin protection, and pain relief (Holgado *et al.,* 2021). Sunflower seed oil is characterized by a relatively high proportion of unsaturated fatty acids, especially linoleic acid and oleic acid, conferring high nutritional value (Yang *et al.,* 2022). Furthermore, the residual meal from sunflower seeds after oil extraction contains rich protein (40 to 50%), with a high nutritional value and balanced amino acid composition, thus rendering it a high-quality plant protein resource (Subasi *et al.,* 2022). A substantial decline in sunflower cultivated area has been observed in recent years, primarily attributed to a limited availability of high-quality hybrid cultivars. This scarcity underscores the significant potential for harnessing sunflower genetic diversity to identify superior accessions (Reddy *et al.,* 2024). These elite accessions can serve as valuable resources for the development of advanced hybrid cultivars, thereby enhancing sunflower production and yield. Biometrical techniques like Mahalanobis's D^2 statistic offer a quantitative approach to assess genetic divergence among breeding lines and provides a valuable index of genetic diversity, allowing the grouping of genotypes based on D^2 values. The genetic diversity analysis in the studied inbred lines help in identifying most diverse and distantly related inbred lines which can be further crossed ensuring better manifestation of heterosis.

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Yield is a complex quantitative trait influenced by a multitude of genetic and environmental factors. Its polygenic nature, coupled with significant genotype-environment interactions, complicates the prediction and understanding of yield variation. To disentangle the intricate relationships between yield and its component traits, path coefficient analysis was employed. This statistical method quantifies both direct and indirect effects of multiple variables on yield, providing insights into the relative importance of each trait in determining final output (Mariyam *et al*., 2024). By elucidating the causal pathways underlying yield, this study contributes to the development of efficient breeding strategies for sunflower improvement. By combining these approaches, this research contributes to the identification of diverse inbred lines and the elucidation of yield components will facilitate the development of superior hybrids with enhanced yield potential and improved nutritional quality.

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MATERIAL AND METHODS

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The experimental material comprised of 45 sunflower inbred lines, including five checks. An Alpha Lattice Design with three replications is employed for cultivation of 45 sunflower inbred lines. The field experiment was carried out during the *Rabi* 2023 at the Regional Agricultural Research Station, Nandyal, Andhra Pradesh, India, geographically located at 15^{0}29’ north latitude and 78^{0}29’ east longitude at an altitude of 211.76 m above mean sea level. Each genotype was cultivated in two rows with a row length of 3m, with plot size of 3.0 × 1.8 m^2 per genotype maintaining a row spacing of 60 cm and plant spacing of 30 cm. All the agronomic practices recommended by Acharya N.G. Ranga Agricultural University were carried to raise a healthy crop. Data were collected on 11 traits days to 50% flowering, days to maturity, final plant stand, plant height, head diameter, volume weight, 100-seed weight, seed yield (g/plant), seed yield (kg ha^{-1}), oil content, and oil yield from five randomly selected plants per genotype in all replications. Analysis of variance (ANOVA) was conducted on the Alpha Lattice Design using the Variability package in R Studio. The collected data is subjected to statistical analysis employing Mahalanobis D^2 distance (Mahalanobis, 1928) and Tocher's method (Rao, 1952) to identify group clusters. Path coefficient analysis as suggested by Dewey and Lu was employed to estimate direct and indirect effects of yield attributing traits on seed yield (kg ha^{-1}). Statistical analysis was calculated using INDOSTAT 9.2 software and R Studio.

