Received: 13 May 2021 | Revised: 30 Aug 2021 | Accepted: 13 Sep 2021

Electronic Journal of Plant Breeding

Research Article

Association analysis in chia (*Salvia hispanica* L.)

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Abstract
The present study was carried out in a set of germplasm accessions for yield related traits in Chia. Results of the correlation coefficient revealed a highly significant and positive effect of the number of primary branches, harvest index and oil content on seed yield per plant on both genotypic and phenotypic levels, while plant height had a positive significant correlation with seed yield only at the genotypic level. Through analysis of path coefficients, the positive and significant direct effect of harvest index on genotypic as well as phenotypic levels was inferred, whilst days to 50% flowering and plant height illustrated positive direct effects on seed yield per plant only at the genotypic level. Moderate direct and positive effects of days to maturity at the genotypic level along with the number of primary branches at the phenotypic level were observed on seed yield per plant. The study revealed that harvesting index, days to 50% flowering and fruiting branches could be used as selection criteria traits, which will be very useful in future breeding programmes to enhance yield in Chia.

Key words: Chia, Correlation coefficient, Path analysis, Selection criteria, Yield improvement.

INTRODUCTION

Chia (*Salvia hispanica* L.) is an annual herbaceous plant, which originated from the land of Northern Guatemala and Southern Mexico (Coates and Ayerza, 1996). It belongs to the family of Lamiaceae. The species of this family are known for their medicinal properties and aromatic compounds (Singh and Gaurav, 2015). Throughout the world, Central Mexico, Guatemala, Australia, South America and Argentina are amongst the major producers of this crop. Chia crop is gaining popularity in this era as consumption of its seeds provides enormous health benefits. Its seeds are rich in omega acids, vitamins, fibres, PUFA (Polyunsaturated fatty acids) MUFA (Monounsaturated fatty acids), iron, magnesium and manganese (USDA, 2004). It has proven its importance since time immemorial. It was found to be one of the four generic components in the traditional food habit of Central American colonies in the pre-Columbian era and-now-a-days it is being recommended into the food habits of occident countries because of its countless healthy properties.

Chia seeds are cultivated on a small scale in their ancestral homeland of Central Mexico and Guatemala. At present Chia is cultivated in Australia, Bolivia, Colombia, Guatemala, Mexico, Peru, and Argentina (Busilacchi *et al*., 2013). The largest production centre is located in Mexico. It currently exports seeds to Japan, the USA, and Europe (Alanbrant *et al*., 2014), with the highest average seed yield (1.1 tonne/ ha). However, the cultivation of Chia in India has been initiated in small pockets in states like Rajasthan, Karnataka, Madhya Pradesh and Gujarat. About 80 per cent of the world's supply of Chia comes from South America. Though commercial production of Chia was only concentrated in a specific area such as Bolivia and Paraguay, in the last decade, cultivation of Chia has been initiated in other countries like Mexico, Australia and Argentina. Argentina has maximum acreage (1,20,000 ha) followed by Paraguay (1,00,000 ha), Bolivia (80,000 ha) and Mexico (50,000 ha) under Chia. Mexico ranked fourth while Australia was the fifth largest producer of Chia (Peperkamp, 2014).

The optimal seed yield depends on different criteria such as variety, climatic conditions and cultivation practices. The productivity of Chia in Bolivia was around 650 kg/ ha, lower than Argentina and Paraguay around 800-900 kg/ ha-(Peperkamp, 2014). However, if optimal agronomic conditions are provided, yield up to 2500 kg/ ha has also been reported (Cahill, 2004; Coates and Ayerza, 2011; Ullah *et al*., 2015). Despite being such a good and versatile crop with good returns and high market value, very limited studies have been carried out on association analysis.

