Australian Government

Australian Centre for International Agricultural Research

Final report

Project full title	**Survey of lentil viral diseases in Nepal**
project ID	CROP/2024/106
date published	April 2025
prepared by	Joop van Leur, Safaa Kumari, Ram Khadka
co-authors/ contributors/ collaborators	
approved by	
final report number	FR2024-038
ISBN	978-1-923261-35-8
published by	ACIAR GPO Box 1571 Canberra ACT 2601 Australia

Contents

1 Acknowledgments

The Governments of Nepal and Australia (through the Australian Centre for International Agricultural Research, ACIAR) collaboratively funded the project. Support was also received from the New South Wales State Government, which allowed the NSW Department of Primary Industries to take the administrative project lead. The International Center for Agricultural Research in the Dry Areas (ICARDA) provided expertise on virus survey techniques and virus diagnostics.

The Nepal Agricultural Research Council greatly supported the project by making staff and research facilities available. Students of the Agricultural and Forestry, Tribhuvan and Purbanchal universities and NARC staff enthusiastically participated in survey and laboratory activities. Without their contributions it would have been impossible to cover the large survey area in such a short time.

The project would not have been possible without the backing by Dr Eric Huttner, Program Manager for Crop Improvement Management, ACIAR, who wholeheartedly supported an untested initiative at very short notice.

2 Executive summary

Lentil (*Lens culinaris*) is the most widely grown legume crop in Nepal and a major source of protein for the Nepalese people. It is also an ancient crop, most likely introduced to the Indian subcontinent around 2,000 BC. Nepalese lentils are popular in the south-Asian market because of their taste and nutritional quality, and lentil has been a main export commodity for Nepal in the past. Increasing production to meet the needs of a growing population is hampered by a range of abiotic and biotic stresses. Lentils are a preferred host for several aphid species, but while aphid feeding damage is frequently reported in Nepali lentil crops, little attention has been given to their role as virus vectors. Virus-like symptoms have been reported to be common in crops, but no systematic surveys were undertaken to identify and quantify virus species. Fungal diseases like Stemphylium blight and Fusarium wilt have been recognised as yield limiting factors and the national breeding program is focused on incorporating resistance to these two diseases. However, breeding for virus resistance has yet to be addressed.

The aim of the project was to address this information gap and to assess whether viruses are a current or potential threat to the Nepali lentil production. Most lentil growing environments in the country were covered with 90 lentil farmers' fields surveyed in relatively short time by both random sampling and sampling of symptomatic plants. In addition, lentil breeding lines in four research stations were sampled as well as 7 chickpea fields and 6 faba bean fields.

Virus presence of sampled plants was diagnosed with Tissue blot immuno assay (TBIA). TBIA is a reliable, cost effective and relatively simple virus diagnostic tool, which is particularly suited to the evaluation of large numbers of individual plants against virus-specific antibodies. TBIA testing can be complemented by molecular diagnostics for those virus species for which highly specific antibodies are not available.

Symptomatic plants were tested against 15 virus species or virus group specific antibodies and randomly collected plants against the 3 antibodies that showed the highest incidences when testing the symptomatic samples. The main viruses found were luteoviruses, largely not yet specified to species level, and Pea seed-borne mosaic virus (PSbMV). Incidences of both viruses reached yield damaging levels in 11% and 22% of the surveyed fields for luteovirus and PSbMV respectively. PSbMV can be readily seed-transmitted in lentils, which is of particular concern in Nepal where lentils are mainly grown by smallholders who retain their own seed. Very large differences in PSbMV infection levels were recorded among fields at close distance, which could be explained by differences in varieties or seed stock. Three other viruses that are considered important in other lentil growing countries, Alfalfa mosaic virus, Cucumber mosaic virus and Faba bean necrotic yellows virus, were only found in minor incidences.

The survey only yielded a single season of data and follow-up activities are needed to properly ascertain the risk that viruses pose to the Nepalese lentil industry. To do this, a virus diagnostic capacity needs to be developed within the national program. Access to virus diagnostics is also necessary to undertake seed testing for virus presence and to support the national lentil breeding program in efforts to release varieties with adequate resistance to the main viruses.

3 Background

Nepal is among the world's largest lentil producers; 6th in terms of both area and production (United Nations Food and Agriculture Organization, 2024). Lentil is the most important pulse crop in Nepal, accounting for 62% of the total legume area and 64% of the total legume production. Lentils are grown mainly in the lower altitudes of the terai, but also in the mid-hills of the country (Magar *et al.* 2014*)*.

Cultivation of lentils provides a range of benefits to farming systems in terms of soil health and nitrogen fixation, and they are an important source of income for farming families. Nepalese lentils are reputed to be highly nutritious and tasty and form the main ingredient in many traditional Nepalese dishes. Nepalese lentils are popular in the south-Asian market and lentil has been a major export commodity for Nepal in the past. There is potential to expand this capacity and therefore improving lentil production is a high priority for the Nepalese government and for the Nepal Agricultural Research Council (NARC).

