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Field Screening and Molecular Characterization of Urdbean Genotypes for Yellow Mosaic Disease

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ABSTRACT

Background: Urdbean [*Vigna mungo* (L.) Hepper] is an important pulse crop in India as well as in South East Asia. It is an excellent source of easily digestible, good quality protein. Mungbean Yellow Mosaic Virus causing Yellow mosaic disease (YMD) is one of the most devastating diseases affecting urdbean production and productivity to a large extent. Identification of resistant genotypes is one of the most important aspects in the management of MYMV diseases. The current study aimed to identify the MYMV resistant urdbean genotypes in order to manage and control the MYMV disease.

Methods: In present investigation, a total 100 urdbean genotypes were screened against MYMV under natural field conditions in the augmented design by planting 2 rows of two test entries each alternated with one row of susceptible check. Further, selected genotypes which showed disease free, highly resistant, resistant, moderately resistance and susceptible reaction in field screening were used to tag RAPD markers associated with MYMV resistance in urdbean.

Result: Out of 100 genotypes tested, only one genotype, NDU 12-1 was found to be disease free, twenty seven genotypes found to be highly resistance and four genotypes showed resistance consistently for four consecutive seasons (two summer and two *Kharif)* during 2017-2018. However, four genotypes were found to be superior for seed yield among all the genotypes tested as well as resistant to MYMV. The RAPD analysis clearly grouped all the MYMV resistant genotypes studied in cluster I whereas the susceptible genotypes were clustered together in the cluster II. The present work has successfully identified the MYMV resistant urdbean genotypes which can be used in MYMV resistance breeding programme.

Key words: MYMV, Resistance, Seed yield, Urdbean [*Vigna mungo* (L.)].

INTRODUCTION

Urdbean [*Vigna mungo* (L.) Hepper], an Indian native pulse crop, is grown throughout India and Southeast Asian countries. In India, urdbean is also referred to as black gram or urad dal and about 70% of its global production is produced there. Due to its wide adaptability and climate resilience, it is grown in all the cropping seasons in India. Despite being the largest producer and consumer of urd beans, India has been unable to keep up with the demand of the world's population, which is expanding quickly. In India, it is cultivated on an approximately 3.19 million hectares, producing about 1.95 million tonnes with an average yield of 596 kg per hectare (Raju, 2019). Pulses in general and urdbeans in particular have very poor global production when compared to cereals, which compromises the nutritional security. The productivity of urdbean oscillates upon various biotic and abiotic stresses. The occurrence of several environmental stresses, the production and productivity of urdbean is in declining trend in majority of urdbean growing areas (Patel *et al*., 2020; Sah *et al*., 2021). The yield losses (5-100%) in urdbean reported due to various biotic stresses which are responsible for the fluctuation in the average yield depending upon disease severity, susceptibility of cultivars and population of whitefly (Nene, 1972; Sahni *et al*., 2016; Equbal *et al.,* 2016; Prema and Rangaswamy 2018). The biotic stresses like yellow mosaic, powdery mildew, cercospora leaf spot and web blight are major limiting factors for high yield (Kumari *et al*., 2020). Among these, Yellow mosaic disease (YMD), caused by mungbean yellow mosaic virus (MYMV), is a most destructive viral disease of urdbean not only in India, but also in Pakistan, Bangladesh, Srilanka and contiguous areas of South East Asia (Bashir *et al*., 2006). In India, this virus cause more severe yellow mosaic disease in urdbean than mungbean (Williams *et al*., 1968). In addition to dramatically reducing yield, the MYMV infection also negatively affects grain size and quality (Singh and Shrivastva, 1985; Sahni *et al*., 2016). The primary factors

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affecting yield decline are the decrease in seed weight and the number of pods/plant seeds/pod (Nene, 1973; Sahni *et al*., 2016; Kumari *et al*., 2020). Even though India has a sizable region dedicated to urdbean farming, MYMV infections are causing low productivity levels (Sahni *et al*., 2016). The chemical management of the vector is not financially viable since controlling whiteflies necessitates frequent sprays of insecticide. Sprayings that are repeated put the environment and human health in peril (Nariani, 1960). Instead, if virus resistant cultivars are available, using them is the greatest strategy to reduce the frequency of YMD in regions where the infection represents a significant productivity bottleneck. The use of resistant crop types is considered the reasonable, powerful and ideal approach of reducing viral infections.

