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Genetic Analysis and Identification of Linked SSR Markers for Erectness in Forage Cowpea [*Vigna unguiculata* (L.) *Walp*.]

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ABSTRACT

Background: Cowpea is an important fodder legume crop due to its high protein content and fast growing nature. Cowpea can be grown as sole crop or it can also be grown as intercrop with some non legume such as maize, pearl millet or sorghum. Mostly the farmers prefer erect or semi erect high yielding cowpea varieties as an intercrop because they are easy to harvest. The transfer of erectness along with other forage traits can be done with proper knowledge of gene action controlling that particular trait.

Methods: To improve the fodder yield, two sets of crosses along with their segregating generations were analysed for erectness along with other forage traits to estimate the type of gene action through generation mean analysis. Identification of SSR primers associated with erectness was also carried out in F_2 population of a Cross (C-88 × TNFC 6926).

Result: The scaling test showed significant results for most of the traits indicating the presence of epistatic interactions. The six-parametric model test was performed and the results indicated the significant overall mean and traits were inherited quantitatively. The high magnitude of additive × additive gene effect for green fodder yield suggests the pedigree method is most suitable breeding programme for development of fodder cultivars with better quality traits. Out of 151 SSR primers, 15 SSRSs showed polymorphism between two parental lines. Out of these 15 SSR primers, three primers showed linkage with erectness.

Key words: Bulked segregant analysis, Fodder cowpea, Generation mean analysis, SSR markers.

INTRODUCTION

Cowpea is an important leguminous crop which can be cultivated as a fodder, vegetable, grain and also as green manure crop (Roy *et al.,* 2016). Cowpea is mainly grown in Asia, Africa, Central and South America. Around 84% of the global production area under cowpea falls in Africa, which contributes 83.4% of its global production (Kebede and Bekeko, 2020). The estimated area under cowpea in India is about 3.9 million ha with a production of over 2.21 million tonnes (Giridhar *et al.,* 2020). There is very less information available about genome or chromosome structure of cowpea. It has a chromosome number 2n=2×=22 with the genome size of 640.6 Mb (Lonardi *et al.,* 2019). Cowpea is a fast growing fodder crop and it can also be stored as a hay, so as to utilize it during fodder scarcity period. Its leaves are rich source of protein and make a high quality fodder for livestock (Kulkarni *et al.,* 2018). The important objective in fodder cowpea is the development of high yielding genotypes. Yield being a complex trait is dependent on other forage traits such as vine length, number of branches, number of leaves, leaf area index. So, the selection for high green fodder yield is performed for these traits because these traits showed positive correlations with green fodder yield (Mary and Gopalan, 2006; Imran *et al.,* 2010; Sahai *et al.,* 2013).

Before initiating any breeding programme, it is important to identify the gene effects controlling that particular trait. Gene effects can be estimated using three parameter test or simple additive-dominance model (Kearsy and Pooni, 2004) or six-parameter test with the ability to determine epistatic gene effects. The three parameter test is used for assessing the effect of additive [d] and dominant [h] gene actions. However, it does not explain the non-allelic gene actions. Mather and Jinks (1982) gave the six-parameter test with the ability to determine epistatic gene effects (dominance×dominace [l], dominance×additive [j], additive×additive [i]) for quantitative traits. The generation mean analysis in cowpea was conducted by various researchers to estimate the type of gene action (Adeyanju *et al.,* 2012; Sobda, 2018; Gupta *et al.,* 2017; Pallavi *et al.,* 2019).

Molecular markers, especially PCR-based markers revolutionized the genetic analysis of crop plants. The variation between small DNA sequences can be determined using molecular markers. SSR markers are most efficient for assessing the variation between genotypes. SSR markers are used in Bulk Segregant Analysis method (BSA) to identify the linked markers to the gene of interest

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(Michelmore *et al.,*1991). Ambika *et al*.(2021) conducted the experiment for identification of indeterminate type plants in chickpea using SSR markers. The BSA in cowpea has been done by various researchers for identification and tagging genes of interest (Uma *et al.,* 2016; Tyagi *et al.,* 1978).

Thus the current investigation was aimed to analyse the genetic control of forage and its quality traits in fodder cowpea using generation mean analysis, to identify the most suitable breeding strategy for the improvement of fodder yield and quality. In addition, bulk segregant analysis based on SSR markers was carried out in F_2 generation so as to identify the associated markers with plant architectural traits viz. erectness, plant height and vine length.

