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Effect of the Site of Infection Source and Some Onion Genotypes on Management of Onion Yellow Dwarf Virus in the River Nile State, Sudan

Abdelmagid Adlan Hamad[1] and Abdalla Hussein Nouri[2]

[1]Gezira Research Station, Wad Medani, Sudan
[2]Shambat Research Station, Khartoum North, Sudan

Abstract: Field experiments were carried out at Hudeiba Research Station Farm during 2004/ 05 and 2005/06 seasons to evaluate the effect of the location of infection source on the spread of onion yellow dwarf virus (OYDV) and to screen 18 onion genotypes for resistance to the virus. Disease incidence was assessed in blocks of 60 x 6 m planted with disease-free onion seedlings and located upwind and downwind of the disease source at distances of 0, 6, 12, 18, 24 and 30 m. Average incidence of 33.7% and 57.8% was recorded in downwind blocks compared to 8.0% and 9.9% in upwind blocks, irrespective of the distance from the disease source, in 2004/05 and 2005/06, respectively. The blocks located downwind and adjacent to the disease source had the highest disease incidence of 93.4% and 95.5% compared with 7.8% and 18.9% in blocks located at a distance of 30 m away from the source during the two seasons, respectively. In upwind blocks, disease incidence of 15.4% and 20.2% was recorded in blocks adjoining the disease source, while incidence of 3.7% and 4.85% was recorded in blocks located at a distance of 30 m in the two seasons, respectively. None of the genotypes tested was resistant to the OYDV infection; however, Baftaim Red genotype proved to be tolerant to the virus.

Key words: Onion; OYDV; disease source direction; block

INTRODUCTION

Onion (*Allium cepa* L.) is the most important vegetable crop in the Sudan; it is grown on an estimated annual area of 48 000 ha. Onion yellow dwarf virus (OYDV) (family Potyviridae, genus *Potyvirus*) is one of the constraints for onion production in the Sudan, particularly in northern

Sudan where a large area is allotted to this crop. OYDV is aphid-transmitted, first described in U.S.A. by Melhus *et al*. (1929), and has a narrow host range including onion, shallot, garlic and few ornamental *Allium* spp. (Van Dijk 1993). It survives in bulbs and sets and is not transmitted by true seeds. It is transmitted mechanically and in a non-persistent manner by over 50 aphid species (Drake 1933). The general symptoms of OYDV disease, which are more pronounced in seed onion crops, include yellow striping, stunting and drooping of the plant and flaccidity of the leaves.

In Sudan, the disease was reported for the first time in 1982 in a commercial onion field north of Khartoum (El Hassan and Morgan 1992). It was recorded at Hudeiba Research Station, Shendi and Kassala in the end of 1970s (Mohamed Ali 2009). Disease incidence of 69% -99% in onion, grown from bulb, was reported by Morgan (1987) in Khartoum area, and infection with OYDV Hudeiba isolate led to significant reduction of bulb and seed yield (Annual Report, Hudeiba Research Station 2002). A reduction in onion productivity of 12-20 tons/ha was reported in Khartoum area, due to the spread of the disease in the last decade (Mohamed Ali 2009).

Generally, management measures of OYDV based on insecticides are unlikely to be successful, because the aphid vector quickly transmits the virus in non-persistent manner before it is killed by insecticides. Short retention periods of the virus within the vector body make OYDV spread more dependent on the distance and direction of onion fields in relation to the source of infection, so wind regime and isolation of onion crops from the infection sources are most likely helpful as an efficient control measure. Thus, control measures of the disease using cultural practices are highly needed, particularly in northern Sudan where the disease is widespread in onion fields.

The objectives of this work were to evaluate the effect of the direction and distance from the disease source on the spread of OYDV and to determine the levels of resistance of some onion genotypes against OYDV Hudeiba isolate.