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RESULTS AND DISCUSSION

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In the present study, genetic diversity among 45 sunflower inbreed lines was assessed using D^2 statistics of Mahalanobis (1928) followed by clustering of genotypes using tocher's method. The mean values of 45 inbred lines were subjected to analysis of variance (ANOVA) and the mean sum of square for each trait was calculated and represented in Table 1. The significance of mean sum of squares across all studied traits indicates presence of variability in these traits and also suggests a focus on these traits for further improvement (Mariyam *et al.,* 2024). The analysis of dispersion based on the Wilk's criterion presented in Table 2 revealed highly significant difference among inbred lines for 11 traits. Group constellation was carried out following tocher's method (Rao, 1952) and grouped 45 inbred lines into six clusters. The cluster composition is represented in Table 3 and Fig.1. Cluster I was the largest comprising of maximum number of 34 genotypes followed by cluster II with seven genotypes the remaining clusters are monogenic with single genotype. Lakshman *et al*, (2021) and Kumar *et al*. (2024) also observed similar clustering pattern of genotypes among cluster as some clusters were unique having only single genotype.

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The intra and inter cluster distance were displayed in Table 4 and Fig.2. Maximum intra cluster value was observed for cluster I (15.17) followed by cluster II (15.13). Cluster III, IV, V and VI has zero intra cluster distance since they are monogenic clusters. The maximum inter cluster distance was found between cluster V and cluster II (79.75) followed by cluster IV and cluster II (70.78) and cluster VI and cluster V (57.87). Crossing between inbred lines of these clusters will result in higher hybrid vigour. Inter cluster distance was found minimum between cluster V and cluster III (9.39) suggesting close relation between them and low level of diversity. The inter cluster distances were greater than intra cluster except in some clusters and same is reported by Lakshman *et al*. (2021) and Kumar *et al*. (2024).

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A perusal of cluster means for 11 traits per cluster was presented in the Table 5. The Cluster VI reported maximum cluster means for the traits *viz.,* days to 50% flowering (56.67), plant height (117.87), head diameter (13.00), 100 seed weight (4.50), seed yield (g/plant) (26.87), seed yield (kg ha^{-1}) (1493.57). Highest cluster mean values for final plant stand (18.66) and volumetric weight (47.00) was displayed in cluster II. Cluster IV recorded desired highest cluster mean for oil yield (kg ha^{-1}). The desired values for days to 50 % flowering (52.67), days to maturity (86.33) *i.e.,* earliness was reported in cluster III and the same cluster exhibited higher value for oil content (37.80). Considering the mean performance of the clusters, the cluster VI had highest mean value for most of the studied traits and it can be used as one of parents to get higher yield. Genotypes of this cluster can be used for generating variability.

Relative contribution of different traits in the present study is represented in the Table 6. Among the traits studied, seed yield (g/plant) (50.81%), contributed maximum to the total variation followed by seed yield (kg ha^{-1}) (12.02%) and head diameter (11.92 %). The traits that contributed least to the genetic diversity were final plant stand (0.2 %) and days to maturity (0.3 %). Rani *et al.* (2016) reported that seed yield (g/plant) contributed maximum towards diversity in their study. Lakshman *et al*. (2021) reported that head diameter contributed maximum towards diversity in their study.

Path analysis is used to analyse all details of relationship between yield and its component traits with examining completely the direct and indirect effects for constituting of main yield traits (Singh and Choudhary, 1979). Therefore, to understand yield trait relationship in sunflower path coefficient analysis is performed and the path coefficient values are displayed in Table 7. Phenotypic and genotypic path diagram for seed yield (kg ha^{-1}) are represented in Fig. 3 and Fig. 4 respectively. In the current study, the residual effect observed was 0.012 and 0.087 at both phenotypic level and genotypic levels, respectively. This implies its significance in measuring influence of unexamined independent variables.

