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Trait Association Studies in Early Segregating Generations of Cowpea [*Vigna unguiculata* (L.) Walp]

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ABSTRACT

Background: Cowpea is a major pulse crop in India. Developing cowpea cultivars with high yield and sustainable protein content contributes to food and nutritional security. Hybridization followed by careful selection of genotypes from the early segregating populations may produce a final cultivar with high yield coupled with high protein content.

Methods: The genetic material used in the present investigation were derived from crosses of three cultivars *i.e.*, H10 (Anaswara × PKB 3) and H11 (Anaswara × PKB 4). The crosses were evaluated in the Department of Plant Breeding and Genetics, Kerala Agricultural University, Thrissur during cowpea season 2015-2017. Trait association based on correlation and path analysis was carried out with phenotypic data from F_2 and F_3 generations of the crosses.

Result: In F_2 generation of cross H10, number of pods per plant and number of seeds per pod displayed significant positive correlation with seed yield per plant. In F_3 generation of cross H10, number of pods per plant and 100-seed weight per plant exhibited significant positive correlation with seed yield per plant. In cross H11, number of pods, pod length, single pod weight, number of seeds per pod and 100-seed weight revealed significant[1] positive correlation with yield in segregating generations. On path analysis, various quantitative characters showed positive direct effects on seed yield. Selection of segregants based on the yield contributing characters is likely to be desirable for yield improvement in cowpea.

Key words: Correlation analysis, Cowpea, F_2 and F_3 generations, Indirect selection, Path analysis.

INTRODUCTION

Cowpea [*Vigna unguiculata* (L.) Walp; 2n=2x=22] is an ancient crop, originated from the African continent which was widely grown in Africa, South East Asia, Latin America and Southern parts of the United States in Neolithic times (Agyeman *et al*., 2014). In India, cowpea is known since the Vedic period and commercially used for tender green pods as vegetables, dried seeds as pulses, foliage as fodder and as cover crops. The botanical species *Vigna unguiculata* has three cultivated and two wild subspecies. *Vigna unguiculata* subsp. *unguiculata* has the widest distribution and most diverse cultigroup of the cultivated subspecies of *unguiculata*. Culti group *biflora* is used as dry seeds and fodder. Sub-species *sesqupedalis* is known as yard long or asparagus beans (Lo *et al.,* 2018). Cowpea is cultivated around the world in tropical, sub-tropical and warm-season regions. About 15.06 m ha of area is covered by cowpea with a global production of 8.90 mtn, with an average yield of 0.59 mt ha^{-1}. Average yields on a global scale increased largely from 0.38 mt ha^{-1} in 1900 to 0.59 mt ha^{-1} at present (FAO, 2020). The development of improved cultivar and good agronomic practices boosted the production of this crop.

Cowpea seed has 17.60 to 25.71 per cent of average protein, form an accompaniment in the diet along with staple cereals and starchy foods (Morris *et al*., 2020). The crop is of importance for food and nutrition security, particularly for poverty-stricken consumers and can potentially improve health, reduce hunger and stabilize ecosystem resilience (Munoz-Amatriain *et al*., 2017). In cowpea, the protein improvement approach is inappreciable because yield attributes are negatively correlated with protein content and breeding programs are focused primarily on producing cultivars with high yield (Iseki *et al*., 2020). Knowledge of genetics of storage proteins and their character association is a pre-requisite to utilize the potential in enhancing the functional quality of the crop. Variations in protein content among cultivars were found to be large (Dakora and Belane, 2019). Incorporation of variants in the breeding program may result in high protein cultivar with other desirable characters correlated to yield. Seed yield of a crop can be enhanced by indirect selection through other associated traits. Correlation studies give a better understanding of the contribution of various traits towards seed yield and also a possible relationship among the traits. Contrarily, path coefficient analysis partition the direct and indirect effects of various traits on seed yield through cause-effect

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regression analysis. The present investigation is based on phenotypic correlation and path coefficient analysis of segregating generations (F_2 and F_3 generations) in cowpea.

