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EST-SSR based Genetic Divergence and Prediction of Heterotic Combinations of Hybrid Parents in Pigeonpea

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A B S T R A C T

Cytoplasmic-genic male sterile based F_1 hybrid is a revolutionary approach to break yield stagnation in pigeonpea. Hybrid vigor is positively correlated to genetic distance and allelic divergence of the parents. This study estimated genetic divergence of 8 'A' lines, 2 'B' lines and 19 'R' lines of A_2 and A_4 cytoplasmic sources, and predicted best parental combinations using molecular markers. Among 390 EST-SSR markers screened, only 60 markers found polymorphic indicating very low degree of polymorphism (15.4%). Average allelic count was 2.26 with a range of 2-4 per loci. Mean PIC was 0.358 with a range of 0.5885 (ASSR236) and 0.1780 (ASSR9). Cluster and factorial analyses were performed based on marker dissimilarity matrix. The best parental combinations identified from the present study were, ICPA2047 X ICPR4013; ICPA2043 X ICPR4013 for A_4 cytoplasm, and GT290A X GTR9, GT33A X GTR9, MS01A X GTR 9, MS10A X GTR9 for A_2 cytoplasm in pigeonpea.

Introduction

Pigeonpea (*Cajanus cajan* (L.) Millsp.) is a versatile legume crop widely cultivated in semi-arid regions of Southeast Asia, Africa and Central America (FAOSTAT 2014). It is an important crop containing about 20-30% protein, contributing hugely to nutritional as well as economical security to small and marginal farmers. India stands first in both area and production of pigeon pea (FAOSTAT 2016). Its productivity is halted to less than 1 Tonn/hectare since last six decades. Initially efforts were made to improve productivity through pure line selection and recombination breeding. This was unsuccessful because of insignificant genetic gain due to narrow genetic base among cultivated pigeonpea (Kassa *et al.*, 2012). Moreover, many breeders ignored the

fact of 25-35 % of heterosis and also cross pollination up to 70 % by insect vectors, and thus handled it as self pollinated crop (Saxena *et al.,* 1990, Saxena *et al.,* 1992). As results, the focus was shifted towards exploitation of heterosis for higher productivity through development of F_1 hybrids.

Initially, genetic male sterile (GMS) based hybrids were developed, however, they failed to commercialize due to cumbersome nature of hybrid seed production (Dalvi *et al.,* 2010). Subsequently, identification of stable cytoplasmic male sterile sources and suitable male fertility restorers, resulted into development of world's first cytoplasmic-genic male sterile (CGMS) based F_1 hybrid in pigenpea, which was commercially feasible and high yielding (Saxena *et al.,* 2013). Further identification of number of CMS sources out of cultivated gene pool and their successful transfer into cultivated background, has provided much needed impetus for hybrid breeding programmes in pigeonpea (Ariyanayagam *et al.,* 1995, Wanjari *et al.,* 1999, Saxena and Kumar, 2003, Mallikarjuna and Saxena, 2005, Saxena *et al.,* 2005, Mallikarjuna *et al.,* 2006, Saxena *et al.,* 2010a, Saxena, 2013).

The CGMS based hybrid development is a three line breeding programme, which involves cytoplasmic male sterile line (CMS or 'A' line) as a female parent, cytoplasmic male sterile maintainer line ('B' line) and male fertility restoration line ('R' line) as a male parent. However, genetic distance and allelic divergence between 'A' and 'R' lines will determine the hybrid vigor and performance of hybrids. Although, in pigeonpea, an extensive morphological diversity was observed within *Cajanus* genus as whole and cultivated species in particular, however, diversity analysis by molecular markers revealed low polymorphism in cultivated species than wild species (Odeny *et al.,* 2007, Saxena *et al.,* 2010b). This created assumption of abundance of genetic diversity within the cultivated species. Thus, selection of diverse parental combination only based on morphological diversity may sometimes mislead the breeders. However, a successful prediction of heterosis by molecular diversity studies was reported in other crop species such as rice (Zhang *et al.,* 1994), maize (Lanza *et al.,* 1997, Barbosa *et al.,* 2003) and sorghum (Jordan *et al.,* 2003). In pigeonpea, application of SSR markers in diversity analyses of germplasm, varieties, hybrid parental lines ('A', 'B' & 'R' lines) and hybrid purity test was reported (Saxena *et al.,* 2010c, Sheikh *et al.,* 2014, Njung'e *et al.,* 2016). Thus, present study was initiated to assess molecular diversity of CGMS systems of *C. scarabaeoides* (A_2) and *C. cajanifolius* (A_4) cytoplasmic sources and their heterotic combinations in pigeonpea.

