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Quantification of variability, Character Association and path analysis for Opium poppy (*Papaver somniferum* L.)

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ABSTRACT

Opium Poppy was important medicinal Plant for investigation to Genetic variability and nature and amount of genetic variability, correlation, and path coefficient for the thirteen characters among forty accessions of opium poppy (*Papaver somniferum* L.) grown was 2021-22. Morpho-metric variation detected flower colure white to red and pink, fringed and non-fringed, leaf narrow and broad, capsule green to brown color in maturity stage, and number of races. Correlation coefficient high significantly positive associated capsule index correlated with seed yield (0.678 0.688), days to maturity correlated with Papaverine (0.641 0.639), Thebaine correlated with Papaverine (0.588 0.587), Morphine correlated with Codeine (0.542 0.541) at both genotypic and phenotypic level. The Peduncle length correlated with Capsule index (0.423 0.279) show that the only at genotypic level. Days to Maturity was positive significantly associated with Morphine (0.347 0.346), Straw yield associated and Days to Maturity associated with Thebaine (0.312 0.308) (0.308 0.307) at both level genotypic and phenotypic. On the other hand Maximum direct positive effect on capsule index, Thebaine content, Morphine content, plant height and Codeine content. The results unambiguously showed that variation in significant economic variables might assist in the selection of O. poppy genotypes for additional genetic improvement.

Introduction

Opium poppy isa valuable medicinal plants is a self-pollinated crop, Poppy seed is a biological source of *Papaversomniferum*,

belonging to the family of papaveraceae. Depending on the kinds of cultivars, wind speed, bee visits, insects, and weather, out crossing can range from 2 to 70% (Khanna and Shukla, 1983) and (Bhandari, 1990). Poppy seeds are a fantastic source of energy since they contain fixed oil, which may also be utilized as edible oil. In pharmaceutical preparations, it used to create emulsions. Here, we sought to cultivate, harvest, and extract poppy for medical and pharmacological purposes.(Varro E.Tyler, 2011). The opium poppy is distributed in the temperate and subtropical regions, the opium poppy was cultivated in India, USSR, Turkey and Australia. Now India it is cultivated largely in Rajasthan,Madhya Pradesh and Uttar Pradesh, other parts minor cultivation is also practiced. It is also cultivated as an ornamental plant in many part of the world including Turkey, India, Burma, Thailand and Australia (Tetenyi, 1997). Among the fifty species in the genus Papaver, only six are found in India. According to CK. Kokate (2014), they are P. somniferum (Opium poppy), P. nudicaule (Ice land poppy), P. rhoeas (Corn poppy), P. orientale, P. argemone, and P. dubium. The genus Papaver, which is part of the Papaveraceae family, contains a large number of annual and perennial herb species. Indian land races of opium poppies have been described in a variety of sources and have been shown to exhibit significant heterogeneity in regard to alkaloids production as well as seed and seed oil quality (Sharma *et al*., 1981; Singh *et al*., 1995;). Besides pharmaceutical importance of opium poppy seeds are also rich source of herb based protein supplement to community consumption (Ozcan and Atalay, 2006). Poppy seeds having no narcotic influence, are very useful owing to presence of high protein content (up and around to 24%) and edible oil (50%) having linoleic acid (up to 68%), which helps in lowering blood cholesterol in human system (Singh *et al*., 1998;). The opium is obtained by lancing semi-ripe capsules of opium poppy and it is having approximately 12% morphine and low quantities of other alkaloids like thabaine, codeine, papaverine, noscapine, etc.(Dingermann*et al*., 2004). Morphine, the major opium morphinane alkaloid, is present in the root, stem, leaf and the fruit wall of the *Papaver somniferum* L. plant (Anonymous, 1966; Hoffman and Menary, 1979). Opium poppy seed are free from narcotic constituents and they used for foodstuff and bakery productions. Seeds are quite nutritious and have a pleasant nutty flavor and are often added to cakes or

sprinkled on breads. The increasing demand for opium alkaloids can be met by the development of high opium yielding varieties able to produce the specific alkaloids It is used in the treatment of diarrhea, dysentery and scalds. For successful breeding in opium poppy, sufficient genetic diversity/genetic variability are required. Increase the seed yield and alkaloids content in poppy straw is one of the main objective of poppy breeding and the productivity is affected by weather conditions, particularly temperature and rainfall at the time of germination, flowering, capsule lancing and capsule drying (Lal *et al*., 2014; Lahiri *et al*., 2017).The successful development of high-yielding cultivars would be facilitated by knowledge of the relationship between yield and its contributing features (Mary and Goalie, 2006). The selection of particular attributes to be utilized as indirect selection criteria for heritable yield improvement depends on the nature of the relationship between yield and its contributing traits (Guljar and Patil, 2016). By Ahmad Zadeh et al. (2012), path analysis is used to assess the direct and indirect impacts of several variables on the dependent variable.

