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Occurrence and management of Chickpea chlorotic dwarf virus in chickpea fields in northern Sudan

Abdelmagid A. Hamed[1] and Khaled M. Makkouk[2]

[1]Hudeiba Research Station, ARC, Ed-Damer, Sudan
[2]International Center for Agricultural Research in the Dry Areas (ICARDA), P.O. Box 5466, Aleppo, Syria

Summary. A field survey of chickpea for viruses was carried out in River Nile State, northern Sudan, in the 1996–1997 and 1999–2000 growing seasons. A total of 42 fields (24 in the first season and 18 in the second) were visually inspected. The fields surveyed covered the main areas of chickpea production. On the basis of the main symptoms observed, stunting and yellowing, the range of virus incidence was 7–25% in the first season and 25–62% in the second. Tissue-blot immunoassay (TBIA) of 264 chickpea samples with symptoms, and collected in 1996–2000, indicated that Chickpea chlorotic dwarf virus (CpCDV, Family *Geminiviridae*) was the most common (72.7%). Field trials at Hudeiba Research Station in northern Sudan in the 1999–2000 and 2000–2001 growing seasons to evaluate the influence of cultivar, sowing date and irrigation interval on the natural spread of CpCDV in chickpea fields revealed that virus incidence in "Shendi" was lower than that in "ICCV-2", regardless of planting date. Delayed sowing reduced CpCDV incidence in the three growing seasons from 1999 to 2002. Virus incidence was also reduced by short irrigation intervals during the growing seasons 1999–2000 and 2001–2002. Therefore, the combined effect of partial resistance, delayed planting and irrigation at short intervals proved useful in chickpea stunt management in chickpea fields in northern Sudan.

Key words: Chickpea stunt, Geminiviruses, leafhopper-borne viruses, disease management, Sudan.

Introduction

Chickpea is one of the most important cool-season food legume crops in Sudan; it is mainly grown in River Nile State, northern Sudan. A virus causing chickpea stunt has been reported to occur in Sudan's most important chickpea producing areas (Mohamed and Van Rheenen, 1991). During the last few years, there has been a remarkable increase in the incidence of this disease in chickpea and lentil. A number of viruses have been associated with chickpea stunt; *Bean leaf roll virus* (BLRV, Family *Luteoviridae*) and *Beet western yellows virus* (BWYV, Genus *Polerovirus*, Family *Luteoviridae*) (Carazo *et al*., 1993). Chickpea chlorotic dwarf virus (CpCDV, Family *Geminiviridae*), a leafhopper-transmitted virus and a tentative species of the genus *Mastrovirus* (Horn *et al.,* 1993), was also found to be associated with chickpea stunt in India and Pakistan (Horn *et al.,* 1996), and CpCDV in chickpea and faba bean was reported in Sudan by Makkouk *et al*. (1995). Accordingly, a systematic survey was undertaken in 1996–2000 to determine the virus or viruses causing yellowing and stunting in chickpea. In addition, a study was carried out at the Hudeiba Research Station to evaluate the influence of host resistance, sowing date and irrigation interval on the spread of these virus(es).

Corresponding author: K. Makkouk
Fax: +963 21 2213490
E-mail: K.Makkouk@cgiar.org

Materials and methods

Field observations

Chickpea fields in Sudan's most important chickpea-producing areas, at Wad Hamid (Salwa-North, Salwa-South, Hagr Eltir), Berber, Rubatab (Bushtanab, Harasha, Zooma) and Shendi, and fields at the Hudeiba Research Station Farm (HRSF) were surveyed for virus infection in February 1996 (1995–1996 growing season), when the crop was at the flowering stage. Another survey, which covered the areas of Berber, Shendi, Salwa and Hudeiba, was conducted during the 1999–2000 growing season. The fields surveyed ranged in size from 5 to 15 acres. Incidence of chickpea stunt was determined on the basis of the symptoms of stunting and yellowing, and by visually identifying and recording the percentage of infected plants in 1-m^2 plots at 5–10 locations picked randomly in each field visited. The fields inspected were randomly selected, 5–10 km apart.

Sample collection and laboratory tests

A total of 264 chickpea samples with stunting and yellowing were collected during the period 1996–2000. Samples were blotted on nitrocellulose membranes and the blots were processed at the Virology Laboratory of the International Center for Agricultural Research in the Dry Areas (ICARDA) for virus identification by tissue-blot immunoassay (TBIA; Lin *et al.,* 1990; Hsu and Lawson, 1991). Each sample was blotted in four replicates on nitrocellulose membrane and tested against CpCDV, BWYV BLRV and *Faba bean necrotic yellows virus* (FBNYV, Genus *Nanovirus*) antibodies. CpCDV polyclonal antibody was provided by J. Vetten, Biologische Bundesanstalt fuer Lund- und Forstwirtschaft (BBA), Braunschweig, Germany; BWYV specific monoclonal antibody was from ATCC (ATCC PVAS-647); BLRV specific monoclonal antibody (4B10) was provided by L. Katul, BBA, Braunschweig, Germany (Katul, 1992); and FBNYV monoclonal antibody was provided by A. Franz, BBA, Braunschweig, Germany (Franz *et al*., 1996).