Further increases in production are partly hampered by biotic stresses. Fusarium wilt and Stemphylium blight are considered the major diseases and are a main focus of the Nepali National Lentil Breeding Program (Pokhrel *et al.* 2018) and both received attention in an earlier ACIAR project (CIM-1999-064). Various insect pests are reported on lentils in Nepal, with aphids, particularly pea aphids, considered to be the most damaging and can cause complete crop failure (Neupane *et al.* 2020). The potential of aphids to vector viruses in Nepal is mentioned in several reports, but there are no data on virus presence in lentils, except for a short publication by Joshi *et al*. (1993), who reported the presence of Pea seed-borne mosaic virus (PSbMV) in 2 out of 8 seed lots tested. Seed-borne viruses such as PSbMV can be particularly important in Nepal, as the vast majority of Nepalese lentil growers use their own, untested, seed (Magar *et al.* 2014). Dr Ram Khadka (*pers com*) observed high incidences of virus-like symptoms when surveying lentils for fungal diseases, but this could not be confirmed due to a lack of plant virus diagnostic facilities in the country. To progress lentil virology research in Nepal surveys are needed to document virus identity, presence and severity in the highly diverse agro-environments where the crop is grown.

In Asia, Africa and Australia, 13 viruses have been reported to infect lentils naturally (Makkouk *et al.* 2014). Proper diagnostics are essential to identify most of these viruses as similar symptoms can be caused by unrelated viruses and even by abiotic stresses. Breeding for virus resistance can be highly effective and is the most economical method to control viruses, but virus resistance is virus species specific and therefore requires suitable diagnostics in order to be effective.

In Australia, lentil is a relatively new crop that is mainly grown in the southern regions which have a Mediterranean type climate. Australia is currently the 3rd largest producer of lentils in the world with a 3-year (2020-22) average production of 793,000 ton (United Nations Food and Agriculture Organization, 2024). There is potential to increase the area under lentils from the current 500,000 ha to over 700,000 ha, by expanding the crop from the winter rainfall dominated environments into the subtropical climate of central and northern NSW (Dr Garry Rosewarne, *pers com*), an environment that is highly favourable for the development of viruses and aphid vectors. Current Australian lentil varieties have a very narrow genetic base, which needs to be widened to support an expansion (Sadras *et al.* 2021). Lentils have been a major crop in Nepal since ancient times and its traditional farming systems and germplasm have adapted to a wide range of environmental and biological stresses. The Australian lentil industry will therefore greatly benefit from collaboration with Nepalese researchers in pathology, virology and breeding.

4 Objectives

- Identification and quantification of lentil viruses by surveying lentil fields in different Nepali agro-environments.
- Provide training in virus detection using Tissue Blot Immuno Assay (TBIA) to the Nepali national program.

5 Methodology

Survey Methodology

Because of the short time to prepare for the survey and the large area to be covered, the survey was carried out by 6 teams of 3-6 members each, with a total of 21 persons (14 students and 7 NARC staff members) involved. Information such as sowing date, variety name and seed source (own seed or purchased) was obtained from farmers where possible. Observations on crop growth and incidence of virus-like symptoms were taken. Fields were sampled both randomly (50 – 100 plants depending on field size) and for virus-symptomatic plants by taking the top 10 cm of each sampled plant. Plant samples were kept in cool boxes during transport to the laboratory.

Tissue Blot Immuno Assay

Tissue Blot Immuno Assay (TBIA) is a reliable, cost effective and relatively simple virus diagnostic tool, which is particularly suited to surveys of pulse crops as it allows for the evaluation of large numbers of individual plants to a wide range of virus species (Makkouk and Kumari 1996).

Symptomatic samples were blotted using 10 replicates, while random samples were blotted over 4 replicates. The symptomatic samples were tested against 15 antibodies (Tables 1 and 2). Based on the results of the symptomatic samples, random samples were tested for the presence of luteovirus, PSbMV and CMV.

Table 1: Monoclonal (MAb) or polyclonal (PAb) antibodies used for detection of pulse viruses in Nepal, 2024 Survey.