MATERIALS AND METHODS

Plant Material

Two biparental hybrid populations derived from a cross between Anaswara × PKB 3 and Anaswara × PKB 4, designated as cross H10 and H11, respectively, were used for evaluation of segregating progenies to derive breeding lines with superior yield coupled with high protein content. Anaswara, the female parent is a high yielding variety of Kerala with moderate protein content and it showed trailing growth habit. PKB 3 and PKB 4 varieties were released by UAS Bangalore have high protein content with stable yield showing bushy and semi-dwarf type of growth, respectively. F_2 and F_3 generations of these crosses were evaluated for high yield and high protein content in the present investigation.

Growth conditions

The research was conducted at the Field Block of Department of Plant Breeding and Genetics, College of Horticulture, Kerala Agricultural University, Thrissur, Kerala (latitude 10° 32′ N and longitude 76° 16′ E, elevation 40 MSL) during cowpea season 2015-2017. The parents and crosses were grown in field condition, following the package of practices recommended for the crop. The annual rainfall was 1751.60 mm. The mean maximum and minimum temperature during the growing season was 30.17°C and 22.54°C, respectively.

Phenotypic data collection and analysis

A total of 200 progenies in a cross segregating in the F_2 population were evaluated for twelve quantitative characters. The traits were observed in individual plants of crosses H10 and H11. Seed yield was determined by the weight in grams of total seeds per plant. Protein content was estimated biochemically by Lowry's method (Sadasivam and Manickam, 1992) and expressed as per cent protein. Other quantitative characters under study are evaluated by standard procedures. A hundred plants from selected lines of the F_2 population along with their parents were evaluated for quantitative characters in the F_3 generation.

Data analysis

The phenotypic data of quantitative characters observed in F_2 and F_3 generations were subjected to phenotypic correlation and path coefficient analysis using IBM SPSS statistic version 2.0 software.

RESULTS AND DISCUSSION

Interrelationship study in F_2 and F_3 generation of crosses

In cross H10 (Table 1), number of pods per plant and number of seeds per pod displayed significant positive correlation with seed yield per plant. Days to first flowering, number of

Table 1: Phenotypic correlation between quantitative characters of cowpea in F_2 generation of cross H10 (Anaswara × PKB 3).

Traits	Plant height (cm)	No. of branches/ plant	Days to 1st flowering	Days to 1st harvest	Days to last harvest	No. of pods/ plant	Pod length (cm)	Single pod weight (g)	Number of seeds/ pod	100-seed weight (g)	Seed yield/ plant (g)	Protein content (%)
Plant height (cm)	1											
No. of branches/plant	0.055	1										
Days to 1st flowering	-0.125	-0.017	1									
Days to 1st harvest	-0.157*	-0.009	0.914**	1								
Days to last harvest	0.010	0.101	0.175*	0.213**	1							
No. of pods/plant	-0.010	0.191**	-0.020	-0.033	0.053	1						
Pod length (cm)	0.027	0.044	-0.002	-0.032	0.018	0.103	1					
Single pod weight (g)	0.073	-0.231**	0.223**	0.196**	-0.195**	-0.167*	0.037	1				
Number of seeds/pods	-0.036	-0.099	-0.020	-0.079	-0.221**	-0.018	0.247**	0.317**	1			
100-seed weight (g)	-0.053	-0.133	0.093	0.043	-0.253**	0.050	0.094	0.299**	0.382**	1		
Seed yield/ plant (g)	-0.062	0.080	0.011	-0.031	-0.055	0.268**	-0.051	0.063	0.286**	0.079	1	
Protein content (%)	0.016	0.177	-0.167*	-0.130	-0.049	-0.192**	0.178	-0.249**	-0.042	-0.142*	-0.129	1

*Correlation is significant at the 0.05 level. **Correlation is significant at the 0.01 level.