Materials and Methods

Plant materials

The experimental materials consisted of 8 male sterile lines ('A' lines), 2 male sterility maintainer lines ('B' lines) and 19 male fertility restorers ('R' lines) derived from A_2 and A_4 cytoplasmic sources (Table 1). These genotypes are extensively used as parental lines in the hybrid breeding programme at IARI, New Delhi, India.

Genic-SSR markers

In total, 390 Simple Sequence Repeat (SSR) primers designed from Expressed Sequence Taq (EST) of pigeonpea varieties Asha and UPAS120 were used (Datta *et al.,* 2011).

Isolation of genomic DNA

Leaves samples were collected from one month old plants and stored at -80ºC in aluminum foils. Genomic DNA was isolated by using CTAB method as procedure

described by Murray and Thompson (1980) and additional, 1% polyvinylpyrrolidone and 0.2% 2-mercaptoethanol were added in the extraction buffer. After the purification was over, the isolated genomic DNA was checked by electrophoresis on 0.8% agarose gel for quality and quantity. The quantification was also done by UV spectrophotometer at 260 nm.

PCR amplification and Gel electrophoresis

Each PCR reaction was prepared by: 1.5µl of 10× buffer, 0.20µl of 10mM dNTPs, 1.5µl each of forward and reverse primers (10pmol), 2.5µl of template genomic DNA (30ng/µl), 0.15µL of Taq DNA polymerase (5U/µl) (Vivantis Technologies) in a final reaction volume was made to 15µl. The PCR reaction profile for amplification DNA was followed: pre-denaturation at 94ºC for 5 min. followed by 35 cycles of 94ºC for 1min., 55ºC for 1min., 72ºC for 1min. and finally, 72ºC for an extension of 7 min. The PCR reactions were performed using PTC225 gradient cycler (MJ Research).

The electrophoresis was done to separate the amplified PCR products on high resolution agarose gels. To prepare high resolution agarose gels, 4% Metaphor agarose have been used and slab gels (containing 0.1µg/ml ethidium bromide) were prepared in 1X TBE buffer. The electrophoresis was done at 130 V for 3 h in TBE buffer. The gels were stained in ethidium bromide (1µg/ml) solution for 20 minute. After electrophoresis, PCR amplified products were visualized and photographed on a UV transilluminator (Alpha innotech Corporation, USA) for analysis.

Statistical analysis

The markers profiles on resolution agarose gels were scored manually. Thus, allelic data obtained was used to prepare a dissimilarity matrix and to construct a two dimensional (2D) plot using the factorial analysis method with DARWIN V5.0.128 software (Perrier *et al.,* 2003). Genetic distance matrix was subjected to cluster analysis by UPGMA, to generate dendrogram using NTSYS-PC (Rohlf, 2000). Based on number of detectable alleles per marker and the distribution of their frequency, the value of a marker for detecting polymorphism within a given germplasm was determined. This value is known as the polymorphic information content (PIC). In the present study, the PIC value of markers was calculated using the following formula (Anderson and Lubberstedt 2003).

$$PIC = 1 - \sum_{i=1}^{k} P_i^2$$

Where *k is* the total number of alleles detected for a given marker locus and *Pi* is the frequency of the i^{th} allele in the lines analyzed.

Results and Discussion

Out of 390 EST-SSR markers screened, only 60 markers showed polymorphism in 29 test genotypes (Table 2). These polymorphic markers amplified a total of 136 alleles, with an average of 2.26 alleles per marker (Table 2). Majority of the markers (46) amplified two alleles, while a maximum of four alleles were amplified by two markers (ASSR17 and ARRS108). The PIC values calculated for these 60 polymorphic markers were in the range of 0.1780 (ASSR9) and 0.5885 (ASSR236) with an average of 0.3587 per marker (Table 2). The pattern of polymorphic profile produced by EST-SSR among pigeonpea genotypes was exemplified by two SSR markers, namely ASSR229 and ASSR236 in Figure 1. Overall polymorphism among test genotypes was 15.3%, indicating a very low level of diversity among ‘A’ ‘B’ and ‘R’ lines. The present results were in agreement with earlier SSR-marker based

diversity studies among A, B and R lines of A_1, A_2 and A_4 cytoplasmic sources in pigeonpea (Saxena *et al.,* 2010c, Petchiammal *et al.,* 2015). Besides, other SSR marker based diversity studies also indicated low polymorphism in different cultivated germplasm of pigeonpea (Burns *et al.,* 2001; Odeny *et al.,* 2007; Saxena *et al.,* 2010b,d). In addition to SSR Markers, the narrow genetic base within cultivated pigeonpea was also evident from analyses based on other DNA marker systems such as RAPD(Ratnaparkhe *et al.,* 1995), RFLP (Nadimpalli *et al.,* 1993), AFLP (Panguluri *et al.,* 2006), DArT (Yang *et al.,* 2006), ISR (Kudapa *et al.,* 2012), and SNP (Kassa *et al.,* 2012).

