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COMPARATIVE ANALYSIS OF GENETIC DIVERSITY IN BOTTLE GOURD (*Lagenaria siceraria* L.) USING RAPD, ISSR AND SSR MARKERS FOR DEVELOPING CROP IMPROVEMENT STRATEGIES

BHIMANI, DHARTI R. AND *MEHTA, D. R.

DEPARTMENT OF BIOTECHNOLOGY
COLLEGE OF AGRICULTURE
JUNAGADH AGRICULTURAL UNIVERSITY
JUNAGADH – 362 001, GUJARAT, INDIA

EMAIL: drmehta@jau.in

ABSTRACT

A genetic analysis of six parents of diverse bottle gourd (Lagenaria siceraria L.) and their fifteen crosses were performed using 10 RAPD, 10 ISSR and 12 SSR markers. RAPD primers yielded total of 28 bands/alleles, out of which 21 bands were polymorphic with an average of 2.3 bands per primer. RAPD amplicons per primer ranged from 0 (OPW-8) to 5 (OPW-3, OPC-13, OPW-07), and varied in size from 210 bp to 4036 bp. A total of 74 bands were obtained from the ten ISSR primers, out of which 48 bands were polymorphic with an average of 4.8 bands per primer. The average ISSR polymorphism was 64.86 per cent. Polymorphic ISSR markers ranged from 4 (UBC-854) to 11 (UBC-808, UBC-825) and ranged in size from 206 bp to 3972 bp. Out of twelve markers, seven SSR primers amplified a total of 16 bands, out of which 12 were polymorphic with an average of 75 per cent polymorphism and 1.74 fragments per primer. The PIC value for each primer was ranged from zero to 0.675. The phylogenetic tree constructed by UPGMA method generated two main clusters, cluster I and II, with only one cross Pusa Naveen x JBOGL-01-6 in cluster II. Nevertheless, the concordance among bottle gourd accession groupings after cluster analysis was relatively high (r = 0.88, 0.76, 0.91), respectively, indicating that RAPD, ISSR and SSR-based diversity assessments in this germplasm array were generally consistent and these differences provided for the development of strategies for genetic analyses and crop improvement.

KEY WORDS: Bottle gourd, ISSR, Molecular diversity, RAPD, SSR

INTRODUCTION

Bottle gourd (*Lagenaria siceraria* L.) is an economically important member of the Cucurbitaceae that is widely cultivated in Africa, South America, China, Malaysia, Philippines, Indonesia, Sri Lanka and India (Heiser, 1979; Decker-Walters *et al*., 2001). The bottle gourd is a hairy, rapid growing trailing or climbing annual herb, extending 3 to 15 meters in length. Bottle gourd is a highly cross pollinated crop (60 to 80 %) due to monoecious plant which has resulted into large variation in shape and size of fruits. It is easily digestible even by patients and recommended during convalescence. The decoction made from the leaves act as medicine for curing the jaundice. The fruit has a cooling effect and is a cardiac tonic and diuretic. The pulp is effective for overcoming constipation, cough, night blindness and as antidote against certain poison (Prajapati *et al.*, 2010). The juice of bottle gourd fruit is a valuable medicine for excessive thirst due to severe diarrhea, diabetes and excessive use of fatty or fried foods. It is used in the treatment of stomach acidity, indigestion and ulcers. Fiber helps in preventing constipation and other digestive disorders like flatulence and piles (Chauhan, 1972).

The diverse morphological characters (i.e., sex expression, growth habit, maturity, and fruit shape, size, colour and surface texture of bottle gourd in India provide relatively broad phenotypic species variation (Robinson and Decker-Walters, 1999). Genetic diversity assessments and linkage map construction can increase the effectiveness of breeding programs (Paterson *et al*., 1991; Fan *et al*., 2006). Among the various DNA marker-assisted techniques available, the randomly amplified polymorphic DNA (RAPD) technique (Williams *et al.*, 1990) has been most popular because of speed, low cost and the use of only minute amounts of plant material for analysis. It is less restrictive than the restriction fragment length polymorphism (RFLP) technique (no hybridization and no use of radioisotope). Similarly, ISSR markers are useful in detecting genetic polymorphisms among accessions by generating a large number of markers that target multiple microsatellite loci distributed across the genome. They are simpler to use than the SSR technique as prior knowledge of the target sequences flanking of the repeat regions is not required (Reddy *et al.*, 2002). Yang *et al.* 1996 found lower relative costs for ISSR as compared to RAPD, since RAPD present extra costs due to the relatively low frequency of reproducible polymorphisms. Although DNA marker analysis can assist in such analyses (Staub *et al.*, 2005), relatively few polymorphic markers have been identified in bitter gourd (Dey *et al.*, 2006). Inter-simple sequence repeat (ISSR) and random amplified polymorphic DNA (RAPD) markers have, however, been successfully used in genetic diversity analysis of various cucurbits (Lee *et al.*, 1996; Meglic *et al.*, 1996; Garcia *et al.*, 1998; Horejsi and Staub, 1999; ; Gwanama *et al*., 2000; Decker-Walters *et al*., 2001; Levi *et al.*, 2001; Chiba *et al*., 2003; Paris *et al*., 2003; Levi *et al*., 2004; Ritschel *et al.*, 2004; Levi *et al.*, 2005; Staub *et al*., 2005; Sureja *et al.*, 2005; Dey *et al.*, 2006; Dje *et al*., 2006; Singh *et al*., 2007; Verma *et al*., 2007).

Simple sequence repeats (SSRs), also referred to as microsatellites, is 1-6 bp long, tandmly repeated sequences. Since microsatellites are abundant in the genome, mostly co-dominant and hyper allelic, they are ideal tools for a broad application in basic and applied plant biology (Kashi *et al*. 1997; Gupta and Varshney, 2000; Li *et al.* 2002; Anderson and Lubberstedt, 2003; Varshney *et al.* 2005; Torada *et al.* 2006; Saha *et al.* 2006). ESTs are important sources for SSR development in many plants. SSR flanking sequences are often conserved among species and even genera (Katzir *et al*. 1996; Gaitan-Solis *et al.* 2002; Saha *et al.* 2006; Downey and Iezzoni 2000; Yamamoto *et al.* 2001; Kuleung *et al*. 2004). Therefore, a study was designed to assess the genetic diversity among 21 diverse bottle gourd genotypes (six parents and their hybrids) using RAPD, ISSR and SSR markers to identify potentially useful germplasm for crop improvement.