Material and Methods

In spite of its favorable production in cold climates, it is successfully grown in sub-tropical regions during the wintertime. At the CSIR-Central Institute of Medicinal and Aromatic Plants (CIMAP) research farm in Lucknow, India, which is situated at 26.5° N latitude and 80.50° E longitude and 120 meters above sea level, 40 genotypes of opium poppies, including various breeding lines and landraces, were cultivated in 2021–2022 using randomized block designs (RBD) with three replications. Plants were planted in rows of 4m long and 50 cm apart and normal cultural procedures, irrigation, and fertilizer treatments (120 kg nitrogen, 80 kg phosphorus, and 60 kg potassium (hectare). For each of the thirteen features listed below, morpho-matric data was collected from five randomly chosen plants in every line of plants as well as Days to 50% flowering, plant height (cm), number of capsule/plant, Peduncle length (cm), Capsule index, Seed yield/plant (gm), Straw yield/plant (gm), Days to maturity,Morphine (%), Codeine (%), Thebaine (%), Papaverine (%), Narcotine (%) obtain from each selected plants.

Chemical analysis

For preparation for chemical analysissamples were taken for HPTLC analysis once 1g of

dry grinded capsule husk powder was first soaked in methanol, sonicated for 30 minutes in an ultrasonic bath and then solution was centrifuged at 10,000 rpm for 10 minutes.Every standard was weighed separately, and a stock solution was made. Created a working standard, an equal volume of each standard stock solution has been taken and pooled. TLC-densitometric procedure were using analyzed the five main opium alkaloids morphine, codeine, thebaine, papaverine and narcotine (Gupta and Verma, 1996). Toluene-acetone-methanol-ammonia (40:40:6:2) was used as a mobile phase. Silica gel plates 60 F254 were scanned after derivatization using Dragendorff reagent no. llC which is used to detect alkaloid content (Wagner and Bladt, 1996) at 540 nm.

Statistical analysis

Statistically analyzed the overall mean data of two consecutive years CSIR-CIMAP, Lucknow developed Statistical Software 4.0, which is usable in the institute's Genetics and Plant Breeding Division Singh and Chaudhary (1979) and Panse and Sukhatme (1976). The mean, standard error, ranges were determined subsequent (Singh and Chaudhary, 1979). Study of variance was carried out following the procedure given by Panse and Sukhatme (1976). The data arranged 13 morpho-metric characters were ANOVA, mean value, range, standard error, variability parameters, relationships, character contributions through path analysis, heredity are all included in the analysis of variances permitting to the conventional technique (Dewey and Lu, 1959; Singh and Chaudhary, 1979; Lal, R.K, 2011)

Result & Discussion

At the 0.01% and 0.05% significant levels, the augmented design's analysis of variance ANOVA showed highly significant and exploitable variation among the analyzed genotypes for each of the thirteen characters Table 1. Higher diversity in the initially developed breeding sources improves the probability that they will produce desirable recombinants for crop development. Genotypic and phenotypic correlation coefficient between all possible combinations was estimated. The successful outcome of selection in any breeding effort is influenced by genetic correlations. The direction and strength of the genetic association between and throughout the various attributes determine the breeding program's subsequent outcome after selection. The level of relationship between