Bohra *et al.,* (2017) gave an overview of SSR-based diversity analyses in pigeonpea. In which, the average number of alleles per polymorphic SSR markers ranged 2.7 to 6.25 from different diversity studies, and the average PIC count was in the range of 0.3 to 0.63. Though allelic count in the current study (2.26) was lower than earlier reports, but average PIC (0.3587) was in line with previous studies. The reason for low polymorphism and less allelic diversity may be the high selection pressure operated on pigeonpea genome for selection of genotypes from narrow germplasm for morphological traits and thus, pigeonpea genome is highly conserved. Thus, the future breeding strategies in pigeonpea must aim at introgression of favorable alleles from landraces and wild relatives to broaden the genetic base in order to maximize selection gain (Kassa *et al.,* 2012).

Out of 60 polymorphic markers, 56 in 'A' lines and 59 in 'R' lines were polymorphic (Table 2). Earlier, Saxena *et al.,* (2010c) also reported similar kind of a low diversity in 'A' lines. Whereas, Souframanien *et al.,* (2003) reported contrasting results of higher diversity in 'A' lines. However, it is important to note that, 'A' lines in the study of Souframanien *et al.,* (2003) were derived from *C. sericeus* (A_1)and *C. scarabaeoides* (A_2), but in the study of Saxena *et al.,* (2010a), the 'A' lines were derived from *C. cajanifolius* (A_4). In current study, 'A' lines are derived from *C. scarabaeoides* (6) and *C. cajanifolius* (2). Thus, source of male sterile cytoplasm has no relevance in determining genetic divergence of 'A' lines. This was evident by observing comparatively higher level of genetic diversity in 'R' lines (15.12%) than 'A' lines (14.3%).

Similarly, the PIC values in 'A' lines ranged from 0.1780 (ASSR108) to 0.5786 (ASSR5) with an average of 0.3062 per marker (Table 2). In case of 'R' lines, the PIC values varied from 0.1046 (ASSR9) to 0.5786 (ASSR108) with an average of 0.3614 per marker (Table 2). Surprisingly, the SSR Marker ASSR 108, which provided lowest PCI value of 0.1780 for 'A' lines also provided the highest PCI value of 0.5786 for 'R' lines (Table 2). Thus, the SSR marker ASSR108 could be more useful in studying diversity among 'R' lines than 'A' lines. However, other 6 markers, namely ASSR5, ASSR15, ASSR17, ASSR93, ASSR229 and ASSR236 showed PCI value >0.500 (Table 2). Thus, indicating possible usefulness of these markers in diversity studies among 'A', 'B' and 'R' lines in pigeonpea. Petchiammal *et al.,* (2015) reported PIC value range from 0.071(CcM1602) to 0.787 (CcM2977) with an average PIC value of 0.147 for 40 SSR markers among 28 'A', 'B' and pollen parental lines. While, Odeny *et al.,* (2007) and Saxena *et al.,* (2010c) reported PIC values of 0.39 and 0.43, respectively.

The relationship among 'A', 'B' and 'R' lines was deduced by cluster analysis using similarity matrix data of polymorphic SSR markers. The generated dendrogram clearly showed that genotypes fell broadly into two

major clusters, namely 'A' and 'B' (Figure 2). Seven restores (ICPR2673, ICPR2740, ICPR2671, ICPR3472, ICPR3461, ICPR3462 and ICPR3494) and two CMS lines (ICPA2043 and ICPA2047) of A_4 cytoplasm system, and one restorer of A_2 cytoplasm GTR9, were grouped in major cluster 'A' (Figure 2). Whereas, six restores (ICPR3359, ICPR3337, ICPR4013, ICPR2751, ICPR3381 and ICPR3341) of A_4 cytoplasm system, and six CMS lines (MS01A, MS04A, MS010A, GT33A, GT288A and GT290A) and four restores (AKPR9, AKPR100, AKPR417 and GTR11) of A_2 cytoplasm systems, were grouped in major cluster 'B'. However, one genotype ICPR3760 formed as out group member. The major clusters 'A' and 'B' were further divided into four sub-major clusters (AI. AII, BI and BII), eight sub-clusters (AIa, AIb, BIa, BIb, AIIa, AIIb, BIIa and BIIb) (Figure 2). Grouping of genotypes was consistent with the genetic background of the lines as well as comparable to the factorial analysis.