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Several traits *viz.,* days to 50% flowering (p_p= 0.0011, p_g= 0.0054), head diameter (p_p= 0.0015, p_g= 0.0212), 100 seed weight (p_p = 0.0024, p_g = 0.0028), seed yield per plant (p_p= 0.9589, p_g= 0.7695) and oil yield (p_p=0.0420, p_g=0.2346) exhibited positive and direct effects on seed yield (kg ha^{-1}), indicating their direct contribution to higher yield. These findings corroborate previous research by Kaur and Kaila (2023) for days to 50% flowering; Radic *et al*. (2021) for head diameter; Gopi *et al*. (2023) for 100 seed weight; Hassan *et al*. (2013) for seed yield (g/plant); Lakshman *et al*. (2021) for oil yield. In contrast, several traits exhibited negative direct effects on seed yield (kg ha^{-1}). Days to maturity (p_p= -0.0019, p_g= -0.0119), final plant stand (p_p= 0.0018, p_g= -0.0046), plant height (p_p= -0.0012, p_g=-0.0162), volumetric weight (p_p=-0.0002, p_g=-0.0013) and oil content (p_p = -0.0139, p_g = -0.0673) negatively influenced seed yield (kg ha^{-1}) These findings align with previous research by Kang and Ahmed (2014) for days to maturity; Kaur and Kaila (2023) for plant height; Radic *et al*. (2021) for oil content; Radic *et al*. (2021) and Kaur and Kaila (2023) for volumetric weight. The results suggest that these traits can show detrimental impact on seed yield. Additionally, oil content displayed a negative direct and indirect effect on seed yield (kg ha^{-1}). Moreover, most traits showed significant positive indirect effects mediated by seed yield (g/plant) and often by oil yield as well. This highlights the importance of these traits in enhancing individual plant productivity, which ultimately drives overall seed yield. Seed yield (g/plant) and oil yield emerged as key drivers of total seed yield, with strong direct and indirect positive effects.

CONCLUSIONS

The present study exhibited high differences among the genotypes for seed yield and almost all the yield components which may favour selection and its utilization in recombination breeding programme. The findings of genetic diversity studies emphasize need for prioritizing traits seed yield (g/plant), head diameter, volumetric weight that contribute maximum towards genetic diversity. Genetically diverse sunflower inbred lines identified from the present study could be utilized in development of diverse inbred lines which may be utilised in future heterosis breeding. Path coefficient analysis reinforced the central role of seed yield per plant in directly affecting seed yield (kg ha^{-1}). These findings highlight the importance of selection of traits seed yield (g/plant) and oil yield for achieving improvement in seed yield (kg ha^{-1}). Further research and validation of these findings across diverse environments and genotypes will be crucial for the successful implementation of breeding programs aimed at enhancing sunflower productivity.

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Table 1. Analysis of variance for yield and yield attributing traits in sunflower

S. No	Traits	Mean squares			
		Replications (df:2)	**Genotypes (df:44)**	**Blocks (df:24)**	**Error (df:64)**
1	Days to 50% flowering	1.49	9.60**	1.98	2.63
2	Days to maturity	0.59	14.82**	5.97	6.04
3	Final plant stand	0.77	5.27**	3.28	2.64
4	Plant height (cm)	1.20	606.00**	57.80	75.40
5	Head diameter (cm)	1.15	8.67**	0.90	0.82
6	Volumetric weight (g $100ml^{-1}$)	2.55	92.19**	2.02	2.50
7	100 seed weight(g)	0.06	1.22**	0.15	0.14
8	Seed yield (g/plant)	0.29	30.96**	5.27	3.03

9	Seed yield (kg ha^{-1})	3947.00	109698.00**	26871.00	15457.00
10	Oil content (%)	2.53	8.17**	2.94	2.30
11	Oil yield (kg ha^{-1})	680.00	13364.00**	2724.00	1410.00

*, ** Significant at 5% and 1% levels, respectively

Table 2 . Analysis of variance for dispersion in sunflower genotypes

Source of Variations	DF	Mean Squares
Varieties	44	3.69**
Error	87	1.87
Total	131	0.048