MATERIALS AND METHODS

The plant material consists of four cowpea genotypes *viz*. C-88 (short plant height and long vine length),CL400 (short plant height and long vine length), C-74 (tall plant type, short vine length) and TNFC 6926 (tall plant type, short vine length), their F_1s (C-88 × TNFC 6926), (CL 400 × C74), their F_2s and their BC_1F_1 and BC_2F_1 long vine length) (Fig 1,2) The morpho-agronomic traits were recorded at the experimental field area of Forage, Millets and Nutrition Section of Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana during *Kharif* 2020. Forage quality of the experimental material were estimated in Forage Evaluation Laboratory, Department of Plant Breeding and Genetics, PAU, Ludhiana, Punjab. BSA was done on bulks of F_2 generation of C-88 × TNFC 6926 in the molecular biology laboratory of School of Agricultural Biotechnology, Punjab Agricultural University, Ludhiana, Punjab.

Generation mean analysis

Parental lines and their segregating generations were used to study the inheritance and determination of genetic components which control the fodder yield and its quality. The genotypes were sown in randomized complete block design with three replications and the data was recorded for fodder and quality traits. Fodder related traits include plant height (PH, cm), vine length (VL, cm), number of leaves (NOL), leaf length (LL, cm), leaf width (LW, cm), green fodder yield (GFY, q/ha), dry matter yield (DMY, q/ha) and quality related traits include crude protein (CP, %), acid detergent fibre (ADF, %), neutral detergent fibre (NDF, %) and *in-vitro* dry matter digestibility (IVDMD, %). The data for fodder traits were recorded on 10 plants from each parental line and F_1, 110 plants for F_2 and 20 plants from both the backcrosses. Five random plants from each plot were harvested at flowering stage and cut into small pieces for biochemical and quality analysis. Fodder samples were dried in hot air oven and then grinded for further analysis. Crude protein was estimated as per AOAC (2005). The dried grinded samples were analysed for nitrogen content using kjeldahl digestion procedure. The per cent crude protein content was estimated using the relationship:

$$\text{Crude protein \%} = \text{N\%} \times 6.25$$

Cell wall components were analysed by the method of Van Soest (1991). *In-vitro* dry matter digestibility content was estimated from protocol given by Tilley and Terry (1963).

Data analysis

The collected data for all the morpho-agronomic traits was subjected for the scaling tests to identify the presence of epistatic interactions as suggested by Mather and Jinks (1982). The significant results of scaling tests indicate the presence of epistatic interactions and the data was subjected to six parameter model test.

Bulked segregant analysis

The parental lines C-88 (short plant height and long vine length) (Fig 1) and TNFC 6926 (tall plant height and short



Fig 1: Cowpea genotype C-88.



Fig 2: Cowpea genotype TNFC-6926.

vine length) (Fig 2) along with bulks of segregating F_2 population was used to identify the genetic markers associated with erectness. SSR markers were selected from published resources (Badiane *et al.,* 2012; Gupta and Gopalkrishna, 2010). The genomic DNA of F_2 and parental lines was isolated using the CTAB (Cetryl Trimethyl Ammonium Bromide) method (Murrey and Thompson, 1980). DNA quantification was done on 0.8 per cent agarose gel electrophoresis. Amplification reaction was carried out in 20 µL volume, containing (6.6 µl dH_2O, 2.0 µl of 10 × PCR buffer, 4.0 µl of dNTPs (1 µM each), 1.2 µl of $MgCl_2$ (25 mM), 2.5 µl forward primer, 2.5 µl reverse primer (5 µl), 0.2 µl *Taq-polymerase*. The PCR amplification reaction consisted of initial denaturation at 94°C for 5 min, followed by 43 cycles of denaturation at 94°C for 45 seconds, annealing at 50-65°C (depending on the primer) for 45 seconds and extension at 72°C for 90 seconds, final extension step at 72°C for 10 min and the product was kept on hold at 4°C after the PCR completion. The 2.5 per cent agarose gel was used to resolve PCR products (Table 1).

RESULTS AND DISCUSSION

The mean performance for all the traits under investigation for both the crosses is presented in Table 2. This indicated that the mean performance of F_1 was higher for VL, CP, ADF, NDF and IVDMD for cross between C-88 and TNFC 6926 (Cross-I) and for cross between CL 400 and C-74 (Cross-II), the higher values for F_1 was observed for LL, GFY, DMY, CP and IVDMD traits. The presence of transgressive segregants can be observed for all the traits under investigation for both Cross I and II. The performance for BC_1 outperformed for traits like VL, ADF and NDF for Cross-I and GFY, DMY, ADF and IVDM for Cross-II. BC_2 performed better for VL and IVDMD for Cross-I and PH, VL, GFY, DMY, ADF and IVDM for Cross-II. There was large degree of variation present among the morpho-agronomic traits under study. This suggests the heritable variation present can be utilised by the breeders for the improvement of genotypes. Similar results have been reported for fodder traits by Jatasara *et al.* (1982); Tyagi *et al.* (1978) and Nehru and Manjunath (2000). The breeding strategies to be adopted for the improvement of traits largely depend on the type of gene action. The simple selection should be more promising if the trait is controlled by additive gene action, whereas in the presence of inter-allelic interactions (complimentary or duplicate gene action) different strategies

Table 1: Temperature profile used for amplification.