The genetic dissimilarity of all test genotypes varied from 0.40 to 0.81 with a mean genetic dissimilarity of 0.60 (Figure 2). Among 'A' lines, the mean genetic dissimilarity was 0.57 with a range of 0.40 to 0.75. Among 'R' lines, the mean genetic dissimilarity was 0.61 with a range of 0.41 to 0.81. However, the genetic dissimilarity among 5 restorers of A_2 cytoplasm varied from 0.52 (AKPR9 and AKPR100) to 0.81 (AKPR100 & AKPR9 and GTR9) with mean genetic dissimilarity of 0.66 (Figure 2). In restorers of A_4 cytoplasm, the genetic dissimilarity varied from 0.41 (ICPR3341 and ICPR3381) to 0.75 (ICPR3341, ICPR3494 and ICPR2751, ICPR3760) with mean genetic dissimilarity of 0.54. This indicated that, the restorer lines of A_2 cytoplasm sources were genetically more diverse than A_2 cytoplasm sources. Earlier, Saxena *et al.,* (2010c) reported genetic dissimilarity of 67% (ICPA 2042 and ICPB 2042) to 94% (ICPA 2039 and ICPB 2039) with an average of 78% among 'A', 'B' and 'R' lines, respectively. Whereas, Bohra *et al.,* (2017) reported decadal trend of mean genetic diversity of Indian pigeonpea cultivars over 4 decades (1975-85 to 2005-15) in the range of 0.51 to 0.56.

Besides, by factorial analysis, two dimensional scatter plot was generated based on genetic dissimilarity of test genotypes (Figure 3). The plot consisted of X axis and Y axis, based on which it was divided into four co-ordinates (Co-1, Co-2, Co-3, and Co-4). Genotypes grouped in cluster A in dendrogram analysis (Figure 2) clearly distributed among coordinate 1 & 4 in factorial analysis (Figure 3). Whereas, genotypes grouped in cluster B in dendrogram analysis (Figure 2) distributed among coordinate 2 & 3 (Figure 3).

Few genotypes namely ICPR 2751, ICPR3381, ICPR3341 & AKPR417 belonging to cluster B in dendrogram distributed to coordinate 1 in factorial analysis (Figure 3). Genotype ICPR 3760 found as outgroup member in dendrogram was grouped in coordinate 4 in factorial analysis (Figure 3). Dendrogram broadly divided genotypes in two clusters, which was further distinguished into four groups by factorial analysis. Interestingly, a close agreement was observed between the results arising from dendrogram and factorial analysis. Bohra *et al.,* (2017) reported a similar kind of findings, in which results of structure and factorial analyses were in close agreement. Thus combination of dendrogram and factorial analysis will help to choose heterotic combinations of hybrid parents. Genotypes placed in coordinate 1 and coordinate 3; coordinate 2 and coordinate 4 are genetically highly distant each other, thus they may produce best hybrid combinations.

Table.1 Details of hybrid parental lines of pigeonpea used in the current study

Sl. No.	Parental lines	Sterile cytoplasm source	Source
Group1			
1	GT33A	*Cajanus scarabaeoides* (A_2)	SKNAU, Dantewara, Gujarat, India
2	GT288A	-do-	-do-
3	GT290A	-do-	-do-
4	MS01A	-do-	PDKVV, Akola, Maharastra, India
5	MS04A	-do-	-do-
6	MS010A	-do-	-do-
7	ICPA2043	*Cajanus cajanifolius* (A_4)	JNKVV, Jabalpur, Madhya Pradesh, India
8	ICPA2047	-do-	-do-
Group2			
9	ICPB2043	*Cajanus cajanifolius* (A_4)	JNKVV, Jabalpur, Madhya Pradesh, India
10	ICPB2047	-do-	-do-
Group3			
11	AKPR9	*Cajanus scarabaeoides* (A_2)	PDKVV, Akola, Maharastra, India
12	AKPR100	-do-	-do-
13	AKPR417	-do-	-do-
14	GTR9	-do-	SKNAU, Dantewara, Gujarat, India
15	GTR11	-do-	-do-
16	ICPR2438	*Cajanus cajanifolius* (A_4)	ICRISAT, Hyderabad, India
17	ICPR2671	-do-	JNKVV, Jabalpur, Madhya Pradesh, India
18	ICPR2673	-do-	-do-
19	ICPR2740	-do-	-do-
20	ICPR2751	-do-	-do-
21	ICPR3337	-do-	-do-
22	ICPR3341	-do-	-do-
23	ICPR3359	-do-	-do-
24	ICPR3381	-do-	-do-
25	ICPR3461	-do-	-do-
26	ICPR3462	-do-	-do-
27	ICPR3472	-do-	-do-
28	ICPR3494	-do-	-do-
29	ICPR4013	-do-	-do-