The correlation coefficient would help in determining the strength of the relationship between different variables under study and will provide information that selection for one character results in progress or deterioration for other traits. Improvement in yield and uniformity and different traits such as flowering, plant height, germination and harvesting is necessary to popularise this crop among farmers for cultivation. Although it is very important in quantifying the size and directions of trait associations, correlation can be inaccurate if the high correlation among two traits is the repercussion of the direct effect of other traits (Bizeti *et al*., 2004). To overcome this, path analysis was used which bifurcated the correlation coefficient to find out the direct and indirect causes of association of each independent variable and it provided a thorough investigation of distinct forces contributing which results in a given correlation (Miller *et. al.,* 1958).

Successful cultivation of this crop will require potential information on the genetic background to select different characters based on their correlation and path analysis coefficients for further exploitation, high yield, enhancement in quality and developing a strategy for carrying out a sound breeding programme in the near future. Association studies help to understand the relationship between traits of economic importance that helps to devise selection criteria. Limited information is available on these aspects in the Chia crop. Therefore, the present study was conducted with an objective of association analysis (correlation along with direct and indirect effects on seed yield), from the set of germplasm accessions for selection criteria for yield improvement.

MATERIALS AND METHODS

The experiment was conducted at Agricultural Research Station (ARS), Mandor, Jodhpur, Rajasthan, India in *Rabi,* season with set 26 germplasm accessions of Chia (**Table 1**). The design used in this experiment was Randomized Block Design (RBD) with three replications in irrigated conditions with a spacing of 30 x 10 cm (row to row and plant to plant, respectively). A typical arid climate and erratic rainfall were experienced on the experimental site. Observations on ten traits *viz*., days to 50% flowering, days to maturity, plant height (cm), the number of primary branches per plant, main - inflorescence length (cm), the number of inflorescence per plant, seed yield per plant (g), test weight (g), oil content (%) and harvesting index (%) were recorded by randomly selecting five plants from each genotype. Oil extraction was carried out using the Soxhlet apparatus, in which the seeds were grounded and subjected to be washed in *n*-hexane at 60^{0}C for a continuous cycle of six hours (Jensen 2007; Noshe *et. al.,* 2017 and Silva *et. al.,* 2016). The percentage of oil extracted was calculated using the following formula

$$Oil\ content\ (\%) = \frac{Initial\ weight\ of\ seed - Final\ weight\ of\ seed}{Initial\ weight\ of\ seed}\ x\ 100$$

The data collected from observations were subjected to statistical analysis with the help of correlation coefficients (Al-Jibouri *et al*., 1958) and path coefficients analysis (Dewey and Lu, 1959).

Table 1. List of Chia germplasm accessions used in present investigation

S. No.	Accession name	S. No.	Accession name
1.	MCS 4	14.	MCS 38
2.	MCS 21	15.	MCS 39
3.	MCS 23	16.	MCS 41
4.	MCS 34	17.	MCS 42
5.	MCS 35	18.	MCS 47
6.	MCS 37	19.	MCS 52
7.	MCS 40	20.	Jalore White 1
8.	MCS 43	21.	Jalore Black 1
9.	MCS 49	22.	Jalore White 2
10.	MCS 3	23.	Jalore Black 2
11.	MCS 14	24.	MCS 53
12.	MCS 19	25.	Bulk White
13.	MCS 32	26.	Bulk Black

https://doi.org/10.37992/2021.1204.172

RESULTS AND DISCUSSION

The knowledge on the direction of relation between various yield related attributes plays a very important role in the genetic improvement of this crop. The development of high yielding varieties is very necessary through systematic breeding methods for the selection of desirable genotypes. In plant breeding, the correlation coefficient determines the mutual relationship between various plant characters and estimates the fundamental traits on which selection can be established upon for enhancement of dependant variables. A positive correlation between two characters indicates that improvement in both the characters can be done in the same direction, while a negative correlation indicates that improvement in one character would lead to deterioration of other characters involved.