** Significant at 1% level

Table 3. Cluster composition of sunflower genotypes based on Tocher's method

Cluster Group	No. of Genotypes	List of Genotypes
Cluster I	34	NDLR-36,NDLR-4,NDSI-3,NDI-47,RHA-1096,COSF-7B,RCR-39,PB-120,EC-601768,NDLR-27,OPH-74,RSFH-11,RHA-172,EC-601829,PS2023B,RHA-95C-1,NDI-50,NDSI-2,IC-502039,RHA-6D-1,NDLR-40,RP-16,PB-110,NDI-55,NDLB-5,RHA-859,CMS-30B,NDLR-32,NDLR-1,COSF-6B,KBSH-44,LTRR-341,NDI-43 and CMS-104B
Cluster II	7	ARM-243B, PM-81, R-64, NDI-20, RP-10, CMS-249B and CMS-302B
Cluster III	1	RSFH-5
Cluster IV	1	R X R-2-38
Cluster V	1	R-106
Cluster VI	1	RHA-1013

Table 4. Average intra and inter cluster distances for the sunflower genotypes

Cluster Distances						
	Cluster I	**Cluster II**	**Cluster III**	**Cluster IV**	**Cluster V**	**Cluster VI**
Cluster I	15.17					
Cluster II	30.51	15.13				
Cluster III	26.33	54.73	0.00			
Cluster IV	27.72	70.78	35.39	0.00		
Cluster V	33.84	79.75	9.39	25.19	0.00	
Cluster VI	24.10	41.92	53.60	36.61	57.87	0.00

Table 5. Cluster means with respect to yield and its attributes among sunflower genotypes

S. No.	Character	Cluster number					
		I	II	III	IV	V	VI
1.	Days to 50% flowering	53.78	54.57	52.67	56.33	54.67	56.67
2.	Days to maturity	88.08	87.19	86.33	92.00	87.00	91.33
3.	Final plant stand	18.10	18.66	17.94	18.00	18.54	17.86
4.	Plant height (cm)	94.66	97.18	70.07	103.40	72.53	117.87
5.	Head diameter (cm)	9.76	9.51	6.38	9.27	5.43	13.00
6.	Volumetric weight (g 100ml^{-1})	37.03	47.00	33.60	29.67	27.57	34.93
7.	100 seed weight (g)	3.78	4.03	2.97	2.70	3.63	4.50
8.	Seed yield (g /plant)	22.61	23.24	14.77	25.80	18.73	26.87
9.	Seed yield (kg ha^{-1})	1256.03	1290.54	820.43	1433.37	1040.67	1493.57
10.	Oil content (%)	35.52	36.46	37.80	37.53	37.37	30.73
11.	Oil yield (kg ha^{-1})	446.54	469.78	310.00	539.33	388.27	459.80

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Table 6. Contribution of yield and its attributes towards total diversity in sunflower genotypes

S. No.	Source	Contribution %	Times ranked 1st
1	Days to 50% flowering	3.84	38
2	Days to maturity	0.30	3
3	Final Plant Stand	0.20	2
4	Plant height (cm)	7.58	75
5	Head Diameter (cm)	11.92	118
6	Volumetric weight (g 100ml^{-1})	9.09	90
7	100 seed weight (g)	0.51	5
8	Seed yield (g/plant)	50.81	503
9	Seed yield (kg ha^{-1})	12.02	119
10	Oil content (%)	2.42	24
11	Oil yield (kg ha^{-1})	1.31	13

Table 7. Phenotypic and genotypic path coefficients for yield and its attributes in sunflower genotypes.

