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Genetic diversity study of Odisha landraces of blackgram based on agromorphological and RAPD markers for YMV resistance

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ABSTRACT

Vigna mungo (L.) Hepper commonly known as black gram is an important pulse with high protein value cultivated through out India. Ten genotypes of Odisha and three released check varieties of black gram were analyzed for genetic diversity using agronomical characters and RAPD markers. Yellow Mosaic Virus (YMV) % showed significant variation of 1% to 85% of infection among the black gram genotypes. Cluster analysis of agronomic characters showed sub-cluster-I with high (~3.0% YMV) and sub-cluster-II with moderate resistance (~15.5% YMV) forming cluster-I. The highly susceptible TAU-1, Kaska local and Balangir local separating as cluster-II. A total 47.98% bands were polymorphic out of the 189 RAPD bands amplified. RAPD analysis showed cluster-I and cluster-II consisted of 10 genotypes and 3 genotypes respectively. Highly susceptible (sub-cluster-I) and moderately susceptible (sub-cluster-II) formed a cluster-I. All the high resistance four varieties namely Deogan local, Cheripalli local, Pendibari local, Kendrapara local (1% to 3% YMV) formed cluster II showing resistance and grain yield like of TU942, a released check variety. Thus, RAPD could distinguish a separate resistance varieties of Odisha genotypes recommended for breeding programme. Genetically heterogeneous group sub-cluster II of cluster I genotypes might the potential breeding partner to cross with susceptible varieties of sub- culture I of cluster I for black gram genetic improvement.

1. Introduction

The cultivation of black gram in India was recorded from ancient times and is one of the most highly prized pulses of India. It is very widely used in Punjabi cuisine and is often referred to as *maah di daal* in the native language by Punjabis. The coastal region in Andhra Pradesh is famous for black gram. The Guntur District ranks first in Andhra Pradesh for the production of black gram. Black gram has also been introduced to other tropical areas such as the Caribbean, Fiji, Mauritius, and Africa, mainly by Indian immigrants. It is grown in different agro-ecological cropping systems that occupy 5.44 mha in India with a an annual production of 3.56 mton having an average yield of 653 kg ha^{-1} during 2017-2018 (Anonymous, 2018). Black gram is a good source of protein (20.8 to 30.5%) and carbohydrate (56.5 to 63.7 %) and is a rich source of dietary protein in south Asian people (Bhaskara Reddy *et al.*, 2015, Suvan *et al.*, 2020).

Yellow Mosaic Disease (YMD) inflicts heavy yield losses in five economically important food legumes including black gram (*Vigna mungo*), soybean (*Glycine max*), mungbean (*Vigna radiata*), French bean (*Phaseolus vulgaris*) and moth bean (*Vigna aconitifolia*). Black gram, which is mainly cultivated in India, Myanmar, Thialand, Phillipines and Pakistan, is highly prone to YMD. The disease is caused by representative species of the genus *Begomovirus*. Based on several studies, it has been confirmed that at least two virus species causing YMD are prevalent in Indian sub-continent. One of these species, Mung-bean Yellow Mosaic India Virus (MYMIV) is commonly occurring in northern part of Indian sub-continent, while Mungbean Yellow Mosaic Virus (MYMV) is mostly

