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X = 25 mm on the ramp, where the mean flow speed nearby the wall is zero. Figure 8 c and d shows the mean velocity distributions in the U (left) and V (right) components of the same region. It can be seen from the U-component velocity cloud chart that shear layer is gradually close to the ramp, and the recirculation region which is covered by shear layer decreases gradually. The distributions of the V-component velocity are a continuation of the oblique V in the separation region.

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Prevalence of *Wheat dwarf India virus* in wheat in India

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Wheat dwarf India virus (WDIV) is the first mastrevirus reported to have subgenomic molecules called satellites. To establish association of the satellites with WDIV across a variety of ecoclimatic conditions, a countrywide survey was carried out. WDIV and its associated satellites (alphasatellite and betasatellite) were identified in plant samples collected from each of the 14 field locations surveyed in the study. Though there were location- and variety-related differences in disease scale, most of the infected wheat cultivars in fields across the country carried both the satellites. The wide occurrence of WDIV disease complex in India suggests the need to assess how the spread of WDIV and its satellites can be limited in wheat fields.

Keywords: Alphasatellite, atypical mastrevirus, betasatellite, symptom severity.

WHEAT dwarf India virus (WDIV) is a leafhopper (*Psammotettix* sp.; family Cicadellidae) transmitted mastrevirus (family Geminiviridae) that infects wheat in India¹. Dwarfing or stunting is the typical symptom of *WDIV*, but yellowing of leaves is also associated with field infection, which may be due to other factors². Two alphasatellites (Cotton leaf curl Multan alphasatellite and Guar leaf

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curl alphasatellite) and a betasatellite (Ageratum leaf curl betasatellite) have been reported as associated with WDIV in field-infected wheat plants that do not show any begomovirus². The presence of WDIV and the absence of begomovirus indicated the association of the satellites with WDIV. This was ascertained by inoculating WDIV in wheat, with or without satellites and detecting them in systemically infected leaves. Wheat plants inoculated with WDIV and any of the satellites showed more severe stunting in comparison to those inoculated with WDIV alone, thus establishing the role of the satellites in WDIV infection of wheat². In a subsequent study, the associated Ageratum leaf curl betasatellite was reported to act as pathogenicity determinant in the infection by $WDIV^3$.

Wheat dwarf virus (WDV), another member of the genus Mastrevirus, is a causal agent of dwarfing, mottling, yellowing or reddening in wheat across the globe⁴. WDV is a ubiquitous wheat virus and has become a serious pathogen of wheat in Europe, Asia and Africa⁴⁻⁸. In spite of the ubiquitous nature of WDV, no sequence suggesting the presence of WDV was identified in the symptomatic (stunted) wheat plants during our study. Except WDIV, no mastrevirus has been reported to be associated with satellites. More studies are desirable to find the association of satellites with other members of the genus Mastrevirus (for example WDV, Maize streak virus) and their role in pathogenesis.

The ubiquitous nature of WDV and the identification of WDIV associated with satellites in India, propelled us to explore if WDIV along with the satellites was ubiquitous in the field infections of wheat in the country. We surveyed a number of geographically and agroclimatically widely separated wheat fields across the country. The present study reports the results of sampling done at 14 locations across India. Varietal effect on the disease severity and the prevalence of WDIV and its associated satellites (alphasatellite and betasatellites) are also reported.

Samples were collected from wheat fields in different parts of the country during 2011–2013 (Figure 1). Fourteen geographically distinct locations were surveyed. Leaf samples were collected from plants showing the primary symptom, i.e. dwarfing, without or with additional phenotypes such as sterile spikes and yellowing of leaves. Pathogenicity on the basis of plant height and other symptoms was determined on 0–9 scale (Table 1). Plants at scales 8 and 9 contained sterile spikes with no grain formation. At scale 3, 4 and 6, in addition to dwarfing, fungal infection was noticed. A total of 1005 samples were analysed, comprising 963 symptomatic (scale 1–9) and 42 asymptomatic (scale 0) plants. The leaf samples were labelled by variety and disease scale, and stored at -80° C till analysis.