		Days to 50% flowering	Days to maturity	Final Plant Stand	Plant height (cm)	Head Diameter (cm)	Volumetric weight (g 100ml^{-1})	100 seed weight (g)	Seed yield (g/plant)	Oil content (%)	Oil yield (kg ha^{-1})	Seed yield (kg ha^{-1})
Days to 50% flowering	p_p	**0.0011**	0.0004	0.0000	0.0001	0.0000	0.0000	0.0000	0.0002	-0.0002	0.0001	0.1740*
	p_g	**0.0054**	0.0026	-0.0012	0.0014	0.0006	-0.0001	-0.0005	0.0014	-0.0012	0.001	0.2540*
Days to maturity	p_p	-0.0006	**-0.0019**	0.0000	-0.0002	-0.0002	0.0001	0.0003	-0.0001	0.0004	0.0000	0.0610
	p_g	-0.0057	**-0.0119**	0.0002	-0.0029	-0.0042	0.0009	0.0044	-0.0006	0.0039	0.0003	0.0510
Final Plant Stand	p_p	0.0000	0.0000	**0.0018**	0.0004	0.0004	0.0003	0.0002	0.0003	0.0000	0.0003	0.1647
	p_g	0.0011	0.0001	**-0.0046**	-0.0027	-0.0029	-0.002	-0.0006	-0.0018	0.001	-0.0016	0.3960**
Plant height (cm)	p_p	-0.0002	-0.0001	-0.0002	**-0.0012**	-0.0007	-0.0002	-0.0002	-0.0002	0.0003	-0.0001	0.1960*
	p_g	-0.0042	-0.004	-0.0096	**-0.0162**	-0.0129	-0.0031	-0.0036	-0.0047	0.006	-0.0031	0.2890**
Head Diameter (cm)	p_p	0.0000	0.0002	0.0003	0.0009	**0.0015**	0.0003	0.0001	0.0004	-0.0003	0.0003	0.2480*
	p_g	0.0022	0.0075	0.0133	0.0169	**0.0212**	0.0055	0.0019	0.0073	-0.0058	0.0054	0.3430**
Volumetric weight (g 100ml^{-1})	p_p	0.0000	0.0000	0.0000	0.0000	0.0000	**-0.0002**	0.0000	0.0000	0.0000	0.0000	0.2030*
	p_g	0.0000	0.0001	-0.0006	-0.0003	-0.0003	**-0.0013**	-0.0002	-0.0003	0.0001	-0.0003	0.2570*
100 seed weight (g)	p_p	-0.0001	-0.0004	0.0003	0.0003	0.0002	0.0002	**0.0024**	0.0006	0.0001	0.0006	0.2490*
	p_g	-0.0002	-0.001	0.0004	0.0006	0.0003	0.0004	**0.0028**	0.0008	-0.0001	0.0007	0.2760*
Seed yield (g/plant)	p_p	0.1665	0.0604	0.1563	0.1880	0.2371	0.1946	0.2369	**0.9589**	-0.0440	0.8981	0.7430**
	p_g	0.197	0.0419	0.3038	0.2228	0.263	0.197	0.2106	**0.7695**	-0.0402	0.7385	0.7430**
Oil content (%)	p_p	0.0024	0.0026	0.0000	0.0038	0.0024	0.0004	-0.0004	0.0006	**-0.0139**	-0.0042	-0.0449
	p_g	0.0151	0.0223	0.0146	0.0249	0.0185	0.0032	0.0029	0.0035	**-0.0673**	-0.0153	-0.0502
Oil yield (kg ha^{-1})	p_p	0.0045	0.0000	0.0063	0.0041	0.0074	0.0079	0.0101	0.0393	0.0127	**0.0420**	0.9370**
	p_g	0.0438	-0.0066	0.0799	0.0446	0.0602	0.0562	0.0582	0.2251	0.0533	**0.2346**	0.9600**
Seed yield (kg ha^{-1})	p_p	0.1740*	0.0610	0.1647	0.1960*	0.2480*	0.2030*	0.2490*	0.7430**	-0.0449	0.9370**	
	p_g	0.2540*	0.0510	0.3960**	0.2890**	0.3430**	0.2570*	0.2760*	0.7430**	-0.0502	0.9600**	

Fig.1. Dendrogram showing relationship among sunflower genotypes in six clusters based on D^2 values