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confined to peninsular region of India (Malathi and John, 2008). Although, Madhya Pradesh, Rajasthan and Bihar states of India produce a certain amount of black gram, but yield potential of this crop is incredibly low and is overburden with a variety of diseases. Blackgram is susceptible to diseases like the yellow mosaic virus, powdery mildew, and *Cercospora* leaf spot. Crop loss due to powdery mildew (Singh, 2080). Mungbean yellow mosaic virus (MYMV) disease can cause severe yield losses which infects a number of legumes like french bean, black gram, pigeon pea, soybean and mung bean. The insect vector transmitted this disease by sweet-potato whitefly (*Bemicia tabaci*). It causes annual yield loss of 300 million USD damaging young leaves with faint yellow specks/ spots, flowers and pods (Malathi and John, 2008, Behera *et al.*, 2020). The dominant warm tropical climate in India favors year-round survival of the polyphagous whitefly vector in overlapping host range (Borah and Das Gupta, 2012) but the information in the Odisha context is very scanty. Management of this disease is the biggest challenge and research priority in MYMIV for mungbean crop is very important globally (Mishra *et al.*, 2020). Thus, evaluation of genotypes having resistance against MYMV is needed for successful breeding and improvement. Only a few genes have been reported which can be used for breeding begomovirus resistance and its use to introgress into popular black gram varieties. Hence, needs for genetic diversity analysis of natural YMV-resistant genotypes is needed for crop protection and improvement (García-Neria and Rivera-Bustamante, 2011). Hence, molecular markers guided breeding strategy for resistant genes is very important in increasing the accuracy and potency of choice of suitable parents in black gram breeding program.

The molecular genetic mechanisms controlling the organization and evolution of genomes of *Vigna* species are not clearly understood. Black gram is a self-pollinating plant and mostly diploid (2n=2x=22) having genome size of ~ 574 Mbp (Arumuganathan and Earle, 1991). RAPD and ISSR marker analysis for genetic diversity and SCAR marker for marker-assisted breeding in black gram and its use as resistance against YMV was reported earlier (Selvi *et al.*, 2006; Souframanien and Gopalakrishna, 2006, Prasanthi *et al.*, 2011, Vishalakshi *et al.*, 2017). RAPD and SSR analysis of different genotypes of black gram (Lavanya *et al.*, 2008; Vyas *et al.*, 2018, Veni *et al.*, 2019) and mungbean (Kaur *et al.*, 2018, Tripathy and Das, 2018) reported high genetic variation in Indian accessions. Crossability studies among cultivated black gram varieties on the basis of pollen fertility along with SSR markers was reported (Tondonba *et al.*, 2018). AFPL mapping of Indian germplasm showed high genetic variability even in a narrow agroclimatic zone (Sivaprakash *et al.*, 2004; Gupta and Gopalakrishna, 2009). Studies on disease-resistant gene have indicated a high level of polymorphism and presence of SSRs at certain loci (Yu and Saghai Maroof, 1996). Although genetic diversity of black gram through microsatellite markers was reported in different genotypes with little information on Odisha germplasm **(**Suvan *et al.*, 2020; Behera *et al.*, 2020; Sreethi Reddy *et al.*, 2008, Wang *et al.*, 2012, Naik *et al.*, 2017). Despite the efforts to spread improved released varieties by Indian Council of Agricultural Research, New Delhi of black gram varieties, local landraces are largely grown in Odisha for their wider adaptability. Such landraces have played an important role in the local food security and sustainable development of agriculture and thus use of genetic resources for genetic improvement in very important. Assessment of genetic diversity can provide information on the genetic distinctiveness. Thus, the present study deals with characterization of 10 landraces of black gram from Odisha and 3 released varieties were characterized for agro-morphological characters including YMV occurrence percentage clubbed with RAPD markers to investigate the genetic diversity, for the potential use of Odisha landraces to use as breeding partner in develop YMV resistant high yielding black gram varieties.

2. Materials and methods

2.1. Plant materials

Ten black gram landraces of Odisha and four check varieties of India were grown in on-field trial for study of adaptive variations during rabi seasons in two successive years (November, 2017 and 2018) at the Department of Botany, Utkal University, Odisha, India (Table 1). Seeds were treated with 0.002% fungicides for 2 h and showed in the plot and materials were grown in randomized block design. Agronomic practices were followed through various stages of crop growth with a basal dose of NPK (20 kg N + 40 kg P_2O_5 + 20 kg K_2O per ha) before seed showing and 7 to 10 interval watering. Agronomic characters were were recorded and depicted in (Table 1).