Amplification of *WDIV* and associated satellites was done in polymerase chain reaction (PCR) using specific primers – MF1_FOR/REV and MF2_FOR/REV (ref. 1). Primer pairs β 01/04 for the betasatellite and 'nanofor'/

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'nanorev' for the alphasatellite were used⁹. The PCR products were cloned into pDRIVE vector (Qiagen GmbH, Germany) and then sequenced using automated sequencer (Applied Biosystem 3730x1 DNA Analyser, USA).

Nucleotide sequence search was done using BlastN to retrieve homologous sequences from the database. These were analysed using pairwise global alignment (<u>http://www.ebi.ac.uk/Tools/psa/emboss_needle/nucleotide.html</u>).

For calculating prevalence, total number of plants was counted in an area of 10 sq. ft. Samples were collected from all the locations for studying the presence of *WDIV* and the satellites in the suspected plants. The prevalence was calculated on the basis of total samples in a unit area and the number of virus-positive samples identified in that area.

Out of the 963 symptomatic plants collected during the survey, 791 yielded an amplicon of ~2.8 kb using *WDIV*-specific primers (MF1_FOR/REV and MF2_FOR/REV), thus indicating the presence of the virus (Table 2). Out of the 42 asymptomatic plants taken as negative controls, two gave an amplicon of ~2.8 kb. The two plants at scale 0 belonged to cv Sonalika, which has been reported to show mild symptoms upon infection by *WDIV* and the satellites². Sequencing of the fragment of ~2.8 kb established the presence of *WDIV* in all the PCR-positive samples. PCR amplification using the alphasatellite and betasatellite-specific primers ('nanofor'/'nanorev' and β 01/04) yielded an amplicon of ~1.3 kb in the samples found positive for *WDIV*.

The viral genomes detected from the samples were 99.5–100% identical to *WDIV* (accession nos JF781306,



Figure 1. Map of India showing locations from where wheat plant samples were collected. A total of 14 collection centres are shown.

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Table 1. Pathogenicity scale and phenotypes							
Pathogenicity scale	Plant mean height* (mean height of three plants in cm ± SD)	Primary (dwarfing) phenotype	Additional phenotype				
0	72.6 ± 1.52	Healthy looking plants	_				
1	67.7 ± 2.08	Slight dwarfing	_				
2	65.5 ± 2.56	Slight dwarfing	Yellowing of leaves				
3	65.6 ± 1.52	Slight dwarfing	Leaf rust				
4	63.5 ± 2.08	Slight dwarfing	Leaf rust and stripe rust				
5	48.6 ± 1.52	Moderate dwarfing	_				
6	48.1 ± 2.08	Moderate dwarfing	Yellowing of leaves				
7	46.6 ± 3.51	Moderate dwarfing	Leaf rust and stripe rust				
8	24.6 ± 1.73	Extreme dwarfing	Sterile spikes				
9	23.9 ± 1.52	Extreme dwarfing	Yellowing of leaves and sterile spikes				

*Height of a representative cultivar (C306) at different disease scales.

-, No additional phenotype.

Table 2. Geographical coordinates of sample collection sites and prevalence of wheat dwarf India virus disease complex in the wheat fields

