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Viral diseases of cultivated legume and cereal crops in Tunisia

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Summary. A survey to identify virus diseases affecting legume (chickpea, faba bean and lentil) and cereal (bread and durum wheat and barley) crops at different locations in Tunisia was conducted in April, 2000. Thirty-eight legume fields (34 faba bean, 3 chickpea and one lentil) and 43 cereal fields (21 durum wheat, 7 bread wheat and 15 barley) were randomly selected. The identity of the viruses and virus disease incidence was determined on 100-200 randomly collected samples and 20-25 symptomatic samples from each field by testing samples against antisera of 12 legume and 6 cereal viruses. In the faba bean fields, *Broad bean mottle virus* (BBMV) and *Beet western yellows virus* (BWYV) were the most common followed by *Faba bean necrotic yellows virus* (FBNYV). In cereal fields, *Barley stripe mosaic virus* (BSMV) was the most common followed by *Barley yellow dwarf virus* (BYDV) and *Barley yellow striate mosaic virus* (BYSMV). Two faba bean fields had a virus disease incidence of 21% or higher at the time of the survey and two cereal fields had a virus disease incidence of more than 6%. The highest incidences found were 24.5% (BWYV and/or FBNYV) in the Siliana region and 23% (BBMV and/or BWYV) in the Jendouba region, both in faba bean fields, and 10.5% (BSMV) in a barley field in the Cap-Bon region. Other viruses, such as *Broad bean stain virus* (BBSV), *Bean leaf roll virus* (BLRV) and *Cucumber mosaic virus* (CMV) in legumes, and *Wheat dwarf virus* (WDV) in cereals were rare.

Key words: Tunisia, cereals, legumes, viruses, survey.

Introduction

Cereal and legume crops (particulary faba bean) are important and widely cultivated field crops in Tunisia and are good sources of protein and calories for a large part of the population. In Tunisia, the total area under cereal cultivation during the 1999-2000 cropping season was around 857,510 ha of durum wheat, 133,440 ha of bread wheat (*Triticum aestivum* L.) and 594,520 ha of barley (*Hordeum vulgare* L.). In food legumes, around 34,600 ha of faba bean (*Vicia faba* L.), 12,450 ha of winter and spring chickpea (*Cicier aritenum* L.) and 1400 ha of lentil (*Lens culinaris* Medik.) were planted.

Worldwide, legumes can become naturally infected with more than 45 viruses, 12 of which have been reported from West Asia and North Africa (WANA) (Makkouk, 1994). In Tunisia, eight viruses have so far been identified to affect faba bean: *Broad bean mottle virus* (BBMV), *Broad been stain virus* (BBSV), *Broad bean true mosaic virus* (BBTMV), *Broad bean wilt virus* (BBWV), *Bean yellow mosaic virus* (BYMV), *Cucumber mosaic virus* (CMV), *Pea enation mosaic virus* (PEMV) and *Pea seed-borne mosaic virus* (PSbMV) (Makkouk *et al*., 1988). These viruses are seed-borne in legumes (Makkouk and Azzam, 1986; Bos *et al*.,1988; Mak-

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kouk *et al.,* 1988, 1990b; Fortass and Bos 1992; Jones and Coutts, 1996).

Many viruses are reported as affecting cereal crops, a few of which also occur in WANA. *Barley yellow dwarf virus* (BYDV) affects cereals in many North African countries including Tunisia (El-Yamani and Hill, 1990; Makkouk *et al*., 1990a). *Barley yellow striate mosaic virus* (BYSMV) has been detected in Morocco (Lockhart and El-Yamani, 1983), while *Barley stripe mosaic virus* (BSMV) and *Wheat streak mosaic virus* (WSMV) were detected in Algeria (Boubetra *et al*., 1999).

Viral diseases of cereal and legume crops in Tunisia have not been extensively identified and no information is available on the incidence of viruses affecting these crops. The main objective of this study was to determine which viruses affect legume and cereal crops in the major production areas of Tunisia and to determine their incidence and importance.