Phenotypic and genotypic correlations between seed yield per plant and various yield component traits *viz.*, days to 50% flowering, days to maturity, plant height, the number of primary branches per plant, the number of inflorescence per plant, main inflorescence length, test weight, harvest index and oil content are presented in **Table 2**. The results of the investigation showed that seed yield had a highly significant genotypic and phenotypic positive correlation with harvest index (0.9209, 0.7257), followed by the number of primary branches (0.4244, 0.3394) and oil content (0.3281, 0.2630). Oil content showed positive highly significant genotypic and phenotypic correlation with the number of primary branches (0.4717, 0.3517), with plant height (0.2996, 0.2249), main inflorescence length (0.3347, 0.2209), harvest index (0.2843, 0.2170) and seed yield per plant (0.3281, 0.2630). Hence, selection criteria of these traits might result in higher seed yield per plant and might help a breeder in selecting good breeding material. Singh *et al.* (2015) also reported a positive significant correlation of oil content, plant height and the number of branches with fresh herb yield in *Ocimum basilicum.*

Path coefficient analysis bifurcates the correlation coefficient into direct and indirect effects which lead to a better understanding of the contribution of various attributes on yield or any other desired character.

Table 2. Correlation coefficients of Chia germplasm accessions for seed yield and it's attributes

Traits		Days to maturity	Plant height	Number of Primary Branches	Number of inflorescence per plant	Main inflorescence length	Harvest Index	Test weight	Oil content	Seed yield per plant
Days to 50% flowering	P	0.1730	-0.2470	0.0030	-0.1580	0.0570	0.0440	0.2350*	-0.0350	0.0960
	G	0.4715**	-0.4000	-0.1380	-0.1970	0.0530	0.0470	0.3400**	0.0200	0.1670
Days to maturity	P	1.000	-0.2630	0.0180	0.1896*	-0.2510	0.0810	-0.0890	-0.1130	-0.0400
	G	1.000	-0.3740	0.2517*	0.4470**	-0.3180	-0.012	0.0370	-0.2020	-0.0750
Plant height	P		1.000	0.3768**	0.2781**	0.4233**	-0.0010	-0.1950	0.2249*	0.1430
	G		1.000	0.4917**	0.3204**	0.6813**	0.0730	-0.1710	0.2996**	0.1935*
Number of Primary Branches	P			1.000	0.4376**	0.2210*	0.1923*	-0.2840	0.3517**	0.3394**
	G			1.000	0.5279**	0.1120	0.3906**	-0.4300	0.4717**	0.4244**
Number of inflorescence per plant	P				1.000	0.0070	0.0500	-0.3550	0.1470	-0.0900
	G				1.000	-0.0460	-0.0970	-0.4400	0.1480	-0.1030
Main inflorescence length	P					1.000	-0.1010	-0.0320	0.2209*	-0.0300
	G					1.000	-0.2280	-0.0920	0.3347**	-0.1100
Harvest Index	P						1.000	-0.1690	0.2170*	0.7257**
	G						1.000	-0.0510	0.2843**	0.9209**
Test weight	P							1.000	-0.0370	0.0290
	G							1.000	-0.0070	0.0380
Oil content	P								1.000	0.2630*
	G								1.000	0.3281**
Seed yield per plant	P									1.000
	G									1.000

P =Phenotypic correlation coefficient and G = Genotypic correlation coefficient
*Significant at 5% and **Significant at 1% level of significance

The correlation coefficient was split into direct and indirect effects by using path coefficient analysis, proposed by Wright in 1921 and expanded by Dewey and Lu in 1959. Yield is a very complex trait that can be governed by multiple factors whether direct or indirect. In this experiment, yield is considered as a dependant variable and the rest of the traits as an independent variables. The highest positive direct effect (genotypic level) on seed yield per plant was exhibited by harvesting index (0.7600), followed by plant height (0.3500) and primary branches per plant (0.1378). Days to maturity showed a negative direct effect on seed yield per plant at phenotypic as well as the genotypic levels (-0.0490, -0.2448) along with main inflorescence length (-0.1014, -0.2812). Days to 50 per cent flowering had a positive direct effect at the genotypic level. Harvesting index (genotypic level) also exhibited positive indirect on seed yield per plant *via*the number of primary branches (0.2969), followed by oil content (0.2161). Selection criteria purely based on correlation studies can be misleading. For example, oil content showed highly significant genotypic and phenotypic correlation with yield but path coefficient analysis revealed that genotypic direct effect on yield of oil content was negative, while the phenotypic direct effect was positive but very low.