Effect of sowing date and cultivar on chickpea stunt incidence

During the 1999–2000 growing season, two chickpea cultivars, "Shendi" and "ICCV-2" were evaluated. These were planted at three sowing dates (25 October, 22 November and 20 December). The treatments were laid out in a randomized complete block design (RCBD) with three replicates. Plot size was 28 m^2, with a spacing of 60 cm between ridges and 10 cm between holes. All plots were hand-weeded once and received 43 kg N-ha^{-1} in the form of urea. Stunt disease incidence (%) was recorded. During the 2000–2001 growing season, three sowing dates (23 October, 6 November and 20 November) were evaluated following the same experimental design. During the 2001–2002 season, 5 sowing dates (29 October, 5, 12, 19 and 26 November) were evaluated for the same two chickpea cultivars.

Effect of irrigation intervals and cultivar on chickpea stunt incidence

During the 1999–2000 growing season, two cultivars (Shendi and ICCV-2) were each grown at two irrigation intervals (10 or 20 days) and laid out in a RCBD with four replications. Chickpea was sown in the second week of November. The effect of these two factors on stunt incidence was determined by counting the percentage of infected plants in each plot. During the 2000–2001 growing season, the same chickpea cultivars were grown with three irrigation intervals (10, 15 and 20 days) and laid out in a RCBD with three replications. The sowing date was 15 November. Stunt incidence was determined as in the previous experiment. The effect of four irrigation intervals (7, 14, 21 and 28 days) on the spread of chickpea stunt was evaluated in the same two cultivars sown on November 1 in the 2001–2002 growing season.

Results

Field survey

Stunt incidence was highest at Wad Hamid (Salwa-North), where virus incidence was 25.6 and 30% in the 1996–1997 and 1999–2000 growing seasons respectively. At a location in Berber, stunt incidence reached 25% in the 1999–2000 growing season. In the Shendi and Hudeiba locations, stunt incidence in the 1999–2000 season reached 30 and 62%, respectively. In the other locations disease incidence ranged from 0 to 10% (Table 1).

Laboratory tests

Laboratory testing revealed that 64.7% of chickpea samples collected during the 1996–1997 grow-

ing season were infected with CpCDV; 40% of samples collected and tested during the 1997–1998 growing season were positive for CpCDV and 8% were infected with BWYV (Table 2).

During the 1998–1999 season, 24 out of 26 samples (92.3%) gave a positive reaction to CpCDV antiserum, whereas in the 1999–2000 season 59 out of 60 samples tested (98.3%) were positive to CpCDV antiserum. None of the chickpea samples tested in the three seasons reacted positive to FBNYV-specific or BLRV-specific monoclonals.

Effect of sowing date and cultivar on chickpea stunt incidence

During the 1999–2000 growing season, chickpea stunt occurred naturally at a high level, and enabled the effect of sowing date and cultivar on stunt incidence to be studied. Plots with an early sowing date (25 October) had a disease incidence of 54.8%, whereas those with late sowing (20 December) had an incidence of 19.4% (Table 3). The reduction in stunt incidence between the second and third sowing date was not significant. Although both cultivars were susceptible to CpCDV infection, Shendi was less susceptible than ICCV-2.

During the 2000–2001 growing season, high infection with wilt and root rots wiped out the plots planted with Shendi and data were collected only from ICCV-2 plots. Although stunt incidence was very low at Hudeiba during this season, plots sown early had a higher incidence of this disease (Table 3).

In the 2001–2002 season stunt incidence was significantly higher in the crop sown on 29 October (17.3%) than in the crop sown on 26 November (1.7%; Table 3).

Effect of cultivar and irrigation interval on chickpea stunt incidence

Results from the 1999–2000 growing season showed that ICCV-2 was more susceptible to stunting/yellowing associated with CpCDV infection (mean incidence 23.9%) than Shendi (mean incidence 0.5%; Table 4), and also that plots irrigated every 20 days had a higher disease level (20.2%) than plots irrigated every 10 days.

Similarly, during the 2000–2001 growing season, ICCV-2 was more susceptible to CpCDV infection than Shendi, and here too longer irrigation intervals significantly increased stunt incidence. The same trend continued in the 2001–2002 growing season, when disease incidence was higher with irrigation intervals of 28 days (28.1%) than with intervals of 7 days (5.9%). ICCV-2 was again more susceptible than Shendi (Table 4). Although more frequent irrigation increased the cost of production, gross benefit outweighed the cost because of yield increase (Table 5).