Virus name or virus group	Abbr	Type	Origin
Alfalfa mosaic virus	AMV	PAb	ICARDA (SC10-86)
Bean yellow mosaic virus	BYMV	PAb	ICARDA (SV205-85)
Bean leaf roll virus	BLRV	MAb	BBA, Germany (6G4)
Beet western yellows virus	BWYV	MAb	Agdia, USA (Clone 510H)
Broad bean stain virus	BBSV	PAb	ICARDA (SV173-85)
Chickpea chlorotic dwarf virus	CpCDV	PAb	ICARDA
Chickpea chlorotic stunt virus	CpCSV	MAb	BBA, Germany (Mixed ETH & SYR Mabs)
Cucumber mosaic virus	CMV	PAb	ICARDA (SV36-86)
Faba bean necrotic yellow virus	FBNYV	MAb	BBA, Germany (3-2E9)
Pea enation mosaic virus	PEMV	PAb	DSMZ, Germany (AS-0017)
Pea seed-borne mosaic virus	PSbMV	PAb	ICARDA (SP9-88)
Soybean dwarf virus	SbDV	MAb	Agdia, USA (Clone 29D19)

Table 2: Monoclonal (MAb) antibodies used for detection of pulse virus groups in Nepal, 2024 Survey.

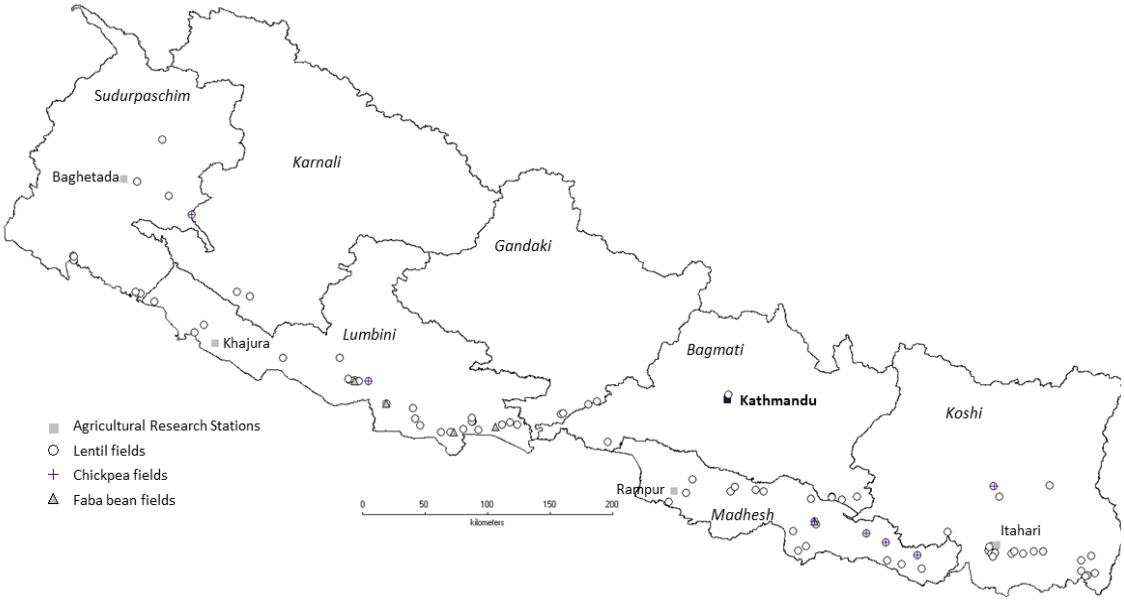
Virus group	Type	Origin
Tospoviruses	MAb	Bioreba, Switzerland (Serogroup I, II, III)
Luteo- & Poleroviruses	MAb	BBA (Katul, 1992), 5G4
Potyviruses	MAb	Agdia, USA (CAB 27200/1000)

6 Achievements against activities and outputs/milestones

Objective 1: The identification and quantification of lentil viruses in different Nepali agro-environments.

A total of 90 lentil famers' fields throughout Nepal's growing regions have been surveyed for virus presence during December 2023 – January 2024 when most crops were in the full flowering – early podding stage. Apart from commercial fields, the presence of viruses was also recorded in experimental lentil fields at four research stations. In addition to lentil crops, 7 chickpea and 6 faba bean crops were surveyed (Figure 1). Virus presence was checked on randomly taken samples and on samples taken from virus-symptomatic plants (Picture 1).

Figure 1. Location of 107 sampled sites, Nepal pulse virus survey, 2024



Picture 1. Sample collection, Nepal pulse virus survey, 2024

Objective 2: To deliver training in virus detection using Tissue Blot Immuno Assay

Training courses on virus diagnostics using Tissue Blot Immuno Assay (TBIA), were given to a total of 40 participants, NARC staff and university students (Picture 2).

Picture 2. Training course in virus diagnostics, participants and instructors, May 2024

7 Key results and discussion

Viruses identified in lentil fields

Out of the 90 lentil farmers' fields, both symptomatic and random samples were taken from 77 fields, from 11 fields only symptomatic samples and from 2 fields only random samples.