MATERIALS AND METHODS

Seed materials and DNA extraction

The seed materials of bottle gourd genotypes (six parents and their fifteen hybrids) were collected from previous work done on the parents of bottle gourd in Department of Genetics and Plant Breeding, J.A.U., Junagadh. The experimental material comprised of twenty-one genotypes of bottle gourd which are listed in Table 1. The total genomic DNA was extracted by using CTAB method (Doyle and Doyle, 1987) from young leaf tissue (i.e., terminal whorl) ground to a fine powder. DNA sample concentration was determined using a Spectrophotometer (Nano Drop, USA), and the DNA samples were diluted to 50ng/µl prior to polymerase chain reaction (PCR) amplification.

Molecular analysis

PCR DNA amplification was performed using 10 random decamer primers (Bangalore GeNei Pvt. Ltd., Bangalore, Karnataka) selected based on their performance in a preliminary screen of the RAPDs that were successfully used in other cucurbits (Behera *et al.*, 2008). Amplifications were performed according to Williams *et al*. (1990) in a 25 µl reaction volume containing 50 ng/µl template DNA, 0.5 µl 3U TaqDNA polymerase, 1.5 µl 2.5mMdNTP, 2 µl 25 pmoles primer, 1.5 µl in 10xTaqbuffer, and 2.5 mM $MgCl_2$ (Bangalore Genei, Bangalore, India). Amplification conditions (thermo cycler Eppendorf, Germany) were initial denaturation at 94 °C for 4 min and 45 cycles at 94 °C for 1 min and then 38 °C for 1 min, a ramp to 72 °C for 2 min, followed by 7 min at 72 °C and hold at 4 °C. Amplicons were separated on 1.5% agarose gel in 1x TBE buffer (100 mMTris–HCl, pH 8.3, 83 mM boric acid, 1 mM EDTA) at 75 V. Gels were visualized by illumination under UV light, and then documented using a gel documentation and image analysis system (Uvitech Ltd., U.K.).

Ten ISSR primers were obtained from the Bangalore GeNei, Bangalore, India and PCR was carried out in 25µl volume containing 50 ng/µl template DNA, 0.5 µl 3U TaqDNA polymerase, 1.5 µl 2.5mMdNTP, 2 µl 25 pmoles primer, 1.5 µl in 10x Taqbuffer, and 2.5 mM $MgCl_2$ (Bangalore GeNei, Bangalore, India). ISSR primers were chosen based on their performance in a preliminary screen of the ISSRs that were successfully used in other cucurbits (Behera*et al.*, 2008; Levi *et al*., 2005, 2004; Paris *et al*., 2003). Amplification protocols were same as those stated for RAPD analysis. Amplified products were loaded on1.5% agarose gel and separated in 1xTBE buffer at 75 V, and then gels were visualized under UV by image analyses.

Out of twelve SSR primers, ten were selected based on their performance in a preliminary screen of the SSRs (Bangalore GeNei, Bangalore, India.) that were successfully used in other cucurbits (Danin-Poleg *et al*., 2001; Hu *et al*,. 2010) and two were designed from genome of bottle gourd by using e-portal (http://wsmartins.net/websat/). PCR amplification was performed in 25 µl reaction containing 1x PCR buffer, ~50 ng of sample DNA, 0.5 µM of each primer, 200 µM of each dNTP, 1.5 mM $MgCl_2$ and 0.75 unit of Taq DNA polymerase (Bangalore GeNei, Bangalore, India). All the amplifications were carried out in a PCR thermal cycler (thermo cycler Eppendorf, Germany) as follows: 5 min at 94°C, followed by 30 cycles of 40s at 94°C, 40s at annealing temperature (Ta) and 1 min at 72°C, and 8 min at 72°C for final extension. Amplified products were loaded on 2.5% agarose gel and separated in 1x TBE buffer at 75 V, and then gels were visualized under UV by using a gel documentation and image analysis system (Uvitech Ltd., U.K.).

Data analysis

Only consistent, bright, reproducible (i.e., band absence was randomly verified) RAPD, ISSR and SSR bands were scored as present (1) or absent (0), where each character state was treated independently. Genetic similarity and cluster analyses were performed by subjecting character state data to empirical examination using the NTSYS-PC software version 2.0 (Exeter Software, NY, USA; Rohlf, 2000), where the SIMQUAL program was used to calculate Jaccard's coefficients ofsimilarity as follows:

$$\text{Jaccard's coefficient} = \frac{N_{AB}}{N_{AB} + N_A + N_B}$$

Where,

N_{AB} is the number of bands shared by samples, N_A the amplified fragments in sample A, and N_B represents amplified fragments in sample B. Coefficients values were then used to create similarity matrices. Correlation between each similarity matrix (i.e., RAPD and ISSR) was estimated using Mantel's matrix correspondence test which yields a product moment correlation(r) that defines the relatedness between the two matrices (Mantel, 1967). In addition, genetic relationships were compared by visual examination of dendrograms derived from clustering procedures using similarity matrices. Dendrograms were constructed using the UPGMA (unweighted pair group method with arithmetic average) procedure (Rohlf, 2000). In order to estimate the congruence among dendrograms (derivedfrom RAPD, ISSR, and SSR as well as combined RAPD + ISSR + SSR marker information), cophenetic matrices for each marker and index type were computed and compared using the Mantle test (Mantel, 1967).