The residual effect calculates the dependency of the dependent character (seed yield) on independent characters for variability. The residual effects were calculated as 0.2450 for genotypic level and 0.5950 for phenotypic level (**Table 3**). The value of residual effect infers some more characters should be involved in future studies. The cause-effect relationships at phenotypic and genotypic levels are diagrammatically presented in **Fig. 1 and Fig. 2,** respectively. Similar conclusions were reported by Patel (2005) in *Ocimum* for the direct positive effect of plant height and oil yield on seed yield. In an experiment conducted by Kumar *et al.* (2012), the yield traits *viz*., herb yield, oil content, and oil yield exhibited a strong genetic correlation and displayed a positive direct path of herb yield and oil content.

Correlation and path coefficients were calculated for yield and its attributing traits. Harvest index, the height of the

Table 3. Path coefficients of Chia germplasm accessions for seed yield and it's attributes

Traits		Days to 50% flowering	Days to maturity	Plant height	Number of Primary Branches	Number of inflorescence per plant	Main inflorescence length	Harvest Index	Test weight	Oil content	Seed yield per plant Correlation coefficient
Days to 50% flowering	P	**0.0530**	-0.0085	-0.0370	0.0009	0.0222	-0.0058	0.0297	0.043	-0.0011	0.0960
	G	**0.4049**	-0.1154	-0.1399	-0.019	0.0041	-0.0147	0.0359	0.011	-0.0004	0.1670
Days to maturity	P	0.0092	**-0.049**	-0.0395	0.0052	-0.0266	0.0254	0.0554	-0.0164	-0.0034	-0.0400
	G	0.1909	**-0.2448**	-0.1308	0.0347	-0.0094	0.0893	-0.0092	0.0012	0.0037	-0.0750
Plant height	P	-0.0131	0.0129	**0.1499**	0.1047	-0.0391	-0.0429	-0.0006	-0.0357	0.0067	0.1430
	G	-0.1618	0.0915	**0.3500**	0.0678	-0.0067	-0.1916	0.0553	-0.0055	-0.0054	0.1935*
Number of Primary Branches	P	0.0002	-0.0009	0.0565	**0.2779**	-0.0615	-0.0224	0.1310	-0.0519	0.0105	0.3394**
	G	-0.0559	-0.0616	0.1721	**0.1378**	-0.0111	-0.0314	0.2969	-0.0139	-0.0085	0.4244**
Number of inflorescence per plant	P	-0.0084	-0.0093	0.0417	0.1216	**-0.1405**	-0.0007	-0.0341	-0.065	0.0044	-0.0900
	G	-0.0796	-0.1094	0.1122	0.0727	**0.0210**	0.0129	-0.0734	-0.0142	-0.0027	-0.1030
Main inflorescence length	P	0.003	0.0123	0.0635	0.0614	-0.0009	**-0.1014**	-0.0689	-0.0058	0.0066	-0.0300
	G	0.0212	0.0778	0.2385	0.0154	0.0010	**-0.2812**	-0.1735	-0.0030	-0.0061	-0.1100
Harvest Index	P	0.0023	-0.0040	-0.0001	0.0535	0.0070	0.0103	**0.6812**	-0.0309	0.0065	0.7257**
	G	0.0191	0.0030	0.0255	0.0538	0.0020	0.0642	**0.7600**	-0.0017	-0.0051	0.9209**
Test weight	P	0.0125	0.0044	-0.0293	-0.0788	0.0499	0.0032	-0.1151	**0.1829**	-0.0011	0.0290
	G	0.1377	-0.009	-0.0597	-0.0593	0.0093	0.0258	-0.0389	**0.0323**	0.0001	0.0380
Oil content	P	-0.0019	0.0056	0.0337	0.0978	-0.0206	-0.0224	0.1478	-0.0068	**0.0298**	0.2630*
	G	0.0082	0.0495	0.1049	0.0650	-0.0031	-0.0941	0.2161	-0.0002	**-0.0181**	0.3281**