two variables, which translates into the genetic gain obtained from a correlated response to selection, determines the magnitude of the direct selection responses. For the majority of the variables analyzed shows that the genotypic correlations were greater than the phenotypic correlations (Table 2).The correlation coefficients between the thirteen characters shown that capsule index positive and highly significant correlated with seed yield (0.678** 0.688**) followed by days to maturity correlated with Papaverine (0.641** 0.639**), Thebaine correlated with Papavervine (0.588** 0.587**), Morphine correlated with Codeine (0.542 ** 0.541**) at both genotypic and phenotypic level. The Peduncle length correlated with Capsule index (0.423** 0.279) show that the only at genotypic level. The Days to Maturity was positive significantly associated with Morphine (0.347* 0.346*) followed by Straw yield associated with Thebaine (0.312* 0.308*) and Days to Maturity associated with Thebaine (0.308* 0.307*) at both genotypic and phenotypic levels. The number of capsule/plant show that the negative significantly associated with Straw yield (-0.345* -0.207) and Number of capsule/plant correlated with Thebaine (-0.335* -0.234) at only genotypic level. All the thirteen characters correlated with all possible combinations at both level genotypic and phenotypic. In genetics, the coupling phase of linkage leads to a positive correlation, however the genes' repulsion linkage results in a negative correlation. Additionally, it has been found that environmental factors having little impact on the appearance of attributes, indicating that the relationship between individual characteristics is frequently controlled by genetics. Later, these outcomes are a good benchmark for the selection. (Kumar, M 1994;)

The path coefficient exploration has been exploited to observe the proportional strength of direct and indirect association between dependent and independent variables for selection in plant breeding programmes Renu, et al. 2018 Table 3 & fig 2. Seed yield (gm/pl.) was as a taken dependent character and other character was a independed. The Maximum direct positive effect on capsule index (0.4170) fallowed by Thebaine content (0.2598), Morphine content (0.2455), plant height (0.1589) and Codeine content (0.1532) and another traits were negative direct effect was Straw yield (-0.3746) followed by Papaverine conent (-0.1927), days to 50 % flowering (-0.1563),

Peduncle length (-0.0968), Narcotine content (-0.0779), days to maturity (-0.0372) and number of Capsule/ plant (-0.0114) but their indirect contribution was invariably maximum and positive capsule index (0.1763) and Thebaine (0.1409). The maximum indirect contribution was negative via Straw yield (-0.2542) and Capsule Index (-0.1107) although residual effect was 0.8457.

Conclusion

The justification underlying the current study could be enhanced in order to develop strategies aimed at increasing seed production. The investigation of the variance of all the characters studied was significant and shown a wide range of variability among genotypes of Opium poppy. Breeders and agrarians may be able to improve the cultivar alkaloids yield and seed yield of this crop by taking into mean, range, and all genetic standards for selection based on the necessity of initiative for numerous agro environments. Morpho-metric variation was also studying flower color was whitish to red and pink, fringed and non-fringed different flowering stage, leaf had fringed and non-fringed, narrow and broad leaf in different vegetative stage green and capsule green to brown color in maturity (harvesting) stage and different number of races. Fig 1. The coefficient correlation was shows that the genotypic correlations were large than phenotypic correlations for almost traits studied among the characters. Correlation coeffecient significantly positive and highly correlated capsule index with seed yield, days to maturity correlated with Papaverine, Thebaine correlated with Papavervine, Morphine correlated with Codeine at both level, Peduncle length correlated with Capsule index at only genetic level. Days to Maturity was positive significantly associated with Morphine, Straw yield with Thebaine and Days to Maturity with Thebaine at both level. Maximum direct positive effect on capsule index, Thebaine content, Morphine content, plant height and Codeine content. Characteristics that directly increase seed yield have been considered to be appropriate selection criteria for developing genotypes that have high yields.

Table1 : Analysis of variance (ANOVA) of thirteen traits of Opium poppy.

Sl. No.	Traits	Treatments (MSS) df=39	Replication (MSS) df=2	Error (MSS) df=78
1.	Days to 50% flowering	15.015**	173.233	0.815
2.	Plant height (cm)	86.420**	253.425	0.211
3.	No. of capsule/ plant	2.533**	5.308	0.693
4.	Pedicel length (cm)	27.674**	14.658	5.146
5.	Capsule index (capsule width/length)	4.068**	5.351	1.105
6.	Seed yield (gm/plant)	1.768**	3.069	0.738
7.	Dry capsule husk/straw yield (gm/plant)	1.435**	0.123	0.009
8.	Days to maturity (days)	71.759**	718.725	0.588
9.	Morphine content (%) in capsule husk/straw	2.178**	0.047	0.0037
10.	Codeine content (%) in capsule husk/straw	0.133**	0.002	0.00009
11.	Thebaine content (%) in capsule husk/straw	0.049**	0.001	0.00008
12.	Papaverine content (%) in capsule husk/straw	0.316**	0.002	0.00010
13.	Narcotine content (%) in capsule husk/straw	1.200**	0.007	0.00038

*** =p < 0.05; ** = p < 0.01, respectively.**

Table 2: Genotypic (r_g), Phenotypic (r_p) and environmental correlation (r_e) among thirteen traits of Opium poppy accessions.