MATERIALS AND METHODS

Field experiments were conducted at Hudeiba Research Station, River Nile State, using OYDV Hudeiba isolate for inoculation and *Aphis craccivora* as a vector.

Distance from OYDV- affected block

This experiment comprised a central rectangular block (60 x 6 m) planted with OYDV- affected onion bulbs in two consecutive growing seasons, on 20.11.2004 and on 30.11.2005. One month later, when the OYDV-affected bulbs were well established and the number of the aphid vectors started to increase, disease- free seedlings of cv. Saggai were planted in blocks each 60 x 6 m north (upwind) and south (downwind) of the diseased block either along side it or 6, 12, 18, 24 or 30 m away on ridges of 60 cm apart and 15 cm between plants, and each ridge contained three rows. The experiment was replicated three times in 500-m isolated fields. Because disease symptoms were clearly revealed in seed onion crop grown from infected bulbs, onion crop was harvested from all blocks, stored and replanted next season for better recording of the disease incidence based on visual symptoms. An average yield (kg/2m^2) was estimated from six plots each of 2 m^2 randomly selected in each block. Data on disease incidence and yield were subjected to analysis of variance using MSTATC software.

Reaction of some onion genotypes to OYDV

Eighteen genotypes were tested for their reaction to OYDV infection (Table 1). Seedlings of each genotype were planted on 10 to 15 ridges, each 6 m long, on 15.12. 2005 and 20.12. 2006 in blocks already planted with diseased onion bulbs one month earlier. The seedlings were planted one month later in well established OYDV- affected onion bulbs to secure high number of vectors and high disease pressure. Since symptoms were not well expressed on the crop grown from seedlings, bulbs of all tested genotypes were harvested and stored to be planted next year for better recording of disease incidence based on visual symptoms. Resistance was assessed by disease incidence and symptom severity. Severity of the disease was recorded according to the following arbitrary scale: M = mild drooping of the leaves, while the whole plant was green; Mo = moderate drooping, mild yellowing and flaccidity without plant stunting; S = stunting of the plant and yellowing, flaccidity and severe drooping of the leaves.

Serological assay

When infected with OYDV, Karadah genotype always showed severe symptoms, while Baftaim Red showed none or very mild symptoms. Thus, it was necessary to determine whether Baftaim Red is resistant, and to what level, to OYDV. Large number of *Aphis craccivora,* the most prevalent aphid vector, was given an acquisition access period of one hour on onion diseased plants. Then, seedlings from Karadah and Baftaim Red were inoculated for two hours with the viruliferous aphid using 10-20 individuals per one seedling. Three weeks later, leaf samples from both genotypes were serologically tested for the detection of the virus using double sandwich enzyme-linked immunosorbent assay (DAS- ELISA) as described by Clark and Adam (1977). OYDV IgG antisera and conjugate were provided by Dr. H. Josef Vetten, Julius Kuehn Institute, Germany. Samples having optical density (OD) absorbance values three times more than that of the healthy control were considered positive for the virus.

RESULTS

Distance from OYDV- affected blocks

Overall, the effects of block location, down wind or up wind, of the disease source on the incidence of the disease were significant irrespective of the distance of the disease source (Table 1). Disease incidence of 33.7% and 57.8% were recorded in blocks located down wind of the disease source during 2004/05 and 2005/06, respectively. In upwind blocks, the average disease incidence was 8.0% and 9.9% in the two seasons, respectively.

There was significant decline in disease incidence with increasing distance from the disease source in both seasons (Table 1; Fig. 1a and 1b). The incidence was 93.4% and 95.5% in downwind blocks located adjacent to the disease source in 2004/05 and 2005/06, respectively. The blocks located 6, 12, 18, 24 and 30 m downwind from the disease source had average disease incidence of 63.3%, 16.3%, 10.9%, 9.5% and 7.8%, respectively, in 2004/05 and 80.6%, 72.8%, 50.3%, 28.4% and 18.9% in

2005/06. In upwind blocks, disease incidence of 15.4%, 10.5%, 8.4%, 5.2%, 4.5%, and 3.7% was, respectively, recorded in blocks at distance of 0, 6, 12, 18, 24 and 30 m in 2004/05 season and incidence of 20.2%, 12.8%, 8.5%, 7.3%, 5.9%, and 4.8% was recorded during 2005/06.