pods per plant, single pod weight and 100-seed weight exhibited a significant negative correlation with protein content. It is evident that the yield contributing characters are negatively associated with protein content. In cross H 11 (Table 2), number of pods per plant, pod length, single pod weight and 100-seed weight revealed significant positive correlation with seed yield per plant. Protein content had significant negative correlation with days to last harvest, indicating more the crop duration the protein constitution is affected. The characters associated with seed yield in F_3 generation (Table 3 and 4) were similar to F_2 generation indicating that duration of crop, number of pods per plant, pod length, pod weight, number of seeds per pod and 100-seed weight was identified as major yield contributing characters. In the present study, negative correlation between seed yield per plant and protein content of seeds was not detected, indicating a possibility of improving and stabilizing seed protein content while maintaining the yield and yield related quantitative attributes. These results are confirming with past studies (Vir and Singh., 2014, Meena *et al*., 2015, Mbuma *et al*., 2021) suggesting that, in cowpea, the above-mentioned traits are helpful for selecting a superior line in segregating population, simultaneously, fixing the level of protein content. Study by Throat and Gadewa (2013) reveled that seed yield per plot showed strong positive significant correlation with number of pods per plant and clusters per plant. Plant height exhibited significant positive correlation with number of branches per plant in segregating generations, indicates plant develops more branches when grows taller. The results coincide with the ones reported by Srinivas *et al*., 2017. Days to first flowering exhibited significant positive correlation with days to first harvest, indicates in nature when the plants are early to flower is early to harvest. The segregating generations did not show any significant relationship between seed yield and protein content. This provides a confidence for developing a cultivar with high yield and superior protein content in cowpea through combination breeding.

Path coefficient studies in F_2 and F_3 generation:Seed yield is a complex character directly and indirectly associated by contributing traits. The direct and indirect effects of quantitative characters towards yield is analyzed by path coefficient analysis and grouped as per Lenka and Mishra (1973). Plant height, number of branches per plant, days to first harvest, days to last harvest, pod length, number of seeds per pod and 100-seed weight displayed positive direct effect on seed yield per plant (Table 5). In F_3 generation plant height, days to first flowering, number of pods per plant, single pod weight and 100-seed weight displayed positive direct effect on seed yield per plant (Table 6). Protein content showed negative direct effect towards seed yield per plant per plant in both generations. Santos et al. (2014) reported that days to flowering, days to last harvest, pod length and pod weight showed direct effect on seed yield. A study conducted by Neema and Palanisamy (2001); Bhardu and Navale (2011) reveled that number of pods per plant showed highest positive direct effect on seed yield.

Table 2: Phenotypic correlation between quantitative characters of cowpea in F_2 generation of cross H11 (Anaswara × PKB 4).

Traits	Plant height (cm)	No. of branches/ plant	Days to 1st flowering	Days to 1st harvest	Days to last harvest	No. of pods/ plant	Pod length (cm)	Single pod weight (g)	Number of seeds/ pod	100-seed weight (g)	Seed yield/ plant (g)	Protein content (%)
Plant height (cm)	1											
No. of branches/plant	0.268**	1										
Days to 1st flowering	0.103	-0.056	1									
Days to 1st harvest	0.128	-0.040	0.843**	1								
Days to last harvest	0.024	-0.153*	0.221**	0.127	1							
No. of pods/plant	-0.066	0.030	-0.202**	-0.237**	-0.249**	1						
Pod length (cm)	0.061	0.231**	-0.065	-0.120	-0.142*	0.247**	1					
Single pod weight (g)	-0.011	-0.060	0.016	-0.025	0.105	-0.045	0.237**	1				
Number of seeds/pods	0.055	0.109	-0.023	-0.008	-0.017	0.014	0.159*	0.178*	1			
100-seed weight (g)	0.035	-0.002	0.087	0.065	0.151*	-0.031	0.230**	0.753**	0.144*	1		
Seed yield/ plant (g)	-0.059	-0.006	-0.060	-0.139	0.004	0.518**	0.169*	0.217**	0.012	0.250**	1	
Protein content (%)	-0.062	0.108	-0.011	-0.102	-0.164*	0.372	0.275	0.076	0.110	0.082	0.226	1

* Correlation is significant at the 0.05 level. **Correlation is significant at the 0.01 level.

