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Field response and molecular detection of yellow dwarf disease in onion genotypes

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Abstract
An experiment was conducted at Bihar Agricultural University, Sabour, Bhagalpur, Bihar during 2020-21 to evaluate the effect of yellow dwarf disease in different genotypes of onion. Thirty genotypes were screened against the yellow dwarf disease inducing symptoms like dwarfing, bending, streaking, curling, leaf flattening and abnormal bulb growth etc. Incidence of disease ranges between 0-27.86% and disease index >50% was found in seven genotypes e.g. W-364, W-464, W-182, ALR, B. Shweta, B. Dark Red, R-1606. Maximum genotypes were observed in category of moderately susceptible whereby none of them were found under highly susceptible category. For confirmation, molecular detection of plant samples of all genotypes was done using RT-PCR. Most of genotypes were confirmed for the OYDV where maximum number of samples were found positive (70%) in genotype W-500. This study will be beneficial for farmers to choose less susceptible varieties of crop and acquiring crop protection strategies based on field response of disease.

Keywords: Genotypes, disease index, disease incidence, RT-PCR, OYDV

Introduction
Onion (*Allium cepa* L.) is a short duration bulb crop that belongs to the family Alliaceae under Order Asparagales. Onion commonly known as "Queen of the kitchen" due to its high valued flavour, aroma and medicinal compounds (Brewster, 1990) [6]
Onion is commonly propagated through vegetative bulbs. Onion and garlic plants belonging to Alliaceae group are more prone to biotic as well as abiotic stress that includes diseases, insects, pests, nematodes, drought, waterlogging conditions (Schwartz and Mohan, 1995) [13]. Viruses that affects the crop includes Onion yellow dwarf (*Onion yellow dwarf virus*-OYDV*)* (Ghosh and Ahlawat, 1997) [3], leek yellow stripe (*Leek yellow stripe virus*- LYSV) (Van Dijk, 1993) [16], garlic mosaic (*Garlic mosaic virus*- GMV), Iris yellow spot (*Iris yellow spot virus*-IYSV) and shallot latent disease (*Shallot latent virus*- SLV). These viruses belong to various genera like *Allexivirus*, *Carlavirus*, *Potyvirus* and *Orthotospovirus* are known to infect the onion crop and cause severe economic losses (Dovas *et al*. 2001) [9]. Among the virus groups, Potyvirus is the largest genus causing considerable economic loss that belongs to family Potyviridae having diverse host range including major crops like potato, tomato, sugarcane, banana, papaya, pepper etc (Vishwanathan *et al*. 2017) [17]. Onion is also infected with other filamentous virus particles suspected to be Allexivirus (Kumar *et al*. 2010) [11]. In India OYDV was first reported in onion seed crop (Dhingra and Nariani 1963) [8].
OYDV exhibits characters like dwarfing, stunting, irregular yellow striping to almost complete yellowing, reduction in number of flowers, seeds and impairment of seed quality (Bos *et al*. 1976) [5]. The OYDV genome consists of single stranded positive sense RNA (about 10 kb) and has a terminal UTR region (Celli *et al*. 2013) [7]. Variability in N-terminal region of viral coat protein of different OYDV isolates resulted in the occurrence of different strains (Barg *et al*. 1997) [4]. The virus can be tested molecularly easily in bulbs and less in leaves and inflorescences (Winiarczyk *et al*. 2014) [18]. The aim of this research was to visually estimate the virus infection incidence and severity and detecting virus molecularly by suspecting plant samples using RT-PCR.

Material and Methods
Field response of different onion genotypes against virus
The present study was undertaken to assess natural response of different onion genotypes against onion yellow dwarf disease in natural field condition at Vegetable Research Field, Bihar Agricultural University, Sabour, Bhagalpur conducted in Rabi season (2020-21) in

in randomized block design (RBD) with 3 replications. The plots were fertile having well drained sandy loam soil with neutral pH of 7.2 having dimension of 1.5 m× 2.0 m. Transplanting of onion seedlings was done in second fortnight of January 2021. Under this study, 30 onion genotypes were assessed, observation of disease and tagging based on the typical symptoms was done at periodic interval of 15, 30, 45, 60, 75 and 90 days after transplanting.