RESULTS AND DISSCUTION

Polymorphism detected by RAPD analysis

Out of 10 RAPD primers screened, nine primers amplified a total of 28 bands/alleles in which 21 bands/alleles were polymorphic with average 2.3 bands per primer and seven were monomorphic. One unique polymorphic band was observed in genotype VR-2 by OPW-6. Three primers *viz*., OPW-3, OPC-13 and OPW-07 produced the highest five bands (alleles), while OPW-6 produced four bands; OPW-13 produced three bands (Figure 1A); OPX-1 and OPW-18 produced two bands; and OPF-13 and OPW-5 produced only one band. The average percentage of polymorphism recorded was 75 per cent per primer for all the nine RAPD primers (Table 2). The primer OPW-6, OPX-1, OPW-13, OPF-13, OPW-5 and OPC-13 gave the maximum polymorphism (100%), while primer OPW-18 exhibited 50 per cent polymorphism (Table 2). Behera *et al.* (2008) reported 208 amplicons by examining 38 *Momordica charantia* accessions with 29 RAPD primers. Amplicon numbers per primer ranged from 3 (OPE-19, OPW-09) to 15 (OPW-05) and varied in size between 200 bp and 3000 bp. Of the 208 amplified bands, 76 were polymorphic, with an average of 2.6 polymorphic fragments per primer. Hadia *et al*. (2008) also examined the polymorphisms among and within the fourteen *Cucurbita* genotypes using six RAPD primers which gave 463 total number of reproducible fragments, out of them 405 (87.5%) were polymorphic fragments. Farriol *et al*. (2003) examined twenty-one RAPD primers on accessions of *Cucurbita maxima* which gave 92 consistent and differential amplification products. The number of fragment amplified per primer was between 2 and 10 fragments with an average of 4.4 bands per primer. Among them 52 bands were polymorphic (57%). The polymorphic information content was recorded from zero (OPF-13 and OPW-07) to 0.799 (OPW-3) (Table 2). Jaccard's coefficient of similarity between 21 bottle gourd genotypes ranged from 40 to 100 per cent. Chang *et al*. (2003) classified twenty pumpkin cultivars into three large categories and identified genetic distance of cluster ranging from 38 to 100 per cent. Song *et al*. (2010) recorded that genetic similarity between wax gourd and chiehqua germplasm was in the range of 60 to 99 per cent. The dendrogram was generated by using Jaccard's coefficient values showed that twenty-one bottle gourd genotypes were grouped into two main clusters I and II with an average similarity of 57 per cent (Figure 2A). The cluster I consisted of twenty genotypes and these were divided into two sub-cluster IA and IB, while cluster II consisted only one hybrid (PBOG-90xNDBG-104).

Polymorphism detected by ISSR analysis

All ten ISSR primers screened, amplified a total of 74 bands, out of which 48 bands/alleles were polymorphic with an average of 4.8 bands per primer. Out of 48 polymorphic bands, 44 bands were shared polymorphic and four unique polymorphic bands were observed; one band in genotype JBOGL-01-6 by UBC 856 and three bands in NDBG-104 x JBOGL-01-6 by UBC 825 (Figure 1B). UBC 808 and UBC 825 primers produced the highest 11 bands (alleles), while UBC 890 and UBC 809 produced 8 bands; UBC 880 and UBC 861 produced 7 bands; UBC 855, UBC 856 and UBC 840 produced 6 bands; and UBC 854 produced 4 bands. The average percentage polymorphism of 64.86 per cent per primer was recorded in all the ten ISSR primers. The highest polymorphism was obtained with three primers *viz.*, UBC 856, UBC 890 and UBC 880 (100%) (Table3). Behera *et al*. (2008) examined fifteen ISSR primers which produced 125 bands in the bitter gourd accessions, of which 94 (74.7%) were polymorphic. The number of amplicons per accession varied from 0 (UBC-841) to 12 (UBC-890). The amplified ISSR fragments were in the range of 128 bp to 3972 bp. The largest fragment and the smallest fragment were amplified by UBC 855 and UBC 854, respectively (Table 3). Behera *et al.* (2008) observed band size between 150 bp and 2700 bp. Sikdar *et al*. (2010) observed that out of the ten (ISSR) primers, eight produced informative data for the phylogenetic analysis in *Cucurbita* species. These eight primers produced 139 ISSR fragments, an average of 17.37 bands per primer. Amplified product sizes ranged from 599 to 2399 bp. The polymorphic information content ranged between 0.732 and 0.908 with an average of 0.829 per primer (Table 3). Similar findings also reported by Huang *et al*. (2010). They observed the lowest and the highest genetic similarity coefficients were 0.508 and 0.938, respectively. Jaccard's coefficient of similarity between 21 bottle gourd parents and their hybrids ranged from 47 to 94 per cent. Similar findings were also reported by Behera *et al*. (2008). They reported Jaccard's coefficient of similarity between bitter gourd accessions ranged from 48 to 91 per cent. Twenty-one bottle gourd genotypes were grouped into two main clusters I and II with an average similarity of 69 per cent (Table 3 and Figure 2B). The cluster I consisted of sixteen genotypes and cluster II consisted of five genotypes.

Polymorphism detected by SSR analysis

Out of twelve SSR primers screened, seven primers amplified a total of 16 bands/alleles in which 12 bands/alleles were shared polymorphic with an average of 1.7 bands per primer. Out of 12 polymorphic bands, 11 bands were shared polymorphic and one was unique polymorphic band as observed in NDBG-104 x JBOGL-01-6 by CMGA 165 (Figure 1C). From the data, 25 per cent monomorphic bands and 75 per cent of polymorphic bands were observed. CMGA 165 primer produced the highest five bands (alleles), while EC 28 produced 3 bands; EC 34, EC 49 and PRIMER 1 produced two bands and EC 39 and PRIMER 2 produced one band. The average percentage of polymorphism of about 75 per cent per primer was recorded for all the seven SSR primers (Table 4). The highest (100%) polymorphism was obtained with three primers (EC 49, EC 28 and PRIMER 1), while primer EC 39 and PRIMER 2 did not give any polymorphism. Kohpayegani and Behbahani (2008) observed that microsatellite markers showed polymorphisms in Iranian melon and 63 alleles have been identified. The percentage of genetic loci having polymorphism was 87.67 per cent. The amplified fragments were in range of 95 bp to 4439 bp. The largest fragment of 4439 bp was amplified by CMGA 165 and the smallest fragment of 95 bp was amplified by EC 28. The highest

PIC value of 0.675 was noticed with CMGA 165, while the lowest PIC value of zero was noticed in EC 39 and PRIMER 2 with an average of 0.372 per primer (Table 4). Katzir *et al*. (1996) observed four out of the seven SSRs detected polymorphism among the11 cucumber genotypes, with gene diversity values ranging between 0.18 and 0.64. Watcharawongpaiboon and Chunwongse (2007) observed maximum polymorphism information content (PIC) 0.78 with an average of 0.47 in sixteen *C. sativus* accessions. Jaccard's coefficient of similarity among 21 bottle gourd genotypes species ranged from 42 to 100 per cent. Six parents and fifteen hybrids of bottle gourd were grouped into two main clusters I and II with an average similarity of 53 per cent. The cluster I consisted of twenty genotypes and these were farther divided into two sub cluster IA and IB with fifteen and five genotypes, respectively (Figure 2C). The cluster II grouped into one hybrid (Pusa Naveen x JBOGL-01-6).