		No. of					Average no. per unit area*			
	Geographical	symptomatic samples	No. of	positive sa	mples	No. of units*	No. of	No. of plants	Positive	Prevalence of infected
Location	coordinates	tested	WDIV	Alpha	Beta	tested	plants	tested	samples	plants (%)
Mohali	30°47′N, 76°41′E	317	263	257	245	5	261 ± 3	63 ± 1	52 ± 1	19.9
Meerut	28°58'N, 77°42'E	67	59	56	45	3	240 ± 5	22 ± 1	20 ± 1	8.3
Kanpur	26°45'N, 80°31'E	30	23	21	17	1	246	30	23	9.3
Gorakhpur	29°45'N, 75°66'E	24	18	17	15	1	275	24	18	6.5
Samastipur	25°80'N, 85°67'E	40	36	35	29	2	253 ± 4	20	18 ± 1	7.1
Hajipur	25°68'N, 85°22'E	20	17	13	15	1	270	20	17	6.2
Bilaspur	22°4′N, 82°9′E	45	33	33	32	3	244 ± 6	15	11 ± 1	4.5
Jagdalpur	20°37'N, 81°35'E	40	28	24	21	2	236 ± 8	20	14 ± 2	5.9
Wellington	11°22'N, 76°47'E	78	71	69	55	3	233 ± 5	26 ± 1	24 ± 1	10.3
Pune	18°6′N, 74°18′E	75	69	61	59	3	237 ± 7	25	23 ± 1	9.7
Indore	22°43′N, 75°49′E	60	54	50	51	3	242 ± 6	20	18 ± 1	7.4
Bhopal	23°12′N, 77°27′E	20	15	10	9	1	247	20	15	6.1
Udaipur	24°34'N, 73°38'E	77	55	46	49	3	251 ± 4	26 ± 1	18 ± 1	7.1
Jaipur	26°5′N, 75°47′E	70	50	48	47	3	245 ± 6	23 ± 1	17 ± 1	6.9

One unit means an area of 10 sq. ft each.

Prevalence, Positive samples in a unit area × 100 ÷ no. of plants in a unit area.

JQ361910 and JQ361911) reported earlier from wheat¹. It exhibited typical mastrevirus genome organization. The betasatellites detected from the samples exhibited an identity of 98% to Ageratum yellow leaf curl betasatellite (AYLCB) reported earlier from wheat². Nucleotide sequence analysis of alphasatellites from different wheat samples revealed that one alphasatellite was close to Cotton leaf curl Multan alphasatellite (CLCuMA) with identity ranging from 95% to 98%. The other molecule resembled Guar leaf curl alphasatellite (GLCuA) with an identity of 93%. CLCuMA was detected in wheat samples collected from all the 14 centres, whereas GLCuA was found only at two centres, Mohali and Wellington.

The prevalence of WDIV and the associated satellites in field samples of wheat plants varied at different locations, being the lowest at 4.5% and highest at 19.9% (Table 2). Both the alphasatellite and betasatellite were found at all the locations (Table 2) in WDIV-positive samples, but not in the samples that tested negative for WDIV.

The disease severity scale was recorded for each cultivar at each location. The cultivars, WL-711, K-65, C-306 and WH-291 were most susceptible among the studied wheat genotypes (Table 3).

The diseased samples, categorized into different disease scales, showed the presence of WDIV and associated satellites. The number of diseased plants across the collection centres was largely at scales 1–4, followed by plants at scales 5-7 (Table 4). A few diseased plants were found at scales 8 and 9 (Table 4).

The number of tillers formed was less in the disease scales 8 and 9. The length of the ear was also reduced in these scales (Table 4). Significant variations were observed in thousand grain weight - measured as described by Singh et al.¹⁰ – at different disease scales. Thousand grain