Stages	Temperature (°C)	Time	Number of cycles
Initial	94	5 min.	1
Denaturation	95	45 sec.	43
Denaturation	48-55	45 sec.	
Annealing	72	1 min. 30 sec	
Extension	72	10 min.	1
Final extension Hold	4	-	-

Table 2: Mean performance for different morpho-agronomic and quality traits in Cross-I and Cross II.

		P_1	P_2	F_1	F_2	BC_1	BC_2
Plant height	C 88 × TNFC 6926	50.4	85.7	52.4	75.04	41.7	57.6
	CL 400 × C-74	69.7	89.7	41	61	76.7	114.9
Vine length	C 88 × TNFC 6926	175.77	117.5	220	121.44	228	206.8
	CL 400 × C-74	188.6	149.2	183.9	148.8	141.5	206.8
No. of leaves	C 88 × TNFC 6926	253.1	411.2	331.5	305.78	248	294
	CL 400 × C-74	151	373.4	293.3	224.5	283.5	286.3
Leaf length	C 88 × TNFC 6926	11.45	8.94	11.05	9.05	10.63	10.53
	CL 400 × C-74	10.76	7.17	11.31	9.01	9.12	8.61
Leaf width	C 88 × TNFC 6926	8.34	6.28	7.9	6.73	7.72	7.62
	CL 400 × C-74	9.17	5.56	8.43	7.15	6.58	7.11
GFY	C 88 × TNFC 6926	3.13	3.6	3.14	2.20	2.83	3.13
	CL 400 × C-74	3.21	3.12	4.56	2.5	3.31	3.56
DMY	C 88 × TNFC 6926	0.43	0.50	0.45	0.37	0.41	0.42
	CL 400 × C-74	0.45	0.44	0.72	0.37	0.52	0.52
CP	C 88 × TNFC 6926	15.8	16.7	17.5	16.9	15.9	15.4
	CL 400 × C-74	14.9	15.4	16.7	15.8	14.7	15.1
ADF	C 88 × TNFC 6926	34.5	33.8	37.1	27.4	38.4	33.9
	CL 400 × C-74	35.6	34.1	34.7	35.9	36.2	35.8
NDF	C 88 × TNFC 6926	47.8	47.5	50.3	43.5	48.4	46.1
	CL 400 × C-74	39.7	42.2	40.9	44.4	40.1	40.3
IVDMD	C 88 × TNFC 6926	50.4	51.9	52.9	53.9	50.1	53.4
	CL 400 × C-74	52.4	52.4	54.1	53.9	52.7	53.3

such as crossing followed by selection to be adopted. Thus the estimation of additive, dominance and epistasis components are paramount for planning and execution of any breeding programme. The generation mean analysis was performed to check the nature of gene action involved in controlling the traits under investigation.

Scaling test

The scaling tests A, B, C and D were performed to check the adequacy of simple additive-dominance model. The significant values of scaling tests indicate the presence of epistatic interactions. In the present investigation the significant values were observed for all fodder yield and quality related traits for both Cross-I and II.

In Cross-I, A, B, C and D were important for VL, NOL, and GFY. A, B and C were important for LL and DMY. A, B and D were important for PH and CP. B, C and D were important for NDF. A and C were important for LW. B and C were important for ADF. C and D were important for IVDMD. In Cross-II, A, B, C and D were important for PH, VL, LL, LW, GFY, DMY, ADF and IVDMD. B and D were important for NOL and CP. B, C and D were important for NDF (Table 3, 4). These results provide the evidence for the failure of simple additive-dominance model and can be concluded that di-genic interactions were present for traits under investigation. Therefore, the six parametric model analyses could be done to estimate the interactions between these traits.

Estimate of gene effects and components of variances

Usually, additive [d] and additive × additive [i] effects are positive in all study traits and the sum of additive [d] and additive × additive [i] effects are of greater magnitude in comparison with dominance [h] and dominance × dominance [j] effects (Adyenju *et al.,* 2012). This suggests the presence of additive variation which favours the selection at early generations.

For Cross-I (Table 5), the mean [m] for morpho-agronomic traits under study was significant for all traits except GFY and ADF which indicates the contribution due to mean, locus effects and interaction. Additive gene effects [d] were significant for all traits except CP, ADF and NDF. This indicates the effectiveness of additive gene action [d] for improving such traits. The dominant gene effects [h] were significant for all traits under investigation except DMY. This reflects the importance of dominance gene effects for the

Table 3: Scaling test for Cross-I (C-88 × TNFC 6926).