Percent disease incidence (PDI) was calculated using formula PDI= No. of infected plant/total number of plants in individual plot. Disease index (DI) was also calculated using formula DI= {Sum of all disease rating/(total no. of rating x maximum grade)}x 100.

DI was calculated on rating scale (0-6) and on that basis genotypes were classified into categories viz: Immune, Highly resistant, resistant, moderately resistant, moderately susceptible and susceptible and highly susceptible.

Table 1: Rating scale (0-6) used for calculating DI

Rating	Category	Percentage diseased area	Description
0	I	0	No symptom.
1	HR	0.1-5	Slight twisting of tips, minor appearance to streaking.
2	R	5.1-15	Twisting, light appeared streaks.
3	MR	15.1-25	3-4 leaves twisting, prominent striking1/4th reduction in plant height.
4	MS	25.1-50	Twisting of >5 leaves, with yellowing, 1/3rd reduction in plan height
5	S	50.1-75	Twisting of all leaves, flattening of 4-5leaves,complete yellowing, streaking throughout leaves, ½ reduction in plant height
6	HS	>75	Complete bending and flattening of all leaves with severe yellow streak, wilting of plants

Detection of virus through RT-PCR

Onion leaf samples from the genotypes were collected. Total RNA extraction of all samples were done using Wizard Genomic SV total RNA isolation kit (Promega, USA).One step RT-PCR was performed using Titanium One-Step RT-PCR Kit (Clontech, USA). Also, cDNA of the extracted and purified RNA was prepared by using GoScriptTM Reverse Transcription System Kit (Promega, USA). A set of potyvirus specific primer Oligo (n) TGGTHTGGTGYATHGGARAAYGG,Oligo (2n) TGGTHTGGTGYATHGGARAAYGG of fragment length of 300 bp (Marie-Jeanne *et al*. 2000) [12] and OYDV specific primer OYDV (F) ATGATTGAAGCTTGGGGTTA, OYDV ® ACATCTTAATACCAAGCAACG of fragment length of 1015 bp.RT-PCR was performed in thermocycler (Mastercycler, Eppendorf, Germany) with thermal programme of total 35 cycles consisting 50 °C for RT-PCR, 94 °C for initial denaturationfor 5 minutes and 94 °C for denaturation for 30 seconds, 49 °C of annealing for 30 seconds, 68 °C for 1 minute for extension. Finally gel electrophoresis of amplified products with 1% agarose gel was done on UV tech Cambridge.

Result and Discussion

Fields response of different onion genotypes

PDI in different onion genotypes

Field response of each onion genotype was assessed by disease incidence in (Table 2) at periodic interval. In observations at 15 DAT, maximum incidence was recorded in genotype ALR (3.58%) followed by Bhima Red (3.07%). Again after 30-DAT, maximum disease incidence was shown by genotype ALR (5.12%) followed by B. Red (5.11%). Few genotypes showed late symptoms (Fig.1) expression (45-DAT) e.g.W-210, B. Super, R-1606, R-1628. Maximum disease incidence was shown by genotypes ALR (7.19%) followed by W-182 (7.17%). Genotypes W-390, W-500, R-1626 showed the delayed symptoms (after 60-DAT). Maximum disease incidence was shown by genotype B. Dark red (18.97) followed by W364 (13.33%). In the observation, majority of genotypes showed the highest disease incidence after 90-DAT. There was no any symptom noticed in six genotypes e.g.W444, W-203, B. Shankar, B. Light Red, B. Shakti, R-1639. As per our observation of investigation, major symptoms i.e., twisting, curling and flattening of leaves, streaking throughout the leaves with yellowing of plants were observed (Abd- El-Wahab *et al*. 2009) [1]. A limited literature is available about screening of onion genotypes against viral diseases. However, Few garlic genotypes were screened against OYDV where G-282 genotype showed the highest incidence of 20.7% followed by genotype G-1 having incidence of 15.0% at 90 DAS. Among all the 20 entries, 3 genotypes i.e. 499, 516 and 493 were non-symptomatic (Aditya *et al*. 2020) [2].