Relation between common parentage and genetic diversity

The efficiency of heterosis breeding programme can be increased by making crosses between divergent parents. For this, genetic diversity among the parents for the prediction of hybrid performance is of considerable importance and has gained much importance in breeding programmes. Genetic divergence of six parents of bottle gourd, based on RAPD, ISSR and SSR analysis, was investigated for the association with the hybrids, for fruit yield per plant and its attributing traits, by using Pearson's simple correlation coefficient procedure (Table 5). The results of correlation of genetic diversity of RAPD with heterobeltiotic effects were non-significant at 1% and 5% level of significance in all the characters. The correlation of genetic diversity of ISSR with heterosis over better parent (heterobeltiosis) were significant and positive at 5% level of significance for node number at which male flower appeared and fruit length, while non-significant at 1% and 5% level of significance in rest of the characters.

Comparison assessment of RAPD, ISSR and SSR moleculer markers

Given the proliferation of genetic markers, comparison between various marker systems is inevitable. Such comparison is necessary to identify the technique which is best suited to the issue being examined. In this study, three PCR-based systems *viz*., RAPD, ISSR and SSR were used to profile six parents and their fifteen hybrids of bottle gourd. Each reaction not only differs in principle, but also in the type and the amount of polymorphism detected. The total number of fragments amplified by nine RAPD primers was 28; ten ISSR primers was 74 and seven SSR primer pairs was 16. The average number of polymorphic fragments amplified per primer was calculated for each marker system and it was observed that ISSR amplified more number of fragments (4.8) followed by RAPD (2.3) and SSR (1.7). Similar results were also found for polymorphism information content in each marker system. Several studies have shown that ISSR generated more number of fragments in many crops of *cucurbitaceae* family (Dje *et al*., 2006; Yeboah *et al*. 2007; Huang *et al.* 2010). The use of agarose gels resulted in low resolution of fragment in the SSR analysis. Moreover, SSR assay exhibited the lowest number of fragments per reaction, while ISSR showed the highest number of fragments per reaction. However, SSR gave the highest per cent polymorphism per primer (10.71 %) followed by RAPD (8.33 %) and ISSR (6.49 %). Powell *et al*. (1996) stated that AFLPs, ISSRs and RAPDs are considered to be random markers and are capable of detecting single nucleotide mutation as well as insertion/deletion. However, their relative sensitivity to these types of mutations is expected to vary, depending on the assay for polymorphism with differing length of the genomic sequences and each exhibit its own sensitivity and level of resolution of different in band size. From the above discussion, it has been concluded that data revealed by RAPD (75%) and SSR (75%) produced higher percentage of polymorphic loci, greater range of genetic distance for discriminating among genotypes than that of ISSR (64.86%). However, more number of polymorphic fragments was amplified by ISSR as compared to RAPD and SSR markers. High level of genetic distance means low similarity which is helpful to choose the genotypes with diverse characters for crop improvement program (Table 2D). Large numbers of single type of markers could be screened for further genetic improvement of bottle gourd genotypes.

CONCLUSION

All the techniques used in the present investigation for DNA fingerprinting clustered 21 bottle gourd genotypes (six parents and their 15 hybrids) in different way but the overall specific grouping was observed in all the three techniques. The average Jaccard's similarity coefficient of 21 bottle gourd genotypes was 57%, 69% and 53% in RAPD, ISSR and SSR, respectively which were more or less acceptable. All the three stated techniques of DNA fingerprinting used in this investigation revealed highly genetic diversity in 21 bottle gourd genotypes. The pooled study of molecular marker through RAPD, ISSR and SSR used to confirm the differences and similarity among the 21 bottle gourd genotypes. The similarity coefficient ranged from 0.592 to 0.920 and showed the highest similarity of 92 per cent between two parents, Pusa Naveen and VR-2 and the lowest 59.2 per cent similarity between NDBG-104 and PBOG-90 x NDBG-104. The dendrogram consisted of two main clusters with an average of 66 per cent similarity. The present study indicated that the RAPD and SSR markers resulted higher percentage of polymorphic loci and greater range of genetic distance among 21 genotypes. If the most important criteria for determining choice of assay are number of polymorphic alleles amplified per primer; polymorphism information content (PIC); primer index values and easy of genotyping, ISSR proved to be the most powerful tool as compared to RAPD and SSR. Hence, the ISSR is one of the methods of choice for genotyping in bottle gourd.

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Table 1: List of bottle gourd genotypes used in present study

No.	Name of Genotypes	No.	Name of Genotypes	No.	Name of Genotypes
1.	ABG-1	8.	ABG-1 x PBOG-90	15.	Pusa Naveen x JBOGL-01-6
2.	Pusa Naveen	9.	ABG-1 x VR-2	16.	PBOG-90 x VR-2
3.	PBOG-90	10.	ABG-1 x NDBG-104	17.	PBOG-90 x NDBG-104
4.	VR-2	11.	ABG-1 x JBOGL-01-6	18.	PBOG-90 x JBOGL-01-6
5.	NDBG-104	12.	Pusa Naveen x PBOG-90	19.	VR-2 x NDBG-104
6.	JBOGL-01-6	13.	Pusa Naveen x VR-2	20.	VR-2 x JBOGL-01-6
7.	ABG-1 x Pusa Naveen	14.	Pusa Naveen x NDBG-104	21.	NDBG-104 x JBOGL-01-6

Table 2: Primers sequence, size of amplicons, total no. of amplicons, polymorphic amplicons, monomorphic amplicons, percentage of polymorphism and polymorphic information content, as revealed by RAPD analysis