Characters	A	B	C	D
Plant height (cm)	-2.78±6.95**	-3.57±6.40**	4.86±12.18**	10.35±4.90**
Vine length (cm)	2.24±29.87**	3.38±21.50**	-6.68±37.0**	-14.40±13.44**
No. of leaves	-1.54±6.54	-3.50±4.09**	-1.27±2.02	1.85±3.20*
Leaf length (cm)	-2.47±0.50**	1.78±0.60**	-8.38±0.35**	-8.62±0.35**
Leaf width (cm)	-2.15±0.37**	2.53±0.41*	-6.74±0.51**	-6.95±0.26**
GFY	-4.00±0.15**	-3.50±0.13**	-15.42±0.27**	-13.05±0.11**
DMY	-1.92±0.03*	-2.69±0.04**	-4.19±0.08**	-2.35±0.03*
CP (%)	-2.18±0.68*	-7.79±0.43**	0.10±0.96	2.83±0.28**
ADF (%)	6.05±0.85**	-5.53±0.56**	-5.45±1.29**	-4.11±0.72**
NDF (%)	-0.97±1.33	-9.00±0.62**	-3.83±1.58**	-6.11±0.46**
IVDMD (%)	-5.24±0.59**	4.27±0.46**	5.35±1.40**	7.17±0.59**

*,**Significant at 5 and 1 per cent respectively.

Table 4: Scaling test for Cross-II (CL 400 × C 74).

Characters	A	B	C	D
Plant height (cm)	4.26±10.00**	19.76±5.01**	0.19±13.11	-8.57±8.11**
Vine length (cm)	-3.77±3.20*	5.04±5.96**	-1.98±5.52*	-1.84±7.94*
No. of leaves	1.82±6.80*	-1.22±7.17*	-1.56±16.48*	-1.75±8.71*
Leaf length (cm)	-5.77±0.66**	-3.91±0.32**	-4.22±1.05**	0.52±0.58
Leaf width (cm)	-7.86±0.56**	0.78±0.29	-2.63±1.13**	1.01±0.60
GFY	-5.29±0.21**	-2.39±0.23*	-7.52±0.72**	-5.06±0.37**
DMY	-5.24±0.02**	-4.24±0.02**	-2.34±0.04*	-1.31±0.01
CP (%)	-6.99±0.20**	-7.98±0.23**	-1.29±0.38	2.73±0.14**
ADF (%)	1.55±1.34	2.27±1.23*	1.89±2.37*	-0.17±1.15
NDF (%)	-0.34±1.16	-1.96±1.27*	6.07±2.28**	4.49±0.98**
IVDMD (%)	-1.31±0.83	0.59±0.50	2.68±0.96**	3.28±0.51**

*,**Significant at 5 and 1 per cent respectively.

Table 5: Six-parametric test analysis, chi-square test and epistasis interactions for Cross-I.

Characters	m	[d]	[h]	[i]	[j]	[l]	χ^2	Interaction
Plant height	15.9613±10.6277**	-4.3153±4.0901**	-10.3506±5.2270**	-10.3558±9.8091**	0.3756±4.6597	9.5256±5.1033**	90.1410**	D
Vine length	-8.6862±7.9152**	3.1801±7.5311**	12.0750±8.9630**	14.4095±6.8801**	-0.1899±4.4800	-8.5326±1.7898**	11.6604**	D
No. of leaves	6.1522±6.4404**	-4.5249±7.4700**	-2.6403±16.6450**	-1.8561±4.4173	1.0821±1.0051	2.8986±11.2460**	12.3607**	D
Leaf length	5.6921±0.7204**	8.8440±0.1413**	6.3844±2.0165**	8.6292±0.7036**	-3.4765±0.332**	-4.1871±1.4149**	22.2092**	D
Leaf width	6.4386±0.5542**	7.7251±0.1333**	5.4950±1.5166**	6.9526±0.5379**	-3.6286±0.2563**	-3.9370±1.0165**	25.0641**	D
GFY	1.0599±0.2516	-2.8000±0.0833*	8.2134±0.2374**	3.0584±0.2374**	-0.6953±0.1009	-5.6581±0.3547**	4.7653**	D
DMY	3.6466±0.0782**	-2.2514±0.0155*	0.9896±0.1768	2.3501±0.766*	1.1088±0.0225	-0.0944±0.1060	2.8919**	D
CP	3.9174±0.6456**	-1.4473±0.3109	-8.2056±1.6653**	-8.8378±0.5658**	2.7050±0.3512**	8.4885±1.1663**	8.8769**	D
ADF	-0.5782±1.4701	1.5038±0.2327	2.9653±3.5797**	4.1123±1.4515**	8.3399±0.4976**	-1.9953±2.1830*	7.7082**	D
NDF	4.1686±1.1194**	0.2412±0.6219	8.5861±2.9991**	6.1165±0.9307**	3.0931±0.6951**	-4.0246±2.0126**	7.0353**	D
IVDMD	4.5984±1.2295**	-2.7548±0.2723*	-6.2433±2.6508**	-7.1730±1.1989**	-8.0455±0.3169**	6.2839±1.5436**	3.0210**	D

***Significant at 5 and 1 per cent respectively, D: Duplicate gene action.

Table 6: Six-parametric test analysis, chi-square test and epistasis interactions for Cross-II.

