Complex Viral Diseases Threatening Lentil and Chickpea Production in Ethiopia

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Abstract

In Ethiopia, lentil and chickpea have many of uses as sources of income and in the preparation of various dishes and nutritional security of millions of Ethiopians. Farmers have well recognized the importance of lentil and chickpea as rotation crops in improving soil fertility. However, several biotic agents of viral origin are threatening the production and productivity of chickpea and lentil. The current study was carried out to assess the relative distribution and importance of viruses infecting lentil and chickpea in major production areas to prioritize and design focused and action-oriented research to develop effective management. Two consecutive surveys were carried out in 2018 and 2019 main cropping seasons, and a total of 635 (chickpea, lentil, grasspea and fenugreek) and 676 (lentil and chickpea) samples were collected, respectively, from 57 and 81 fields in central, north, and northwest regions. The study revealed that Pea seed-borne mosaic virus (PSbMV, genus Potyvirus, family Potyviridae) and Chickpea chlorotic stunt virus (CpCSV, genus Polerovirus, family Solemoviridae) are the most-wide spread and frequently recovered viruses from lentil samples, while CpCSV had the highest incidences and was a more prevalent viruse in chickpea fields assessed. Based on this result, integrated management options can be developed targeting factors aggravating the epidemics of the dominant viruses along with continuous disease and vector monitoring.

Keywords: Chickpea chlorotic stunt virus, legume diseases, Pea seed-borne mosaic virus, *Potyvirus, legume virus incidence, prevalence*

Introduction

Lentils and chickpeas play major role in Ethiopian Agriculture, serve as an important source of income, and provide food and nutritional security for millions of people in Ethiopia. The importance of chickpea and lentil are also well recognized by Ethiopian farmers as a rotation crop, for improving soil fertility and reducing the use of chemical fertilizer for cultivation of cereals in subsequent farming system (Kebede, 2020). Among major constraints so far identified for lentil and chickpea production in Ethiopia, diseases caused by biological agents appear to take the lion's share that severely threatening its production and productivity (Fig. 1).

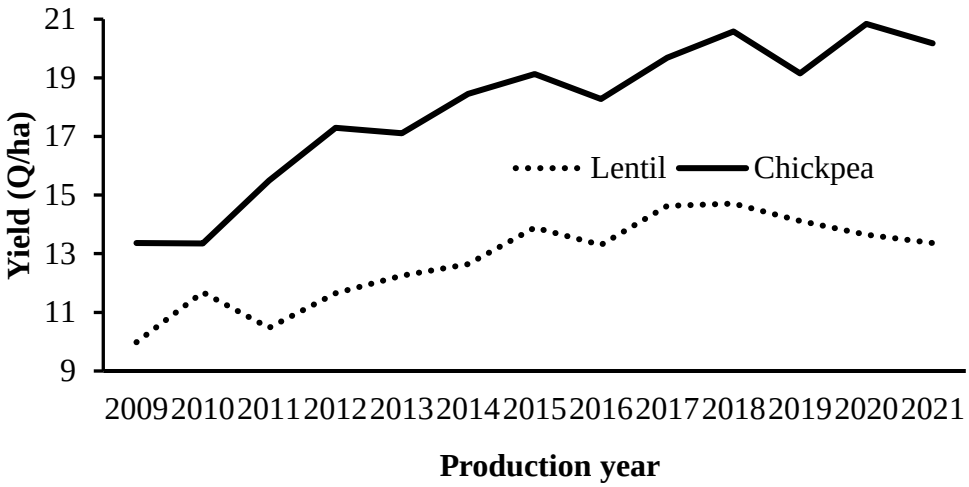

Figure 1. Lentil and chickpea productivity trend over thirteen years (2009-2021) in Ethiopia(source: CSA, 2009-2021)

Among biotic stresses, diseases caused by fungi (Fusarium wilt, Ascochyta blight and Rusts) and viruses are of major importance and widespread constraints in major lentil and chickpea production areas in Ethiopia (Tadesse*et al.*, 2008).