RAPD Primers	Sequence 5'-3'	Amp. Size (bp)	Total Amp.	Poly. Amp.	Mono. Amp.	% Polymorphism	PIC Value
OPW-6	AGGCCCGATG	2763 -283	4	4	0	100	0.638
OPX-1	CTGGGCACGA	1572 – 1326	2	2	0	100	0.444
OPW-3	GTCCGGAGTG	4036 – 548	5	4	1	80	0.799
OPW-18	TTCAGGGCAC	897 – 310	2	1	1	50	0.498
OPW-13	CACAGCGACA	991 -237	3	2	1	66.66	0.665
OPF-13	GGCTGCAGAA	258	1	1	0	100	0
OPW-5	GGCGGATAAG	466	1	1	0	100	0
OPC-13	AAGCCTCGTC	2195 - 535	5	4	1	80	0.646
OPW-07	CTGGACGTCA	1491 - 210	5	2	3	40	0.742
		Total	28	21	7		
		Average	3.1	2.3	0.7	**75.00**	0.492

Poly.= Polymorphic Mono.= Monomorphic Amp. = Amplicons PIC= Polymorphic Information Content

Table 3: Primers sequence, size of amplicons, total no. of amplicons, polymorphic amplicons, monomorphic amplicons, percentage of polymorphism and polymorphic information content, as revealed by ISSR analysis

ISSR Primers	Sequence 5'-3'	Amp Size (bp)	Total Amp.	Poly. Amp.	Mono. Amp.	% Polymorphism	PIC Value
UBC 854	TCTCTCTCTCTCTCTCAGG	1662 – 128	4	2	2	50	0.749
UBC 855	ACACACACACACACACCTT	3972 – 245	6	5	1	83	0.799
UBC 856	ACACACACACACACACCTA	2600 – 288	6	6	0	100	0.732
UBC 861	ACCACCACCACCACCACC	3185 – 397	7	5	2	71	0.847
UBC 890	ACGACTACGGTGTGTGTTTGTGT	2076 – 145	8	8	0	100	0.841
UBC 840	GAGAGAGAGAGAGAGACTT	2940 - 615	6	2	4	33	0.818
UBC 808	AGAGAGAGAGAGAGAGC	1395 - 235	11	3	8	27	0.908
UBC 825	ACACACACACACACACT	2863 – 269	11	4	7	36	0.879
UBC 880	GGAGAGGAGAGGAGA	1598 – 262	7	7	0	100	0.852
UBC 809	AGAGAGAGAGAGAGAGG	1026 - 206	8	6	2	75	0.870
		Total	74	48	26		
		Average	7.4	4.8	2.6	64.86	0.829

Poly.= Polymorphic Mono.= Monomorphic Amp. = Amplicons PIC= Polymorphic Information Content

Table 4: Primers sequence, size of amplicons, total no. of amplicons, polymorphic amplicons, monomorphic amplicons, percentage of polymorphism and polymorphic information content, as revealed by SSR analysis

SSR Primers		Sequence 5'-3'	Amp Size (bp)	Total Amp.	Poly. Amp.	Mono. Amp.	% Polymorphism	PIC Value
CMGA 165	F R	CTTGTTTCGAGACTATGGTG TTCAACTACAGCAAGGTCAGC	4439 - 152	5	4	1	80	0.675
EC 34	F R	GATCCCCATCATAATCACCC CAAAGGGCTACAATAACAAAC	235 - 115	2	1	1	50	0.268
EC 49	F R	CGTGTTTTCTCAGATTTCCCA CACTTCCCTTATCAACCCCA	297 - 201	2	2	0	100	0.5
EC 28	F R	CTGAGTTATGGGGAAAGCAA TGTTAGTGATGTTGTTGGACC	276 - 95	3	3	0	100	0.663
EC 39	F R	CCAAGTTTAAGTTATTTAGGAG GAAGAGGACGATAAAGATGA	210	1	0	1	0	0.000
PRIMER 1	F R	AAAAGGATCAGGGAAGAGGAAG GGGGAAGGCTAATGTAATGGAT	385 - 139	2	2	0	100	0.498
PRIMER 2	F R	GAGAAGCAAAGAACTCCAGCAT CTAGGACCCTCTACCCGAAAAC	347	1	0	1	0	0.000
			Total	16	12	4		
			Average	2.3	1.7	0.6	75.0	0.372

Table 5: Correlation between heterobeltiotic effects with Jaccard's similarity coefficient of molecular markers in bottle gourd

Characters	RAPD	ISSR	SSR
Days to first female flower	-0.1115	0.0747	0.5476*
Days to first male flower	-0.1172	0.0336	0.5571*
Node at female flower appeared	-0.1550	-0.2630	0.3275
Node at male flower appeared	-0.1558	0.5049*	0.2594
Vine length (m)	-0.1097	0.0329	-0.5904*
Days to first fruit picking	-0.1233	0.0758	0.4747
Number of fruits per plant	0.2044	-0.1873	-0.0740
Fruit length (cm)	-0.3500	0.5871*	-0.7464*
Fruits equatorial diameter (cm)	-0.1881	-0.0284	0.2363
Average fruit weight (g)	-0.3811	0.2272	-0.2793
Days to last fruit picking	0.1424	-0.1286	0.0838
Fruit yield per plant (kg)	-0.0085	0.0276	-0.3138