Table 1. Effect of plot direction and distance from disease source on the incidence (%) of OYD disease in Hudeiba during 2004/2005 and 2005/06 seasons

Distance (m)	Block location		
	Downwind	Upwind	Mean
	2004/05 Season		
0	(93.4) 75.4	(15.4) 23.1	(54.4) 49.3
6	(64.0) 57.1	(10.5) 18.8	(40.2) 37.9
12	(16.3) 23.7	(8.4) 16.8	(12.4) 20.3
18	(10.9) 19.2	(5.2) 12.8	(8.0) 16.0
24	(9.5) 17.9	(4.5) 11.9	(7.0) 14.9
30	(7.8) 16.1	(3.7) 10.9	(5.8) 13.5
Mean	(33.7) 34.9	(8.0) 15.7	
SE±	0.80		1.39
S. level	***		***
	2005/06 Season		
0	(95.5) 79.1	(20.2) 26.7	(57.8) 52.9
6	(80.6) 64.0	(12.8) 20.8	(46.7) 42.4
12	(72.8) 58.7	(8.5) 22.4	(44.2) 40.6
18	(50.3) 45.2	(7.3) 15.5	(28.8) 30.3
24	(28.4) 32.1	(5.9) 14.1	(17.2) 23.1
30	(18.9) 25.7	(4.8) 12.5	(11.9) 19.1
Mean	57.8)50.8	(9.9)18.7	
SE±	0.998		1.728
S. level	***		***

Incidence percentages were transformed to arcsine.
Values in parentheses are actual incidence percentages.
***=Significant at P = 0.001