From the 88 fields with symptomatic samples, a total of 1,488 plant samples were taken and tested by TBIA against 12 virus specific antibodies and three antibodies that react with virus groups (Table 3). Positives to PSbMV, CMV, FBNYV, BWYV and CpCSV were found as well as positives to luteoviruses, that appear different from the 4 luteoviruses (BLRV, BWYV, CpCSV and SbDV) for which specific antibodies were used. Out of the five identified viruses, only PSbMV was earlier reported in lentils in Nepal (Joshi *et al*. 1993). There are no previous reports of FBNYV, BWYV or CpCSV on lentils or any other pulse crop in Nepal, while CMV has only been reported in cowpea (Acharya and Regmi 2020).

Table 3. Field average (Ave) and highest (High) percentage positives when testing symptomatic lentil samples from 88 farmers' fields for the presence of 15 pulse viruses or virus groups[1].

Province	Sites	Plants	Luteovirus		BWYV		CpCSV		PSbMV		CMV	
			Ave	High	Ave	High	Ave	High	Ave	High	Ave	High
Koshi	26	480	21	73	8	65	6	30	12	85	1	10
Madesh	21	527	21	62	18	50	7	21	17	62	3	52
Bagmati	2	55	14	29	13	25	4	7	20	39	0	0
Gandaki	4	34	16	38	9	25	0	0	6	25	9	38
Lumbini	24	218	7	44	5	29	3	17	10	60	2	27
Karnali	3	44	10	31	6	19	2	6	4	13	0	0
Sudurpaschim	8	130	14	73	9	36	3	11	12	34	1	7
All	**88**	**1488**	**16**	**73**	**10**	**65**	**5**	**30**	**12**	**85**	**2**	**52**

1 Only a single plant in a symptomatic sample from Koshi province was found to be FBNYV positive. None of the symptomatic lentil samples collected from farmers' fields reacted positive to AMV, BLRV, BBSV, CpCDV, PEMV and SbDV. Testing against the general potyvirus antibody showed that PSbMV was the only potyvirus present. Testing of a sample of 160 symptomatic plants for TSWV (tospovirus) did not yield any positives.

A high proportion (70%) of symptomatic lentil samples did not yield positives to any of the antisera used. Differentiating symptoms caused by viruses from those caused by disease and abiotic stresses is difficult in the field, particularly with lentils, but it is well possible that viruses are present that could not be detected with the range of antibodies used in the TBIA tests.

Based on the testing results of the symptomatic samples, the 4,711 random samples taken from 79 lentil farmers' fields were tested for the specific PSbMV and CMV antibodies and for the general luteovirus antibody. Only minor CMV infections were identified, but a surprisingly high level of CMV (30%) was found in one field near Chandrapur, Madesh Province. Infection reached high (>15% infection) and potentially yield damaging levels for luteovirus in 9 fields and for PSbMV in 17 fields (Table 4). Two fields had both high luteovirus and PSbMV infection, one irrigated field near Urlabari, Koshi Province (18% luteovirus and 82% PSbMV) and one rainfed field near Chandrapur, Madesh Province (80% luteovirus and 40% PSbMV).

Table 4. Luteovirus and PSbMV infection levels in randomly collected samples from 79 lentil fields[1].

Province	Sites	Plants	Luteovirus					PSbMV				
			% infection Average (range)	Sites / Infection class				% infection Average (range)	Sites / Infection class			
				0%	>0% ≤5%	>5% ≤15%	>15%		0%	>0% ≤5%	>5% ≤15%	>15%
Koshi	26	1,450	4.9 (0-18)	7	8	9	2	14.3 (0-82)	15	4	1	6
Madesh	19	1,170	9.6 (0-80)	11	1	3	4	18.2 (0-100)	10		2	7
Bagmati	1	50	0	1				0	1			
Gandaki	4	162	0	4				3.5 (0-8)	1	2	1	
Lumbini	20	1,224	2.4 (0-30)	15	2	2	1	6.7 (0-50)	12	2	4	2
Karnali	2	100	17.0 (16-18)				2	2 (0-4)	1	1		
Sudurpaschim	7	555	2.1 (0-12)	4	2	1		13.7 (0-56)	2	1	2	2
All	**79**	**4,711**	**5.1**	**42**	**13**	**15**	**9**	**12.2**	**42**	**10**	**10**	**17**

[1] CMV was only detected in 6 random samples; 3 from Koshi province (4, 4 and 6%), 2 from Madesh province (2 and 30%) and 1 from Lumbini province (4%).