2.2. Agronomic parameters

The yield attributes, morphological and yield parameters of all genotypes of black gram were recorded by selecting randomly five plants in each replication for each genotype. Observation on days to 50% flowering and days to maturity was recorded on a plot basis. The agronomic characters recorded are shown in Table 1. Plant height, total number of main branches per plant, the number of days for pod maturity, the number of fully matured pods, the number of seeds per pod, the weight of grains per plant and 100-seed weight in (g) was taken randomly. YMV % scoring was done in different genotypes using a 0-9 scale (Lal *et al.*,

2005). Tolerance index was calculated as high tolerance (1-10% infection), moderately tolerant (21-30% infection) and moderately susceptible (31-50 % infection) and highly susceptible (>50% infection).

2.3. DNA extraction and quantification

DNA was isolated from leaves using CTAB method (Williams et al. 1090). Young leaves were ground in liquid nitrogen using mortar and pestle and suspended in CTAB extraction buffer [100 mM Tris HCl (pH 8.2), 20mM EDTA, 0.5M NaCl, 2% CTAB and 2% beta-mercaptoethanol] and was incubated in a water bath at 60°C for 1½ h. An equal volume of chloroform:isomayl alcohol was added after cooling down to room temperature; the mixture was emulsified for 15 min with gentle mixing and centrifuged at 10, 000 rpm for 20 min. The top aqueous phase was transferred into a new tube and DNA was precipitated with S! of its volume of chilled isopropanol. The thread of DNA was pooled out with a sterile glass loop or pellet was washed within 70% ethanol after centrifugation at 10,000 rpm for 10 min. The pellet was dried in a vacuum concentrator and dissolved with $T_{10}E_1$ (10 mM Tris HCl, 1 mM disodium EDTA, pH 8.0) and treated with RNase A (10 mg ml^{-1}). The DNA was precipitated with an equal volume of ethanol with 3 mM sodium acetate, air-dried, and dissolved in $T_{10}E_1$ buffer; concentration of DNA was checked in nanodrop machine. The DNA was diluted into a final concentration of 25 ng μl^{-1} using $T_{10}E_1$ buffer for use as a template for PCR amplification with different primers (Table 2).

2.4. PCR reaction and RAPD analysis

Randomly amplified polymorphic DNA (RAPD) profiles were generated by using different primers following the polymerase chain reaction (Saghai-Maroof et al. 1984). Each amplification reaction mixture contained 25 ng of template DNA, 200 µM of each dNTPs, 25 ng of primer, 0.5 unit of Taq DNA polymerase (Bangalore Genei Pvt. Ltd., Bangalore, India), and 10 × PCR assay buffer (50 mM KCl, 10 mM Tris HCl, 1.5 mM $MgCl_2$, pH 9.0) in a reaction volume of 25µl. The PCR reaction was carried out in a Gene amplification PCR system thermal cycler (GENEAMP-9700; Applied Biosystems, USA) with an initial denaturation at 94°C for 5 min, followed by 45 cycles of denaturation at 94°C for 1 min, annealing at 42°C for 1 min, and an extension at 72°C for 2 min; then a final extension at 72°C for 8 min and the amplification were stored at 4°C. The amplified products were separated in 1.5% agarose gel containing 0.5 ìg ml^{-1} ethidium bromide in TAE buffer (40 mM Tris-acetate, pH 8.0; 2 mM EDTA) at a constant 55 V for 60 to 80 min. A gel loading buffer [20 % (w/v) sucrose; 0.1 M EDTA, 1.0 % (w/v) SDS; 0.25 % (w/v) bromophenol blue; 0.25 % (w/v) xylene cyanol] was used as tracking dye. Amplified DNA fragments were visualized by staining gel with ethidium bromide solution and images were captured using a gel documentation system (Geldoc XR system, Biorad, USA) and photographed. The sizes of the amplified products were determined using Gene ruler 100 bp DNA ladder as the size standard to determine the DNA fragments.