Characters	m	[d]	[h]	[i]	[j]	[l]	χ^2	Epistasis
Plant height	-3.6486±16.3075**	-6.1488±1.6263**	9.4469±4.3838**	8.5787±6.2262**	-5.1286±5.4985**	-11.3460±4.7665**	21.7723**	D
Vine length	1.1795±5.9543	7.3588±2.6771**	1.7246±12.8571	1.8411±5.8902	-7.0968±1.8505**	-1.3252±2.2156	5.7722**	D
No. of leaves	0.1482±9.0008	-5.3524±2.7757**	1.6143±5.9283	1.7579±7.4394	2.3965±5.2326**	-1.2758±10.8528	5.9458**	D
Leaf length	8.1095±1.1813**	12.3176±0.1457**	-1.4234±2.7926	-0.5246±1.1723	-3.6238±0.3546**	3.4132±1.6714**	23.2250**	D
Leaf Width	7.1012±1.2090**	13.8836±0.1300**	-2.0475±2.7277*	-1.0150±1.2019	-7.7233±0.3023**	3.4455±1.5760**	14.3764**	D
GFY	-3.7861±0.7442**	3.7493±0.0645**	4.4649±1.6100**	5.0626±0.7414**	-2.0766±0.1469*	0.8980±-2.2681	12.8916**	C
DMY	-4.1491±0.0366**	0.7143±0.0117	4.4707±0.0947**	7.3198±0.0346**	-0.4789±0.0174	-5.2492±0.0654**	13.0726**	D
CP	6.2472±0.3012**	-2.4020±0.1041*	-2.9841±0.7509**	-2.7359±0.2827**	-1.1337±0.1323	5.1988±0.5066**	1.6551**	D
ADF	4.6945±2.3444**	1.9471±0.3852	0.9691±5.7269	0.1730±2.3125	-0.4404±0.7948	-1.4503±3.6544	3.9526**	D
NDF	8.5764±2.0210**	-2.9739±0.4203**	-7.4345±4.9163**	-8.4984±1.9768**	1.4848±0.7072	6.1042±3.2273**	4.7292**	D
IVDMD	5.3735±1.0654**	0.0354±0.2482	-2.1001±2.8094*	-3.2815±1.0361**	-1.5234±0.4595	2.3024±1.8242*	5.7983**	D

***Significant at 5 and 1 per cent respectively, D: Duplicate gene action, C: Complimentary gene action.

improvement of such traits. The magnitude of [d] compared to [h] indicated that for the traits like LL and LW, [d] is predominant and [h] is predominant for trait like VL.

However, Additive × Additive gene actions [i] were significant for all traits except NOL. The positive and significant values indicated the presence of associating gene pairs for VL, LL, LW, GFY DMY, ADF and NDF. Additve × Dominance [j] were significant for LL, LW, CP, ADF, NDF and IVDMD; and Dominance × Dominance [l] were significant for all traits except DMY. These indicate the importance of three types of interactions for the improvement of traits like LL, LW, CP, ADF, NDF and IVDMD. [l] is predominant for PH, CP and IVDMD with highly positive and significant values. The opposite signs of [h] and [l] indicated that duplicate gene action was present for all traits under study. This will slow down the process of selection and range of variability should be limited.

In Cross-II (Table 6), overall mean [m] were significant for all traits except VL and NOL. Additive gene effects [d] were significant for all traits except DMY and ADF. Dominant gene effects [h] were significant for PH, LW, GFY, DMY, CP, NDF and IVDMD. This signifies the importance and effectiveness of both additive and dominance gene effects for the improvement of these traits. The magnitude of [d] and [h] were compared, this indicates that [d] was predominant for VL, LL and LW whereas [h] was predominant for PH.

Additive × additive gene actions [i] were significant for PH, GFY, DMY, CP, NDF and IVDMD. The positive and significant values indicated the presence of associating gene pairs for PH, GFY and DMY. Additve × dominance [j] were significant for PH, VL, NOL, LL, LW and GFY; and Dominance × dominance [l] gene effects were significant for PH, LL, LW, DMY, CP, NDF and IVDMD. These indicate the importance of three types of interactions for the improvement of traits like PH. [l] is predominant for CP, NDF and IVDMD with highly positive and significant values. The opposite signs of [h] and [l] indicated that Duplicate gene action was present for all traits under study except GFY. This will slow down the process of selection and range of variability should be limited. However, GFY indicated the presence of complimentary gene action for Cross-II, which supported that selection at early segregating generations would be effective.