Residual effect (G) = 0.2450, (P) = 0.5950 Bold values represents direct effects,
P =Phenotypic path coefficient and G = Genotypic path coefficient
*Significant at 5% and **Significant at 1% level of significance

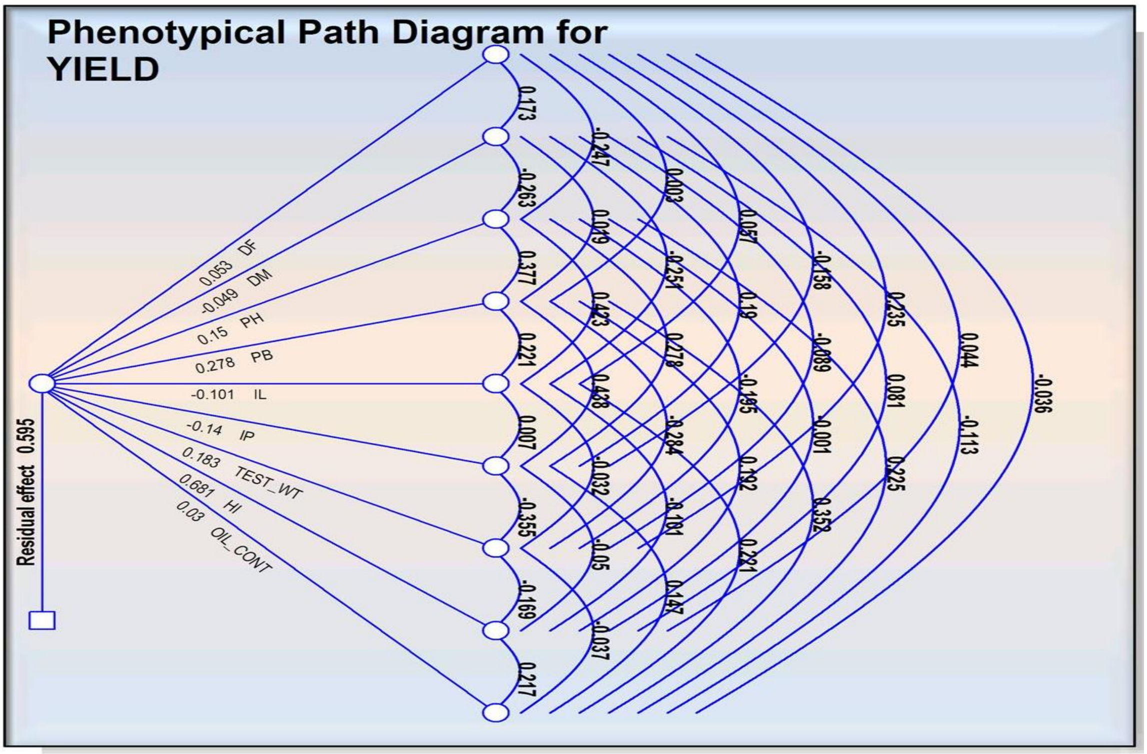

Fig. 1. Phenotypic path diagram of Chia germplasm accessions for seed yield and its attributes