			Table 3.	Varietal effect on di	isease symptom ex	xpression across the	country			
				Pat	hogenicity scale a	it different locations				
Variety/cultivar	Scale 9	Scale 8	Scale 7	Scale 6	Scale 5	Scale 4	Scale 3	Scale 2	Scale 1	Scale 0
WL-711	1, 9, 11, 13, 14	1, 9, 11, 13	1-3, 5, 7-11, 13, 14	I	I	1, 5, 9, 13	1, 5, 7–11, 13, 14	1	2, 3, 5, 7	1-3, 5, 7-11, 13, 14
PBW-343	I	I	1-3, 5, 9, 11, 13, 14	I	4, 12	1, 5, 9, 13, 14	1, 5, 9, 11, 14	4,6	4, 6, 12	1-6, 9, 11-14
K-65	1, 9, 14	1, 9, 14	2, 3, 7, 8, 10, 13	I	I	2, 3, 7, 8, 10	2, 3, 10, 13	I	2, 3, 7, 8	1-10, 13, 14
C-306	6	1, 9	2, 3, 5, 7–11, 14	4, 6, 12, 13	4, 6, 12, 13	2, 3, 5, 7–11	2, 3, 5, 9, 14	4, 6, 12	4, 6, 12	1 - 14
Sonalika	I	I	I	I	I	I	I	7, 9, 11, 14	1-3, 5-7, 9	1-7, 9, 11, 14
LOK-1	I	I	I	1-3, 5, 7-11, 13, 14	1 1, 5, 9, 13, 14	I	I	4,12	4, 6, 12	1 - 14
HD-2329	I	I	1, 5, 9, 11, 13, 14	7, 8, 10	7, 8, 10	1, 5, 9, 11, 14	1, 5, 9, 13, 14	I	7, 8	1, 5, 7–11, 13, 14
HD-2781	I	I	I	3, 9, 14	1-3, 9, 14	I	I	I	1, 9, 13	1-3, 9, 13, 14
HD-3016	I	I	I	1, 9	1, 2, 9, 14	I	I	I	1, 2, 9	1, 2, 9, 14
WH-542	I	I	1-3, 5, 9, 11, 13, 14	I	I	1, 5, 9, 11, 13	1-3, 5, 9, 14	I	1, 3, 5, 9	1-3, 5, 9, 11, 13, 14
NI-5439	I	I	1-3, 5, 7, 9, 11, 14	I	I	1, 5, 9, 11, 14	3, 5, 7, 9	I	2, 3, 5, 7	1-3, 5, 7, 9, 11, 14
HI-1568	Ι	Ι	1, 2, 7–10, 14	I	I	1, 2, 7, 14	1, 2, 9, 14	I	1, 2, 7, 10	1, 2, 7-10, 14
NIAW-917	Ι	Ι	1-3, 5, 7-9, 11, 14	I	I	1, 5, 9, 11, 14	5, 7–9, 11, 14	I	1, 2, 5, 11	1-3, 5, 7-9, 11, 14
RAJ-3765	I	I	I	1, 9, 11, 14	9, 11, 14	I	I	I	1, 9, 11	1, 9, 11, 14
WH-291	1, 9	1, 9	1-3, 5, 7-11, 14	I	I	1-3, 5, 7-11, 14	1-3, 5, 7-11, 14	I	1, 2, 5, 7	1-3, 5, 7-11, 14
GW-366	Ι	Ι	I	1-3, 9, 14	1-3, 9, 14	I	I	1-3, 14	1-3, 5, 7, 14	1-3, 5, 7, 9, 14
GW-391	I	Ι	I	1-3, 9	1, 3, 9	I	I	1–3	1, 2, 9	1-3, 9
GW-322	I	I	I	3,9	1–3	I	I	1–3	1–3	1-3, 9
HD-2985	Ι	I	I	1-3, 9-11, 14	3, 9, 10, 14	I	I	1, 2, 11, 14	1, 11	1-3, 9-11, 14
HD-2932	I	I	I	1-3, 9, 14	1-3, 9, 14	I	Ι	1-3, 9, 14	1-3, 9, 14	1-3, 9, 14
K-8027	I	I	1-3, 9, 14	I	I	3, 14	9, 14	1-3, 9, 14	9, 14	1-3, 9, 14
PBW-299	I	I	I	1-3, 9, 14	9, 14	I	I	I	1, 3	1-3, 9, 14
PBW-550	I	I	I	1-3, 5, 7, 9	1, 9, 13, 14	I	I	7, 9, 13, 14	1, 2, 5	1-3, 5, 7, 9, 13, 14
DBW-17	I	I	I	1-3, 9, 14	1-3, 9, 14	I	I	1, 2, 9, 14	1, 2, 9, 14	1-3, 9, 14
UP-2425	Ι	I	I	1-3, 9, 14	1, 2, 9	I	I	1,2	1, 9	1-3, 9, 14
HD-3007	I	I	1-3, 9, 14	I	I	1, 9	14	I	1, 2, 9	1-3, 9, 14
1, Mohali; 2, Me	erut; 3, Kanpur; ²	4, Gorakhpur; 5	i, Samastipur; 6, Hajipu	r; 7, Bilaspur; 8, Jage	dalpur; 9, Welling	ton; 10, Pune; 11, 1	ndore; 12, Bhopal;	13, Udaipur; 1.	4, Jaipur; –, Ni	il.