The genetic control of PH was not in agreement as estimated by Filho *et al.* (2020) but it showed the similar results to Adeyanju *et al.* (2012) and Shinde *et al.* (2021). As the leaf weight is correlated to number of leaves, leaf length and leaf width, leaf weight is controlled by positive additive and dominance gene effects (Adeyanju *et al.,* 2012). However, for Cross-I, the NOL showed contradicting results but favour the results for leaf length and leaf width and for Cross-II, LL and LW showed that additive gene effect is predominant and positively significant for dominance × dominance [l]. The genetic components for GFY showed varied results depending on the genotypes selected for the breeding programme and is a complex trait reported by many workers (Adeyanju *et al,.* 2012; Grafius, 1956; Mitra *et al.,* 2001; Tyagi *et al.,* 2000).

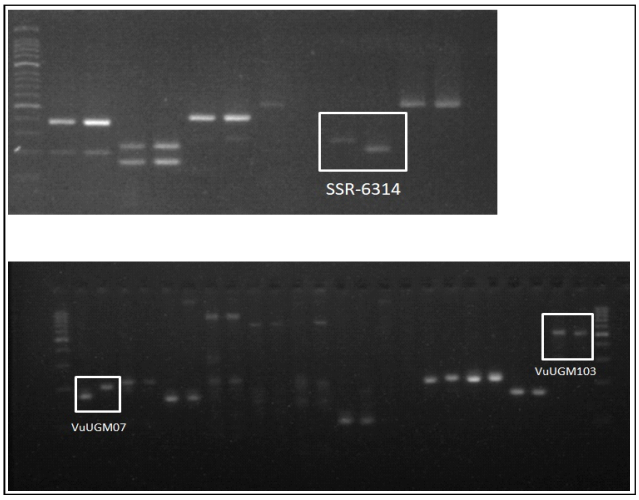


Fig 3: Identification of polymorphic SSR primers on parents of Cross I (C-88 × TNFC 6926) using 2.5% Agarose gel electrophoresis.

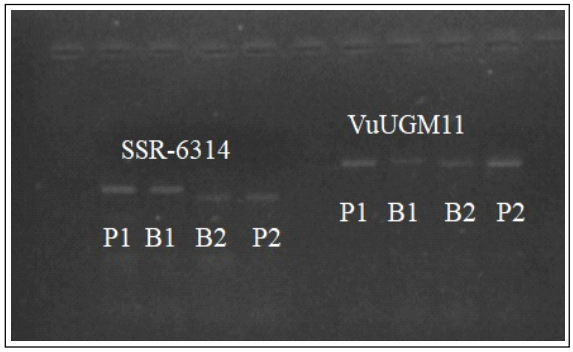


Fig 4: Polymorphic markers amplified with bulks Aof F_2 population along with parents.

Table 7: List of primers showing polymorphism between C-88 and TNFC 6926.

S.no.	Primer codes
1	SSR-6284
2	SSR-6314
3	SSR-6573
4	SSR-6304
5	SSR-6251
6	EST-110
7	VuUGM03
8	VuUGM07
9	VuUGM11
10	VuUGM13
11	VuUGM30
12	VuUGM57
13	VuUGM69
14	VuUGM90
15	VuUGM103

Table 8: List of polymorphic primers associated with plant height identified using BSA.

Primer code		Sequence	Chromosome no.	Annealing temperature (°C)
SSR 6314	F	5′-TGGAGGCATAAAAATGACACCT-3′	5	50
	R	5′-TGAAGCTGATTGTGGAACCAT-3′		
VuUGM 07	F	5′-TGTTTCCAACAGGATTAGCC-3′	6	48
	R	5′-AAGGCCAATAATTGCACAAG-3′		
VuUGM11	F	5′-GGGCAGGAGCTGCATATAAC-3′	9	49
	R	5′-CCTGCAACAACAAAAATGGA-3′		

Hayman (1960) has indicated when epistasis is of major importance in the inheritance of a trait and then it is impossible to obtain unbiased estimates of pooled additive or dominance effects. The presence of both additive and non-additive gene effects in controlling such traits suggested that recurrent selection followed by pedigree method is suitable for these crosses.

Molecular marker analysis

Fodder yield related traits are quantitatively inherited and are controlled by several genetic loci as suggested by generation mean analysis. It is difficult to measure the genetic components for green fodder yield. To speed up the process of breeding programme, marker assisted breeding should be deployed. The major fodder yield related architectural traits include PH, VL, NOL, LL and LW (Tyagi *et al.,* 1978; Chopra and Singh, 1977; Thaware *et al.,* 1991). Identification of associated SSR markers at a major locus contributing to such plant architectural traits contributing to green fodder yield would be useful in identification and selection of plants. SSR markers associated with trait of interest were identified using Bulked Segregant Analysis (BSA) (Michelmore *et al.,* 1991).