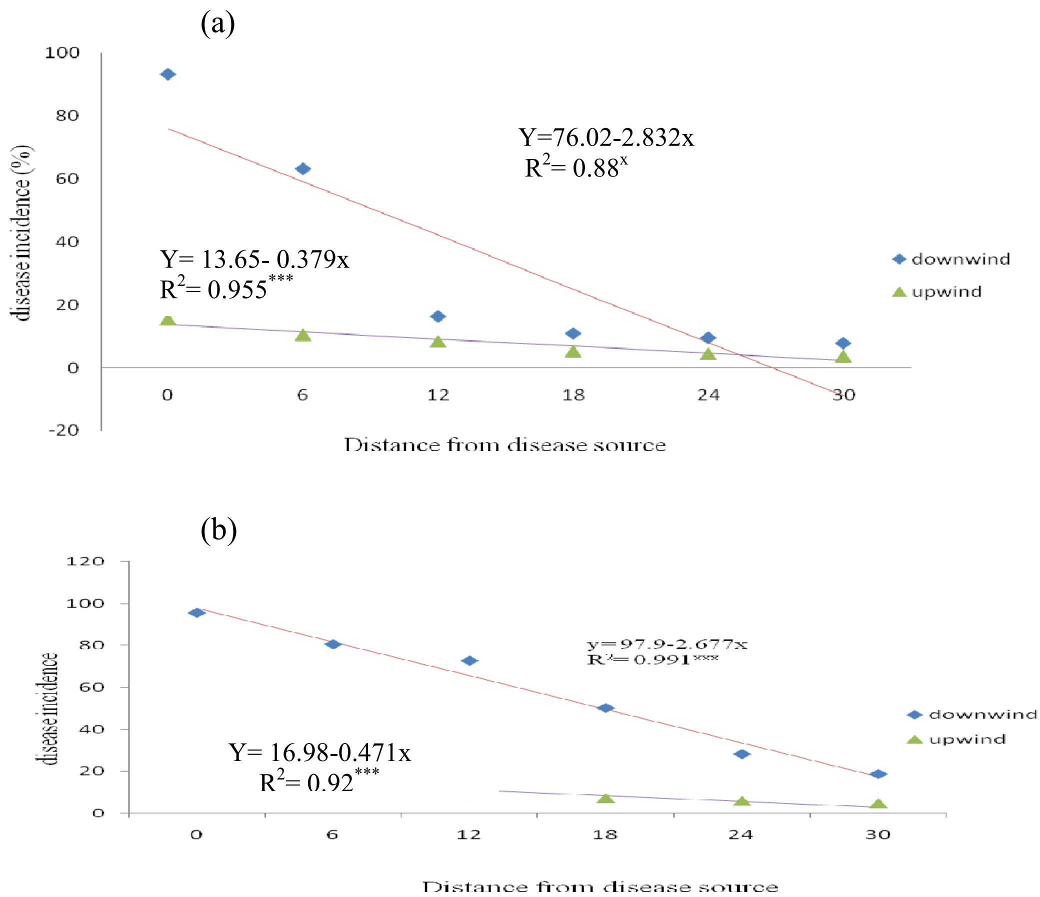

Fig. 1. Spread of OYDV to blocks located downwind or upwind adjacent or 6, 12, 18, 24, 30 m away from an OYDV- affected block, (a) 2004/05 and (b) 2005/06

Effect on yield

A significant reduction of 41.5% in onion yield was obtained in blocks located downwind of the disease source compared with upwind located blocks, regardless of the disease source distance (Table 2). Yield of onion grown downwind in proximity to the disease source was also reduced by 37.1% compared with blocks at distance of 24 m away from the source regardless of wind direction (Table 2).

Table 2. Bulb yield (kg/2m^2) in blocks located upwind and downwind at different distances away from severely infected onion seed crop, 2004/2005

Distance (m)	Block location		
	Downwind	Upwind	Mean
0	1.9	3.3	2.63
6	2.4	3.5	2.95
12	2.3	4.2	3.25
24	2.9	5.4	4.18
Mean	2.4	4.1	
SE$\pm$	0.218		0.31
S. level	***		***

***=Significant at P= 0.001

Reaction of some onion genotypes to OYDV

Of the 18 onion genotypes tested (Table 3) for resistance to OYDV, none was found to be resistant. However, the average disease incidence of Baftaim Red and Baftaim Yellow was 6.5% and 9.4%, respectively, with only mild symptoms expression, and reduction in bulb weight of 8.9% and 17.9%, respectively (Table 3). Kamleen, El- Hilo and Dongla 4 had average disease incidence of 18.2%, 30.5% and 17.2%, respectively, with mild and moderate infection. The reduction in bulb weight in these three genotypes was 20.4%, 26.2% and 20.7%, respectively (Table 3). The other 12 genotypes were highly susceptible and had average disease incidence ranging from 36.9% to71% with moderate or severe infection. The reduction in bulb weight of the most severely affected genotypes was

42.8% - 57.4% (Table 3). ELISA tests revealed no difference in the titer of OYDV in the inoculated Karadah and Baftaim Red genotypes as they

gave similar absorption values (0.584 and 0.523, respectively), although the latter did not develop visible symptoms. Inoculated plants of Baftaim Red reacted positively with the antiserum to OYDV and gave absorption values (0.523) more than three times those of the healthy control (0.014).