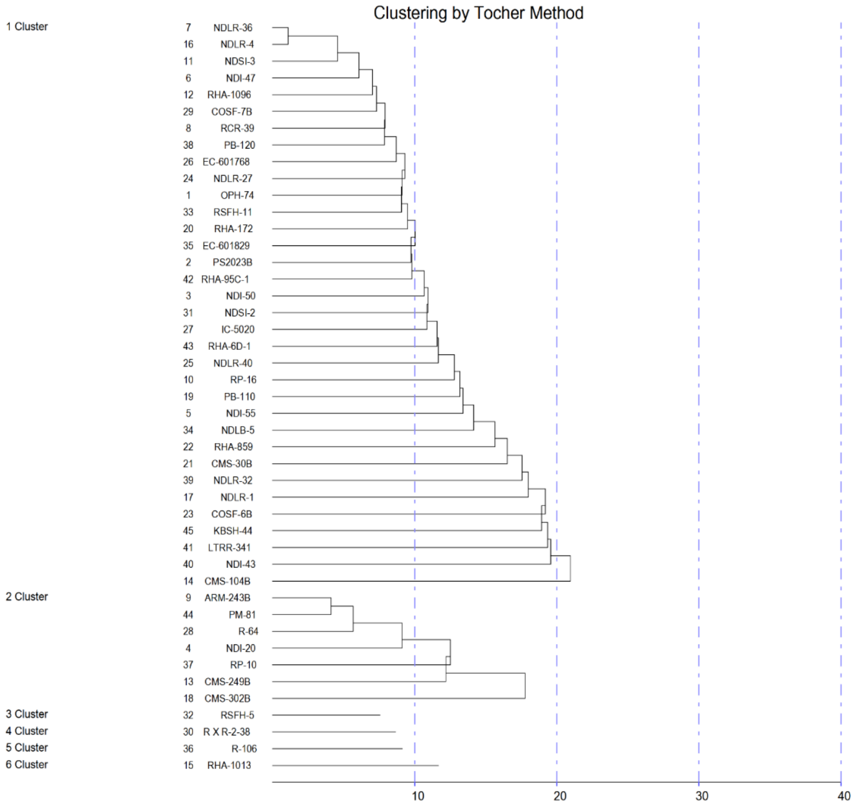

Fig.2. Cluster diagram showing average intra and inter cluster distance for yield and its attributes of sunflower genotypes

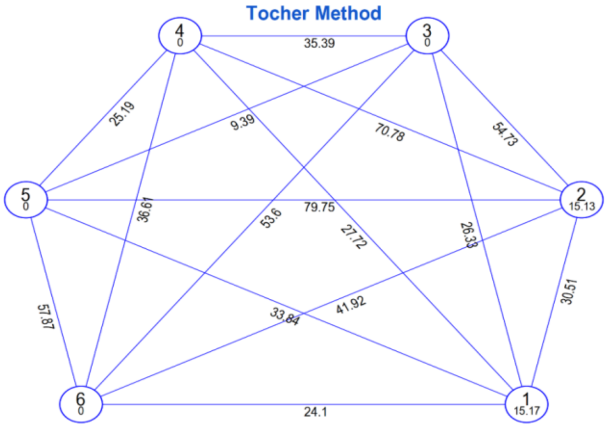

Fig. 3. Phenotypic path diagram for seed yield (kg ha^{-1})

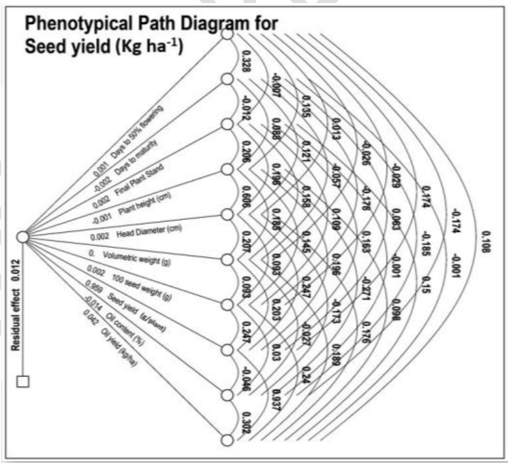

Fig. 4. Genotypic path diagram for seed yield (kg ha^{-1})