In the present investigation, short plant type C-88, tall plant type TNFC 6926 and their F_2 population were subjected to molecular marker analysis to identify the SSR markers associated with erectness in cowpea. A total of 151 SSR markers were used to check the polymorphism between C-88 and TNFC 6926. Out of these, 15 markers turned out to be polymorphic for parental lines. Fig 3 shows the polymorphism for SSR 6314, VuUGM07 and VuUGM103 between C-88 and TNFC 6926. The list of polymorphic markers is presented in Table 7 and 8.

As Tyagi *et al.* (1978) reported that PH and GFY showed positive and significant correlation, two bulks of extreme phenotypes related to plant height were formed by pooling the DNA of 10 individuals of F_2 population for each bulk. These 15 polymorphic SSRs were deployed for Bulk Segregant Analysis (BSA). 3 SSRs showed putative relatedness to the erectness out of 15 parental polymorphic markers.

Fig 4 shows the BSA for two sets of markers, SSR 6314 and VuUGM11, P1 denotes C-88, B1 denotes Bulk 1 with semi-erect plant types, B2 denotes Bulk 2 with prostrate plant types and P2 denotes TNFC 6926 and shows the relatedness of these markers with semi-erectness of the plants and these markers needed to be amplified on whole F_2 population. The more number of markers on a large population is required to identify tightly linked markers associated with erectness.

CONCLUSION

The generation mean analysis suggests that the selection for the improvement of green fodder yield should be more effective if done on sixth or seventh segregating population. The pedigree or recurrent selection should be effective for improvement of fodder and quality traits in cowpea and the breeding strategy should be planned accordingly. The marker assisted breeding will be effective but more SSRs linked with erectness needs to be identified.

Conflict of interest

It is stated on the behalf of all the authors that above said research is conducted ethically and in compliance with relevant guidelines and regulations.

REFERENCES

Adeyanju, A.O., Ishiyaku, M.F., Echekwu, C.A. and Olarewaju, J.D. (2012). Generation mean analysis of dual purpose traits in cowpea [*Vigna unguiculata* [L.] walp]. African J. of Biotechnol. 11(46): 10473-483.

Ambika, Hegde, V., Nimmy, M.S., Bhardwaj, C., Tripathi, S., Singh, R.K. and Kumar, R. (2021). Unraveling genetics of semi determinacy and identifcation of markers for indeterminate stem growth habit in chickpea (*Cicer arietinum* L.). Nature. 11: 21837.

AOAC, (2005). Official Methods of Analysis. 18th ed. Association of Official Analytical Chemists. Maryland, U.S.A.

Badiane, F., Gowda, B.S., Cisse, N., Diouf, D., Sadio, O. and Timko, M. (2012). Genetic relationship of cowpea (*Vigna unguiculata*) varieties from Senegal based on SSR markers. Genetmolres. 11: 292-304.

Chopra, S.K., Singh, C. (1977). Correlation and path coefficient analysis in fodder cowpea [*Vigna unguiculata* (L) Walp]. Forage Res. 2: 97-100.

Filho, F.R.F., Mosta-Son, C.H.A., Gomes, R.L.F., Rocha, M.M., de Almedia, A.C. and Rodrigues, J.A. (2020). Inheritence of traits related to plant architecture in cowpea. Ciencia Rural. 44: 4.

Giridhar, K., Raju, P.S., Pushpalatha, G. and Patra, C. (2020). Effects of plant density on yield parameters of cowpea (*Vigna unguiculata* L.). International Journal of Chemical Studies. 8(4): 344-347.

Grafius, J.E. (1956). Components of yield in oat: A geometrical interpretation. Agronomy Journal. 48: 419-423.

Gupta and Gopalkrishna (2010). Development of unigene- derived SSR markers in cowpea [*Vigna unguiculata* (L.) walp] and their transferability to other *Vigna* species. Genome. 53: 508-523.

Gupta, R.P., Patel, S.R., Modha, K.G. and Wadekar, P.B. (2017). Generation mean analysis for yield and yield components in cowpea [*Vigna unguiculata* (L.) Walp.]. International Journal of Current Microbiology and Applied Science. 6(7): 2231: 2240.

Hayman, B.I. (1960). Maximum likelihood estimates of genetic components variation. Biometrics. 16: 369-381.

Imran, M., Hussain, A., Hussain, S., Khan, S., Bakhsh, A., Zahid, S. and Baig, D. (2010), Character association and evaluation of cowpea germplasm green fodder and grain yield under rainfed conditions of Islamabad. Sarhad J. Agric. 26 (3): 319-323.

Jatasara, D.S., Gupta, P.C., Singh, K. (1982). Studies on forage production potential and quality of different varieties of cowpea. Forage Research. 8: 141.