(a) Mild streaking with light greening

(b) Prominent streaking

Fig 1: Symptoms of OYDV

Disease index (DI) in different onion genotypes

In our investigated findings in (Fig.2), maximum genotypes (30%) were kept under moderately susceptible genotypes followed by immune and susceptible (20%). Moreover, 13.3% genotypes were grouped under resistant and moderately resistant category. In highly resistant group contains 3.3% genotypes. However, the findings showed that no genotypes were fallen under highly susceptible category. Genotypes under different categories are enlisted in Table 3.

Table 2: PDI (%) of different onion genotypes at periodic interval

S. No.	Genotypes	15 DAT	30DAT	45DAT	60DAT	75DAT	90DAT
1	W-364	1.54	4.61	6.66	13.33	16.41	26.15
2	W-210	0	0	3.58	11.28	14.87	18.97
3	W-226	2.05	3.07	5.12	10.25	11.79	14.87
4	W-444	0	0	0	0	0	0
5	W-464	0.51	2.05	5.64	12.82	13.33	19.14
6	W-390	0	0	0	4.61	12.82	14.35
7	W-500	0	0	0	4.61	11.79	15.38
8	W-182	2.05	4.1	7.17	10.25	20	27.867
9	W-117	1.5	1.53	3.58	10.76	15.38	18.97
10	W-405	2.05	2.56	5.6	12.82	16.22	19.48
11	W-203	0	0	0	0	0	0
12	ZIRAT	2.05	2.05	4.1	7.62	9.74	9.74
13	ALR	3.58	5.12	7.19	12.8	17.09	21.02
14	NHRDF	1.55	1.53	3.5	5.6	14.84	18.63
15	B. Shankar	0	0	0	0	0	0
16	B. Shweta	0.51	2.05	5.6	11.11	14.73	19.48
17	B. Kiran	0	0	0	0	0	0.51
18	B. Safed	1.53	3.58	5.12	9.76	11.28	15.89
19	B. Light Red	0	0	0	0	0	0
20	B. Super	0	0	1.53	3.07	8.2	10.76
21	B. Red	3.07	5.11	6.66	9.18	12.82	14.35
22	B. Shakti	0	0	0	0	0	0
23	B. Dark Red	0.51	2.05	6.3	16.41	16.92	18.97
24	B. Raj	4.1	4.1	6.15	12.3	13.333	18.46
25	R-1626	0	0	0	5.64	10.76	15.89
26	R-1664	1.52	3.07	3.07	7.69	11.28	15.89
27	R-1606	0	0	2.05	9.23	14.35	18.97
28	R-1628	0	0	2.05	8.71	11.28	12.82
29	R-1612	0.51	2.05	1.53	10.25	14.87	18.97
30	R-1639	0	0	0	0	0	0
	C.D	0.30	0.451	0.714	2.151	1.982	4.248
	C.V	13.355	12.978	14.186	11.893	11.554	12.181

Table 3: Genotype categorised under different category

Category	DI range	Genotypes
Immune (I)	0	W-444, W-203, B. Shankar, B. Light Red,B. Shakti, R-1639
Highly Resistance (HR)	0.1-5	B. Kiran
Resistance (R)	5.1-15	W-226, W-390, ZIRAT, B. Super
Moderately Resistance (MR)	15.1-25	W-500, W-117, B. Safed, R-1628
Moderately susceptible (MS)	25.1- 50	B. Red, W-210, W-405, NHRDF, R-1612, B. Dark Red, B. Raj, R-1626, R-1664
Susceptible(S)	50.1-75	W-364, W-464, W-182, ALR, B. Shweta, R-1606
Highly susceptible (HS)	>75	-