Traits		Days to 50% flowering	Plant height (cm)	No. of capsule/ plant	Peduncle length(cm)	Capsule index	Seed yield (gm/pl.)	Straw yield (gm/pl.)	Days to maturity (days)	Morphine	Codeine	Thebaine	Papaverine	Narcotine
Days to 50% flowering	r_g r_p	—	0.249 0.234	-0.105 -0.030	-0.027 -0.026	0.017 0.007	0.128 0.041	0.029 0.020	0.053 0.049	0.208 0.193	0.111 0.105	0.279 0.257	0.026 0.027	-0.137 - 0.124
Plant height (cm)	r_e	0.144	—	0.033 0.032	0.030 0.028	-0.266 -0.200	-0.169 -0.107	-0.359 -0.354	-0.029 -0.029	-0.221 -0.220	-0.279 -0.278	-0.131 -0.130	0.005 0.005	-0.006 -0.006
No. of capsule/plant	r_e	0.130	0.157	—	-0.136 -0.029	0.143 0.075	0.009 - 0.013	-0.345* -0.207	0.139 0.092	0.222 0.153	0.200 0.138	-0.335* -0.234	-0.184 -0.125	0.294 0.209
Peduncle length	r_e	-0.028	0.101	0.093	—	0.423** 0.279	0.113 0.079	0.179 0.129	0.113 0.088	-0.087 -0.067	0.244 0.184	0.094 0.077	0.294 0.228	0.077 0.065
Capsule index	r_e	-0.014	-0.300	0.014	0.119	—	0.678** 0.688**	0.240 0.161	-0.101 -0.072	-0.094 -0.069	-0.066 -0.041	0.213 0.145	0.104 0.067	-0.039 -0.021
Seed yield (gm/pl.)	r_e	-0.081	-0.172	-0.022	0.057	0.709	—	-0.002 -0.000	-0.190 -0.107	-0.069 -0.036	-0.023 0.001	0.219 0.125	0.025 0.012	-0.257 -0.134
Straw yield (gm/pl.)	r_e	-0.107	-0.065	0.235	-0.069	-0.018	0.005	—	0.046 0.045	0.194 0.192	0.075 0.074	0.312* 0.308*	0.283 0.280	-0.034 -0.033
Days to maturity (days)	r_e	0.001	-0.052	-0.051	0.025	-0.051	-0.013	0.025	—	0.347* 0.346*	0.191 0.190	0.308* 0.307*	0.641** 0.639**	0.240 0.238
Morphine	r_e	0.102	0.047	0.022	-0.006	-0.132	0.071	0.064	0.101	—	0.542 ** 0.541**	0.294 0.293	0.277 0.276	0.218 0.216

Codeine	r_e	0.123	-0.079	0.010	-0.072	0.085	0.241	0.076	0.055	0.227	—	0.048 0.047	0.085 0.084	0.264 0.262
Thebaine	r_e	-0.008	-0.059	-0.220	0.217	-0.062	0.072	0.091	0.114	0.255	0.359	—	0.588** 0.587**	-0.180 -0.178
Papaverine	r_e	0.325	0.141	0.021	0.072	-0.182	-0.075	0.114	0.164	0.118	0.192	0.304	—	0.025 0.024
Narcotine	r_e	0.027	-0.021	0.100	0.076	0.053	0.082	-0.026	0.062	0.018	0.127	-0.069	0.008	—

Genotypic and Phenotypic correlation = Upper values above diagonal; Environmental correlation =Lower values above diagonal; *, **= $p<0.05$ and $p<0.01$, respectively.

Table 3: Direct (bold) and Indirect effects in details for different yield traits on Seed yield of *Papaver somniferum* L. accessions.