Materials and methods

Field surveys and samples collection

A total of 38 legume and 43 cereal fields were randomly selected, and distance between fields was determined on a daily basis. The fields were located in the following districts: (1) Beja region (Borj El-Amri, Mejez El-Bab, Goubellat, Testour, Beja); (2) Bizerte region (Utique, Bizerte, Bechateur, Nkhilat, Menzel Bourguiba, Khetmine, Mateur, Sebaa Aouinat, Fritissa); (3) Cap-Bon region (Menzel Bou Zelfa, Menzel Temime); (4) Jendouba region (Bou Salem, Fernana, Jendouba, Le Kef); (5) Kairouan region (Le Fahs, Kairouan); (6) Siliana region (Le Krib, Siliana), and (7) Zaghouan region (Mohammedia, Bir Mecherga, Siminja, Zaghouan, Le Fahs). The location of the fields is shown in Fig. 1. Each field surveyed was evaluated using a standard format. Virus disease incidence in each field was determined (i) on the basis of visual symptoms and (ii) by counting the percentage of infected plants at different randomly chosen locations in the field. From each field two types of samples were collected; from 20-25 symptomatic plants, and from 100-200 randomly collected plants. Samples were placed in labelled plastic bags and brought to the Virology Laboratory of INRAT in Tunis for testing.

A total of 778 legume samples (674 faba bean, 44 chickpea and 60 lentil) with symptoms suggestive of virus infection, and 4388 randomly chosen legume samples (4033 faba bean, 195 chickpea and 160 lentil) from 34 faba bean, 3 chickpea and one lentil field were collected. A total of 1430 cereal samples (451 barley, 746 durum wheat and 233 bread wheat) with symptoms suggestive of virus infection, and 5227 randomly chosen cereal samples (1654 barley, 2546 durum wheat and 1027 bread wheat) from 15 barley, 21 durum wheat and 7 bread wheat fields were collected.

Laboratory tests

All the legume and cereal samples were tested for the presence of viruses by using the tissue blot immunoassay (TBIA, Makkouk and Kumari, 1996) at the Virology Laboratory of INRAT, Tunis.

Legume crops

All legume samples were tested against a battery of polyclonal and monoclonal antibodies. Rabbit polyclonal antiserum for chickpea chlorotic dwarf virus (CCDV) was provided by D.V.R. Reddy, ICRISAT, India. BBSV, PSbMV, BYMV, BBWV, BBMV, CMV, *Alfalfa mosaic virus* (AMV) and *Pea early browning virus* (PEBV) polyclonal antisera were supplied by the Virology Laboratory of ICARDA, Aleppo, Syria. In addition, two monoclonal antibodies for the detection of *Faba bean necrotic yellows virus* (FBNYV) and a broad-spectrum legume luteovirus (5G4) were provided by A. Franz and L. Katul, BBA, Braunschweig, Germany.

To identify the individual luteoviruses affecting legume crops in Tunisia, infected samples that gave a positive reaction to the broad-spectrum monoclonal 5G4 were further tested against five specific monoclonals: *Beet western yellows virus* (BWYV) (ATCC PVAS-647), *Soybean dwarf virus* (SbDV) (ATCC PVAS-650), *Potato leaf roll virus* (ATCC PVAS-649), *Bean leaf roll virus* (BLRV) (4B10), and 6F9, a monoclonal that reacts with either BLRV or SbDV (Katul, 1992).

Cereal crops

For the detection of viruses in cereals, five rabbit polyclonal antisera were used: BYSMV was provided by E. Luisoni, IFA, Torino, Italy; *Wheat dwarf virus* (WDV) by J. Vacke, Research Institute of Crop Production, Prague, Czeck Republic; and