Significant at 5%

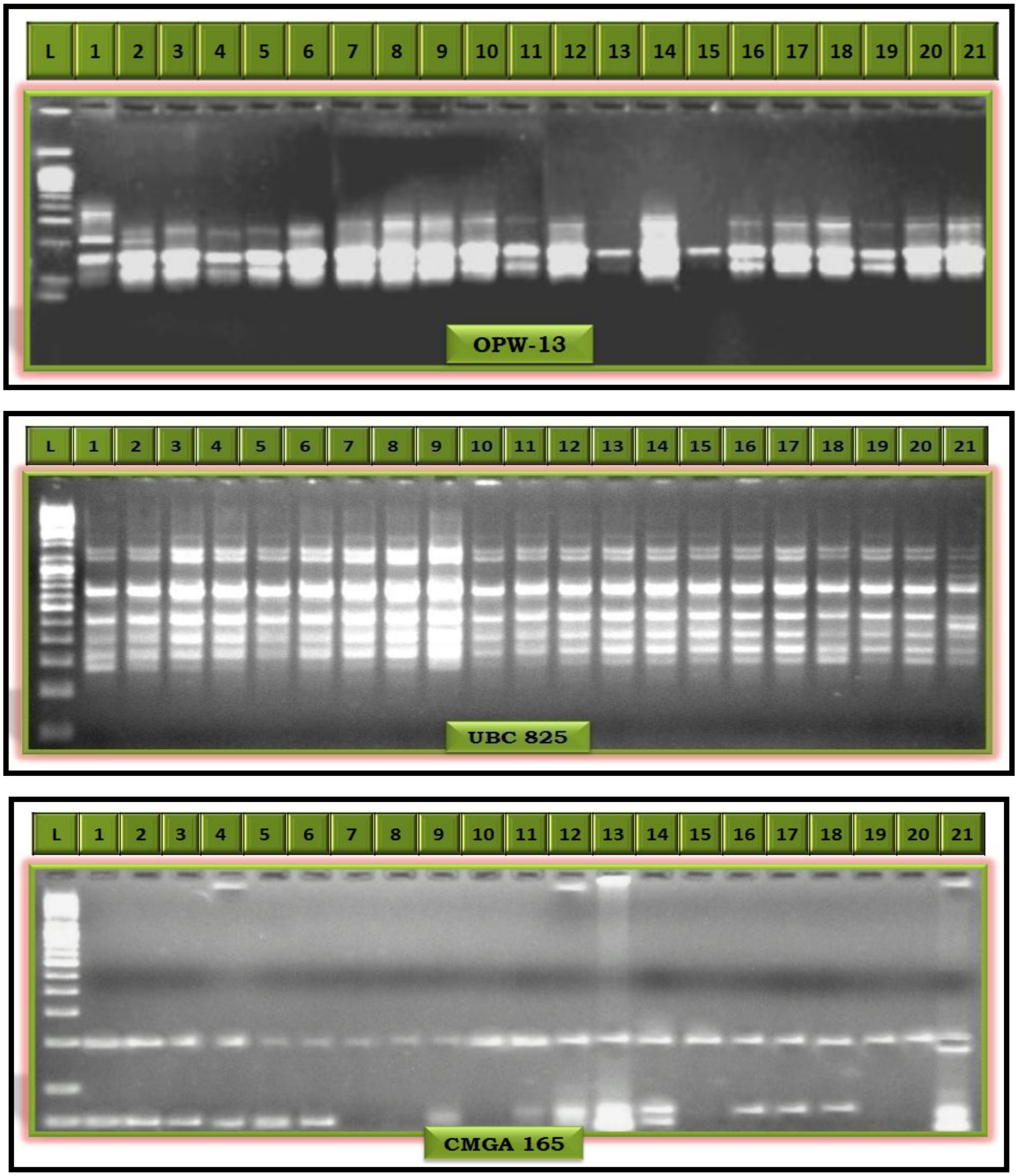
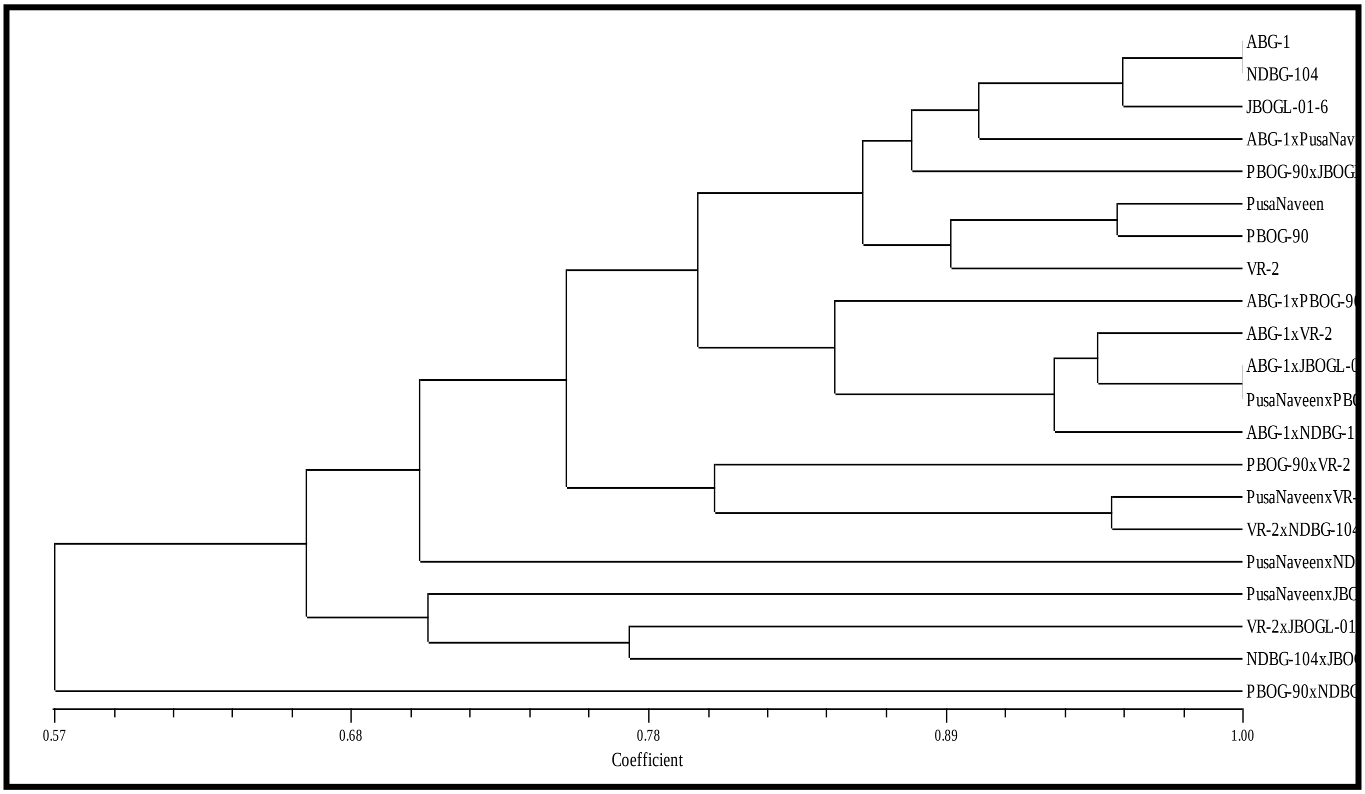
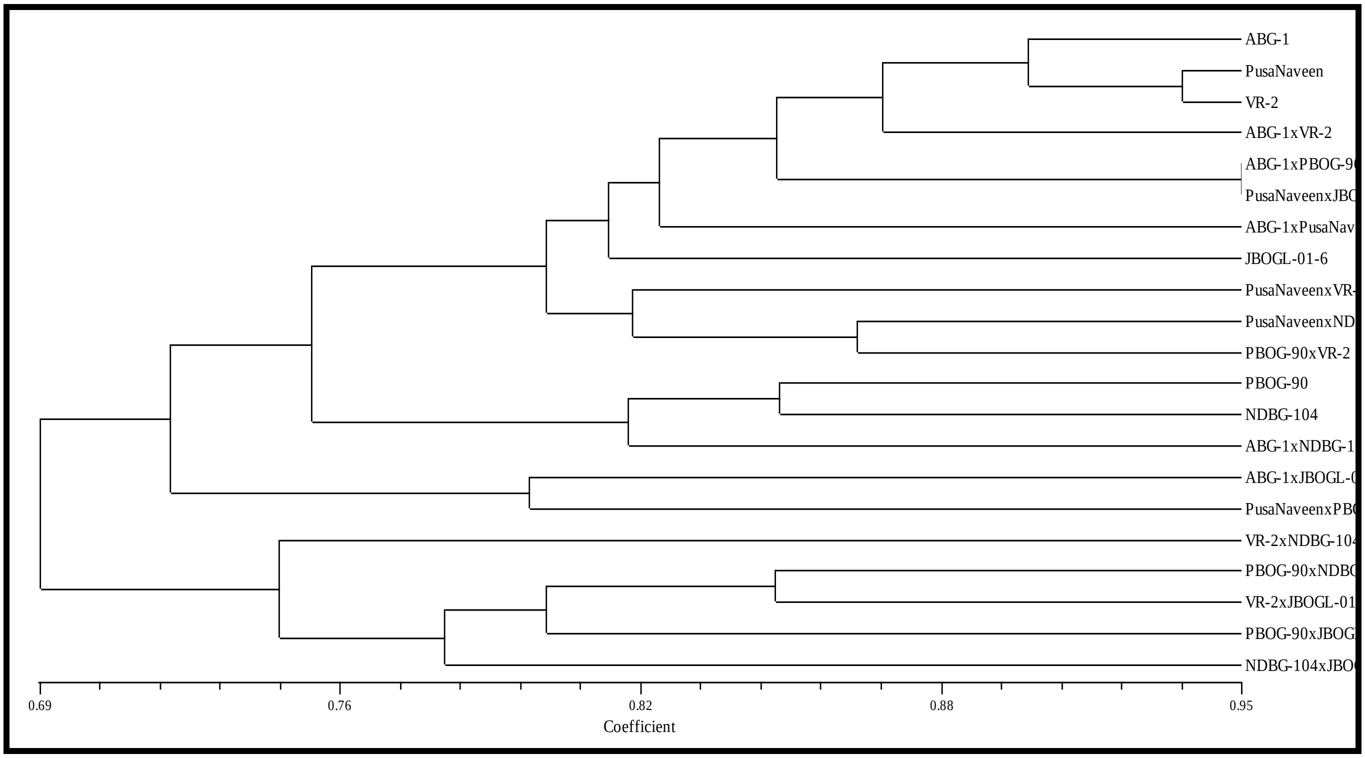