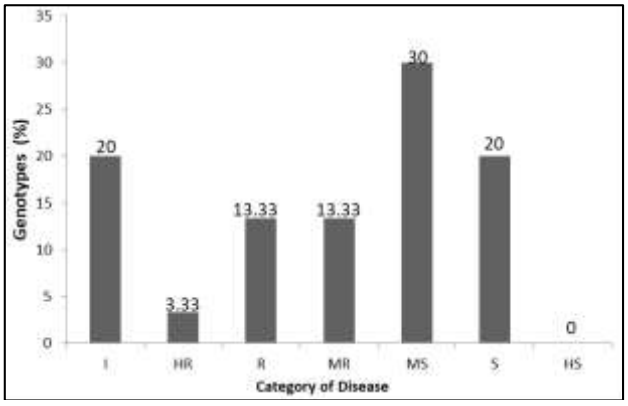

Fig 2: Graph showing genotype percentage under different groups

Molecular detection of yellow dwarf disease through RT-PCR assay

Plants showing typical symptoms of yellow dwarf disease were collected from 24 symptomatic genotypes as shown in table 4, Initially RNA was isolated from different samples and confirmed the virus using Potyvirus specific primer. Each positive samples against potyvirus specific primer, further screened with *Onion yellow dwarf virus* specific primer targeting CP gene. Maximum samples from genotype W-500 where 71.4% and 70% samples were found positive for Potyvirus and OYDV specific primers respectively.

Table 4: Number of positive samples in genotypes against potyvirus and OYDV specific primers

S.No.	Genotype	Potyvirus specific primer (No. positive/ Total tested sample)	OYDV specific primer (No. Positive/Total tested sample)
1	W-364	7/10 (70.0%)	4/7 (57.1%)
2	W-210	3/8 (37.5%)	2/3 (66.6%)
3	W-226	4/9 (44.4%)	3/ 4 (75.0%)
4	R-1612	6/10 (60.0%)	3/6 (50.0%)
5	W-464	3/7 (42.8%)	2/3 (66.6%)
6	W-390	3/6 (50.0%)	1/3 (33.3%)
7	W-500	10/14 (71.4%)	7/10 (70.0%)
8	W-182	5/9 (55.5%)	3/5 (60.0%)
9	W-117	6/9 (66.6%)	4/6 (66.6%)
10	W-405	4/8 (50.0%)	3/ 4 (75.0%)
11	R-1628	2/6 (33.3%)	1/2 (50.0%)
12	ZIRAT	1/6 (16.6%)	1/1 (100.0%)
13	ALR	6/7 (85.7%)	5/ 6 (83.3%)
14	NHRDF	2/5 (40.0%)	1/2 (50.0%)
15	R-1626	1/5 (20.0%)	1/1 (100.0%)
16	B. Shweta	2/6 (33.3%)	2/2 (100.0%)
17	B. Kiran	3/6 (50.0%)	2/3 (66.6%)
18	B. Safed	3/5 (60.0%)	1/3 (33.3%)
19	R-1664	2/6 (33.3%)	2/2 (100.0%)
20	B. Super	5/9 (55.5%)	4/5 (80.0%)
21	B. Red	4/7 (57.1%)	3/4 (75.0%)
22	R-1606	3/8 (37.5%)	2/3 (66.6%)
23	B. Dark Red	2/7 (28.5%)	2/2 (100.0%)
24	B. Raj	4/8 (50.0%)	3/4 (66.6%)

In molecular diagnosis based on coat protein gene sequences and genome organization the characterization of Potyvirus was done and found that OYDV as main virus infecting onion crop (Tsuneyoshi *et al*. 1998; Ahlawat *et al*. 1997) [15, 3]. Smekalova *et al*. (2017) [14] screened 122 accessions of shallot where 65 genotypes resulted in atleast one positive sample for the presence of four different viruses *viz*., OYDV, LYSV, GCLV, SLV etc.