Currently, virus diseases are causing a major problem on chickpea and lentil. Some attempts were made to monitor viruses infecting chickpea and lentil and many viruses were identified, where stunt in chickpea and PSbMV in lentil are more prevalent (Tadesse *et al*., 1999; Bekele *et al*., 2005). Other commonly identified viruses were Beet western yellows virus (BWYV, genus *Polerovirus*, family *Solemoviridae*), Faba bean necrotic yellows virus (FBNYV, genus *Nanovirus*, family *Nanoviridae*), Broad bean mottle virus (BBMV, genus *Bromovirus*, family *Bromoviridae*), Alfalfa mosaic virus (AMV, genus *Alfamovirus*, family *Bromoviridae*) in chickpea; BWYV, FBNYV, BBMV and Bean yellow mosaic virus (BYMV, genus *Potyvirus*, family *Potyviridae*) in lentil, in order of their importance (Bekele *et al*., 2005; Abraham and Makkouk, 2002; Tadesse *et al.,* 1999). When 253 chickpea and 332 lentil samples tested positive to a broad spectrum monoclonal antibody (5G4), which reacted with all members of legume viruses in the families *Solemoviridae* and *Tombusviridae*, were subjected to specific monoclonal antibodies against BWYV, Bean leafroll virus (BLRV) and Soybean dwarf virus (SbDV) (genus *Luteovirus*, family *Tombusviridae*), only BWYV was recovered in 35 (13.8%) chickpea and 12 (3.6%) lentil samples, and no virus was detected in 218 (86.2%) chickpea and 320 (96.4%) lentil samples (Bekele *et al*., 2005), implying the presence of other viruses which could not be detected by the antibodies used. Later, Abraham *et al*. (2006) reported a novel virus named "Chickpea chlorotic stunt virus (CpCSV, genus *Polerovirus*, family *Solemoviridae*)", causing yellowing

and stunting symptoms and exclusively transmitted by only one aphid species '*Aphis craccivora'*. Lack of specific monoclonal antibodies for detecting *luteoviruses* and *poleroviruses* on one hand and the occurrence of a novel virus infecting legumes on the other coupled with the ever-increasing virus-like disease intensity and coverage prompted us to go for further assessment to elucidate the relative distribution and importance of viruses causing yellowing and stunting symptoms to enable search for appropriate disease management strategies.

Materials and Methods

Surveys and sample collections

Two consecutive surveys involving a field visit and leaf sample collection were made in chickpea and lentil growing areas during 2018 and 2019 main cropping seasons. Other legumes encountered in the area such as grasspea and fenugreek were also sampled during the first survey.

During 2018 (1-6 December 2018), survey covered legumes growing areas in northern part of central, north and northwest regions. A total of 635 leaf samples (334 chickpea, 157 lentil, 121 grasspea and 23 fenugreek) were collected from 58 fields(32 chickpea, 11 lentil, 11 grasspea and 3 fenugreeks) in 40 locations (3in north Shewa, 7 in Wollo, 17 in Gondar, and 13 in Gojam). (Fig. 2; Table 1).

Similarly, another survey was conducted in October and November 2019,during main cropping season (Fig. 3) and a total of 676 leaf samples (440 lentil and 236 chickpea) were collected from 47 lentil and 34 chickpea fields in central (East Shewa, North Shewa), north (North Wollo) and north-west (South Gondar and Gojam) regions (Tables 2&3). During survey in 2018, the crop was at full flowering/podding and early maturity stages, while in 2019, it was at full flowering to maturity stages, except in North Gondar zone where most fields were at vegetative stage.

Sampling was done randomly at 5-10 km intervals depending on the distribution and availability of the crops, and samples were collected by walking diagonally along the field, where focus was given to the symptomatic samples. Samples were collected during the day time, kept in labeled plastic bags, and blotted in triplicate onto the nitrocellulose membranes (NCM) during the night time. The blotted NCM was transported to the virology laboratory of Ambo Agricultural Research Centre to determine the status of virus association in the collected samples. Data related to locations,altitude,geographical positions, crop variables such as variety/species, crop age etc. were also collected during the survey and sampling process.