S.No.	Path ways of associations	Direct effects (P)	Indirect effects (P×r)	Correlations (r_g) Seed yield
1.	Days to 50% flowering			
	Direct effect	**-0.1563**		
	Indirect effect via			
	Plant height (cm)		0.0396	
	No. of capsule/plant		0.0012	
	Peduncle length (cm)		0.0026	
	Capsule index		0.0070	
	Straw yield (gm/pl.)		-0.0478	
	Days to maturity (days)		-0.0011	
	Morphine %		0.0130	
	Codeine%		0.0319	
	Thebaine%		0.0288	
	Papaverine%		-0.0538	
	Narcotine %		-0.0020	
	Total effect			-0.1369
2.	Plant height (cm)			
	Direct effect	**0.1589**		
	Indirect effect via			
	Days to 50% flowering		-0.0390	
	No. of capsule/plant		-0.0004	
	Peduncle length		-0.0029	
	Capsule index		-0.1107	
	Straw yield (gm/pl.)		0.0634	
	Days to maturity (days)		0.0134	
	Morphine		-0.0073	
	Codeine		-0.0340	
	Thebaine		-0.0726	
	Papaverine		0.0253	
	Narcotine		-0.0004	

	Total effect			-0.0062
3.	Number of capsule/plant			
	Direct effect	**-0.0114**		
	Indirect effect via			
	Days to 50% flowering		0.0164	
	Plant height (cm)		0.0053	
	Peduncle length		0.0132	
	Capsule index		0.0594	
	Straw yield (gm/pl.)		-0.0003	
	Days to maturity (days)		0.0128	
	Morphine		0.0340	
	Codeine		0.0340	
	Thebaine		0.0521	
	Papaverine		0.0646	
	Narcotine		0.0143	
	Total effect			0.2944
4.	Pedicel length			
	Direct effect	**-0.0968**		
	Indirect effect via			
	Days to 50% flowering		0.0042	
	Plant height (cm)		0.0048	
	Number of capsule/plant		0.0016	
	Capsule index		0.1763	
	Straw yield (gm/pl.)		-0.0424	
	Days to maturity (days)		-0.0067	
	Morphine		0.0279	
	Codeine		-0.0135	
	Thebaine		0.0635	
	Papaverine		-0.0183	
	Narcotine		-0.0230	
	Total effect			0.0777
5.	Capsule index			
	(a) **Direct effect**	**0.417**		
	(b) Indirect effect via			
	Days to 50% flowering		-0.0026	

	Plant height (cm)		-0.0422	
	Number of capsule/plant		-0.0016	
	Pedicel length		-0.0409	
	Straw yield (gm/pl.)		-0.2542	
	Days to maturity (days)		-0.0089	
	Morphine		-0.0250	
	Codeine		-0.0144	
	Thebaine		-0.0174	
	Papaverine		-0.0412	
	Narcotine		-0.0082	
	Total effect			-0.0396
6.	Straw yield (gm/pl.)			
	(a) **Direct effect**	**-0.3746**		
	(b) Indirect effect via			
	Days to 50% flowering		-0.0199	
	Plant height (cm)		-0.0269	
	No. of capsule/plant		-0.0001	
	Peduncle length		-0.0109	
	Capsule index		0.2830	
	Days to maturity (days)		0.0001	
	Morphine		-0.0467	
	Codeine		-0.0106	
	Thebaine		-0.0062	
	Papaverine		-0.0422	
	Narcotine		-0.0020	
	Total effect			-0.2570
7.	Days to maturity (days)			
	(a) **Direct effect**	**-0.0372**		
	(b) Indirect effect via			
	Days to 50% flowering		-0.0046	
	Plant height (cm)		-0.0570	
	No. of capsule/plant		0.0039	
	Peduncle length		-0.0174	
	Capsule index		0.1002	
	Straw yield (gm/pl.)		0.0007	
	Morphine		0.0113	

	Codeine		0.0298	
	Thebaine		0.0193	
	Papaverine		-0.0602	
	Narcotine		-0.0220	
	Total effect			-0.0332
8.	Morphine			
	(a) **Direct effect**	**0.2455**		
	(b) Indirect effect via			
	Days to 50% flowering		-0.0083	
	Plant height (cm)		-0.0047	
	No. of capsule/plant		-0.0016	
	Peduncle length		-0.0110	
	Capsule index		-0.0425	
	Straw yield (gm/pl.)		0.0713	
	Days to maturity (days)		-0.0017	
	Codeine		0.0532	
	Thebaine		0.0498	
	Papaverine		-0.0593	
	Narcotine		-0.0499	
	Total effect			0.2408
9.	Codeine			
	(a) **Direct effect**	**0.1532**		
	(b) Indirect effect via			
	Days to 50% flowering		-0.0325	
	Plant height (cm)		-0.0352	
	No. of capsule/plant		-0.0025	
	Peduncle length		0.0085	
	Capsule index		-0.0393	
	Straw yield (gm/pl.)		0.0260	
	Days to maturity (days)		-0.0072	
	Morphene		0.0852	
	Thebaine		0.1409	
	Papaverine		-0.0565	
	Narcotine		-0.0215	
	Total effect			0.2188
10.	Thebaine			