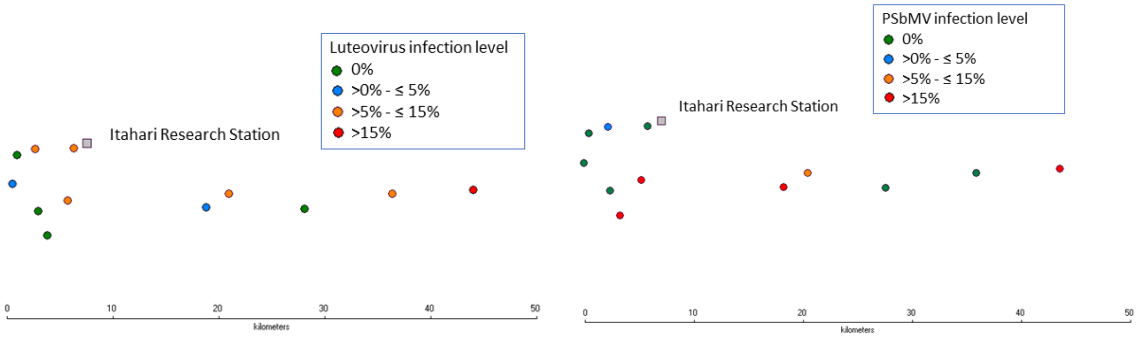
Relation between virus infection and agronomic or geographical factors

For the 79 randomly sampled lentil fields no correlation was found between luteovirus and PSbMV infection levels. There was also no correlation between the infection levels of either virus and the altitude of the surveyed site.

Surveyors classified fields in three categories: “Excellent” (3 fields), “Average” (57 fields) and “Poor” (19 fields). No significant differences were found in virus infection levels among these three categories even though in the “Excellent” rated fields no PSbMV was found, while the “Average” and “Poor” rated fields both averaged 12.7% PSbMV. Luteovirus infection levels were 4.7% for “Excellent”, 4.9% for “Average” and 6.1% for “Poor”.

When asked about the source of their seed, 14 growers used local seed, while in 65 fields an unknown source was used. There was no difference between both seed sources for luteovirus (5.0 vs 5.2% respectively) or PSbMV infection (13.1 vs 12.0% respectively) in the crop.

Figure 2 Luteovirus and PSbMV infection levels in 12 lentil fields in Koshi province.



Although there appeared to be differences among the provinces in both luteovirus and PSbMV infection levels (Table 4), these are not significant because of the wide variation in infection levels among fields. Even at close distance large differences can be noted between fields as is demonstrated in a cluster of 12 fields in the southern part of Koshi province, close to the Itahari Research Station (Figures 2a and 2b).

Viruses identified in Research Stations.

Lentil breeding lines and released varieties grown at the main NARC Pulse Research Station at Khajura, Banke (Lupini Province), were sampled by 10 virus symptomatic plants / entry and tested by TBIA against the same antibodies as symptomatic samples collected from lentil farmers' fields. Luteovirus was found only in the breeding line X2011s-55-9, but large differences were present among the 18 lines for PSbMV (Table 5). Particularly remarkable were the large differences among what appeared to be sister lines from one cross, with two PSbMV free selections (X2011-123-42 and X2011s-55-9 P). Apart from the symptomatic sampling, a random sample of 50 plants was also taken from two lines; HUL-57 showed 2% luteovirus and 50% PSbMV positives; X2011s-55-9 showed 30% luteovirus positives, but no PSbMV.

Table 5. Luteovirus and PSbMV infection levels (based on 10 symptomatic plants / entry) in breeding lines and varieties grown at the NARC Khajura, Banke, Research Station.

Name	%Luteovirus	%PSbMV	Origin / Comments
Khajura Masuro-3	0	0	Released variety
HUL-57	0	0	BHU, Banaras, India
ILL10065	0	0	Poudel *et al*. (2023): high yield
ILL10853	0	20	Poudel *et al.* (2023): high yield
ILL10856	0	0	Neupane *et al* (2020): less susceptible to aphids Poudel *et a*l. (2023): high yield
ILL10947	0	100	IG 156770, no information available
PL-4	0	0	GPPUAT, Pantanagar, India
X2011-123-42	0	0	Local breeding line
X2011s-109-7	0	70	Local breeding line
X2011s-123-26	0	80	Local breeding line
X2011s-163-9	0	40	Local breeding line
X2011s-169-32	0	10	Local breeding line
X2011s-17-6	0	100	Local breeding line
X2011s-183-11	0	90	Local breeding line
X2011s-247-19	0	100	Local breeding line
X2011s-55-9	20	0	Local breeding line
X2011s-55-9	0	40	Local breeding line
X2011s-60-28	0	50	Local breeding line

The variety Khajura Masuro 3 was also sampled for virus presence in the Tajpur Research Station (Madesh Province); no virus was found in 26 symptomatic and 50 random samples.

At the Dipayal Research Station (Sudurpaschim Province) 40% PSbMV positives were detected in 15 symptomatic samples and 10% PSbMV positives in 50 random samples. Sampling of lentils at the Itahari Research Station (Koshi Province) showed 4% luteovirus, 42% PSbMV, 7% AMV and 2% CMV positives in 45 symptomatic samples. AMV has been reported as a virus in chickpea in Nepal, but not in lentils (Acharya and Regmi 2020).