2.5. Data analysis

Mean data, standard deviation and standard error were calculated for all the replicated agronomic and YMV% data. The coefficient of variation was calculated to compare the degree of variation from one data set value to the other data set of various genotypes values applying the formula (standard deviation/mean) × 100 to get a percentage value. ANOVA analysis was done among different agronomic parameters as well as YMV occurrence percentage in the field. The mean data of agronomic characters collected against all the genotypes of black gram were used as set of variables for similarity matrix. Phylogenetic analysis and cluster analysis were made using NTSYS version 1.7, Exeter Software, New York, USA and neighbor-joining methods were adapted (Rohlf, 2008). The visualization of presence or absence of the bands was taken into consideration in RAPD analysis. Amplified bands of RAPD primers were scored as present (1) or absent (0) in each landraces for each set of primers and only clear and reproducible bands were used in this study. A binary matrix was obtained from the RAPD profile using NTSYS-pc programme (Rohlf, 2008). Binary matrix was transformed into a similarity matrix using Jaccard's coefficient. From this matrix a phylogenetic dendrogram was obtained by cluster analysis following the method of Unweighted Pair Group Method with Arithmetic Mean (UPGMA) (Sneath and Sokal, 1973).

3. Result and discussion

3.1. Variation in agronomic characters

The plant height varied from 15.4 cm in Balangir local to 22.2 cm in Sudhasarangi local which is at per with released check variety Pundibari local. Genotype classification on height found semi-dwarf (~15.0 to ~18.8 cm) in 7 landraces and rest varied from ~21.0 to 24.0 cm (Table 1). The mean branch per plant found lowest in TAU-1 (2.1) and highest in Balangir local and Tigiria local (3.2cm). The days of 50% flowering found maximum in Deogaon local (88 days) which was found like a check variety TU 942. The pod length recorded showed a minimum of 3.9 cm in Deogaon local to a maximum of 5.5 cm in LBG-17. The number of seeds per pod found varied from 6 per pod in Balangir local and Deogaon local to 6.6 in Kendrapada local which found at per of Pundibari local, a check variety. Seed yield per plant

also varied from 2.955 g in Balangir local to ~4.6 g per plant in TU942 (a check variety) and 100 seed weight was 1.391 in Kaska local to 5.681 was recorded in a check variety Pundibari local (Table 1). Sudhasarangi local showed 100 seed weight of 5.05 g which was found similar to a check variety LBG-17 a susceptible released variety. The mean YMV was recorded as 25.46%. The genotypes found with high resistance with little infection in the field condition from ~1.0 to 3.0 % of infection in Odisha varieties like Kendrapada local, Cheripalli local, Deogaon local, which were as per the released check varieties like Pundibari local, TU942 with high resistance. The moderate resistant genotypes were Tigiria local (~8 %) and LBG-17 (15 %) infection (Table 1). The moderate susceptible genotypes recorded (Mohona local, Shergarh local) with 30% infection and highly susceptible with 42 to 85% infection (Sudhasarangi local, Balangir local, Kaska local) besides a check variety TAU-1 having 85 % infection.

An insight into phenotypic markers' analysis indicated that there was a distinct pattern of grouping of black gram genotypes based on agronomic characters. Morphological characters could form two clusters with high number of YMV resistance check varieties and landraces of Odisha in cluster-I. The high disease susceptible varieties formed cluster-II i.e. Kaska local and Balangir local with ~60% of disease incidence (Fig. 1). Such type of grouping and comparison of released YMV resistant variety although not reported earlier in black gram genotypes of Odisha except few reported in other genotypes of black gram and in other crops too (Dikshit et al. 2013) which is in accordance to our result. The yield potential and other agronomic characters showed that high to moderate resistance variety of Odisha like Kendrapada local having 5.89 g per 100 seed weight compared to released resistant check variety TU942 with 4.49 g seed weight per 100 seed which might be suitable for breeding purpose with susceptible varieties of Odisha.