Fig. 1: Molecular marker profile showed by (A) OPW-13 in RAPD (B) UBC 825 in ISSR (C) CMGA 165 in SSR in 21 bottle gourd genotypes

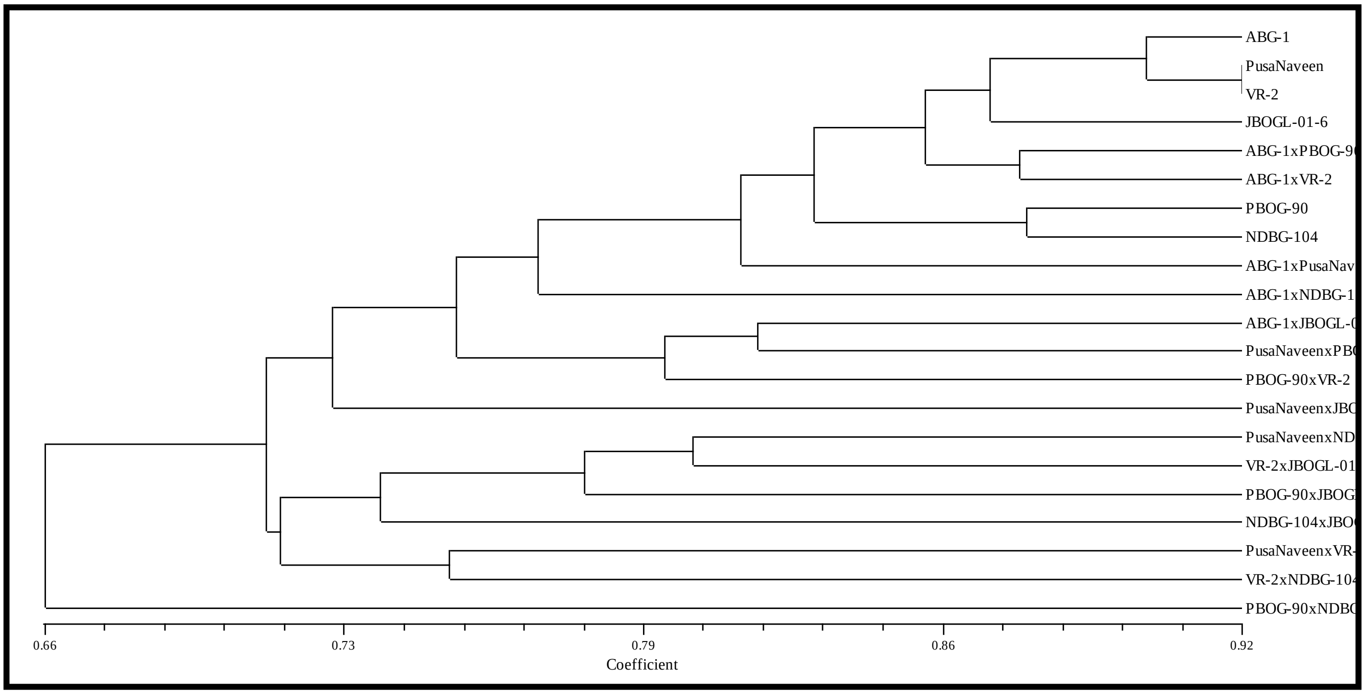
1. ABG-1
2. Pusa Naveen
3. PBOG-90
4. VR-2
5. NDBG-104
6. JBOGL-01-6
7. ABG-1 x Pusa Naveen
8. ABG-1 x PBOG-90
9. ABG-1 x VR-2
10. ABG-1 x NDBG-104
11. ABG-1 x JBOGL-01-6
12. Pusa Naveen x PBOG-90
13. Pusa Naveen x VR-2
14. Pusa Naveen x NDBG-104
15. Pusa Naveen x JBOGL-01-6
16. PBOG-90 x VR-2
17. PBOG-90 x NDBG-104
18. PBOG-90 x JBOGL-01-6
19. VR-2 x NDBG-104
20. VR-2 x JBOGL-01-6
21. NDBG-104 x JBOGL-01-6