SKNAU: Sardarkrushinagar Nagar Agril. University, Dantewara, Gujarat, India; PDKVV: Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola, Maharastra, India; JNKVV: Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur, Madhya Pradesh, India; ICRISAT:International Crops Research Institute for the Semi-Arid Tropics, Hyderabad, India; Group1: Cytoplasmic male sterile lines (CMS lines/A-lines); Group2: Cytoplasmic male sterile maintainer lines (B-lines); Group3: Male fertility restorer lines (R lines).

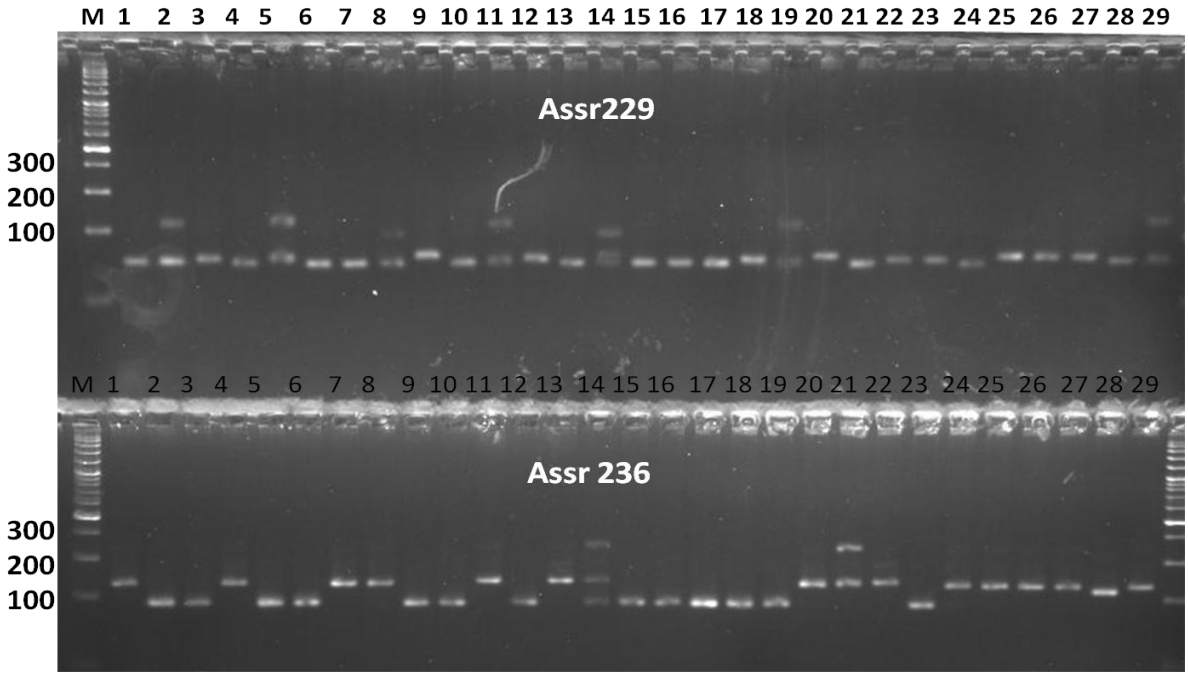
Table.2 Details of polymorphic EST-SSR markers detected among hybrid parental lines of pigeonpea.