Table 3. Disease incidence (%), symptoms severity and average bulb weight (g) of 18 onion genotypes naturally infected by onion yellow dwarf virus

Genotype	Disease incidence (%)		Symptoms severity	Average bulb weight(g)	
	2004/05	2005/06		Healthy	Infected
Mokabrab Red	38.0	86.4	Mo/S	91.2	46.1
Baftaim Yellow	10.2	08.5	M	124.3	102.1
Wad Hamid Asfer	48.6	36.4	Mo/S	85.9	43.2
Kamleen	22.0	14.5	M/Mo	102.1	81.3
Abufrerwia Wad Salim	45.2	78.5	S	85.5	39.4
Abufrewia Makabrab	55.5	78.7	Mo/S	95.3	48.2
Abufrewia El Khilala	63.9	70.5	Mo/S	89.6	50.3
El Hilo	31.6	29.4	M/Mo	102.3	75.5
Graigreeb	45.0	77.0	S	96.5	41.3
Karadah	62.4	78.3	S	100.4	45.2
Saggai	45.5	76.1	S	117.2	61.6
Baftaim Red	09.2	03.8	M	120.5	109.8
Dongla 4	19.1	15.2	M/Mo	98.8	70.3
Abufrewia Wad hamid	88.4	53.6	S	89.7	51.3
Dongla Asfer	33.3	40.5	Mo	95.5	69.8
Saggai Improved	50.3	37.5	Mo/S	101.3	56.4
Baida	50.9	66.0	Mo	91.6	57.8
Dongla 9	33.7	34.9	Mo	107.5	71.8

M= Mild dropping, Mo= Moderate dropping and S= Extreme dropping of the leaves

Fig. 3. Abufrewia El Khilala (left), Baftaim Red (middle) and Saggai (right) plants grown from OYDV naturally inoculated bulbs

DISCUSSION

During 2002-2005 seasons, varied levels of incidence of OYDV disease were observed on onion seed crops in different fields in the River Nile State (Hudeiba Research Station, Annual Reports, 2002-2005). However, these levels were underestimated and do not reflect the actual incidences of the disease spread in the fields, because the disease symptoms were not clearly expressed on onion commercial bulb crops. The vectors of OYDV are large number of aphids that do not colonize onion plants, but are more important in the spread of the virus. This is because non-colonizing aphids are more likely to probe the diseased plants and continue to disperse rather than to settle down and feed (Raccah *et al*. 1985; Atiri 1992; Fereres *et al*. 1993; Dusi *et al*. 2000). Probing by viruliferous aphids is sufficient to transmit the virus. As a result, insecticide application is not an effective means of reducing incidence of infection. Hence, it is important to direct the effort towards the eradication of the disease sources, mainly the infected onion bulbs. The natural source of OYDV in Sudan is most likely the neighbouring infected onion seed crop as there are very few other plant species that act as virus reservoirs (Salih 1990). It is most likely that the main, if not the only, source of infection would be the onion bulb since the other sources like garlic, shallot and leek are not widely grown. As it is unlikely for insecticides to be useful and there is no onion cultivar, up to now, known to be resistant to OYDV, cultural practices seem to be a feasible measure for the management of the disease. Worldwide, elimination and avoidance of infection sources, such as infected onion sets, have controlled the disease effectively in onion in U.S.A. (Henderson 1935) and New Zealand (Chamberlain and Baylis 1948). The fact that OYDV is not seed-transmitted in onion (Melhus *et al*. 1929; Henderson 1935) supports such control. However, despite this early control of the virus, the disease has remained of great importance in other countries, for example high incidence of up to 50% occurred in onion crops in Chile in1977 (Alvarez *et al*. 1977).

In this study, conducted to determine the effect of distances of primary infection sources on the spread of the disease, the blocks were adjacent to each other and were not isolated from the infection sources by bare soil or barrier crops. Thus, theoretically, each block should have represented a secondary source of infection for the next neighbouring one and, therefore, there might have been no difference in disease incidence in all blocks how far away from the primary sources. However, the results showed that the highest virus disease incidence was recorded in blocks at the proximity of the disease source, while the lowest incidence was recorded in blocks located at a distance of 30 m away from the source. This may indicate that distances from infection sources could be the major factor in the spread of OYDV. The relatively short retention period of OYDV in their aphid vectors and the tendency for wingless aphid, the most common form in the River Nile State, to make short flights probably explains the proximity effect observed in this study. The success of distance trials in restricting the spread of OYDV indicates the importance of avoiding nearby sources of infection and confirms that the spread of OYDV is mainly local.