The high level of PSbMV infection in breeding material grown in the main Pulse Breeding Research Station at Khajura is concerning, however this could also provide a great opportunity to identify potential PSbMV resistance in breeding material. Seed harvested from the experimental plots this year should be tested for PSbMV seed transmission before the next season.

Of particular interest is the absence of PSbMV in the variety Khajura Masuro 3 at both the Khajura and Tajpur Research Stations. The variety, selected from a cross involving parents from Argentina, Canada, Bangladesh, was released in 2016, is high yielding, has tolerance to Stemphylium blight and its grain has a high content of zinc and iron. As it is likely sown widely there will be opportunities to confirm its PSbMV resistance in different growing environments.

Viruses identified in chickpea and faba bean.

Samples taken from 7 chickpea and 6 faba bean fields were processed similar to the lentil samples with symptomatic samples tested against 15 antibodies and random samples tested for the presence of luteoviruses, PSbMV and CMV.

Of the five viruses detected in chickpea (Table 6), only AMV has been previously described in this crop in Nepal (Acharya and Regmi 2020). The number of samples taken in the faba bean fields were very limited (Table 7), but of the five viruses detected in faba bean only PSbMV was previously reported in Nepal.

Table 6. Viruses detected in symptomatic and randomly collected samples from 7 chickpea fields.

Field	Province	Symptomatic samples						Random samples		
		No of Samples	Average % / Field					No of Samples	Avg % / Field	
			Luteo	BWYV	CpCSV	PSbMV	AMV		Luteo	PSbMV
3	Karnali	16	25	25	6	0	0	50	20	4
7	Koshi	20	80	75	10	0	0			
35	Lumbini	8	0	0	0	0	0	50	0	0
67	Madesh	26	0	0	0	4	0			
68	Madesh	25	20	12	4	20	4	50	0	0
69	Madesh	26	0	0	0	8	0	50	0	0
70	Madesh1	31	58	52	0	16	0	50	64	2

[1] Field 70 was part of the Tajpur Research Station.

Luteovirus species identification by molecular tools (work in progress)

Dr Safaa Kumari has started to investigate luteovirus specimens collected during this survey in the ICARDA laboratory at Zahle, Lebanon, using PCR.

So far 12 plant samples (10 lentils and 2 chickpeas) that reacted positive with the TBIA test to one or more luteoviruses were tested using a generic primer (AS3/Pol3870F) with only 8 lentil and 1 chickpea showing clear DNA amplicons.

The 9 positive samples were retested using multiplex primers: Mix-1 (BLRV, SbDV, BWYV and Phasey bean mild yellowing virus, PBMYV) and Mix-2 (CpCSV and Cucurbit aphid-borne yellows virus, CABYV). TBIA results showing the absence of BLRV and SbDV were confirmed with Mix-1 and 4 lentils amplified clear DNA amplicons with BWYV specific primers.

Results of Mix-2, showed no CpCSV but 3 lentils amplified a DNA amplicon similar to the CABYV specific amplicon. In addition, a non-specific DNA amplicon (300-320 bp) was amplified in 8 samples.

The RT-PCR of BWYV, CpCSV and CABYV was repeated using single specific primers to confirm the above results: Four samples amplified with BWYV, two samples amplified very weak with CpCSV and three samples amplified with CABYV. In addition, a non-specific DNA amplicon (300-320 bp) was amplified in 8 samples when CABYV primers was used.

The non-specific (unidentified) DNA amplicon (300-320 bp) using CABYV primers could be a new Luteovirus in these samples. To confirm this, DNA amplicons of the samples need to be sequenced using generic primers.

Further work was interrupted by the current political and social unrest in Lebanon but will be restarted as soon possible.

Table 7. Viruses detected in symptomatic and randomly collected samples from 6 faba bean fields.

Field	Province	Symptomatic samples						Random samples		
		No of Samples	Average % / Field					No of Samples	Avg % / Field	
			Luteo	BWYV	CpCSV	PSbMV	FBNYV		Luteo	PSbMV
36	Lumbini	3	0	0	0	0	0	5	0	80
37	Lumbini	2	50	50	50	0	0	10	0	10
38	Lumbini	6	0	0	0	0	17			
39	Lumbini	6	0	0	0	0	0	3	0	67
40	Lumbini	4	0	0	0	0	0	2	0	0
71	Madesh	20	0	0	0	25	0			

Discussion

The survey identified a luteovirus complex, including some yet to be identified virus species, and PSbMV as the dominant viruses in Nepali lentils during the 2023-24 growing season. Compared to the vast range of viruses reported on lentils worldwide (Makkouk *et al.* 2014) this is a relatively small number. However, Makkouk *et al*. (2001) surveyed 29 lentil fields in a similar environment in northern Pakistan during 1997 and found only two viruses with high incidences; PSbMV and CMV, while FBNYV and CpCDV and unidentified luteoviruses occurred at lower incidences. They also found high PSbMV infection levels in experimental plots in Agricultural Research Stations in Pakistan. This is of importance to the Nepali lentil breeders as several Pakistani breeding lines have been released in Nepal. Surprisingly no surveys of viruses in commercial lentil crops have been published in India or Bangladesh, both large lentil producers, neighbouring Nepal with active lentil breeding programs.