Sl. No.	Marker Id.	(SSR Motif)n	Allele size (bp)	No. of Alleles produced	PIC value		
					A lines	R lines	Overall
1	ASSR1	(GA)10	100-120	2	0.3047	0.2149	0.2755
2	ASSR3	(AGAAAG)5	150-180	3	0.3719	0.3623	0.3633
3	ASSR5	(AAATT)6	120-145	2	0.5786	0.4862	0.5198
4	ASSR8	(AGA)9	140-160	2	0.3750	0.2484	0.3301
5	ASSR9	(AGA)8	140-150	2	0.2859	0.1046	0.1780
6	ASSR12	(AACAC)6	170-180	2	0.3719	0.3524	0.3633
7	ASSR13	(ATTAG)5	150-160	3	NP	0.3047	0.2356
8	ASSR14	(ATC)7	140-150	3	0.5632	0.4303	0.4018
9	ASSR15	(CAA)8	120-150	2	0.3047	0.5280	0.5674
10	ASSR16	(GTT)9	160-170	2	0.2149	0.3648	0.2533
11	ASSR17	(CCTTCT)6	175-210	4	0.3698	0.2188	0.5815
12	ASSR19	(TGTTCA)5	160-170	2	0.2149	0.3648	0.2533
13	ASSR20	(AT)11	130-140	3	0.3589	0.4415	0.4226
14	ASSR23	(CCTTCT)5	130-150	2	0.3047	0.1780	0.2149
15	ASSR38	(CATTGC)7	100-120	2	0.3750	0.3524	0.3707
16	ASSR42	(GTT)8	150-155	2	NP	0.3648	0.3197
17	ASSR43	(CT)12	105-120	2	0.2149	0.3571	0.3750
18	ASSR48	(AAGAGG)6	100-120	2	0.3047	0.3589	0.3735
19	ASSR70	(GGTAGA)6	130-180	3	0.0000	0.4728	0.3298
20	ASSR93	(CATTTG)5	120-180	3	0.5112	0.5243	0.5219
21	ASSR97	(ATGGAC)8	300-320	2	0.3750	0.3719	0.3729
22	ASSR99	(CAC)10	150-155	2	NP	NP	0.0644
23	ASSR100	(GGT)7	130-160	3	0.3402	0.5400	0.4562
24	ASSR108	(GAT)7	170-220	4	0.1780	0.5786	0.4427
25	ASSR109	(GAA)7	130-140	2	0.3047	0.3750	0.3698
26	ASSR120	(CTT)7	170-190	2	0.1948	0.3538	0.3498
27	ASSR146	(CACCAT)6	180-200	2	0.3750	0.3648	0.3729
28	ASSR148	(CAA)7	180-190	2	0.2149	0.3457	0.3047
29	ASSR153	(GAG)8	150-160	2	0.3750	0.3719	0.3666
30	ASSR155	(TGGACA)5	120-140	2	0.3047	0.2484	0.3502
31	ASSR169	(TCA)7	170-180	2	0.2149	0.3290	0.3161
32	ASSR170	(CAT)9	180-185	2	0.2859	0.3388	0.2800
33	ASSR173	(GAT)8	180-190	2	0.2149	0.3457	0.3747
34	ASSR189	(GTT)8	170-185	2	0.2688	0.3318	0.2873
35	ASSR205	(ATGAAG)11	160-180	2	0.3719	0.1706	0.2800
36	ASSR206	(GTAATA)6	170-180	2	0.3249	0.2859	0.2920
37	ASSR221	(TCG)8	180-185	2	0.3047	0.3741	0.3719
38	ASSR229	(TAAGGG)5	150-180	3	0.5676	0.5435	0.5050
39	ASSR230	(GAGCAT)9	160-170	2	0.2392	0.3457	0.3161
40	ASSR236	(ACTAGC)10	190-380	3	0.5547	0.5827	0.5885
41	ASSR237	(GGTGAA)7	180-220	3	0.1948	0.4236	0.4735
42	ASSR247	(CACCAA)6	180-190	2	0.3750	0.2392	0.3103
43	ASSR258	(CCATA)5	280-290	2	0.3457	0.3711	0.3735
44	ASSR259	(TATG)5	180-185	2	0.3698	0.3750	0.3750
45	ASSR273	(CCAA)6	150-160	2	0.2859	0.3687	0.3498