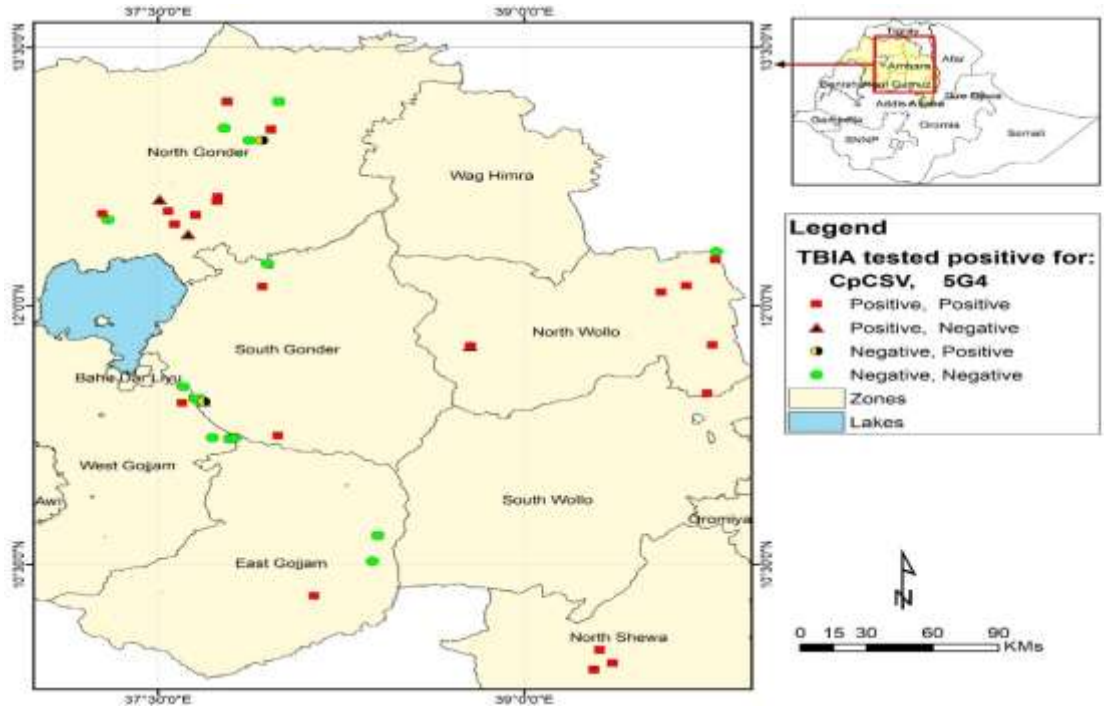

Figure 2. Study areas and test results of chickpea and lentil viruses in Amhara regional state, Ethiopia during the 2018 main cropping season (1-6 December 2018)

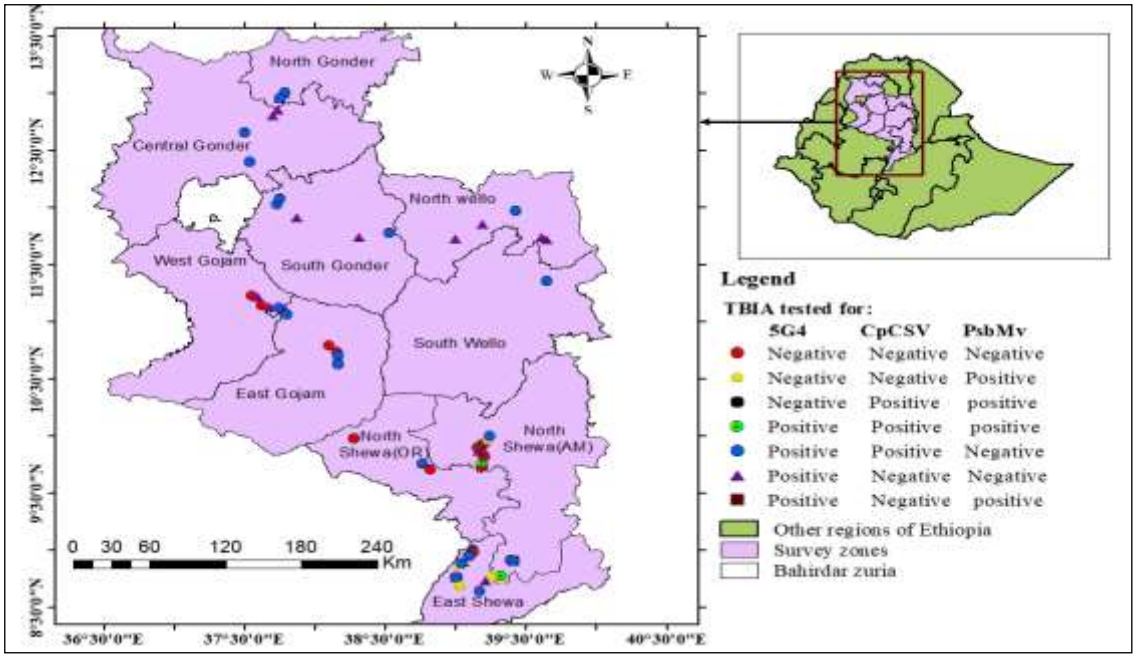

Figure 3. Study areas and distribution of chickpea and lentil viruses in Amhara regional state, Ethiopia during the 2019 main cropping season (October – November 2019).