3.2. RAPD marker derived phylogenetic analysis

Amplification with RAPD primers showed out of the 43 tested generated 189 unequivocal scorable DNA fragments out of which 84 found polymorphic (Table 2, Fig. 2). A total of 20 primers were amplified out of 43 primers tested that exhibited a wide range of variation with 47.98% of polymorphic bands. RAPD markers like 475 bp found monomorphic in OPA08 primer. YMV resistant genotypes like Deogaon local Shergarh local showed 550 bp marker in OPA-8 marker (Fig. 2). Likewise, OPN-04 showed 600 bp and 650 bp markers in LBG-17 and TU-942 check variety (Fig. 2). Cheripalli local, Kendrapada local highly resistant variety could produce some marker band of ~ 670 bp in OPF-12 primer. The moderately resistant variety Mohana local and Sudhsarangi local showed ~ 1200 bp marker in OPN-04 primer (Fig. 2).

Dendrogram constituted with RAPD markers showed a single common ancestry those basically formed cluster-I, and cluster-II forming a distinct group with the majority of moderate to highly susceptible varieties in cluster-I (Fig. 3) while cluster-II formed all the highly resistant landraces of Odisha (1.0 – 3.0% disease incidence) with resistant check varieties like Pendibari local and TU942. Cluster-I formed highly susceptible genotypes as a single group forming sub-cluster-I with 60 to 85% disease incidence in the field. In Odisha condition TAU1 was not able to show its resistance as release variety. Moderately susceptible varieties formed sub cluster-II with a range of YMV resistance from 8.5% in Tigiria local to 42% in Sudhasarangi local. Thus, local varieties like Kendrapara local, Deogaon local and Cheripalli local found highly YMV resistant and formed cluster-II along with check varieties Pendibari local and TU 942 could be used for breeding purpose with other genetically distinct groups (Fig. 3). So, RAPD markers have been used in many instances to assess the genetic variability in genetic resource utilization which is in accordance with our earlier report in black gram (Tripathy and Das, 2018) and other crops (Panigrahi *et al.,* 2015; Shelke and Das, 2015; Sahu *et al.*, 2016). It was evident from the fact that black gram landraces belong to different agro climatic-zones grouped into the same phylogenetic cluster. Powdery mildew resistance loci in mungbean was mapped earlier (Humphry *et al.*, 2003, Gupta *et al.*, 2013) in other genotypes and SSR polymorphism recorded in some Odisha genotypes (Behera *et al.*, 2020) is supported our findings in RAPD polymorphism. It was evident that, narrow genetic variability exists in sub cluster-I and sub cluster-II of cluster-I. High genetic variability of cluster-II with YMV resistance of local landraces found suitable for use in breeding with susceptible varieties to improve yield and YMV resistance. The established genotypes TU942 and Pendibari local further confirm their resistance in the ecological condition of Odisha and formed a distinct cluster-II which can be used for breeding with susceptible varieties of local genotypes of Odisha belongs to cluster-I. BAC and EST library development might be important for gene-based markers analysis (Miyagi *et al.*, 2004; Galeano *et al.*, 2009). A low level of DNA polymorphism was recorded earlier in black gram by (Gupta *et al.*, 2013) also in accordance with our study in genotypes of Odisha might be due to a narrow genetic base with less genetic diversity (Kumar *et al.*, 2011). Identification of a number of YMV markers as described in the result section is in accordance with the discovery of a 445 bp marker linked to the YMV tolerant gene in black gram variety TU942 and *V. mungo* var. *silvestris* using specific

Table 1

Different agronomical characters and YMV% in 13 genotypes of black gram.