Table 1. Average incidence (%) of chickpea stunt based on symptoms observed in some randomly selected chickpea fields in River Nile State, northern Sudan, during the 1996–1997 and 1999–2000 growing seasons.

Location	1996–1997		1999–2000	
	No. of fields surveyed	Average disease incidence (%)	No. of fields surveyed	Average disease incidence (%)
Wad Hamid				
Salwa-North	5	25.6	5	30
Salwa-South	5	8.5	0	-
Hagr Eltir	3	5.7	0	-
Berber	0	-	4	25
Rubatab				
Bushtanab	3	8.3	0	-
Harasha	3	6.8	0	-
Zooma	3	2.2	0	-
Shendi	0	-	5	30
Hudeiba	2	7.0	4	62

Table 2. Virus screening tests on 264 chickpea samples with stunting and yellowing symptoms, collected from fields in River Nile State, northern Sudan, in 1996–2000.

Growing season/Location	No. of samples tested	No. of samples positive for	
		CpCDV[a]	BWYV[b]
1996–1997			
Wad Hamid			
Salwa (north)	32	25	0
Salwa (south)	22	9	0
Hudeiba Research Station	99	65	0
1997–1998			
Hudeiba	15	7	2
Nahr Atbra	10	3	0
1998–1999			
Hudeiba	26	24	0
1999–2000			
Salwa	16	16	0
Berber	13	12	0
Hudeiba	19	19	0
Shendi	12	12	0
Total 1999–2000	60	59	0

[a] Chickpea chlorotic dwarf virus.
[b] *Beet western yellows virus*.

Table 3. Effect of sowing date and cultivar on chickpea stunt incidence and chickpea yield (kg ha^{-1}), at Hudeiba, Sudan, during three growing seasons (1999–2002).

Growing season/ Sowing date	Chickpea cultivar			
	ICCV-2		Shendi	
	Virus incidence (%)	Yield (kg ha^{-1})	Virus incidence (%)	Yield (kg ha^{-1})
1999–2000[a]				
25 October	93.1	177.2	16.5	770.2
22 November	29.2	720.5	4.9	1096.2
20 December	28.4	610.6	1.4	816.3
2000–2001[b]				
23 October	8	950	-	-
6 November	5.6	1275.8	-	-
20 November	0	2050	-	-
2001–2002[c]				
29 October	28.5	875	6.1	1190
5 November	18.9	1005	6.2	1275
12 November	8.1	1703	1.1	2150
19 November	4.7	1911	0.7	2400
26 November	3.4	1116	0	2360

[a] SE for cultivar ± 1.3, for sowing date ± 1.6, and for yield ± 210.5.
[b] SE for sowing date was ± 0.19, and for yield ± 151.2.
[c] SE for cultivar ± 0.4, for sowing date ± 0.6, and for yield ± 121.

Table 4. Effect of irrigation intervals on chickpea stunt incidence at Hudeiba during the 1999–2000 and 2001–2002 growing seasons in two cheackpea cultivars, ICCV-2 and Shendi.

Growing season/ Irrigation interval	Total No. of irrigations	Chickpea stunt incidence (%)	
		ICCV-2	Shendi
1999–2000[a]			
10 days	9	8.5	0.02
20 days	5	39.4	0.9
2001–2002[b]			
7 days	11	10.2	1.7
14 days	5	27.6	2.8
21 days	4	26.8	5.1
28 days	3	46.9	9.2

[a] SE for cultivar was ± 1.4, and for irrigation interval ± 1.4.
[b] SE for cultivar was ± 1.7, and for irrigation interval ± 2.4.

Table 5. Economic benefit obtained by irrigation in controlling the spread of chickpea stunt incidence in chickpea during the 1999–2000 growing season.

Irrigation interval	No. of irrigations	Total cost of irrigation (LS/ha)[a]	Yield (kg ha^{-1})	Gross benefit (LS)[b]	Net benefit (LS)[c]
10 days	9	315,000	1,995	1,995,000	1,680,000
20 days	5	175,000	1,001	1,001,000	826,000

[a] US$ 1= 2500 LS (Sudanese pounds).
[b] Calculated as average yield of the two cultivars ICCV-2 and Shendi (kg ha^{-1}) multiplied by the value of chickpea (LS 1000 kg^{-1}).
[c] Gross benefit minus total cost of irrigation.

Discussion

Surveys and laboratory tests revealed that CpCDV was the most frequent virus in Sudan's main chickpea producing area, and it is probably the main causal agent of chickpea stunt in that region, with yellowing and stunting the main symptoms. The importance of this virus is due to the fact that it can cause more than 90% yield loss in chickpea, even when infection occurs late in the growing season (Hamed, 2000).