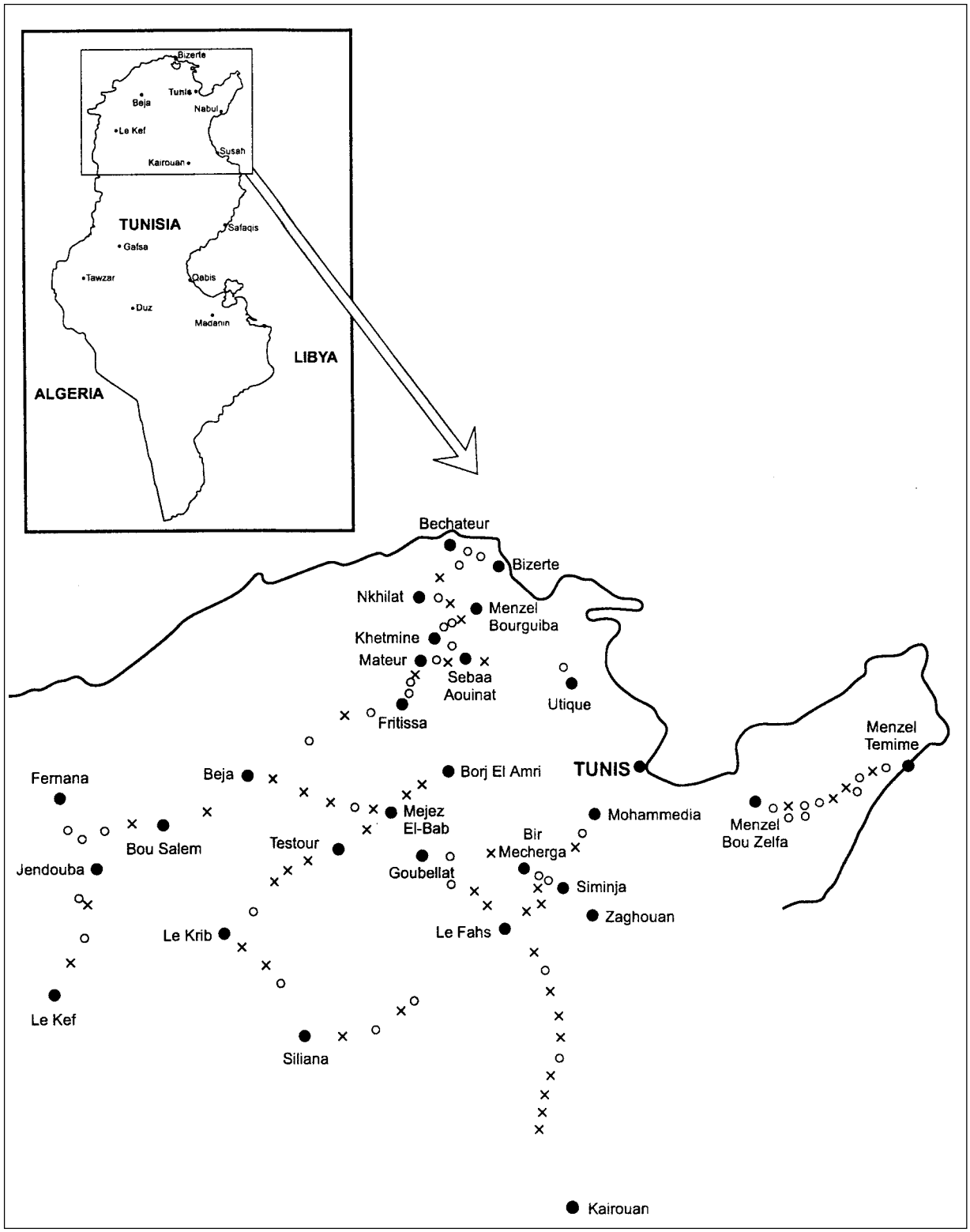

Fig. 1. Map of Tunisia showing the location of cereal (×) and legume (○) fields surveyed, April, 2000.