The survey covered a large area, but the results only represent virus infection in one single season. It is likely that the range of viruses and their infection levels vary between years not only because of seasonal conditions, but also because most viruses depend on the presence of alternative hosts for virus and virus vector survival during the off-season. A reliable picture of the importance of viruses in Nepali lentil cultivation can therefore only be developed from repeated surveys over several years. However, while large fluctuations can be expected for virus species that rely on alternative hosts, like luteoviruses, far less variation will occur for seed-borne viruses. The high incidence of PSbMV is of particular concern. PSbMV in field pea is recognised world-wide as a major threat and in-depth research over many years has revealed its infection processes and resistance mechanisms. PSbMV in lentil has received far less attention, even though it has been shown to be among the most important lentil viruses in countries as diverse as Ethiopia (Abraham and Makkouk 2002), Pakistan (Makkouk *et al.* 2001), Azerbaijan (Mustafayev *et al.* 2011), Syria (Makkouk *et al.* 1992) and Greece (Chatzivassiliou *et al.* 2016). It is possible to control PSbMV by using virus-free seed, but this would be difficult to implement in Nepal where lentil is grown in small fields and most seed is retained. Alternatively, breeding for resistance would provide a permanent solution, but information about the pathogenicity of Nepalese PSbMV strains is needed to identify durable resistance genes. Ongoing research at the Tamworth Agricultural Institute is showing that the PSbMV-lentil pathosystem is more complicated than the PSbMV-pea pathosystem and that lentil seed-borne PSbMV strains show large differences in pathogenicity when using a set of lentil differentials. Complete resistance to the all PSbMV strains, including the most virulent lentil seed derived strains, has been found in late maturing Iranian germplasm. Recent investigations carried out as part of the ACIAR supported project CROP/2020/164 (“Protecting Ethiopian lentil crops”) also identified this level of resistance in Greek and Nepalese germplasm held at the Australian Grains Genebank (AGG). While the Greek germplasm selections are medium-late maturing, the Nepalese germplasm selections mature early and will be of great use to lentil breeding programs in Nepal, Ethiopia and Australia that aim at developing early maturing varieties.

The survey results, even though only data of a single season, support the need for further research in the ecology of lentil viruses and their control. The establishment of a virus diagnostic capacity within the national program is a necessity to support this research. A virus laboratory will allow for testing of survey samples as well as for seed testing of farmers’ seed lots. It will also provide the national lentil breeding program with the necessary diagnostics to screen for virus resistance.

8 Impacts

8.1 Scientific impacts – now and in 5 years

Awareness has been raised within the Nepali agricultural research community about the danger of viruses and the NARC management has expressed interest to expand its virus diagnostics capacity.

8.2 Capacity impacts – now and in 5 years

NARC staff and university students have received basic training in executing virus surveys and have been acquainted with diagnosing viruses using the Tissue blot immunoassay technique.

Further training of technical staff is needed to enable the independent operation of a TBIA laboratory.

8.3 Community impacts – now and in 5 years

8.3.1 Economic impacts

The growing of lentils provides an income to rural communities in Nepal. The project results show the presence of viruses that can affect yield levels. Controlling viruses by improved agronomic practices or by making virus resistant varieties available to farming communities will mitigate these potential losses at no or little costs to the grower.

8.3.2 Social impacts

Community confidence in lentil cultivation will be restored through the release of better yielding virus-resistant lentil cultivars.

8.3.3 Environmental impacts

The ability to differentiate between the impact of aphid feeding damage from their role as virus vectors will help in establishing threshold levels for aphid populations and will reduce the use of aphicides.

Including selection for virus resistance in the Nepali lentil breeding program will have the potential to control viruses in an economic and environmentally sustainable way in the future.

8.4 Communication and dissemination activities

A meeting attended by 50 stakeholders representing research institutes (Plant Quarantine, Pulse Breeding Program, Genebank) and extension services was held at the end of the training course to highlight the importance of pulse viruses to Nepali agriculture, to develop strategies to avoid virus-induced crop losses and to raise awareness of farmers to the importance of using virus-free seed stock. Participants declared their support for further research in lentil viruses.