It was expected that luteoviruses, particularly BLRV, would be detected in the samples since these viruses are the most likely to infect legume crops. Before CpCDV was identified, and before its antiserum had become available, most virus diseases of faba bean and chickpea causing yellowing and leaf roll were assumed to be caused by BLRV. Apparently, however, viruses such as CpCDV and FBNYV, are more likely than BLRV to cause yellowing and stunting in legume crops in this region. The possible involvement of different viruses was suggested by Bos (1982). It is likely that CpCDV has occurred in legume crops in Sudan for a long time, as yellowing and stunting of chickpea and faba bean were previously observed (Bos, 1982).

Field observations indicated that cultivar ICCV-2 was more susceptible to CpCDV infection than Shendi. Two neighboring fields at the HRSF, one planted with ICCV-2 and one with Shendi had natural stunt incidences of 90 and 30% respectively. Moreover, it was noticed that early sown fields containing a lot of weeds had a higher stunt incidence than late sown fields that were free from weed infestation.

Early sowing of chickpea increased the spread of stunt disease. In practical terms, virus spread

started to decrease after the mid-November sowing, probably because of reduced vector activity at that time. A drop in temperature in December was probably responsible for a reduction in the numbers and movement of the leafhopper vector (*Neolimnus aegyptiacus*), which reduced disease spread.

The availability of many leguminous weeds that survive throughout the year and act as a reservoir for CpCDV suggests that it is the leafhopper vector population, and not the plant hosts of the virus, that limits virus spread at Hudeiba. Longer irrigation intervals seem to encourage leafhopper vector activity, but a humid atmosphere depresses it. An alternative explanation could be that longer intervals allow less natural vegetation to spring up and consequently leafhopper vectors have to feed on chickpeas. Results from the three growing seasons (1999–2000, 2000–2001 and 2001–2002) confirmed that CpCDV spread in northern Sudan was reduced by late sowing, partial cultivar resistance, and short irrigation intervals (7 or 10 days). These short irrigation intervals not only reduced CpCDV spread but also led to a substantial increase in yield.

Literature cited

Bos L., 1982. *Virus Diseases of* Vicia faba *and Some Other Crops in the Nile Valley (Sudan and Egypt) and the Involvement of ICARDA*. Report of a consultancy visit, April 24–May 4, IPO/ICARDA. 10 pp.

Carazo G., C. DeBlas, M. Saiz, J. Romero and S. Castro, 1993. Virus diseases of chickpea in Spain. *Plant Disease* 77, 210.

Franz A., K.M. Makkouk, L. Katul and H.J. Vetten, 1996. Monoclonal antibodies for the detection and differentiation of faba bean necrotic yellows virus isolates. *Annals of Applied Biology* 128, 255–68.

Hamed A.A., 2000. *Assessment of Yield Losses in Chickpea due to Stunt Disease*. Agricultural Research Corporation, Hudeiba Research Station, Annual Report 1999–2000, Ed-Damer, Sudan, 13 pp.

Horn N.M., S.V. Reddy, I.M. Roberts and D.V.R. Reddy, 1993. Chickpea chlorotic dwarf virus, a new leafhopper-transmitted geminivirus of chickpea in India. *Annals of Applied Biology* 122, 467–479.

Horn N.M, S.V. Reddy, J.F.J.M. van den Heuvel and D.V.R. Reddy, 1996. Survey of chickpea (*Cicer arietinum* L.) for chickpea stunt disease and associated viruses in India and Pakistan. *Plant Disease* 80, 286–290.

Hsu H.T. and R.H. Lawson, 1991. Direct tissue blotting for detection of tomato spotted wilt virus in *Impatiens*. *Plant Disease* 75, 292–295.

Katul L., 1992. *Characterization by Serology and Molecular Biology of Bean Leaf Roll Virus and Faba Bean Necrotic Yellows Virus*. PhD thesis, University of Göttingen, Germany, 115 pp.

Lin N.S., Y.H. Hsu and H.T. Hsu, 1990. Immunological detection of plant viruses and a mycoplasma like organism by direct tissue blotting on nitrocellulose membranes. *Phytopathology* 80, 824–828.

Makkouk K.M, G. Daffalla, M. Hussein and S.G. Kumari, 1995. The natural occurrence of chickpea chlorotic dwarf geminivirus in chickpea and faba bean in Sudan. *Phytopathology* 143, 465–466.

Mohamed A.S. and H.A. Van Rheenen, 1991. A rapid survey of chickpea cultivation, Wad Hamid, Sudan 1989–1990. *International Chickpea Newsletter* 23, 31.

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