Sl. No.	Genotypes	PH (±S.E.)	BP (±S.E.)	DF (±S.E.)	DM (±S.E.)	CP (±S.E.)	PP (±S.E.)	PL (±S.E.)	SP (±S.E.)	SY (±S.E.)	SW (±S.E.)	YMV% (±S.E.)
1	Balangir local	15.4±0.95	3.2±0.08	37±0.07	77±0.04	3.5±0.09	15.4±0.24	4.2±0.02	6.0±0.05	2.955±0.14	3.976±0.10	60.0 ±1.45
2	Cheripalli local	18.4±0.53	2.7±0.12	37±0.19	77±0.09	4.2±0.05	16.6±0.24	4.6±0.07	6.1±0.02	3.887±0.11	2.872±0.05	2.0 ±0.36
3	Deogaon local	16.3±1.02	2.0±0.31	38±0.15	88±0.03	3.4±0.08	13.2±0.45	3.9±0.02	6.0±0.08	3.384±0.15	3.151±0.10	3.0 ±0.09
4	Kaska local	16.2±0.85	2.1±0.09	38±0.71	77±0.08	3.5±0.03	12.6±0.13	4.8±0.04	6.5±0.02	2.990±0.14	1.391±0.06	60.0±2.15
5	Kendrapada local	24.2±1.35	2.3±0.05	37±0.08	77±0.03	6.1±0.07	22.6±0.21	4.6±0.07	6.6±0.05	3.886±0.11	5.897±0.06	1.0 ±0.08
6	Mohana local	18.8±0.45	2.0±0.04	37±0.07	79±0.03	6.1±0.05	22.5±0.13	4.6±0.05	6.5±0.02	3.898±0.11	5.009±0.06	30.0 ±1.25
7	Shergarh local	22.2±0.56	2.9±0.03	36±0.04	78±0.02	4.9±0.03	21.9±0.13	4.6±0.07	6.5±0.05	3.781±0.08	4.521±0.04	30.0 ±1.88
8	Sudhasarangi local	21.6±1.25	2.2±0.07	35±0.05	78±0.06	5.4±0.03	19.3±0.45	4.8±0.04	6.4±0.04	3.193±0.16	5.058±0.08	42.0 ±1.05
9	Tigiria local	22.0±0.46	3.2±0.04	37±0.11	76±0.09	5.5±0.07	23.5±0.45	4.7±0.02	6.3±0.08	3.857±0.08	2.082±0.05	8.5 ±0.25
10	Pundibari local	22.2±1.42	2.9±0.09	36±0.08	75±0.04	4.8±0.05	19.6±0.13	4.5±0.02	6.8±0.08	3.596±0.14	5.681±0.06	1.8 ±0.05
11	LBG-17	21.5±0.91	3.5±0.08	37±0.03	77±0.02	6.2±0.07	25.2±0.41	5.5±0.04	6.5±0.02	3.745±0.14	5.193±0.05	15.5 ±1.72
12	TU 942	18.2±0.23	2.0±0.02	40±0.16	84±0.04	3.5±0.05	19.1±0.45	4.5±0.06	6.3±0.04	4.613±0.16	4.680±0.08	1.8 ±0.09
13	TAU-1	20.6±0.94	1.2±0.06	37±0.12	76±0.02	3.5±0.05	8.6±0.13	4.8±0.06	6.1±0.04	3.728±0.16	4.496±0.06	85.0 ±1.36
	Average (Mean) ± S.D.	19.81±5.55	2.47±1.29	37.07±2.37	78.38±7.23	4.66±2.23	18.46±9.84	4.62±0.73	6.35±0.49	3.65 ±0.89	4.15±2.77	25.46 ±57.36

PH- Plant height (cm), BP- Number of branches per plant, DF-Days of 50% flowering, DM- Number of days for maturity, CP-Number of cluster of pods per plant, PP-Number of pods per plant, PL-Pod length (cm), SP-Number of seeds per pod, SY-Seed yield per plant (g), SW-100-seed weight (g), YMV %= Yellow mosaic virus infection percentage.

Table 2

RAPD profile and DNA polymorphism data generated in 13 blackgram landraces under study.