Diseases (viral and fungal) symptoms and insect pests encountered were recorded right in the field based on visual assessment, and disease incidence data collected in percent and categorized as low (when less than 20%), medium (20-50%) and high (more than 50%). Collected samples were blotted on nitrocellulose membranes (NCM) and the blotted NCM were sent to ICARDA virology laboratory for identification of the virus.

Tissue-blot immunoassay (TBIA)

All the blotted NCM were tested for the presence of viruses by the standard technique of tissue-blot immunoassay (TBIA) (Makkouk and Comeau, 1994; Makkouk and Kumari, 1996) at ICARDA Virology Laboratory (Terbol station, Lebanon) using broad-spectrum monoclonal antibody (5G4) (Katul, 1992), monoclonal antibody against CpCSV (Abraham *et al.*, 2006, 2009) and polyclonal antibody against PSbMV (Makkouk *et al.*, 1993). However, the samples collected during 2019, and not in 2018, were tested for PSbMV.

Results

Field observation

During survey, the most conspicuous virus-like symptoms recorded in lentil fields were mosaic, mottling, yellowing, reddening, stunting, small leaves, downward leaf curling, and pale yellowing to whitish leaves that starts from lower branches and progresses upward. Moreover, in some cases aborted flowers/pods or suppression of flower development and seed setting was observed (Fig. 4a, b, c, d; 5). Similarly, virus-like symptoms commonly encountered in chickpea fields were leaf yellowing, bronzing, stunting, brown phloem discoloration and shortened internode (Fig. 6). In many of the fields visited, one or more of these symptoms were simultaneously observed. Pea aphids (*Acyrthosiphon pisum*) at intensities ranging from low to high were also recorded in the assessed lentil fields.

Disease incidence in the field based on visual assessment

The altitudes of the study areas during the 2018 main season survey were in the range of 2532-2766 meters above sea level (masl) for lentil, 1471-2766 masl for chickpea, 1835-2602 masl for grass pea and 2589-2766 masl for fenugreek. The most frequently recorded virus-like symptoms in the field were yellowing, stunting and reddening on chickpea and lentil fields with incidences ranging from low (less than 20%) to medium (20-50%), while mosaic symptoms were encountered rarely. Yellowing and stunting were the most common symptoms in grass pea and fenugreek samples although mosaic and purple-colored plants were encountered rarely.

Of fungal diseases recorded, fusarium wilt/root rot was the most prevalent, followed by ascochyta blight, rust and powdery mildew. Among the 32

chickpea fields assessed, low (less than 20%) wilt/root rot incidence was recorded in 15 fields (46.9%), medium (20-50%) in 6 fields (18.8%), high (more than 50%) in 7 fields (21.9%) and none in 4 fields (12.5%). Low incidence was recorded in 5 (45.5%) out of 11 lentil fields visited. Low Ascochyta incidence was recorded in 7 (21.9%) chickpea fields.

Regarding insect pests, pea aphid was more frequently recorded from lentil, at low intensity from 5 (45.5%) fields, medium from 18.28% fields and high from only one field. In grass pea, low incidence of pea aphid was observed from 3 (25%) fields, medium from 2 (16.7%) and high from 3 (25%) fields. Pod-borer was recorded at low levels from 7 (21.9%) chickpea fields.

The surveyed areas during the 2019 main season ranged from 1779-3303 masl for lentil and 1585-2722 masl for chickpea (Tables 2 & 3). Of the 81 fields surveyed, average virus disease incidence ranged from 1.0 to 47.5 based on visual inspection in the lentil field while it ranged from 0.5 to 12.3 in chickpea fields. Highest incidences of virus-like symptoms in lentil were recorded in many locations in east Shewa, followed by north Shewa, south Gondar and north Wollo.