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				Average of 10 independent observations					
Pathogenicity scale	No. of plants collected	No. of virus-positive plants	Percentage of positive plants	No. of tillers/ plant	No. of ears/ plant	Length of ear (cm)	Total grain weight/ plant (g)	Thousand grain weight (g)	
8–9	15	14	93.3	5–6	5-6	5-8	No grain	No grain	
5-7	406	349	85.9	8-15	8-15	7-10	8-15	35 ± 4	
1-4	542	426	78.5	5-15	5-15	7-12	12-37	41 ± 4	
0	42	2	4.7	5-16	5-16	8-14	13-39	44 ± 5	

weight at scale 0 was 44 g (\pm 5 g) that gradually decreased to 35 ± 4 g at higher disease scale (Table 4). Grain formation was nil in the spikes at scales 8 and 9 (Table 4).

Our survey documented the incidence and prevalence of a previously unreported type of virus, i.e. a mastrevirus with satellites. Somewhat unexpectedly, the mastrevirus disease complex, WDIV and the satellites, were present in wheat fields in different ecoclimatic zones in India and showed the presence of both the satellites at all the locations. Prevalence of WDIV disease complex was as high as 19.9% at Mohali. It contrast to what has been reported since the discovery of mastreviruses, all WDIVinfected plants collected from widely separated geographical locations across the country contained both the alphasatellite and betasatellite. The virus has been missed by earlier wheat researchers in India, and the mastreviruses from other countries have not been reported to contain the satellite DNAs.

The satellites (GLCuA, CLCuMA and AYLCB) identified in the present study, have recently been reported by us to be associated with WDIV^{2,3}. These were characterized to enhance symptom development. In laboratory infections, we reported that each of the satellites added incrementally to symptom expression and virus accumulation in the infected plants². However, under natural infection in field, both the satellites were found associated with the diseased plants at all disease scales in all the cultivars tested, and at all the locations. A substantial varietal effect was recorded on disease severity (scale 1-9), suggesting the significance of host-pathogen interactions. At the same time, a given cultivar exhibited different disease scales at different locations, suggesting the effect of environment on varietal susceptibility. Among the 26 cultivars scored at different field locations, Sonalika was the most resistant cultivar and WL-711 was most sensitive to WDIV. The presence of different disease scales in a given cultivar at a given location is possibly because of the effect of plant age and microconditions at the time of infection. However, the detection of WDIV in two plants of cultivar Sonalika at scale 0 suggests that WDIV infection to these plants may have been initiated at a late stage of growth. Also, Sonalika has been reported to show mild symptoms in laboratory inoculation by WDIV and the satellites².

The highest number of plants was found at disease scale 1-4, followed by 5-7. The number of infected plants was few at disease scale 8 and 9. Hence, most of the wheat cultivars investigated during this study may be considered as resistant or moderately susceptible. The incidence and prevalence levels of WDIV disease complex on the basis of observed disease symptoms were established by PCR-based amplification and sequencing. The disease incidence and prevalence of WDIV was highest at Mohali and lowest at Bilaspur. Our study suggests that a number of factors, including environmental conditions, plant developmental stage and varietal type may determine the prevalence of WDIV disease complex in wheat besides the prevalence of the insect vector. The reduction in the number of tillers, length of ear and grain weight per plant in the diseased plants suggests the potential effect of viral infection on yield of wheat crop.

Wide prevalence of WDIV disease complex in India could be a potential threat to wheat production and therefore warrants studies to limit its spread, and also find alternative hosts, vectors and strategies for resistance. At present, the virus does not appear to cause economic loss to wheat yield. However, cultivar-specific field data in different regions of India need to be collected for detailed assessment. The association of the satellites with a mastrevirus opens new possibilities in developing novel vectors for genomic studies in wheat and examining the effect of the satellites on host range and pathogenesis.