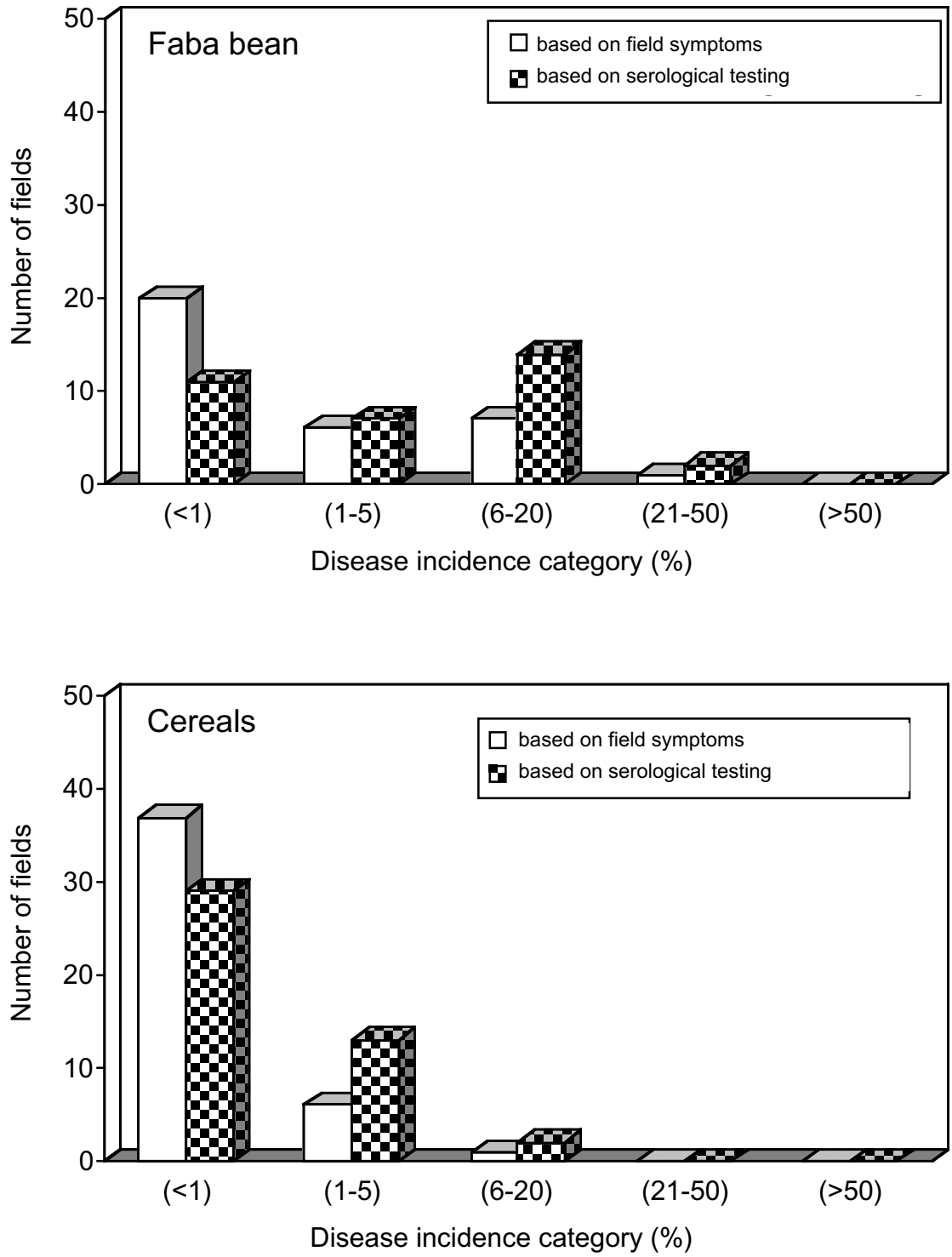

Fig. 2. Virus infection incidence in faba bean and cereal crops based on symptoms observed in the field and laboratory testing of randomly collected samples. Survey conducted in Tunisia, April, 2000.

BYDV-PAV, BSMV and WSMV by the Virology Laboratory of ICARDA, Aleppo, Syria. Infected samples that gave a positive reaction to BYDV-PAV polyclonal antiserum were further tested against two specific monoclonal antibodies, BYDV-MAV (ATCC PVAS-673) and BYDV-RPV (ATCC PVAS-669).

Results

Virus disease incidence observed in the fields

Legume crops

The virus symptoms most commonly observed in faba bean fields were mottling, mosaic, chlorosis, stunting and yellowing. Virus disease incidence based on field inspection is summarized in Table 1. Twenty-eight legume fields (76%) had a virus incidence of 5% or less, and only one faba bean field had a virus incidence more than 20%.

Cereal crops

The symptoms observed in barley and wheat fields were striping, stunting and yellowing of the leaves. In general, virus disease incidence in cereals was lower than in legumes (Table 1). Most cereal fields (84%) had a virus incidence of less than 1%; in the remaining fields the incidence was less than 5% except in one durum wheat field, where it was in the 6-20% category.

Virus identification and incidence based on laboratory testing

Legume crops

Laboratory testing of the 4033 randomly collected faba bean samples indicated that BBMV was the most common (Table 2), with an overall average of 2.3%, followed by the luteoviruses (1.8%), FBNYV (0.9%) and BBSV (0.7%). The incidence of CMV was extremely low (0.02%). The highest incidence in a faba bean field was 24.5% with BWYV and/or FBNYV in the Siliana region and 23% (BBMV and/or BWYV) in a faba bean field in the Jendouba region. Testing of symptomatic samples from faba bean fields also indicated that BBMV was the most common, followed by the luteoviruses and FBNYV (Table 2). BBSV and CMV were detected in 19 and 4 out of 674 symptomatic samples respectively. Of the 44 symptomatic chickpea and 60 symptomatic lentil samples tested, luteoviruses were found in only 7 chickpea and 2 lentil samples.