The breeding for resistance is one of the important strategies for developing disease resistant variety (Gupta *et al*., 2020). However, conventional breeding requires a long time, which can be accelerated using molecular markers. Past few decades, DNA markers are extensively used for resistance breeding. Random amplified polymorphic DNA is one of the simplest and fastest DNA marker technologies which is widely used in characterization of genotypes and tagging of genes associated with specific character. In present investigation as attempt was made to characterize the MYMY resistant and susceptible urdbean genotypes using RAPD markers.

MATERIALS AND METHODS

The present study was undertaken to identify the resistant urdbean germplasms against mungbean yellow mosaic virus disease. Field experiments were conducted for four consecutive seasons (two summer and two *Kharif)* during 2017-2018 in the experimental Farm of Tirhut college of Agriculture, Dholi, Muzaffarpur, Bihar, India. Well-leveled field with satisfactory drainage system was selected for the experiment.

Plant materials

Urdbean genotypes were collected from All India Coordinated Research Project (AICRP) on MULLaRP, T.C.A., Dholi. Urdbean genotypes were selected to identify the MYMV resistant genotypes.

Screening of urdbean genotypes against MYMV under field conditions

The assessment of urdbean genotypes against MYMV were performed under natural field conditions. The seeds were sown in the augmented design by planting 2 rows of two test entries each alternated with one row of LBG 623 as susceptible check. Each test entry was planted in a row that was 4 meters long, with a 30 cm space between rows and a 10 cm space between plants. In order to attract white flies to promote MYMV infection, two rows of spreaders were planted all around the experiment. All agronomic procedures were followed for proper crop growth and development. However, no insecticides were applied to boost the whitefly population in order to promote the spread of MYMV naturally.

The percent disease incidence of MYMV infection at pod formation stage of urdbean genotypes were scored using 0-9 scale as described previously (Kumari *et al*., 2020).

Seed yield analysis

Ripen pods from 20 urdbean plants per row were gradually hand-picked and sundried. Further, the grains were separated and weighed to determine the yield.

Isolation of genomic DNA of Urdbean genotypes

Genomic DNA of urdbean genotypes were isolated from freshly harvested young leaves of urdbean by following modified CTAB method (Anu *et al*., 2015; Mishra *et al*., 2020).

RAPD analysis

Utilizing decamer random primers, we performed RAPD analysis for the characterization of urdbean genotypes. The RAPD system for amplification was made as described previously (Anu *et al*., 2015; Williams *et al.,* 1990). The amplification cycle involved an initial denaturation at 94°C for 5 min followed by 45 cycles of denaturation at 94°C for 1 min, primer annealing at 35°C for 30 sec, primer extension at 72°C for 2 min and final extension at 72°C for 10 min. For each RAPD marker, the presence or absence of DNA bands was scored as 1 or 0, respectively. The dendrogram based on similarity indices was obtained by un-weighted pair-group method using arithmetic mean (UPGMA). Analysis was performed with the help of NTSYS-pc software (Rohlf, 2000).

Statistical analysis

All the data were analysed statistically with the computer software OPSTAT. Significance of differences was analyzed by one-way analysis of variance (ANOVA).

RESULTS AND DISCUSSION

In this study, 100 urdbean genotypes were assessed for their resistance and susceptibility against MYMV under natural field conditions during four consecutive seasons (two summer seasons and two *Kharif* seasons) during 2017-2018). Based on the disease reaction and occurrence of YMD, urdbean genotypes were grouped in different reaction group (Table 1). Out of the 100 genotypes examined, only one (NDU 12-1) was found to be consistently MYMV disease-free in both the summer and *Kharif* seasons, twenty-seven were determined to be highly resistant and four genotypes consistently displayed resistance throughout a two-year period (Table 1). Five genotypes showed moderately resistance reaction (Table 1). Forty-seven genotypes were observed to be susceptible (Table 1). Sixteen genotypes were found to be highly susceptible to MYMV in both seasons over two years, with severe yellow discoloration of leaves covering 50-75 per cent of the foliage, plants stunting and pod size reduction. Forty-seven genotypes were found to be moderately susceptible, susceptible and highly susceptible to MYMV in one of the seasons (Table 1).

The current investigation demonstrated that certain genotypes responded differently to MYMV during the summer and *Kharif* seasons. The variation in temperature and relative humidity, which may have a direct impact on the vector population and its migration, may be the cause of the variation in illness incidence and disease reaction. Earlier reports have noted a similar impact of climate on vector populations (Singh and Gurha, 1994). Several researchers have already recorded similar types of varietal ratings (Pathak and Jhamaria, 2004; Kumari *et al*., 2020). Identification of resistance genotypes was encouraged in a prior work by Kumari *et al*. (2020) and comparable to our discovery, NDU12-1 was also revealed to be the disease-free against MYMV in field conditions (Kumari *et al*., 2020).

Analysis was done on the seed yield and disease incidence of urdbean genotypes that displayed disease-free, HR, R and MR reactions to MYMV in both the season for two years. The range of seed production for urdbean genotypes exhibiting different levels of resistance (disease-free, HR, R and MR) against MYMV varied from 6.43 g to 0.07 g per plant. With a disease incidence of 3.17 per cent, VBN (BG) 7 had the highest seed yield (6.43g/plant), followed by IPU 2-43 (5.40 g/plant), KUG 586 (5.24 g/plant) and KUG 503 (5.84 g/plant). It also demonstrated a high level of resistance, with a rating scale of "2" indicating yellow specks with a constrained spread covering 0.1-5 per cent of leaf area. Kopergaon barely yielded 0.07 g of seeds per plant, despite having a "3" rating for disease resistance and a 96.16 per cent disease incidence rate. Similar to this, ACM 05-007 only yielded 1.16 g of seeds per plant while displaying high resistance (rating scale: 2) and a disease incidence rate of 10.5%. While UG-218 had a 4.36 g seed output per plant, was rated as having a moderate level of resistance and had a disease incidence of 98.00 per cent. Stable genotypes for seed yield per plant in blackgram were also reported previously (Natarajan, 2001; Kumari *et al*., 2020). Previous report suggests that resistance against YMD is rare and scarce (Gill *et al.,*1983). Based on these results, it can be said that only four genotypes namely, VBN (BG) 7, IPU 2-43, KUG 586 and KUG 503 were found to have superior seed yield and MYMV resistance out of a total of 100 genotypes tested.

Table 1: Reaction of urdbean genotypes against MYMV.

Genotypes	Total	Reaction group
NDU 12-1	1	Disease-free
IPU 10-23, KUG 586, Mash-338, NDU 12-300, PU 09-35, UH 07-06, Uttara, VBG 10-008, VBN 6, IPU 2-43, KPU 1-10, KU 363, KUG 503, KUG 540, LRB 332, Mash 1-1, Mash-114, NDU 11-202, P 719, PU 08-5, PU 31, UH 08-05, VBG 10-0024, VBN (BG) 7, ACM 05-007, Naveen, Pant U 30	27	HR
Kopergaon, RUG-44, VBG 09-005, NDU 11-201	4	R
IGKU 02-1, KU 1106, KUG 391, KUG 662, UG-218	5	MR
AKU 10-1, AKU 9804, AKU 9904, B-3-8-8, Barabanki, BDU-1, Birsa Urd-1, CBG 703, CBG-757, CO 6, COBG 10-5, COBG 653, COBG 761, DBG 1, K 851, KKB 05-011, KPU 26-10, KPU 405, KPU 406, KPU-07-06, KPU-07-08, KUG 752, Kullu 4, KKB 20055, LBG 752, LBG 792, LBG 17, LBG-20, LBG-685, Mash 391, NUL 7, OBG 35, P 726, Palampur 93, Pragya, Phule U-0014, RBU-38, RUG-10, RVSU 11-8, RVSU 60, T9, TAU-9, TPU-4, TU 631, TU-94-2, VBN (BG) 3, VBN (BG) 5	47	S
AAU 34, AKU-7-1, AKU 7-4, AKU 10-4, AKU 15, CO 5, LBG-645, NUL 2-5, NUL-138, PDU 1, TAU-1, TAU-4, TU 17-4, TU-26,VBN (BG) 4, LBG 623 (Susceptible check)	16	HS

HR: Highly resistance, R: Resistance, MR: Moderately resistance, S: Susceptible, HS: Highly susceptible.

Table 2: List of RAPD markers used for characterization of tolerant and susceptible urdbean genotypes.

Primers	Primer sequence 5′-3′	Total number of bands	Number of polymorphic bands	Polymorphism (%)
OPA04	AATCGGGCTG	8	6	75.00
OPA14	TCTGTGCTGG	8	4	50.00
OPA18	AGGTGACCGT	8	5	62.50
OPA20	GTTGCGATCC	6	2	33.33
OPB04	GGACTGGAGT	9	5	55.55
OPB07	GGTGACGCAG	9	8	88.88
OPB12	CCTTGACGCA	12	8	66.00
OPC09	CTCACCGTCC	10	6	60.00
OPC14	TGCGTGCTTG	6	2	33.33
OPC15	GACGGATCAG	6	3	50.00
OPD07	TTGGCACGGG	9	2	22.22
OPD17	TTTCCCACGG	11	7	63.63
Mean		8.5	4.83	55.04

In the current study, 24 selected urdbean genotypes that displayed disease-free, highly resistant, resistant, moderately resistant and susceptible reactions during field screening were examined for RAPD markers linked to MYMV resistance. Twelve randomly chosen decamer primers were used to measure the variation between 24 urdbean genotypes (Fig 1). It's interesting to note that all 12 primers examined in this study displayed polymorphism. The range of polymorphism observed in this study varied from 22.22 to 88.88% (Table 2). The degree of polymorphism observed in this study on urdbean is comparable to reports from earlier investigations (Arulbalachandran *et al*., 2009; Binyamin *et al*., 2011). The highest amount of polymorphism (88.88%) was produced by primer OPB-07, whereas the lowest level (22.22%) was seen with primer OPD-07. High values of Jaccord's similarity coefficient, which ranged from 0.727 to 0.965, showed that all of the genotypes under consideration shared a close genetic similarity (Table 3). On the basis of

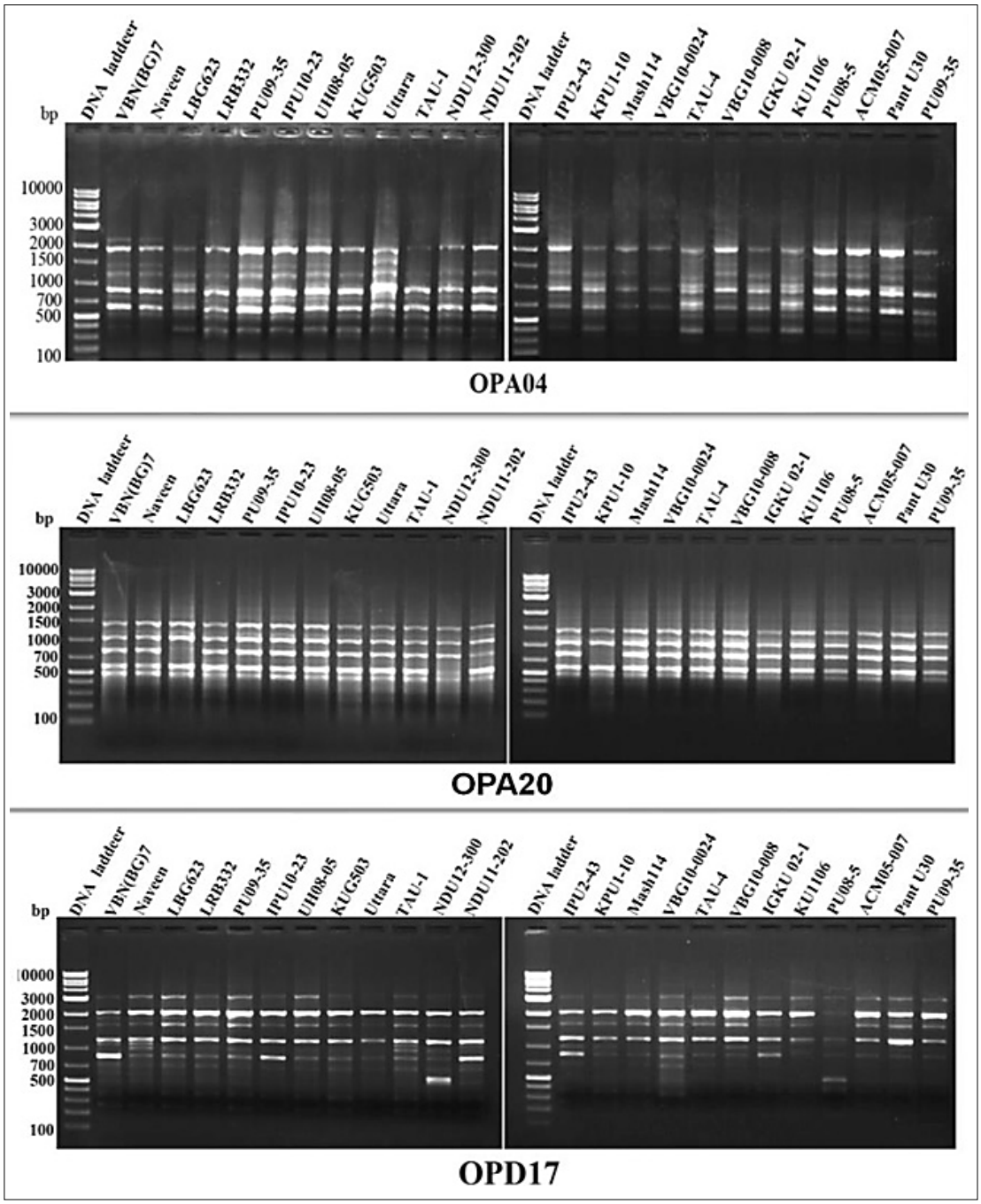

Fig 1: RAPD-PCR of urdbean genotypes using different random primers.

Table 3: Correlation coefficient similarity matrix of RAPD banding pattern obtained from different tolerant and susceptible urdbean genotypes using random primers.

	VBN (BG) 7	Naveen	LBG 623	LBR 332	PU0 9-351	IPU 0-23	UH0 8-05	KUG 503	Uttara	TAU -1	NDU12 -300	NDU11 -202	IPU 2-43	KPU 1-10	Mash 114	VBG 10-0024	TAU -4	VBG10 -008	IGKU 02-1	KU1 106	Pant U30	PU0 8-5	ACM 05-007	PU0 9-35
VBN (BG)7	1.00																							
Naveen	0.87	1.00																						
LBG623	0.80	0.79	1.00																					
LBR332	0.85	0.84	0.73	1.00																				
PU09-35	0.84	0.89	0.80	0.87	1.00																			
IPU10-23	0.85	0.86	0.79	0.83	0.96	1.00																		
UH08-05	0.81	0.86	0.80	0.94	0.84	0.91	1.00																	
KUG503	0.81	0.83	0.76	0.89	0.86	0.85	0.83	1.00																
Uttara	0.80	0.84	0.73	0.81	0.87	0.86	0.88	0.85	1.00															
TAU-1	0.78	0.81	0.78	0.79	0.82	0.81	0.80	0.78	0.80	1.00														
NDU12-300	0.78	0.83	0.80	0.78	0.84	0.83	0.87	0.82	0.83	0.87	1.00													
NDU11-202	0.84	0.86	0.81	0.85	0.89	0.90	0.86	0.87	0.84	0.83	0.89	1.00												
IPU2-43	0.85	0.84	0.82	0.82	0.90	0.89	0.87	0.84	0.82	0.78	0.82	0.87	1.00											
KPU1-10	0.81	0.78	0.80	0.78	0.80	0.78	0.79	0.79	0.76	0.73	0.77	0.80	0.88	1.00										
Mash114	0.79	0.78	0.73	0.80	0.82	0.80	0.79	0.82	0.78	0.75	0.75	0.78	0.86	0.89	1.00									
VBG10-0024	0.86	0.83	0.82	0.80	0.87	0.85	0.86	0.82	0.83	0.80	0.82	0.85	0.91	0.87	0.84	1.00								
TAU-4	0.76	0.73	0.70	0.77	0.79	0.77	0.80	0.81	0.77	0.75	0.75	0.75	0.80	0.78	0.78	0.81	1.00							
VBG10-008	0.86	0.85	0.80	0.85	0.89	0.88	0.88	0.87	0.86	0.79	0.82	0.88	0.93	0.87	0.87	0.94	0.83	1.00						
IGKU 02-1	0.81	0.79	0.78	0.85	0.80	0.79	0.79	0.82	0.77	0.74	0.78	0.81	0.88	0.86	0.84	0.85	0.76	0.88	1.00					
KU1106	0.80	0.77	0.72	0.76	0.80	0.83	0.82	0.80	0.81	0.72	0.78	0.81	0.86	0.82	0.80	0.82	0.81	0.85	0.80	1.00				
Pant U30	0.85	0.82	0.77	0.84	0.86	0.82	0.83	0.83	0.80	0.77	0.79	0.86	0.87	0.85	0.86	0.87	0.80	0.91	0.81	0.81	1.00			
PU08-5	0.84	0.83	0.76	0.82	0.86	0.85	0.83	0.87	0.83	0.76	0.78	0.83	0.88	0.86	0.91	0.87	0.83	0.92	0.82	0.87	0.93	1.00		
ACM05-007	0.88	0.85	0.86	0.82	0.86	0.85	0.85	0.82	0.79	0.76	0.80	0.87	0.90	0.86	0.83	0.86	0.78	0.91	0.86	0.82	0.90	0.88	1.00	
PU09-35	0.83	0.84	0.77	0.82	0.88	0.87	0.85	0.83	0.82	0.80	0.79	0.84	0.85	0.81	0.88	0.84	0.80	0.86	0.79	0.84	0.87	0.93	0.87	1.00