Primer name	Sequence (5'- 3')	No. of bands amplified	No. of poly-morphic	Polymorphic % bands	Size range of bands
OPA08	GTCACGTAGG	14	6	42.84	200-900
OPA11	CAATCGCCGT	11	8	72.72	250-1100
OPA14	TCTGTGCTGG	8	4	50.00	250-1200
OPD-08	GTGTCCCCCA	9	5	55.55	400-950
OPD-12	CACCGTATCC	10	6	60.00	300-850
OPE03	CCAGATGCAC	8	2	25.33	200–1150
OPF08	GGGATATCGG	5	2	40.00	300–1250
OPF10	GGAAGCTTGG	6	1	16.16	200–870
OPF12	ACGGTACCAG	14	6	42.85	340–1250
OPF13	GGCTGCAGAA	12	4	33.33	280–1120
OPF16	GGAGTACTGG	8	3	37.5	300–940
OPW02	ACCCCGCCAA	10	4	40.00	150–1280
OPW03	GTCCGGAGTG	6	3	50.00	230–1300
OPW05	GGCGGATAAG	9	4	44.00	200–1200
OPW06	AGGCCCGATG	6	2	33.33	250–1060
OPW08	GACTGCCTCT	11	6	54.54	270–1050
OPW11	CTGATGCGTG	10	3	30.00	350–900
OPN-04	GACCGACCCA	12	7	58.33	220-930
OPN-11	TCGCCGCAAA	8	3	37.50	150-1150
OPN-15	CAGCGACTGT	12	5	41.66	450-1240
		189	84	47.98	

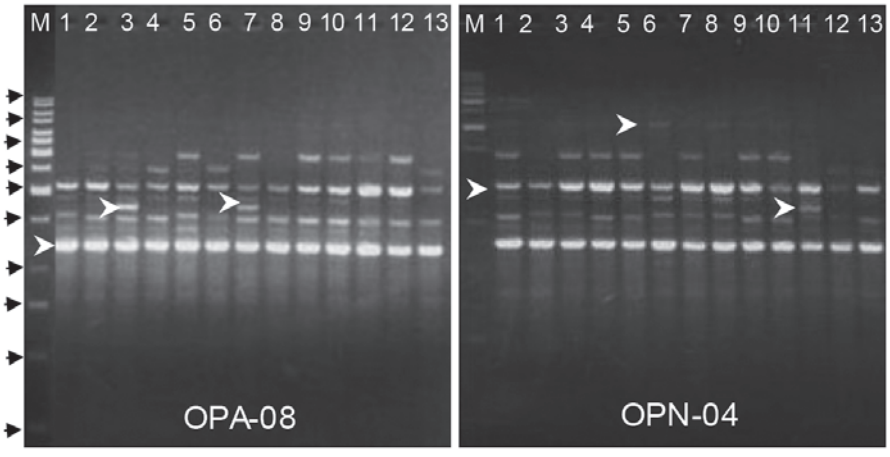

Fig.1 - RAPD Profile of 13 genotypes of black gram amplified with OPA-S and OPN-04 primers M=DNA molecular weight marker; 1-13 lane represent different genotypes as per Sl.No. of Table-1.