	(a) **Direct effect**	**0.2598**		
	(b) Indirect effect via			
	Days to 50% flowering		-0.0173	
	Plant height (cm)		-0.0444	
	No. of capsule/plant		-0.0023	
	Peduncle length		-0.0237	
	Capsule index		-0.0279	
	Straw yield (gm/pl.)		0.0089	
	Days to maturity (days)		-0.0028	
	Morphene		0.0470	
	codeine		0.0830	
	Papaverine		-0.0091	
	Narcotine		-0.0066	
	Total effect			0.2647
11.	Papaverine			
	(a) **Direct effect**	**-0.1927**		
	(b) Indirect effect via			
	Days to 50% flowering		-0.0436	
	Plant height (cm)		-0.0208	
	No. of capsule/plant		0.0038	
	Peduncle length		-0.0092	
	Capsule index		0.0891	
	Straw yield (gm/pl.)		-0.0820	
	Days to maturity (days)		-0.0116	
	Morphene		0.0755	
	Codeine		0.0449	
	Thebaine		0.0122	
	Narcotine		-0.0458	
	Total effect			-0.1802
12.	Narcotine			
	(a) **Direct effect**	**-0.0779**		
	(b) Indirect effect via			
	Days to 50% flowering		-0.0040	
	Plant height (cm)		0.0007	
	No. of capsule/plant		0.0021	
	Peduncle length		-0.0285	

	Capsule index		0.0437	
	Straw yield (gm/pl.)		-0.0094	
	Days to maturity (days)		-0.0105	
	Morphine		0.1574	
	Codeine		0.0424	
	Thebaine		0.0220	
	Papaverine		-0.1134	
	Total effect			0.0246
Residual effects = 0.8457				

Figure 1: Field View and Variation among flower colour of opium poppy accessions

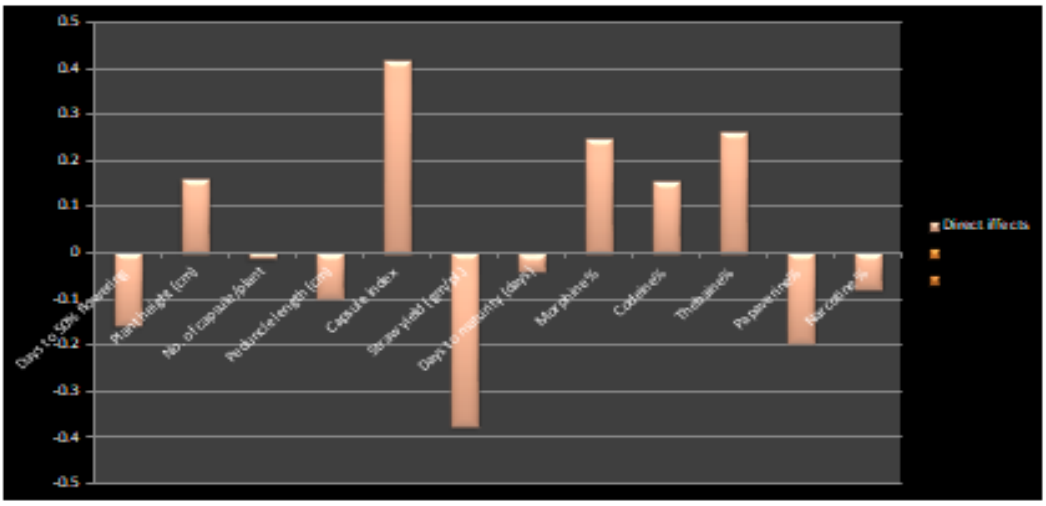

Figure 2 Graphical Estimation of Direct effect (Positive & Negative) for Seed yield

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