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Magnetic fabric studies of sandstone from Jhuran Formation (Kimmeridgian–Tithonian) of Jara dome, Kachchh Basin, northwest India

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Low-field anisotropy of magnetic susceptibility (AMS) study was performed on the clastic sandstones of the Jhuran Formation from the Jara dome in the Kachchh basin. The AMS results consistent with petrographic analysis indicate primary deposition fabric for Arkose, sub-litharenite, wacke and quartz arenite sandstones of the Jhuran Formation. Isothermal remanent magnetization and thermal demagnetization curves indicate that magnetite, titano-magnetite and hematite are the chief magnetic minerals contributing to the AMS. The distribution of K_1 , K_2 and K_3 axes in the stereographic projections suggest depositional fabric development for arkose, sub-litharenite and wacke, whereas dispersed K_3 axes for quartz arenite are inferred to be due to low strain activity. The shape factors T, q confirm the oblate-shaped ellipsoid and horizontal fabric respectively, for all samples. The reconstructed palaeoflow directions for arkose and sublitharenite are NW–SE and for wacke and quartz arenite are NE–SW based on K_1 AMS axis.

Keywords: AMS, magnetic fabric, Kachchh basin, palaeoflow directions.

IN clastic sedimentary rocks, magnetic fabric is produced during physical transportation and deposition of magnetic particles. Studies of the magnetic fabric provide information concerning palaeoflow directions, environment of deposition, influence of tectonism and weak deformation of rock units¹⁻³. The low-field anisotropy of magnetic susceptibility (AMS) is a widely used technique to determine the magnetic fabric and palaeoflow direction of the sediments and sedimentary rocks, particularly sandstone. Generally the shape of the magnetic susceptibility ellipsoids provides insight into the mode of deposition, i.e. in still water, the minimum susceptibility axes of the grains are clustered on the pole, while the maximum and intermediate axes disperse uniformly on the bedding plane. Whereas the flowing water current results in the alignment of susceptibility axes which lie in different directions⁴⁻⁸.

This communication presents AMS results of sandstone of the Upper Jurassic (Kimmeridgian to Tithonian) Jhuran Formation exposed in Jara dome in the Kachchh sedimentary basin.

The Kachchh basin is located in western India (Figure 1). Formation of the basin is linked to the break-up between eastern and western Gondwanaland during Late Triassic/Early Jurassic period⁹⁻¹¹. The rift basin contains several intra-basinal strike faults such as the Island Belt Fault (IBF), the Banni Fault (BF), the Kachchh Mainland Fault (KMF), the Katrol Hill Fault (KHF) and the South Wagad Fault (SWF). A first-order meridional (NNE–SSW) high is found across the middle of the basin¹².

The basin consists of 2000–3000 m thick Mesozoic sediments ranging in age from Lower Jurassic to Lower Cretaceous, 600 m of Tertiary sediments and a thin sheet of Quaternary sediments. The rock outcrops are better exposed in the uplifted regions of the basin, such as Kachchh Mainland, Pachham Island, Khadir Island, Bela Island, Chorar Island and Wagad uplifts. Lower Jurassic to Lower Cretaceous are well preserved in the Kachchh Mainland. The stratigraphic succession of Kachchh Mainland is divided into four formations, namely Jhurio (Bathonian to Callovian), Jumara (Callovian to Oxfordian), Jhuran (Kimmeridgian to Lower Cretaceous) and Bhuj (pre-Aptian to Santonian (?)) Formations in ascending stratigraphic order¹³, are best exposed in a series of domes at Habo, Jhura, Keera, Nara, Jumara and Jara hills (Figure 1). The lithological sequence of these formations consists of clastic sandstone, siltstone, shale and limestone with distinct demarcation boundary, deposited in marine to fluviodeltaic conditions.

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