46	ASSR279	(ACAGGA)7	200-220	2	0.3750	0.3457	0.3666
47	ASSR281	(CAAATG)6	200-240	3	0.5048	0.4917	0.4670
48	ASSR286	(TGTTCA)5	550-555	2	0.3249	0.3750	0.3687
49	ASSR293	(AGA)7	120-140	2	0.3698	0.3687	0.3737
50	ASSR297	(GCCACC)5	200-240	2	0.3047	0.3750	0.3663
51	ASSR304	(GTT)7	150-155	2	0.3457	0.3571	0.3675
52	ASSR317	(GAGCAT)9	160-180	2	0.3457	0.3719	0.3746
53	ASSR350	(CAT)	150-160	2	0.1948	0.3589	0.3353
54	ASSR362	(AG)13	100-120	2	NP	0.1780	0.2078
55	ASSR366	(CGT)8	140-150	2	0.3457	0.3741	0.3719
56	ASSR372	(CACCAT)	130-180	3	0.4918	0.3814	0.3829
57	ASSR379	(TTCATG)6	150-160	2	0.2859	0.3207	0.3737
58	ASSR380	(TTTC)5	180-190	2	0.3589	0.3524	0.3576
59	ASSR381	(GAT)8	150-180	2	0.1948	0.2772	0.2447
60	ASSR383	(GAT)7	170-180	2	0.3249	0.3741	0.3690
Total			136				
Mean			2.26				
Maximum alleles	ASSR17 &ASSR108		4				
Maximum PIC				0.5786(ASSR5)	0.5786 (ASSR108)	0.5885 (ASSR236)	
Minimum PIC				0.1780(ASSR108)	0.1046 (ASSR9)	0.1780 (ASSR9)	
Mean				0.3062	0.3614	0.358	

NP: Non-polymorphic marker

Fig.1 Polymorphism produced by EST- SSR markers ASSR229 and ASSR236 among different male sterile and restorer lines



M – marker, 1 to 6 – R lines, 7-10 - A&B lines, 11-23 R lines and 24 to 29 - A lines

Fig.2 Dendrogram of A, B and R lines based on SSR markers using UPGMA cluster analysis