Kearsey, M.J. and Pooni, H.S. (1998). The genetical analysis of quantitative traits. Stanley Thornes (Publishers) Ltd. Chapman and Hall, UK, ISBN 0-7487-4082-1.

Kebede, E. and Bekeko, Z. (2020). Expounding the Production and Importance of Cowpea [*Vigna unguiculata* (L.) Walp.] in Ethiopia. Cogent Food. Agri. 6: 1769805.

Kulkarni, K.P., Tayade, R., Asekova, S., Song, J.T., Shannon, J.G. and Lee, J.D. (2018). Harnessing the Potential of Forage Legumes, Alfalfa, Soybean and Cowpea for Sustainable Agriculture and Global Food Security. Front. Plant. Sci. 9: 1314.

Lonardi, S., Munoz-Amatria, M., Liang, Q., Shu, S. *et al.* (2019). The genome of cowpea [*Vigna unguiculata* (L.) Walp.]. Plant Journal. 98: 767-782.

Mary, S.S. and Gopalan, A. (2006). Association studies for yield and its related traits of fodder cowpea in F4 generation. Journal of Applied Scientific Research. 2: 584-586.

Mather, K. and Jinks, J.L. (1982). Introduction to Biometrical Genetics. Chapman and Hall Ltd, London.

Michelmore, R.W., Paran, I. and Kesseli, R.V. (1991). Identification of markers linked to disease-resistance genes by bulked segregant analysis: A rapid method to detect markers in specific genomic regions by using segregating populations. Proceedings of the National Academy of Sciences. 88(21): 9828-9832.

Mitra, J., Natarajan, S. and Jain, R.K. (2001). Gene action for fodder yield and its components in clusterbean [*Cyamopsis tetragonoloba* (L.) Taub]. International Journal of Genetics and Plant Breeding. 61(3): 253-255.

Murrey, M.G. and Thompson, W.F. (1980). Rapid isolation of high molecular weight plant DNA. Nucleic Acid Research. 8(19): 4321-4325.

Nehru, S.D. and Manjunath, A. (2000). Genetic variability for yield and accessory characters in cowpea [*Vigna unguiculata* (L.) Walp]. Ind. Agric. 45: 99-101.

Pallavi, Singh, A. and Chaudhary, S. (2019). Generation mean analysis using six parameters genetic model for quantitative traits in cowpea [(*Vigna unguiculata* (L.) *Walp*.]. International Journal of Current Microbiology and Applied Science. 8(2): 1967-1973.

Roy, A.K., Malviya, O.R. and Kaushal, P. (2016). Genetic improvement of fodder legumes especially dual purpose pulses. Indian Journal of Genetics. 76: 608-25.

Sahai, G., Malaviya, D.R. and Singh, U.P. (2013). Morphological traits association with fodder and seed yield in *Vigna unguiculata* (L.). Journal of Environmental Biology. 34: 139-145.

Shinde, R.J., Toprope, V.N., Sargar, P.R., Dhakne, V.R. and Chavan, S.S. (2021). Generation mean analysis studies in Cowpea [*Vigna unguiculata* (L.) Walp]. Int. J. Theor. App. Sci. 13(2): 22-25.

Sobda, G., Atemkeng, F. M., Bouker, O., Fatokun, C., Tongoona, P.B., Ayertey, J. and Offei, S.K. (2018). Generation mean analysis in cowpea [*Vigna unguiculata* (L.) Walp.] under flower thrips infestation. J. Agric. Sci. 10(4): 86-95. doi: 10.5539/jas.v10n4p86.

Thaware, B.L., Birari, S.P. and Jamadagni, B.M. (1991). Genetic parameters and correlation studies in forage yield components of cowpea. J. Maharashtra Agric. Univ. 16(2): 261-262.

Tilley, J.M.A. and Terry, R.A. (1963). A two stage technique for the *in-vitro* digestion of force crops. British Journal of Grassland Society. 18: 104-111.

Tyagi, I.D., Parihar, B.P.S., Dixit, R.K. and Singh, H.G. (1978). Component analysis of green fodder yield in cowpea. Ind. J. Agric. Sci. 48: 646-649.

Tyagi, P.C., Kumar, N. and Agarwal, M.C. (2000). Genetic variability and association of component character for seed yield in cowpea. Legume Research. 23: 92-96.

Uma, M.S., Hedge, N. and Hittalmani, S. (2016). Identification of SSR marker associated with rust resistance in cowpea (*Vigna unguiculata* L.) using bulk segregant analysis. Legume Research. 39 (1): 39-42. doi: 10.18805/lr.v39i1.8861.

Van Soest, P.J., Robertson, J.B. and Lewis, B.A. (1991). Methods for dietry fibre neutral detergent fibre and non-starch polysaccharides in relation to animal nutrition. Journal of Dairy Science. 74: 3583-3597. doi: 10.3168/jds.S0022-0302(91)78551-2.