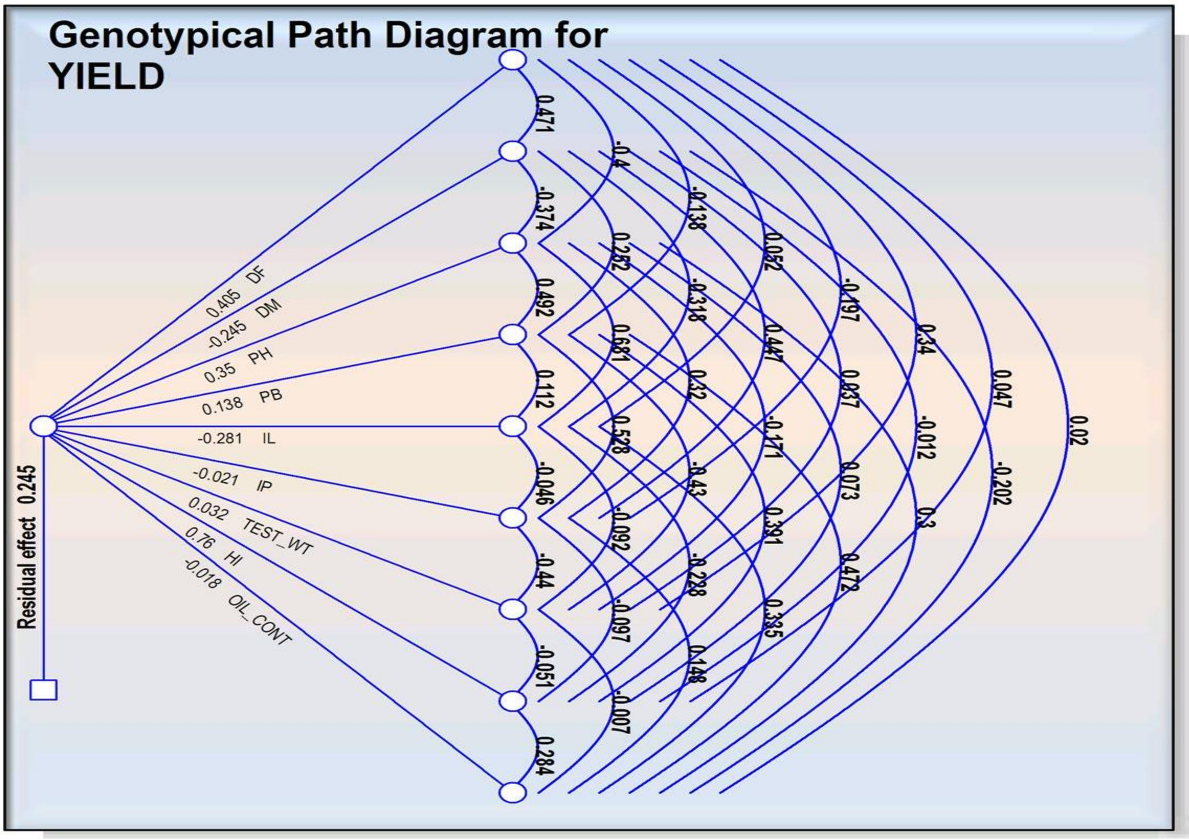

Fig. 2. Genotypic path diagram of Chia germplasm accessions for seed yield and its attributes

plant, and the total amount of primary branches per plant had a significant positive effect on seed yield per plant which can be selected for improving the yield of the crop. Traits namely days to maturity and main inflorescence length illustrated negative significant direct effects which implied that selection for enhancement in these traits would result in a diminishing seed yield. The seeds as well as the oil of Chia seeds have an immense potential for monetisation as it has several important nutritional as well as therapeutic characteristics which can be used for consumption or for medicinal purpose. Genotypes with high seed yield and oil content from the present investigation could be effective selection criteria using direct or indirect effects and correlation among various traits. Also, the traits contributing to high seed yield and high oil content could be selected for exploiting heterosis in a future breeding programme. Further testing of these germplasm accessions for yield related traits will be very useful in future breeding programs to enhance yield in Chia.

ACKNOWLEDGMENT

The authors would like to thank the All India Coordinated Research Network on Potential Crops unit and Zonal Director Research, ARS, Mandor, Jodhpur, Rajasthan, for providing the necessary facilities in successfully conducting the research work.

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