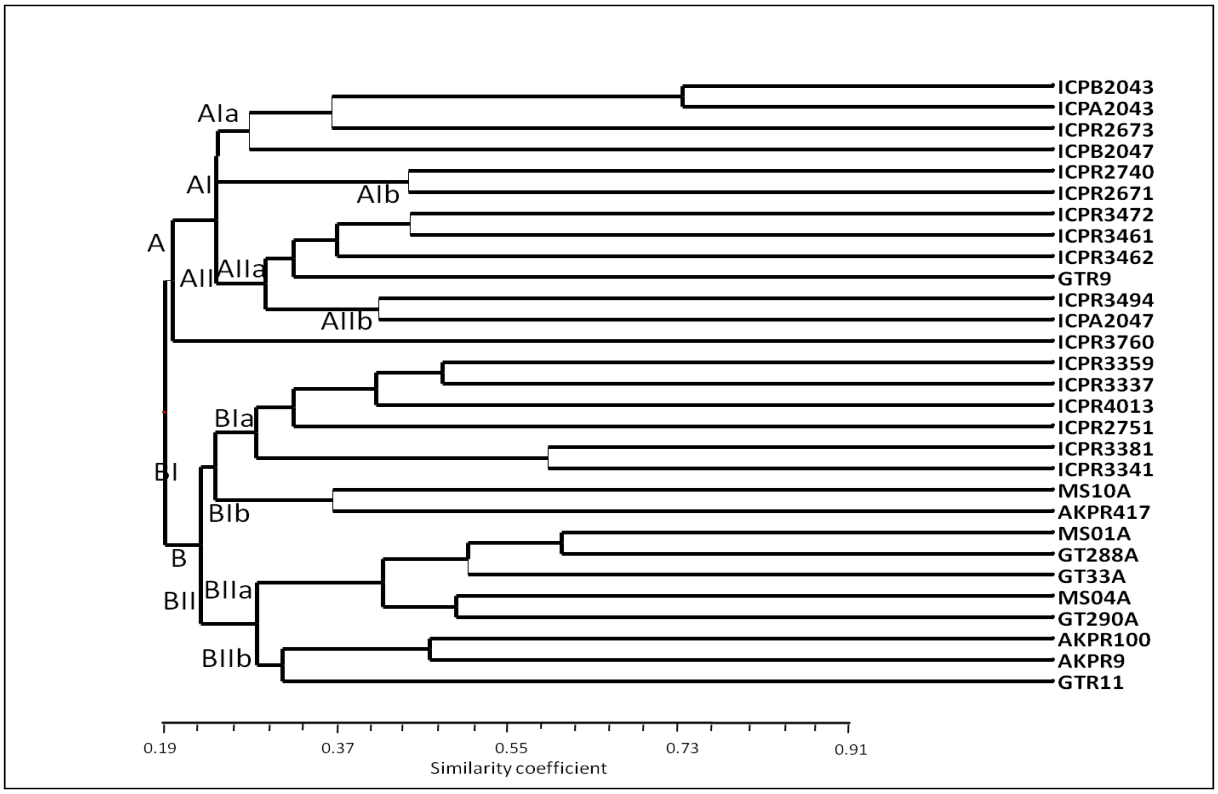
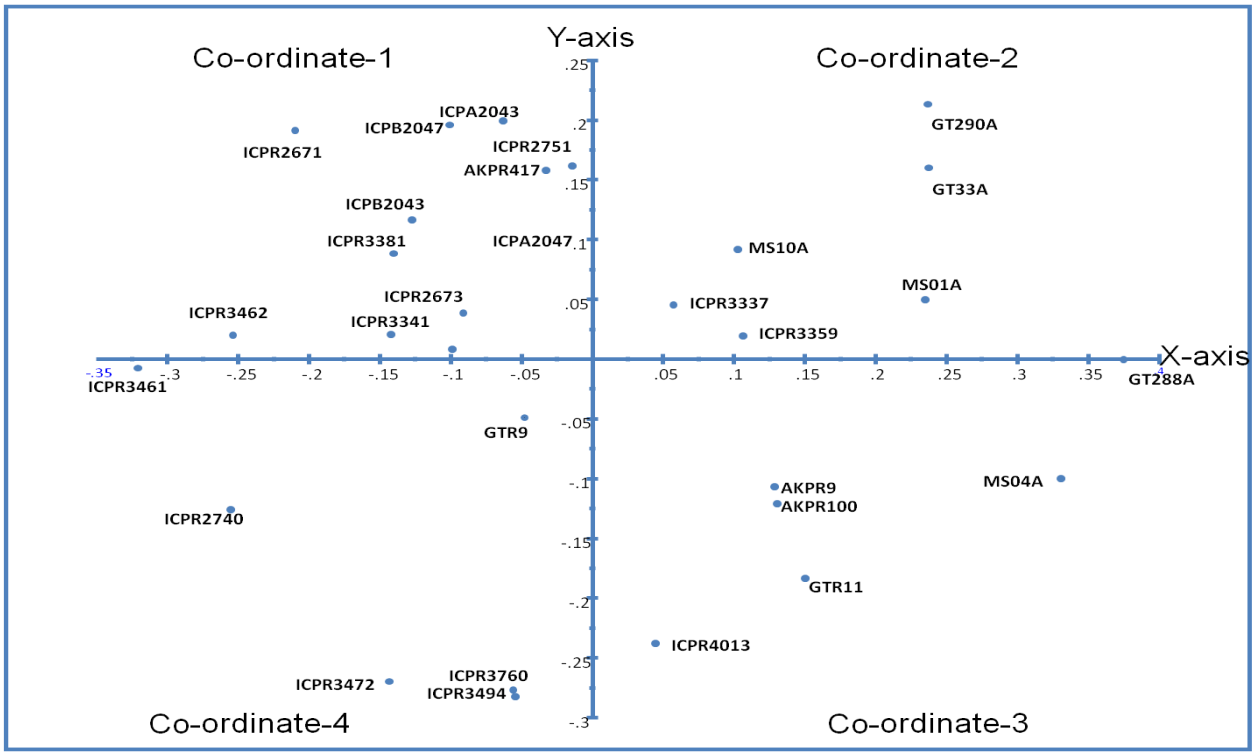


Fig.3 Factorial analysis of A, B and R lines of pigeonpea using DARWIN V5.0.128 software



Present study revealed that 'R' lines of A_4 cytoplasm were genetically close to each other and also to 'A' lines except the 'R' line ICPR4013 (placed in coordinate 3). Thus the parental combinations by using the 'R' line ICPR4013 with two 'A' lines, ICPA2047 and ICPA2043 may produce best hybrids in A_4 cytoplasm. Similarly in A_2 cytoplasm, 'R' line GTR9 (placed in coordinate 4) was genetically highly distant from both 'R' lines and 'A' lines, as rest of the 'R' lines and 'A' lines were placed in coordinates 2&3. Thus parental combination by using 'R' line GTR9 with 'A' lines of A_2 cytoplasm (placed in coordinate 2) may produce best hybrid combinations. The best heterotic combinations identified in present study are ICPA2047 X ICPR4013; ICPA2043 X ICPR4013 for A_4 cytoplasm, and GT290A X GTR9, GT33A X GTR9, MS01A X GTR 9, MS10A X GTR9 in A_2 cytoplasm. These genetically diverse combinations can be effectively used as candidate parental genotypes in hybrid development programme.

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