9 Conclusions and recommendations

9.1 Conclusions

- The project results unequivocally show the importance of viruses in the Nepalese lentil growing environments and the need for further research to develop strategies to control viruses and to mitigate virus-induced crop losses.
- Several viruses infecting lentil, chickpea and faba bean have been reported for the first time in Nepal, some of these require further research by molecular methods to correctly identify them at species level.
- Seed-borne viruses, particularly Pea seed-borne virus, are of particular concern as most Nepali lentil farmers use saved, untested, seed. Lentil varieties and breeding lines grown at the Khajura Research Staition showed remarkable differences in PSbMV infection levels, which could indicate useful resistance levels in current breeding material.

9.2 Recommendations

- Surveys to be repeated regularly to get a full picture of the scope and intensity of viruses in Nepali lentil fields. As part of these surveys, seed samples to be collected from growers to determine the extent of seed transmitted viruses in seed stocks.
- The Nepali National Lentil Breeding Program to commence virus resistance screening of breeding lines and germplasm in close collaboration with virology researchers.
- There is a need for seed testing for virus presence and for extension services to inform lentil growers about the importance of seed quality and health.
- The identification of luteo- or polero viruses to species level by molecular methods is needed. There is potential for an exchange program in which a Nepali researcher gains experience in an Australian virology laboratory.
- Determine PSbMV pathotypes within Nepal to support PSbMV control through breeding
- There is an urgent need to establish serological facilities for virus detection with NARC. As a stopgap arrangement until such a facility is established and technical staff are adequately trained, TBIA membranes could be sent to the virology laboratories of ICARDA or Australia for processing.

10 References

10.1 References cited in report

Abraham A, Makkouk KM (2002) The incidence and distribution of seed-transmitted viruses in pea and lentil seed lots in Ethiopia. Seed Science and Technology 30: 567-574.

Acharaya B, Regmi H (2020) A review on status and prospects of legumes viral disease in Nepal. Journal of the Plant Protection Society 6: 40-52.

Chatzivassiliou EK, Giakountis A, Kumari SG, Makkouk K (2016) Viruses affecting lentil (*Lens culinaris* Medik.) in Greece; incidence and genetic variability of Bean leafroll virus and Pea enation mosaic virus. Phytopathologia Mediterranea 55: 239-252.

Joshi S, Albrechtsen SE, Manandhar HK, Shrestha SK (1993) Virus diseases of lentil, broad bean and black gram identified in Nepal. FAO Plant Protection Bulletin 41 (3-4): 201-204.

Magar DBT, Gauchan D, Darai R (2014) Varietal Adoption and Marketing of Lentil in the Mid and Far Western Terai region of Nepal. Advances in Plants & Agriculture Research 1 (5): 00026.

Neupane S, Subedi S, Darai R (2020) Field Screening of Lentil Genotypes against Aphid Infestation in Inner Tarai of Nepal. Journal of Nepal Agricultural Research Council 6: 79-84.

Makkouk KM, Kumari SG, Al-Daoud R (1992) Survey of viruses affecting lentil (*Lens culinaris* Med.) in Syria. Phytopathologia Mediterranea 31: 188-190.

Makkouk K, Kumari S (1996) Detection of ten viruses by the tissue-blot immunoassay (TBIA). Arab Journal of Plant Protection 14: 3-9

Makkouk KM, Bashir M, Jones RAC, Kumari SG (2001) Survey for viruses in lentil and chickpea crops in Pakistan. Journal of Plant Diseases and Protection 108: 258-268.

Makkouk KM, Kumari SG, van Leur JAG, Jones, RAC (2014) Control of plant virus diseases in cool-season grain legume crops. Advances in Virus Research 90: 207-253.

Mustafayev E, Kumari SG, Attar N, Zeynal A (2011) Viruses infecting chickpea and lentil crops in Azerbaijan. Australasian Plant Pathology 40: 612-620.

Pokhrel A, Aryal L, Poudel P (2018) A Review on Research Work of Grain Legumes Research Program, NARC. Nepal Agricultural Research Council.

Poudel PP, Dhakal K, Darai R, Sah R, Subedi S, Mishra S (2023) Yield Stability and Genotype x Environment Interaction of Lentil. Nepalese Journal of Agricultural Sciences 24: 103-114.

Sadras VO, Rosewarne GM, Lake L (2021) Australian lentil breeding Between 1988 and 2019 has delivered greater yield gain under stress than under high-yield conditions. Frontiers of Plant Science 12: 674327.

United Nations Food and Agriculture Organization, Statistics Division. 2024. https://www.fao.org/faostat/en/#data/QCL Retrieved 6 February 2024

10.2 List of publications produced by project

Khadka RM, Kumari SG, van Leur JAG (2024) Prevalence and incidence of lentil viruses in Nepal. Presentation at the Australian Plant Virology Workshop, Gold Coast, 29-31 October 2024.

A publication with the same title to be submitted to an international journal is currently in preparation.

11 Appendixes

11.1 Appendix 1: