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Status of legumoviruses infecting mungbean and urdbean in Tarai region of India

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Abstract
Yellow mosaic disease of legumes is known to be caused by four distinct viruses in India, together known as Legume Yellow Mosaic Viruses (LYMV's). There is a lack of information regarding prevalence, distribution and identity of LYMVs on different legumes in tarai region of India. Present study was carried out in northwestern tarai region of Uttarakhand and adjoining districts of Uttar Pradesh. A roving survey was conducted for over two seasons during 2013 and 2014 to record the disease incidence, severity, vector population etc. The identity of the LYMV was confirmed by subjecting symptomatic samples PCR amplification using specific primer for each LYMV. The coat protein gene was sequenced for representative isolate from each host for phylogenetic analysis. Among the four LYMV's, only bipartite Mungbean Yellow Mosaic India Virus (MYMIV) was found infecting majority of the *Kharif* legumes *viz*., mungbean, urdbean, soybean, pigeon pea and dolichos bean in the region. Among the various weeds suspected, only *Gomphrena sessilis* with yellowing and vein clearing symptoms was found to have natural infection of MYMIV. Phylogenetic analysis revealed that, all the isolates originating from different locations of tarai region found to have maximum similarity despite being associated with different hosts. Rigorous survey of mungbean and urdbean fields over 70 villages revealed that, the incidence of yellow mosaic disease ranged from 4.30 to 31.20 during 2013 and 4.20 to 31.60 during 2014. The data also revealed that the prevalence of the disease was more in U.S. Nagar during both the years (15.40 and 15.18 during 2013 and 2014 respectively).

Keywords: mungbean, urdbean, LYMVs, gomphrena, yellow mosaic

Introduction
India is the largest producer, about 26% of world's production, and consumer, 30% of total pulses of the world. The domestic production of about 23 million tons during 2016-17 shall be still less than the future estimated demand of 29-30 million tons (Tiwari and Shivhare, 2017) [29]. Mungbean is an important and cheap source of food protein across Asia, especially for the poor, thus plays an imperative role in the alleviation of protein malnutrition especially in the developing countries (Selvi *et al*., 2006) [25]. India is the largest mungbean producer, yielding 2.17 million tons of grains from about 4.32 m ha area. However, the average productivity of mungbean in India is quite low (~502 kg/ha), even lower than most of the other pulse crops (Project Coordinators Report-2018). Among the several biotic and abiotic factors limiting the pulse production, the yellow mosaic virus complex of the genus Begomovirus vectored by whitefly *Bamisia tabaci* Genn. In circulative persistent manner is a greatest emerging threat reducing the productivity (Nagendran *et al*., 2017) [14]. It has been estimated that, the yield loss per annum due to yellow mosaic disease (YMD) is approximately $ 300 million taking blackgram, mungbean and soybean together (Varma and Malathi, 2003) [31]. Yellow mosaic disease (YMD) is characterized by typical yellow mosaic symptoms. Diseased plants will be stunted, with fewer flowers and pods that bear smaller, occasionally shriveled seeds in severe cases, and other plant parts also become completely yellow. Depending on the severity of the infection, the yield penalty may reach up to 85 per cent (Haq *et al*., 2011) [7]. YMD control is often based on limiting the vector population with insecticides, which are ineffective under severe whitefly infestations (Malathi and John, 2008a) [11]. The virus has got a huge host range and affects almost all the legumes crops (Malathi & John 2008b; Ilyas *et al*. 2009; Akthar *et al*. 2011; Ara *et al*. 2012; Akram *et al*., 2016) [12, 8, 2, 3, 1]. At present, the cause of yellow mosaic disease (YMD) in different legumes is attributed to four different viruses *viz*., Mungbean Yellow mosaic India Virus (MYMIV), Mungbean Yellow Mosaic Virus (MYMV), Horsegram Yellow mosaic Virus (HgYMV) and Dolichos Yellow Mosaic Virus (DoYMV) that are together referred to as Legume Yellow Mosaic Viruses (LYMVs) (Qazi *et al*., 2007) [23].

Among them MYMV is thought to be more prevalent in southern India and MYMIV is dominant over northern India. Further, these viruses are known for cross infection, large host range and high variability (Karthikeyan *et al*., 2014; Akram *et al*., 2016) [9, 1]. Although, the viruses are well characterized, the information on extent of their geographical distribution and severity of occurrence in different parts of the countryis not clear. The prevalence of the virus in tarai region has been reported earlier (Nene, 1972) [19], a systematic identification and characterization of the virus occurring in this area is lacking. In this context, the present study was conducted in tarai region of the country to study the extent of severity of YMD on different pulses and to confirm the identity of LYMV's present in tarai region.

Material and Method

Survey in different pulse crops to understand the incidence of YMD

Roving survey was carried out in the mungbean and urdbean growing areas of Kumaon Tarai region of Uttarakhand and adjoining districts of Uttar Pradesh to record the severity of mungbean yellow mosaic virus disease. A total of 70 villages *i.e.*, 35, 20, 10 and 05 villages in Udham Singh Nagar, Nainital, Rampur and Bareilly districts respectively were surveyed. Survey was conducted in the months of July-August when the crop was 1-2½ months old for the two consecutive seasons of *Kharif* 2013 and 2014. In each village 3 to 5 fields and in each field and in each field, three spots $5m^2$ was randomly marked and disease incidence was assessed by recording number of plants infected. In addition, observations on number of whiteflies per leaf, stage of crop, cultivar and conspicuous symptoms etc. were also recorded. Details of the areas selected for the survey are presented in Table 1 (figure 1).Based on the survey data various epidemiological parameters were calculated as per formulae given below:

$$\text{Per cent Disease severity (PDI)} = \frac{\text{No. of plants infected in a row}}{\text{Total no. of plants in a row}} \times 100$$

$$\text{Average PDI of a taluk} = \frac{\text{Sum of DI of fields surveyed in a taluk}}{\text{Number of fields surveyed in a taluk}}$$

$$\text{Average PDI of district} = \frac{\text{Sum of average DS of taluks surveyed in a district}}{\text{Number of taluks surveyed in a district}}$$

Molecular characterization of and distribution of YMD on major crop legumes and weeds hosts in tarai region and collection of virus isolates

Plant Sample of showing typical symptoms of yellow mosaicon various pulse crops *viz.*, mungbean, urdbean, pigeon pea, soybean, dolichos bean and some of the commonly found weeds having yellow mosaic symptoms were collected from the different areas of the Tarai region and subjected to molecular detection by using geminivirus specific degenerate primers (Deng A/B). The samples showing positive results were then subjected to PCR amplification employing specific primers against all the four LYMVs (Table 2). The molecular diversity of virus isolates were determined based on the sequence data of the PCR product.

DNA isolation: Young symptomatic leaves of each isolate was subjected DNA isolation following the standard CTAB (Cetyltrimethyl ammonium bromide) procedure (Doyle and Doyle, 1987) [4].

PCR amplification: The primers specific to DNA A and DNA B of each LYMV reported in previous studies along with geminivirus degenerate primer commercially synthesized from Eurofins Inc., Bengaluru were employed in the present study (Table 3).Initially all samples were subjected to degenerate primers (Deng A/B) and the positive samples were subsequently subjected to primers specific to four LYMV. The PCR reaction was performed with primer specific annealing temperature for 1 min and having total 35 cycles. The reaction was visualized through agarose gel electrophoresis. The PCR products (30 µl) of DNA a partial genome were sent to Sci Genom laboratory, Cochin, Kerala for the direct sequencing. Sequences thus obtained were subjected to NCBI blast and DNA sequence of the amplified product were submitted to NCBI (National Center for Bioinformatics) and obtained the accession numbers.

Phylogenetic analysis: Previously published sequences having maximum similarity to the sequences of the present investigation were downloaded from the NCBI and subjected to multiple sequence alignment using "Clustal W" programme available online to obtain Phylogenetic tree.

Result and Discussion

Mungbean

Survey conducted in mungbean fields over two years revealed that the incidence of yellow mosaic disease ranged from 4.30 to 31.20 during 2013 and 4.20 to 31.60 during 2014 (Figure 2). The average incidence was higher during 2013 in most of the parts surveyed although the overall average was slightly higher during 2014 (11.47) compared to previous year (10.45). The data also revealed that the prevalence of the disease was more in U.S. Nagar during both the years (15.40 and 15.18 during 2013 and 14 respectively). District Rampur of Uttar Pradesh was having lowest incidence of 6.56 and 8.89 during 2013 and 2014 respectively. The average whitefly population recorded during both 2013 and 14 was lowest in district Bareilly (3.34 and 2.74 respectively) and highest was in U.S. Nagar (3.90 to 3.30 respectively).

The survey revealed that PM-5 and PM-3 (data not shown) are most widely cultivated varieties of mungbean in Kumaon Tarai belt of Uttarakhand and adjoining areas of Uttar Pradesh and recorded low levels of disease incidence. In most cases maximum yellow mosaic incidence was found in local cultivars grown by the farmers.

Urdbean

Survey conducted in Urdbean fields over two years also revealed that the disease incidence of yellow mosaic disease ranged from 5.60 to 32.40 during 2013 and 5.60 to 34.23 during 2014 (Figure 3). The average incidence was highest during 2013 in most of the parts surveyed and the overall average was slightly maximum during 2013 (11.96) compared to 2014 (11.60). The data also revealed that the prevalence of the disease was more in U.S. Nagar during both the years (16.54 and 15.40 during 2013 and 14 respectively). District Rampur of Uttar Pradesh was having lowest incidence of 8.89 and 9.31 during 2013 and 14 respectively. The average whitefly population recorded during both 2013 and 14 was lowest in district Rampur (2.95 and 2.75 respectively) and highest was in U.S. Nagar (3.67 and 3.53 respectively).

The survey also revealed that PU-31 and PU-35 and UL-2 were most widely cultivated varieties of mungbean in the area surveyed and recorded low levels of disease incidence. In most cases maximum yellow mosaic incidence was found in local cultivars grown by the farmers.

Several reports from different parts of the country suggest the wide spread occurrence of disease across the country (Singh *et al*., 2000 and Pathak and Jhamaria, 2004) [27, 20]. The earlier information on the prevalence of the disease in tarai region suggests that, there was 5-100 per cent yellow mosaic incidence and 100 per cent damage in early-infected plants (Nene, 1972) [19]. Similar results of survey were found in the southern India where, a recent report of roving survey conducted during *kharif* 2005 in northern Karnataka revealed the incidence of YMD up to 22.64 per cent (Salam *et al*., 2011) [24]. The incidence of YMD in major mungbean growing areas of Southern Karnataka indicated the occurrence of disease ranging from 31.49 to 100 per cent (Manjunath *et al*., 2013) [13]. Pawar and Mahatma (2013) [21] reported that the, occurrence of mungbean yellow mosaic virus (MYMV) in mungbean was noticed in serious proportion causing heavy losses in Navsari, Surat and Valsad district.

The high degree of variation in disease incidence may be attributed to variation in weather parameters that might have direct influence on vector population and its migration. Similar effect of climate on vector population was earlier reported by Nath and Saikia (1995) [18] and Singh and Gurha (1994) [28]. The resistant varieties are also important in reducing the incidence and severity among the population. In present study, we found that farmers cultivating new varieties *viz*., PM-3 & PM-5 of mungbean and PU-31& PU-35 of urdbean did not succumb to high YMD infection and which is major reason behind over all low to moderate disease pressure in tarai region. In all the fields surveyed, infected plants exhibited, varied kind of symptoms *viz.*, irregular yellow and green patches on trifoliate leaves, puckering, reduced leaf size, complete yellowing, stunting of plants bearing few flowers and pods with small and immature seeds. These symptoms are in confirmation with those described by Nariani (1960) [17], Gautam (1990) [5] and Salam *et al.* (2011) [24]. The puckering in lamina which is sometimes reported in YMD (Malathi and John, 2008a) [11].

Molecular identification of legume yellow mosaic viruses in tarai region

Various pulse crops *viz*., mungbean, urdbean, dolichosbean, soybean, Pigeon pea showing typical yellow mosaic symptoms along with weeds *viz*., *Ageratum conizoids*, *Sidarhambifolia*, *Gomphrena sessilis*, *Malvastrum coromandelianum*, *Durranta* spp. Were subjected to molecular detection through specific primers of MYMV, MYMIV, DoYMV, HgYMV. All the crop legumes along with one weed i.e., *Gomphrena sessilis* were positive for both DNA and DNAB of MYMIV. Whereas, none of the tested samples were found to have other Legumoviruses (Table 5).The study clearly indicated that, MYMIV is the predominant Legumoviruses in the region. MYMIV is also previously known to be predominant in northern and central India where as MYMV was known to be predominant in southern India (Karthikeyan *et al*., 2004; Usharani *et al*., 2005) [10, 30].

Coat protein gene of the MYMIV samples from the present study were sequenced and sequences were subjected phylogenetic analysis (Table 4, Figure 4).Phylogenetic tree based on the coat protein sequences of five isolates from each host from the present study along with 14 sequences of MYMIV from different locations crops obtained from NCBI revealed more than more than 97 per cent similarity across the isolates indicating close lineage. However, the tree has revealed 3 major clusters indicating considerable variation among the isolates. Three isolates of MYMIV *viz*., Mungbean, Urdbean and Pigeonpea from tarai region of Uttarakhand (KX655578, KX655576 & KX655579 respectively) were groped in one cluster with 100 percent similarity. Whereas an isolate from *Gomphrena sessilis* has groped separately as sole member indicating its non-crop origin. The soybean isolate (KX655575) has been found to be groped with another MYMIV isolate from same host but from a distant geography. The pattern of clustering was mostly found to be based on host rather than origin except for the isolates from this study. Legume Mosaic Virus is known have huge variability and expanding its host range (Shahid *et al*., 2019) [26]. The mechanism *viz*., pseudo recombination between different Legumoviruses may enhance the variability among the LYMVs (Qazi *et al*. (2007) [23] It is important to keep a constant watch on variability at regional level is very important to develop much required stable resistant cultivars the (Karthikeyan *et al*., 2014) [9].

Table 1: Details of the area of survey conducted during the present study

State	District	Tehsil
Uttarakhand	Udham Singh Nagar	Kashipur, Bazpur, Jaspur, Gadarpur, Kiccha, Sitarganj, Khatima
	Nainital	Lalkuan, Haldwani, Kaladhungi, Ramnagr
Uttar Pradesh	Rampur	Suar, Bilaspur
	Bareilly	Beheri

Table 2: List plant samples subjected to molecular detection of yellow mosaic virus

Sl. No.	Host plant	Symptoms	Location
1.	Mungbean	Yellow mosaic	Pantnagar
2.	Mungbean	Leaf size reduction	Pantnagar
3.	Urdbean	Yellow mosaic	Pantnagar
4.	Soybean	Yellow mosaic	Pantnagar
5.	Pigeon Pea	Yellow mosaic	Sitarganj
6.	Dolichos Bean	Yellow mosaic	Khatima
7.	Mungbean	Yellow mosaic	Kashipur
8.	*Ageratum conizoids*	Yellow vein mosaic	Pantnagar
9.	*Sida rhambifolia*	Yellow vein mosaic	Pantnagar
10.	*Gomphrena sessilis*	Yellow mosaic and vein clearing	Pantnagar
11.	*Malvastrum coromondalianum*	Vein clearing	Pantnagar
12.	*Duranta* spp.	Leaf curling and yellowing	Pantnagar

Table 3: The details of the primers employed in this study

Sl. No.	Name Primer	Virus to be Identified/Primer Specificity	Expected amplicon (bp)	References
1.	Deng (A/B)	Degenerate Primer for begomovirus	530	(Naimuddin and Akram, 2009; Naimuddin *et al.*, 2011; Gautam *et al.*, 2014) [15, 16, 6]
2.	NM1/NM2	MYMIV (DNA A)	950	
3.	MYMIVMPF/ MYMIVMPR	MYMIV (DNA B)	1000	
4.	MYMV-CPF/ MYMV-CPR	MYMV (DNA A)	1000	
5.	MYMV-MPF/ MYMV-MPR	MYMV (DNA B)	900	
6.	HgYMV-CPF/ HgYMV-CPR	HgYMV (DNA A)	900	
7.	HgYMV-MPF/ HgYMV-MPR	HgYMV (DNA B)	900	
8.	DoYMV-CPF/ DoYMV-CPR	DoYMV (DNA A)	900	
9.	DoYMV-MPF/ DoYMV-MPR	DoYMV (DNA B)	900	

Table 4: Details of selected viruses sequences used in the study for comparison

S. No.	Virus	Abbreviation	Accession number*
1	Mungbean yellow mosaic India virus-[India: Pantanagar: 2017]	MYMIV-Soybean-Pantnagar	KX655575
2	Mungbean yellow mosaic India virus-[India: Pantanagar: 2017]	MYMIV-Mungbean-Pantnagar	KX655576
3	Mungbean yellow mosaic India virus-[India: Khatima: 2017]	MYMIV-Dolichos-Khatima	KX655577
4	Mungbean yellow mosaic India virus-[India: Sultanpur: Sitarganj: 2017]	MYMIV-Pigeonpea-Sitarganj	KX655578
5	Mungbean yellow mosaic India virus-[India: Haldwani: Pantnagar: 2017	MYMIV-Urdbean-Pantnagar	KX655579
7	Mungbean yellow mosaic India virus-[India: Kurnool: Andhra Pradesh: 2016]	MYMIV-Urdbean-Kurnool	JN181004
8	Mungbean yellow mosaic India virus-[India: Guntur: Andhra Pradesh: 2016]	MYMIV-Urdbean-Guntur	JN181003
9	Mungbean yellow mosaic India virus-[India: Kadapa: Andhra Pradesh: 2013]	MYMIV-Urdbean-Kadapa	KC243785
10	Mungbean yellow mosaic India virus-[India: Coimbatore: Tamil Nadu: 2015]	MYMIV-Urdbean-coimbatore	KP313758
11	Mungbean yellow mosaic India virus-[India: PAU: Haryana: 2008]	MYMIV-Soybean-Ludhiana	DQ389155
12	Mungbean yellow mosaic India virus-[India: Kanpour: Uttar Pradesh: 2008]	MYMIV-Cowpea-Kanpur	DQ389154
13	Mungbean yellow mosaic India virus-[India: New Delhi: 2008]	MYMIV-Cowpea-New Delhi	DQ389153
14	Mungbean yellow mosaic India virus-[India: Aurangabad: Maharashtra: 2008]	MYMIV-Soybean-Aurangabad	DQ389150
15	Mungbean yellow mosaic India virus-[India: PAU: Ludhiana: 2008]	MYMIV-Urdbean-Ludhiana	DQ400847
16	Mungbean yellow mosaic India virus-[India: Varanasi: Dolichos: 2005]	MYMIV-Dolichos-Varanasi	AY547317
17	Mungbean yellow mosaic India virus-[India: Tirupati: Andhra Pradesh: 2012]	MYMIV-Urdbean-Tirupati	JX110618
18	Mungbean yellow mosaic India virus-[India: Akola: Gandhinagar: 2016]	MYMIV-Frenchbean-Gandhinagar	KP779635
19	Mungbean yellow mosaic India virus-[India: Varanasi: French bean: 2011]	MYMIV-Frenchbean-Varanasi	KC019304

*Sl no. 1-5 are isolates from the present study

Table 5: Molecular identification of legume yellow mosaic virus in tarai region

Sl. No.	Host plant	Symptoms	Location	MYMIV		MYMV		HgYMV		DoYMV	
				DNA A	DNA B	DNA A	DNA B	DNA A	DNA B	DNA A	DNA B
1.	Mungbean	Yellow mosaic	Pantnagar	+	+	-	-	-	-	-	-
2.	Mungbean	Leaf size reduction	Pantnagar	+	+	-	-	-	-	-	-
3.	Urdbean	Yellow mosaic	Pantnagar	+	+	-	-	-	-	-	-
4.	Soybean	Yellow mosaic	Pantnagar	+	+	-	-	-	-	-	-
5.	Pigeon Pea	Yellow mosaic	Sitarganj	+	+	-	-	-	-	-	-
6.	Dolichos Bean	Yellow mosaic	Khatima	+	+	-	-	-	-	-	-
7.	Mungbean	Yellow mosaic	Kashipur	+	+	-	-	-	-	-	-
8.	*Ageratum conizoids*	Yellow vein mosaic	Pantnagar	-	-	-	-	-	-	-	-
9.	*Sida rhambifolia*	Yellow vein mosaic	Pantnagar	-	-	-	-	-	-	-	-
10.	*Gomphrena sessilis*	Yellow mosaic and vein clearing	Pantnagar	+	+	-	-	-	-	-	-
11.	*Malvastrum coromondalianum*	Vein clearing	Pantnagar	-	-	-	-	-	-	-	-
12.	*Duranta* spp.	Leaf curling and yellowing	Pantnagar	-	-	-	-	-	-	-	-

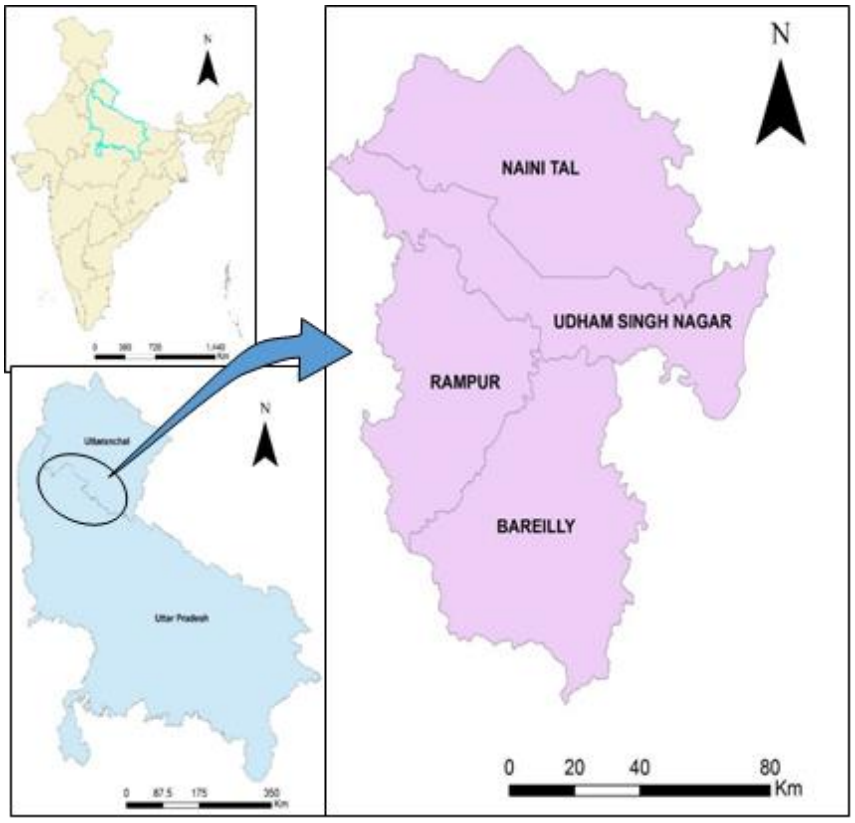

Fig 1: Details of the area of survey conducted during the present study

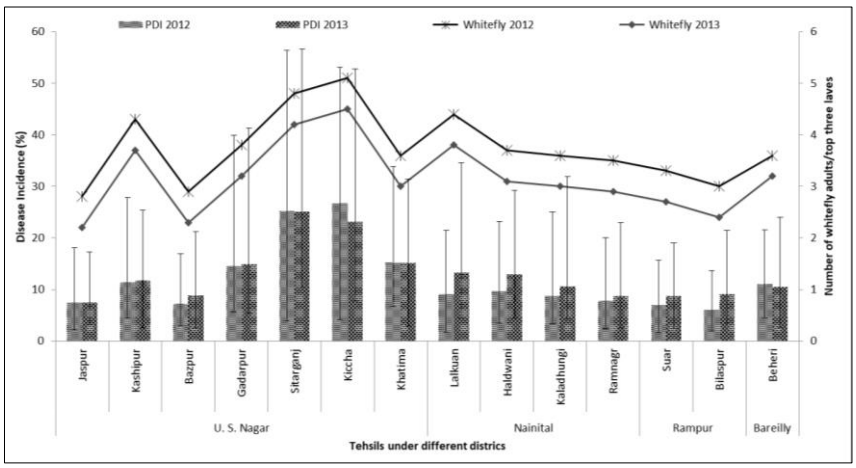

Fig 2: Incidence of yellow mosaic disease of mungbean in different tehsils of Kumaon tarai region of Uttarakhand and adjoining areas of Uttar Pradesh during *Kharif* 2013 and 2014

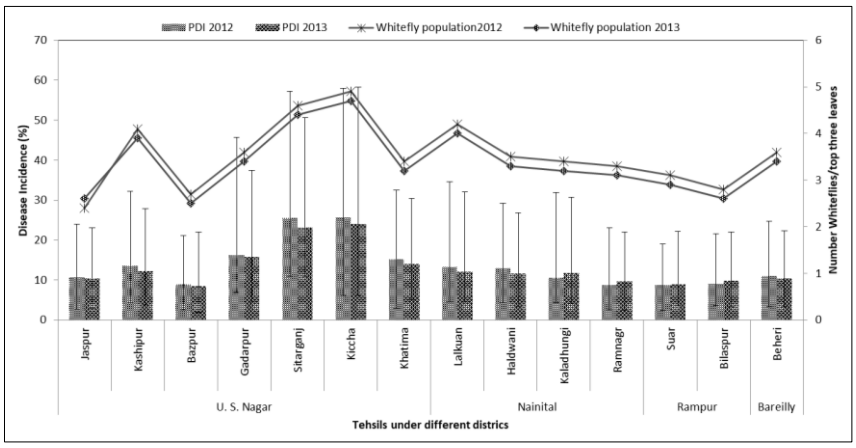

Fig 3: Incidence of yellow mosaic disease of urdbean in different tehsils of Kumaon tarai region of Uttarakhand and adjoining areas of Uttar Pradesh during *Kharif* 2013 and 2014

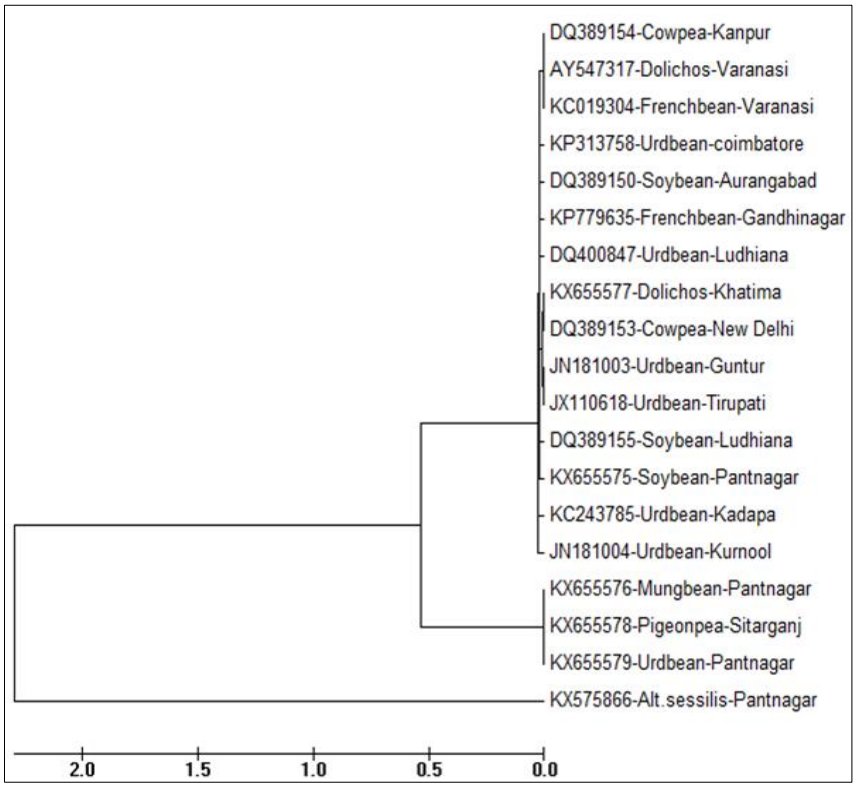

Fig 4: Details of selected viruses sequences used in the study for comparison

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