When 201 samples (192 faba bean, 7 chickpea and 2 lentil) that gave a positive reaction with the broad-spectrum legume luteovirus monoclonal an-

Table 1. Virus infection incidence in legume and cereal crops based on symptoms observed in the field and the laboratory testing of randomly collected samples. Survey conducted in Tunisia, April, 2000.

Crop	No. of fields surveyed	Number of fields with disease incidence category (%) of									
		<1		1–5		6–20		21–50		>50	
		S[a]	L[b]	S	L	S	L	S	L	S	L
Faba bean	34	20	11	6	7	7	14	1	2	0	0
Lentil	1	0	1	0	0	1	0	0	0	0	0
Chickpea	2	2	2	0	0	0	0	0	0	0	0
Legumes subtotal	37	22	14	6	7	8	14	1	2	0	0
Barley	15	12	7	3	6	0	2	0	0	0	0
Durum wheat	21	18	14	2	7	1	0	0	0	0	0
Bread wheat	7	6	7	1	0	0	0	0	0	0	0
Cereals subtotal	43	36	28	6	13	1	2	0	0	0	0
Total	80	58	42	12	20	9	16	1	2	0	0

[a] based on symptoms observed in the field.
[b] based on laboratory testing of randomly collected samples.

Table 2. Results of laboratory tests on legume samples randomly collected and with symptoms suggestive of virus infection from 37 fields in Tunisia in April, 2000. Viral identification was based on serological reactions (Tissue Blot Immunoassay).

Region	Sample collection method	No. of fields surveyed	No. of samples tested	Number of samples found positive to[a]					No. of samples negative to all antisera used	Total incidence (%)[b]
				FBNYV	5G4 Luteov.	BBMV	BBSV	CMV		
Faba bean										
Cap-Bon	Random	6	528	0	4	5	6	0	511	8.2
	Symptoms	6	83	0	28	16	2	0	36	
Beja	Random	5	758	2	7	53	0	0	696	5.7
	Symptoms	5	123	5	5	87	1	0	30	
Jendouba	Random	4	512	8	1	23	0	0	483	4.1
	Symptoms	4	127	9	6	47	0	0	65	
Bizerte	Random	9	1163	6	6	10	22	0	1116	10.7
	Symptoms	9	109	8	6	13	16	0	66	
Siliana	Random	3	280	7	23	0	0	0	250	6.4
	Symptoms	3	63	7	25	0	0	2	29	
Zaghouan	Random	5	561	10	20	0	0	0	525	6.9
	Symptoms	5	80	22	18	6	0	0	45	
Kairouan	Random	2	231	2	13	0	0	1	215	6.9
	Symptoms	2	89	2	30	2	0	2	35	
Subtotal	Random	34	4033	35	74	91	28	1	3796	5.9
	Symptoms	34	674	53	118	171	19	4	306	
Chickpea										
Cap-Bon	Random	2	195	0	0	0	0	0	195	0
	Symptoms	2	35	0	0	0	0	0	35	
Bizerete	Symptoms	1	9	0	7	0	0	0	2	
Lentil										
Bizerte	Random	1	160	0	0	0	0	0	160	0
	Symptoms	1	60	0	2	0	0	0	58	
Total	Random	37	4388	35	74	91	28	1	4151	5.3
	Symptoms	38	778	53	127	171	19	4	401	

[a] All samples were negative to CCDV, BYMV, PSbMV, BBWV, AMV, PEMV and PEBV.

FBNYV= *Faba bean necrotic yellows virus* BBSV= *Broad bean stain virus*
CCDV= *Chickpea chlorotic dwarf virus* PSbMV= *Pea seed-borne mosaic virus*
BYMV= *Bean yellow mosaic virus* BBWV= *Broad bean wilt virus*
BBMV= *Broad bean mottle virus* CMV= *Cucumber mosaic virus*
PEMV= *Pea enation mosaic virus* AMV= *Alfalfa mosaic virus*
PEBV= *Pea early browning virus* 5G4= *A broad spectrum monoclonal reacting with all legume luteoviruses*

[b] Total incidence was calculated only from samples collected at random.

tibody (5G4) were tested with specific monoclonals, at least two luteoviruses BWYV and BLRV were identified (Table 3).