ABG-1
NDBG-104
JBOGL-01-6
ABG-1xPusaNav
PBOG-90xJBOG
PusaNaveen
PBOG-90
VR-2
ABG-1xPBOG-9
ABG-1xVR-2
ABG-1xJBOGL-
PusaNaveenxPB
ABG-1xNDBG-1
PBOG-90xVR-2
PusaNaveenxVR
VR-2xNDBG-10
PusaNaveenxND
PusaNaveenxJB
VR-2xJBOGL-01
NDBG-104xJBO
PBOG-90xNDB
0.57
0.68
0.78
0.89
1.00
Coefficient

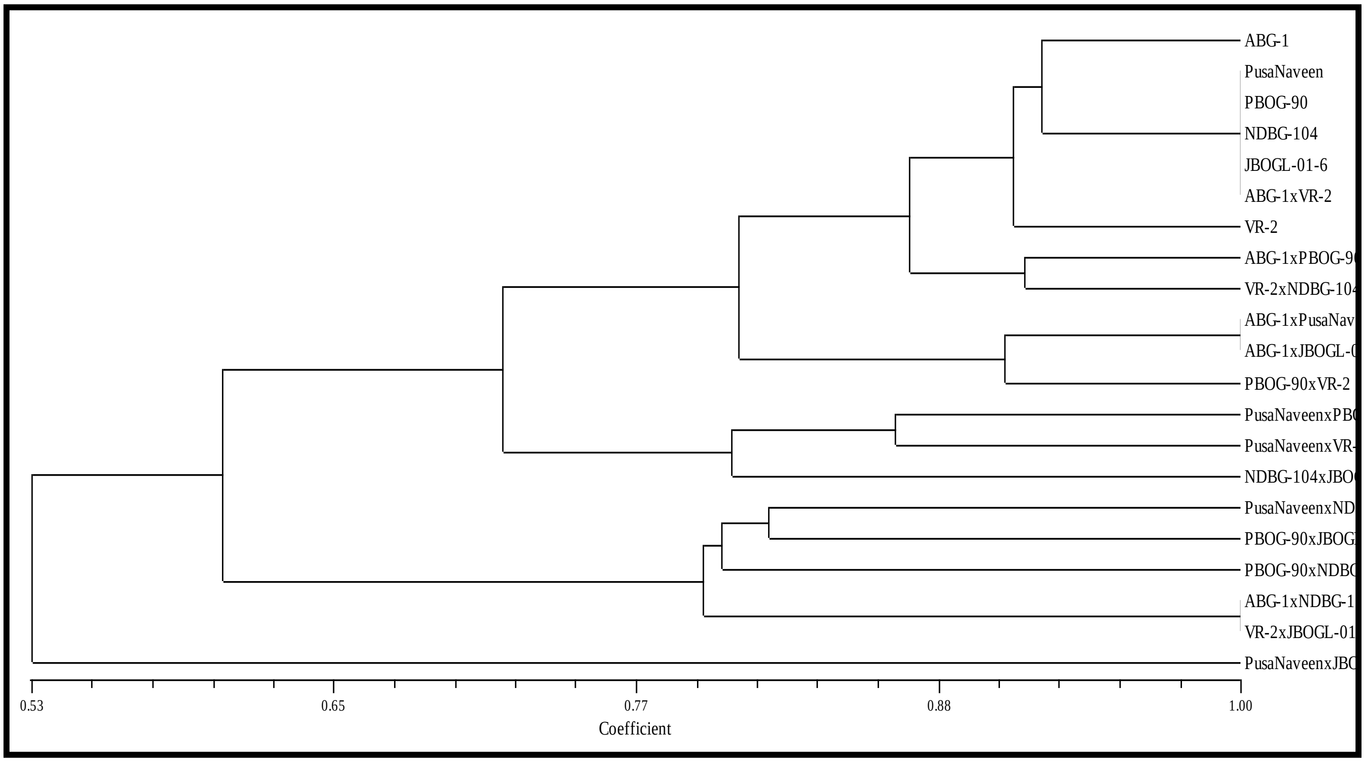
(A)


ABG-1
PusaNaveen
VR-2
ABG-1xVR-2
ABG-1xPBOG-9
PusaNaveenxJB
ABG-1xPusaNav
JBOGL-01-6
PusaNaveenxVR
PusaNaveenxND
PBOG-90xVR-2
PBOG-90
NDBG-104
ABG-1xNDBG-1
ABG-1xJBOGL-
PusaNaveenxPB
VR-2xNDBG-10
PBOG-90xNDB
VR-2xJBOGL-01
PBOG-90xJBOG
NDBG-104xJBO
0.69
0.76
0.82
0.88
0.95
Coefficient

(B)



(C)



(D)

Fig. 2: Dendrograms of the 21 bottle gourd genotypes as revealed by (A) RAPD, (B) ISSR, (C)SSR and (D) combined data

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