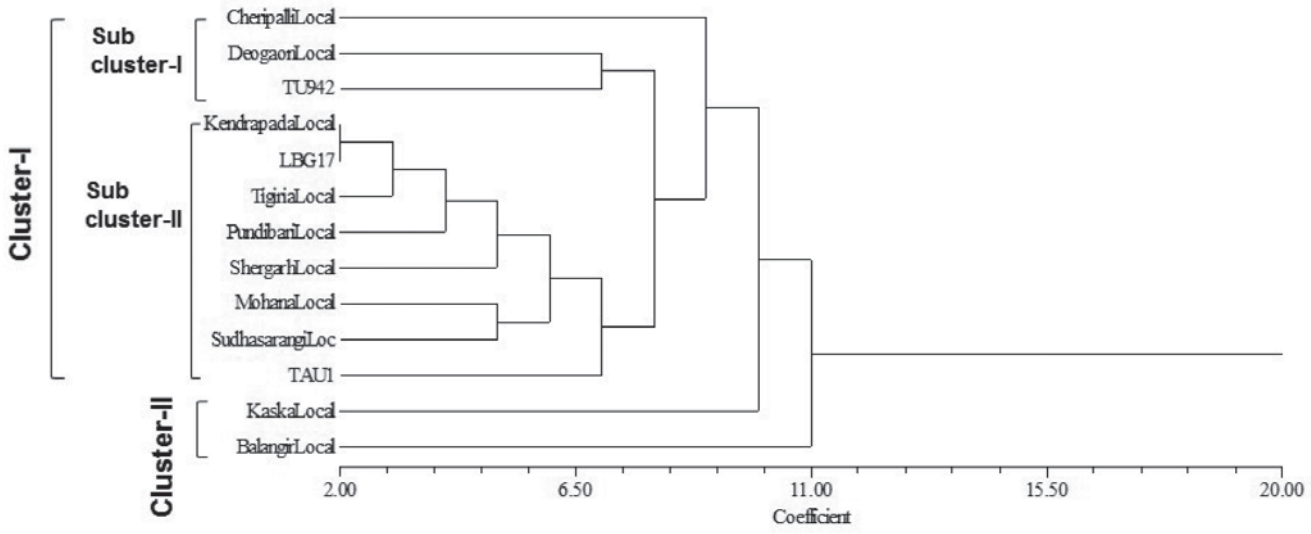

Fig.2 - Phylogenetic tree obtained from apronomic parameters of different genotypes of black gram.

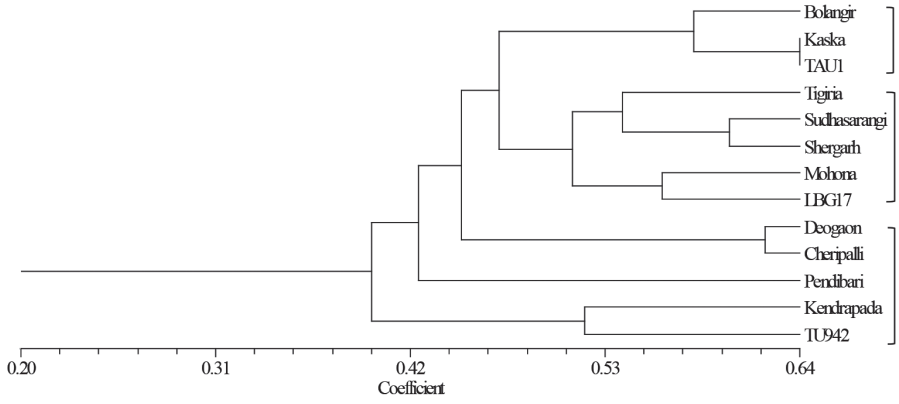

Fig.3 - Dendogram of genotypes of black gram constructed on the basis of RAPD data.

primers of (Basak *et al.*, 2004) also reconfirmed by Souframanien and Gopalakrishna (2006) can be developed through SSR study in Odisha landraces as reported earlier from mungbean, soybean, common bean, red gram, azuki bean (Sudha *et al.*, 2013; Rambabu *et al.*, 2018; Dhole and Reddy, 2013; Kaewwongwal *et al.*, 2015; Tondonba *et al.*, 2018; Kumar *et al.*, 2020; Suvan *et al.*, 2020). The selection of distinct YMV resistant genotypes from the distinct groups could be helpful for plant breeders in selecting parents for hybridization. The RAPD marker may be developed as SCAR marker from putatively YMV resistant genotypes in different individuals might be a potential genetic marker in future identification of registrant genotypes.

5. Conclusion

Genetic fingerprinting of black gram landraces by RAPD markers are useful methods for the analysis of genetic diversity of Indian germplasms particularly from Odisha besides different agronomic parameters and assessment of on-field YMV resistance. On the basis of morphological traits and RAPD markers, few genotypes of Odisha formed separate clusters having high resistance (Deogaon local, Cheripalli local and Kendrapara local) against YMV in Odisha condition compared to check variety TU942. Thus, the putative YMV resistance polymorphic RAPD loci found in this study can be used in developing SCAR markers to identify YMV resistant genotypes. Furthermore, the resistant Odisha landraces can be recommended for use in breeding to develop disease resistant varieties with high yielding.

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