Cereal crops

Laboratory testing of 1654 randomly collected barley samples (Table 4) indicated that BSMV was the most common, followed in order by WDV, BYSMV and BYDV-PAV. When 2546 durum wheat and 1027 bread wheat samples randomly collected were tested in the lab, results showed that there was a slightly higher incidence of virus infection in durum than in bread wheat. In durum wheat, BYDV-PAV was the most common, followed

by BSMV, BYSMV and WDV. In bread wheat, BYDV-PAV and WDV were detected in 0.2% of samples, whereas BSMV and BYSMV were not detected. Testing of the symptomatic cereal plants gave a more or less similar pattern in terms of the viruses identified and their relative occurrence in the field (Table 4). The highest virus disease incidence in cereals was in a single barley field with 10.5% incidence of BSMV in the Cap-Bon region.

Discussion

In general, virus incidence in both cereal and legume crops was low in Tunisia during the 1999/2000 growing season, and slightly lower in cereals than in legumes. However, this growing season was relatively dry, with a reduced vector population, and consequently a lower chance of virus spread. And indeed, in most of the fields surveyed the aphid population was low.

With faba bean average virus incidence was around 6%; BBMV was the most common here with an overall average of 2.3% in randomly collected samples, and it was detected in all seven regions except Siliana. BBMV is seed-transmitted in faba bean (Makkouk *et al*., 1988; Fortass and Bos, 1992) and transmissible by several beetle species, such as *Apion* sp. and *Sitona* sp. (Makkouk and Kumari, 1995). In this survey, around 60% of faba bean fields were found infested with *Sitona* sp.. Under such conditions, using healthy seeds should be an important element in faba bean production. Making virus-free seeds available to the farmers in Tunisia could significantly reduce the damage caused by this virus.

FBNYV has been reported to infect food legumes in many countries in WANA (Katul *et al*., 1993; Horn *et al*., 1995; Makkouk *et al*., 1992a, 1998). Even though this virus reaches epidemic proportions in some countries, such as Egypt (Makkouk *et al*., 1994), its incidence in Tunisia, as revealed by this study, was low. Moreover, the survey was conducted during early April, when only a few fields were approaching maturity and most were still at the flowering stage. Virus incidence levels reported here could be expected to increase further towards the end of the growing season if environmental conditions were favorable for the increase and spread of the aphid vector populations. However, because of the damage this virus can cause, it needs closer monitoring in coming years.

At least two luteoviruses, BWYV and BLRV, were identified in this study. Out of 152 samples that reacted with the specific monoclonal of BWYV, 105 also gave a positive reaction with another monoclonal, 6F9 which is known to react with either BLRV or SbDV (Katul, 1992). This suggests that the BWYV identified in Tunisia could be a slightly different variant of the ones identified earlier by Katul (1992).

Cereals were less affected than faba bean by viruses in this season. Special attention must be given to BSMV in barley. The highest BSMV incidence in cereals 10.5%, was in a single barley field

Table 3. Reaction with specific monoclonal antibodies of legume samples infected by luteoviruses collected from Tunisia during April 3–12, 2000.

Crop	No. of samples tested	Monoclonal antibodies						
		5G4 (broad)	4B10 (BLRV)	ATCC 650 (SbDV)	ATCC 647 (BWYV)	ATCC 649 (PLRV)	3B11 (BLRV or SbDV)	6F9 (BLRV or SbDV)
Faba bean	98	+	-	-	+	-	-	+
Faba bean	47	+	-	-	+	-	-	-
Faba bean	47	+	+	-	-	-	+	+
Chickpea	7	+	-	-	+	-	-	+
Lentil	2	+	+	-	-	-	+	+

Table 4. Results of laboratory tests on cereal samples randomly collected and with symptoms suggestive of virus infection, from 43 fields in Tunisia, during April, 2000. Viral identification was based on serological reactions (Tissue Blot Immunoassay).