Table 3: Phenotypic correlation between quantitative characters of cowpea in F_3 generation of cross H10 (Anaswara × PKB 3).

Traits	Plant height (cm)	No. of branches/ plant	Days to 1st flowering	Days to 1st harvest	Days to last harvest	No. of pods/ plant	Pod length (cm)	Single pod weight (g)	Number of seeds/ pod	100-seed weight (g)	Seed yield/ plant (g)	Protein content (%)
Plant height (cm)	1											
No. of branches/plant	0.170*	1										
Days to 1st flowering	0.145	0.074	1									
Days to 1st harvest	0.282**	0.015	0.724**	1								
Days to last harvest	0.133	-0.068	0.162*	0.123	1							
No. of pods/plant	-0.081	-0.032	-0.055	0.071	-0.067	1						
Pod length (cm)	0.043	0.139	-0.001	0.068	0.038	0.099	1					
Single pod weight (g)	-0.052	0.075	-0.052	-0.037	-0.069	-0.011	0.299**	1				
Number of seeds/pod	-0.079	0.059	-0.099	0.003	-0.128	0.055	0.473**	0.475**	1			
100-seed weight (g)	-0.096	0.065	0.046	0.007	0.061	0.197*	0.306**	0.248**	0.194*	1		
Seed yield/plant (g)	-0.101	-0.117	0.046	-0.105	0.063	0.131*	0.081	0.012	-0.025	0.134**	1	
Protein content (%)	0.095	-0.021	-0.076	-0.089	0.025	-0.303**	0.036	0.050	0.055	-0.074	-0.123	1

* Correlation is significant at the 0.05 level. **Correlation is significant at the 0.01 level.

Table 4: Phenotypic correlation between quantitative characters of cowpea in F_3 generation of cross H11 (Anaswara × PKB 4).

Traits	Plant height (cm)	No. of branches/ plant	Days to 1st flowering	Days to 1st harvest	Days to last harvest	No. of pods/ plant	Pod length (cm)	Single pod weight (g)	Number of seeds/ pod	100-seed weight (g)	Seed yield/ plant (g)	Protein content (%)
Plant height (cm)	1											
No. of branches/plant	0.682**	1										
Days to 1st flowering	-0.024	0.042	1									
Days to 1st harvest	0.009	0.084	0.856**	1								
Days to last harvest	0.059	0.004	0.012	-0.007	1							
No. of pods/plant	-0.010	-0.066	-0.083	-0.054	-0.045	1						
Pod length (cm)	-0.019	-0.032	0.045	0.011	-0.025	-0.051	1					
Single pod weight (g)	0.049	-0.009	0.106	0.054	-0.105	-0.083	0.550**	1				
Number of seeds/pod	0.066	0.018	0.030	-0.032	-0.041	-0.030	0.537**	0.528**	1			
100-seed weight (g)	0.025	0.019	-0.109	-0.121*	-0.023	-0.051	0.078	0.060	0.033*	1		
Seed yield/ plant (g)	0.119	0.085	-0.110	-0.065	0.055	0.032**	-0.070	-0.091	0.112**	0.032*	1	
Protein content (%)	-0.027	-0.006	0.061	0.070	-0.058	-0.012	0.028	0.004	0.065	-0.050	-0.038	1

* Correlation is significant at the 0.05 level. **Correlation is significant at the 0.01 level.

Table 5: Path analysis with direct and indirect effects on seed yield of F_2 generation in cowpea.