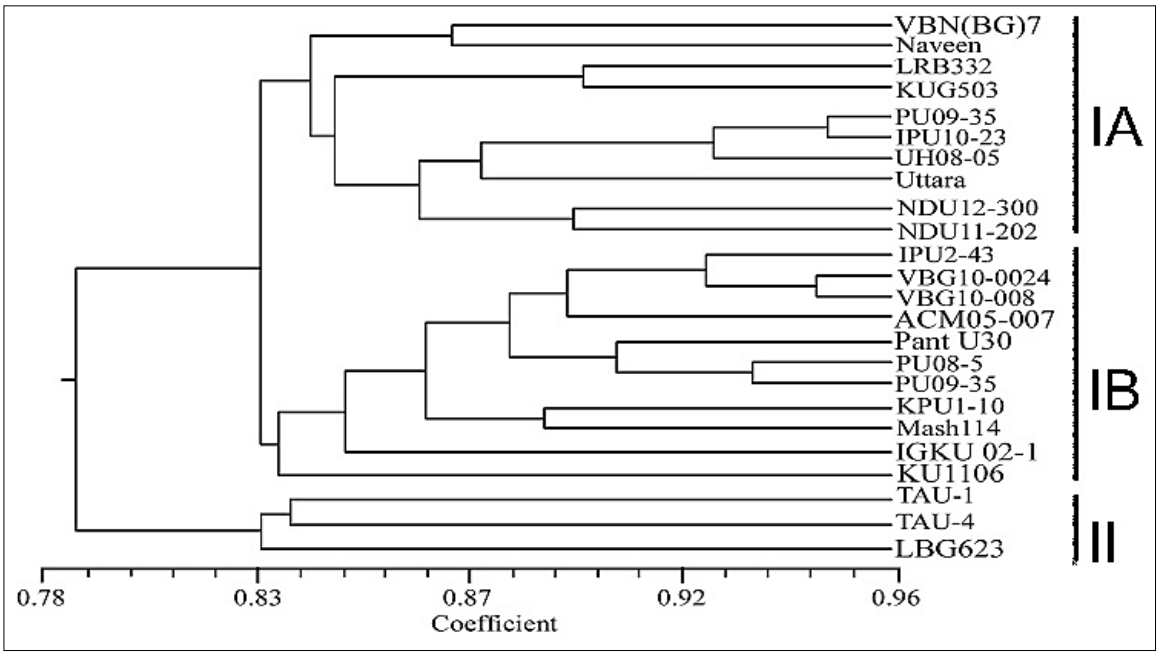

Fig 2: Dendrogram of urdbean genotypes obtained from RAPD analysis. The dendrogram was generated using the similarity indices obtained by un-weighted pair-group method using arithmetic mean (UPGMA). Analysis was performed with the help of NTSYS-pcsoftware.

RAPD data, a dendrogram was created and it revealed two significant clusters (I and II) (Fig 2). Cluster I was further divided in to two sub-cluster (IA and IB) (Fig 2).

Based on our field research, all of the MYMV resistant genotypes were grouped together in cluster I, whereas all of the susceptible genotypes were grouped together in cluster II. Additionally, cluster I was split into the subclusters IA and IB. VBN(BG)7, Naveen (Local resistance check), LRB332, KUG503, PU09-35, IPU10-23, UH08-05 and Uttara (Highly resistance) were the ten genotypes that made up Cluster IA.

The remaining 11 genotypes which made up Cluster IB, of which two (IGKU02-1 and KU1106) were discovered to be moderately resistant in one season, while the remaining nine were discovered to be resistant or extremely resistant in that same season. TAU-1, TAU-4 and LBG623 (Local Susceptible Check), which were extremely susceptible, are included in Cluster II. According to the study's findings, there is a high degree of genetic similarity among the genotypes of urdbean.

CONCLUSION

In present investigation, a total of 100 urdbean genotypes were evaluated against MYMV resistance. Out of 100 urdbean genotypes studied, one genotype (NDU 12-1) consistently showed a disease-free reaction, while thirty one genotypes were found to be resistant to MYMV. These thirty two urdbean genotypes identified as resistant for MYMV may be exploited in further breeding programme aimed at the development of MYMV resistant varieties of urdbean. On the basis of RAPD data, a dendrogram was created and it revealed two significant clusters. Based on our field research, all of the MYMV resistant genotypes were grouped together in cluster I, whereas all of the susceptible genotypes were grouped together in cluster II. Additionally, cluster I was split into the subclusters IA and IB. According to the study's findings, there is a high degree of genetic similarity among the genotypes of urdbean. The outcome of present study may be exploited in future MYMV resistance breeding programmes in urdbean.

Conflict of interest: None.

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