Region	Sample collection method	No. of fields surveyed	No. of samples tested	Number of samples found positive to[a]				No. of samples negative to all antisera used	Total incidence (%)[b]
				BYDV-PAV	BSMV	BYSMV	WDV		
Barley									
Beja	Random	4	460	0	0	3	0	457	0.7
	Symptoms	4	151	1	0	0	0	150	
Cap-Bon	Random	2	183	0	10	0	2	171	6.6
	Symptoms	2	51	4	14	0	0	43	
Kairouan	Random	4	435	0	2	0	3	430	1.2
	Symptoms	4	65	0	1	0	0	64	
Siliana	Random	1	128	0	0	0	0	128	0.0
	Symptoms	1	40	0	0	0	0	40	
Zaghouan	Random	4	448	2	10	0	0	436	2.7
	Symptoms	4	144	0	12	0	0	132	
Sub-total	Random	15	1654	2	22	3	5	1622	1.9
	Symptoms	15	451	5	27	0	0	429	
Durum wheat									
Beja	Random	4	436	0	0	5	0	431	1.2
	Symptoms	4	166	1	3	5	0	157	
Bizerte	Random	6	743	4	4	3	1	735	1.1
	Symptoms	6	215	7	1	0	1	203	
Cap-Bon	Random	2	240	0	4	0	0	236	1.7
	Symptoms	2	55	0	13	0	0	42	
Jendouba	Random	2	215	0	0	0	0	215	0.0
	Symptoms	2	69	3	0	3	1	63	
Kairouan	Random	3	415	7	0	0	0	408	1.7
	Symptoms	3	95	3	0	0	0	92	
Siliana	Random	1	112	0	0	0	0	112	0.0
	Symptoms	1	36	0	0	0	6	30	
Zaghouan	Random	3	385	0	0	0	0	385	0.0
	Symptoms	3	110	0	0	0	0	110	
Sub-total	Random	21	2546	11	8	8	1	2522	0.9
	Symptoms	21	746	14	17	8	8	697	
Bread wheat									
Beja	Random	2	372	1	0	0	2	369	0.8
	Symptoms	2	72	3	0	1	2	66	
Jendouba	Random	2	216	1	0	0	0	215	0.5
	Symptoms	2	54	2	0	2	0	50	
Kairouan	Random	1	195	0	0	0	0	195	0.0
	Symptoms	1	35	1	0	0	0	34	
Siliana	Random	2	244	0	0	0	0	244	0.0
	Symptoms	2	72	0	0	1	0	71	
Sub-total	Random	7	1027	2	0	0	2	1023	0.4
	Symptoms	7	233	6	0	4	2	221	
Total	Random	43	5227	15	30	11	8	5167	1.1
	Symptoms	43	1430	25	44	12	9	1347	

[a] All samples were negative to WSMV.
BYDV-PAV= *Barley yellow dwarf virus* BSMV= *Barley stripe mosaic virus*
WSMV= *Wheat streak mosaic virus* WDV= *Wheat dwarf virus*
BYSMV= *Barley yellow striate mosaic virus*

[b] Total incidence was calculated only from samples collected at random.

in the Cap-Bon region. BSMV is exclusively seed-borne (Makkouk *et al*., 1992b) and using healthy seed reduces the damage caused by this virus. Our field observations suggest that farmers in the Cap-Bon region were in fact using their own seeds.

BYDV was reported in Tunisia during 1985/1986 growing season with an average incidence of 24.5% (Makkouk *et al*, 1990a), but, in the dry 1999/2000 growing season its incidence was extremely low (less than 1%). This was probably due to limited vector activity during this season. Information on the coming 2-3 growing seasons will give a clearer picture of the economic importance of BYDV in cereal crops in Tunisia.

The study showed that visual field inspection tended to underestimate virus disease incidence in both legume and cereal fields. In faba bean, 35% of fields surveyed had a virus disease incidence of 6% or more based on laboratory testing, but by field inspection only 24% of the faba bean fields were in that disease category. In cereals, only one field was placed in the 6-20% incidence category by visual inspection, whereas two fields were found to be in that category by lab testing.

In many of the fields surveyed, especially those with cereals, large sectors of poor growth were observed, suggesting a problem related to the soil (e.g. nematodes, soil-borne fungi or viruses vectored by lower fungi). The stress created by drought conditions made it easy to distinguish between healthy plants and plants infected at the seedling stage (Van Leur *et al*., 1991). It is well known that early season vigour makes the plant more drought resistant. At the seedling stage, when cereals are infected with a soil-borne agent, their root system is reduced and consequently cannot extract enough moisture to maintain good growth. Because of its importance for crop yield at country level, this phenomenon requires further investigation to identify the agents involved.

In this survey a number of viruses were detected in Tunisia for the first time, BWYV and FBNYV in legume and BSMV, BYSMV and WDV in cereals, the last two being vectored by leafhoppers. A preliminary note on this has recently been published (Najar *et al*., 2000a; 2000b). Further monitoring of these viruses will be conducted in coming seasons.

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