The spatial pattern of OYDV was highly dependent on the wind regime and direction of disease sources and was characterized by pronounced downwind oriented disease gradients. It has been recognized for a long time that wind direction is critical for movement of aerial virus vectors (Thresh 1976; Harrison 1981). Infection of the adjoining upwind field was less, because the movement of aphid vectors against the wind seems to be fairly limited. This is consistent with the evidence that wind- borne vectors tend to be carried downwind rather than upwind (Thresh 1076) The spatial spread resulted from the aphid vector carried by the prevailing north/ south oriented wind and, therefore, it is advisable not to plant onion bulb crops downwind of and next to onion seed crop in an area where OYDV is known to be spread. This can be achieved through the isolation of onion seed crops from commercial crops or by planting border or barrier crops between bulb and seed crops to intercept viruliferous aphids (Salih 1990).

Regarding resistance to OYDV, Baftaim Red and most likely Baftaim Yellow were considered tolerant and not resistant to the virus infection, because the virus concentration in the Baftaim Red was the same as in the severely infected Karadah genotype as shown by ELISA tests. Thus, the low incidence and the mild symptoms may be explained by tolerance and not by resistance. So, ELISA tests confirm that Baftaim Red was not resistant but tolerant to virus infection leading to small reduction in bulb weight of 8.8%.

In conclusion, the potential solution to the problem of onion yellow dwarf disease could be (i) not to plant onion bulb crop next to seed crop in an area where OYDV is known to be spread or (ii) to specify certain areas for onion seed production.

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تأثير موقع مصدر المرض وبعض الطرز الوراثية للبصل في مكافحة فيروس إصفرار وتقزم محصول البصل بولاية نهر النيل، السودان

عبد الماجد عدلان حمد [1] و عبدالله حسين نوراى[2]

[1]محطة بحوث الجزيرة، ودمدنى - السودان
[2]محطة بحوث شمبات، الخرطوم بحرى - السودان

المستخلص: إجريت تجارب بمزرعة محطة بحوث الحديبة بولاية نهر النيل لموسمين (05/2004 و 06/2005) لتقييم تأثير اتجاه وبُعد مسافة مصدر المرض علي إنتشار فيروس اصفرار وتقزم البصل ، واختبار مقاومة 18 من الطرز الوراثية للبصل للمرض. تم ذلك بزراعة شتول خالية من المرض في أحواض بمساحة 60 x6 متر جنوب وشمال المصدر وعلى أبعاد 0 و 6 و12 و18 و24 و 30 مترا. كانت نسبة الإصابة بالمرض حوالى 8% في البصل الذي زرع شمال المصدر (عكس اتجاه الرياح) بينما بلغت 33.7% جنوب مصدر المرض (في اتجاه الرياح) وذلك في موسم 05/2004. أما في الموسم 06/2005 فبلغت نسبة الإصابة 9.9% شمال المصدر و57.8% جنوب المصدر وذلك بغض النظر عن بُعد مسافة المصدر. كذلك وجد أن متوسط الإصابة بلغ 93.4% و95.5% في البصل المزروع جنوب المصدر مباشرة في موسمي 05/2004 و06/2005 علي التوالي وعندما كان المصدر على بُعد 30 متراً بلغت الإصابة 18.9% و7.8% في الموسمين علي التوالي. بلغت الإصابة في البصل الذي زرع مباشرة شمال المصدر حوالي 15.4% و20.2% أما المحصول المزروع علي مسافة 30 مترا شمال المصدر فكانت الإصابة فيه حوالي 3.7% و4.9 % في الموسمين علي التوالي. لم تبد كل الطرز الوراثية المختبرة مقاومة للاصابة وأثبت الطراز بافطيم تحملاً للمرض.