Reference

1. Abd El-Wahab AS, Elnagar S, El-Shiekh MAK. Incidence of Aphid- borne Onion Yellow Dwarf Virus (OYDV) in Alliaceae crops and associated weeds in Egypt.4th conference on recent technologies in Agricuture;c2009. p. 21-33.
2. Aditya K,Bhagat AP, Shree S, Ansar M. Molecular Detection of Bulb-AssociatedPotyviruses in Garlic and their Field Response. International Journal of microbiology and applied sciences. 2020;9(11):1938-1945.
3. Ahlawat YS, Verma A. Serological detection of a mixed viral infection in onion seed crop and possible measure for its management. Indian Phytopathology. 1997;50(1):137-140
4. Barg E, Lesemann DE, Vetten HJ, Green SK. Viruses of Allium and their distribution in different Allium crops and geographical regions. Acta Hortic. 1997;433:607-61.
5. Bos. Onion yellow dwarf virus. CMI/AAB Descriptions of Plant Viruses. 1976;158:4.
6. Brewster JL, Rabowitch HD. Physiology of crop growth and bulbing in Onions andAllied Crops. CRC Press: Boca Raton;c1990. p. 53-81.
7. Celli MG, Torricco AK, Kiehr M. Striking differences in the biological and molecular properties of onion and garlic isolates of onion yellow dwarf virus.Archives of Virology. 2013;158:1377-1382.
8. Dhingra KL, Nariani TK. A virus disease of onion. Indian Phytopathology. 1963;16:311-312.
9. Dovas CI, Hatziloukas E, Salomon R, Barg E, Shiboleth Y, Katis NI. Comparison of Methods for Virus Detection in Allium spp. Journal of Phytopathology. 2001;149:731-737.
10. Ghosh DK, Ahlawat YS. Filamentous viruses associated with the mosaic disease of garlic in India. Indian Phytopathology. 1997;50:266-267.
11. Kumar S, Baranwal VK, Joshi S, Arya M, Majumder S. Simultaneous Detection of Mixed Infection of Onion yellow dwarf virus and an Allexivirus in RT-PCR for Ensuring Virus Free Onion Bulbs. Indian Journal of Virology. 2010;21(1):64-68.
12. Marie-Jeanne V, Ioos R, Peyre J, Alliot B, Signoret P. Differentiation of Poacea potyviruses by reverse transcription polymerase chain reaction and restriction analysis. Journal of phytopathology. 2000;148:141-151.
13. Schwartz HF, Mohan SK. Compendium of onion and garlic diseases and pests. The American

phytopathological Society PRESS, 1995, 52.
14. Smekalova K, Stavelikova H, Dusek K. Distribution of viruses in the shallot germplasm collection of the Czech Republic. Horticultural Science (Prague). 2017;44(1):49-52
15. Tsuneyoshi T, Matsumi T, Natsuaki KT, Sumi S. Nucleotide sequence analysis of virus isolates indicates the presence of three *Potyvirus* species in Allium plants. Archieves of Virology. 1998;143:97-113.
16. Van Dijk P. Survey and characterization of potyviruses and their strains of Allium Species. Netherlands Journal of plant pathology. 1993;99:1-48.
17. Viswanathan R, Parameswari B, Nithya K, Jeevalatha A, Rao GP. Potyviruses infecting crop plants in India. A century of Plant Virology in India;c2017. p. 361-404.
18. Winiarczyk K, Solarska E, Sienkiewicz W. Prevalence of infections with Onion yellow dwarf virus, Leek yellow stripe virus and Garlic commom latent virus in plants from the genus Allium . Acta Scientiarum Polonorum, Hortum Cultus. 2014;13(3):123-133