Traits	Plant height (cm)	No. of branches/ plant	Days to 1st flowering	Days to 1st harvest	Days to last harvest	No. of pods/ plant	Pod length (cm)	Single pod weight (g)	Number of seeds/ pod	100-seed weight (g)	Protein content (%)
Plant height (cm)	0.440	-0.003	0.067	-0.057	-0.016	-0.243	-0.086	-0.067	0.134	-0.046	0.008
No. of branches/plant	0.031	0.048	-0.008	0.016	0.054	-0.261	-0.088	-0.007	0.101	0.014	0.007
Days to 1st flowering	-0.075	-0.001	-0.039	0.351	-0.028	0.095	0.044	0.043	0.000	-0.008	-0.019
Days to 1st harvest	-0.062	-0.002	-0.338	0.409	0.014	0.148	0.088	0.077	-0.063	-0.038	-0.003
Days to last harvest	0.035	0.013	-0.055	-0.029	0.200	0.154	-0.003	0.031	0.030	0.101	0.001
No. of pods/plant	0.181	-0.021	0.063	-0.102	0.052	-0.593	-0.110	-0.169	0.071	0.030	0.000
Pod length (cm)	-0.136	0.015	-0.063	0.131	0.002	0.237	0.277	0.106	-0.277	0.024	-0.007
Single pod weight (g)	0.123	-0.001	0.071	-0.131	0.026	-0.415	-0.122	-0.242	0.037	0.000	0.014
Number of seeds/pod	-0.158	0.013	0.000	0.069	0.016	0.113	0.205	0.024	0.374	0.022	0.000
100-seed weight (g)	0.101	0.003	-0.016	0.078	0.100	0.089	-0.033	0.000	0.041	0.202	0.039
Protein content (%)	-0.031	0.003	-0.067	0.012	0.002	0.000	0.017	0.029	0.000	0.069	-0.114

Residual effect: 0.268.

Table 6: Path analysis with direct and indirect effects on seed yield of F_3 generation in cowpea.

Traits	Plant height (cm)	No. of branches/ plant	Days to 1st flowering	Days to 1st harvest	Days to last harvest	No. of pods/ plant	Pod length (cm)	Single pod weight (g)	Number of seeds/ pod	100-seed weight (g)	Protein content (%)
Plant height (cm)	0.240	-0.168	0.144	-0.246	-0.068	0.023	0.003	0.041	0.033	0.004	0.026
No. of branches/plant	0.133	-0.302	-0.108	-0.074	-0.024	-0.033	0.033	-0.020	0.009	0.001	0.003
Days to 1st flowering	0.037	0.035	0.926	-0.953	-0.044	-0.037	0.066	0.061	0.068	0.002	0.049
Days to 1st harvest	0.058	-0.022	0.875	-1.009	-0.026	-0.030	0.069	0.068	0.051	0.002	0.041
Days to last harvest	0.090	-0.039	0.223	-0.143	-0.182	0.009	-0.031	0.215	0.096	0.000	0.016
No. of pods/plant	0.035	0.062	-0.218	0.189	-0.010	0.158	-0.062	0.001	0.108	0.001	-0.023
Pod length (cm)	-0.003	0.040	-0.244	0.276	-0.023	0.039	-0.251	0.187	-0.244	-0.003	-0.032
Single pod weight (g)	0.023	0.014	0.133	-0.160	-0.092	0.000	-0.110	0.428	-0.113	-0.002	0.029
Number of seeds/pod	-0.021	0.007	-0.166	0.138	0.046	-0.045	-0.163	0.129	-0.377	-0.002	-0.018
100-seed weight (g)	-0.127	0.053	-0.213	0.328	0.000	-0.021	-0.090	0.114	-0.100	0.207	-0.006
Protein content (%)	0.046	-0.008	-0.339	-0.311	-0.021	-0.027	0.061	0.093	0.049	0.000	-0.135

Residual effect: 0.300.

CONCLUSION

Seed yield is a complex trait which depends on genetic and environmental factors. Therefore, direct selection based on seed yield in the segregating generations will not be effective always. Direct advancement of breeding lines with high yield and protein content may not be effective, as lines segregates in consecutive generations. Selection based on yield contributing characters such as number of pods per plant, number of seeds per pod, pod length, pod weight and 100-seed weight by fixing protein percentage in early segregating generations are suggested for enhancing the seed yieldalong with protein content of cowpea.

Conflict of interest: None.

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