Of the 47 lentil fields surveyed, about 76% had pea aphid density that ranges from 0.1 to 12 individuals per 130 cm^2; while the remaining (24%) were free of pea aphid (Table 2). Other than pea aphids, thrips (probably *Thaeno thrips*) were found in 50% of the field and their density ranges from 0.1 to 13 individuals per 130 cm^2. Natural enemies of pea aphids were not prevalent in the majority of the fields assessed. The only exception was one field each in Zewelde kebele (Minjar-shenkora district) and Ariselele kebele (Moretnajiru district) where coccinellid predator and cadavers of pea aphids, respectively, were found. In chickpea, only the pod borer (*Helicoverpa armigera*) was found in most chickpea fields.

Virus detection using tissue blot immunoassay

During 2018, among 68 samples (17 of chickpea and 51 of lentil) collected and tested from north Shewa zone, CpCSV and other *luteoviruses* and *poleroviruses* detectable by 5G4 monoclonal antibody were equally detected in 64 (94%) of the samples. Similarly, these groups of viruses were detected nearly equally in areas covered in south Wollo, north Wollo, Gondar and Gojam zones (Fig. 2; Table 1). Overall, across the study areas, out of 635 samples tested during the 2018 main season survey, CpCSV was recovered from 273 (43%) samples, while 264 (41.6%) samples were positive to broad spectrum monoclonal antibody (5G4).

Figure 4. (A, B) Lentil with leaf rolling, pale yellowing, stunting, reduced internode, suppression of flowering and pod setting, pods in the infected plants are reduced in size and poorly filled at DZARC experiment station, 2019. Samples tested positive for 5G4 and CpCSV (Table 2, line 1); (C, D, E)Mosaic, chlorosis and narrowed leaves of lentil samples tested positive for CpCSV and PSbMV, collected from DZARC, 2019 (Table 2, line 5); (F, G, H) Reddening, chlorosis, narrow leaves, leaf curling and stunted lentil plants tested positive for 5G4 and CpCSV. (Table 2, line 6); (I, H) Reddening, stunting, mosaic and small leaves on lentil plants, tested positive for PSbMV (See table 2, line 10).

Figure 5. Above and below ground (foliar and root symptoms) on lentil due to wilt: A) dull, pale yellow, necrosis at leaf tip and leaf shading at late stage; B) dull yellow at early stage; C) dull yellow, chlorosis and leaf shading as observed in the field; D, E, F) Vascular bundle discoloration in the root and collar regions. Tested negative for suspected viruses (Table 2, lines 2, 4, 34)

Figure 6. Severely affected chickpea fields due to stunt and wilt/root rot complex at Enewari Experimental plot in Amhara region. Plots are sparsely populated due to death of plants by either stunt or wilt or both diseases.

Table 1. Survey locations, and TBIA results for chickpea and lentil viruses in central, north and northwest regions of Ethiopia, 1-6 December 2018*

Zone	District	Location	Host	No. of samples tested	No. of samples positive to	
					5G4	CpCSV
North Shewa	Moretena Jiru	DbARC, Enewari Station	Chickpea	17	17	17
North Wello	Habiru	Sirinka RC sub station	Chickpea	17	8	4
North Wello	Kobo	Mariyam Erash	Chickpea	10	0	0
North Wello	Kobo	Kobo R.C. Station	Chickpea	15	2	5
North Wello	Guba Lafto	Gedobere	Chickpea	10	1	1
North Wello	Guba Lafto	Gedobere	Chickpea	10	1	1
North Wello	Meket	Ambaye	Chickpea	11	9	10
North Gondar	Dabat	Dar Abo	Chickpea	25	15	14
North Gondar	Dabat	Gebsoye	Chickpea	13	10	11
North Gondar	Wogera	Amaba Giorgis	Chickpea	13	11	11
North Gondar	Western Dembiya	Efes Bemeda	Chickpea	19	6	7
North Gondar	Western Dembiya	Meskele Kirstos	Chickpea	14	5	5
North Gondar	Eastern Dembiya	Debebaw Demboskie	Chickpea	2	0	1
North Gondar	Eastern Dembiya	Loza Mariyam	Chickpea	5	3	4
North Gondar	Gonder Zuriya	Teda	Chickpea	5	1	2
North Gondar	Gonder Zuriya	Berbuax	Chickpea	8	2	2
North Gondar	Gonder Zuriya	Zanit	Chickpea	7	0	4
North Gondar	Gonder Zuriya	Tsion Mariyam	Chickpea	16	13	13
North Gondar	Gonder Zuriya	Chira Manterno	Chickpea	14	3	7
South Gondar	Libo Kemkem	Selkesa Ginaza	Chickpea	13	9	12
South Gondar	Libo Kemekem	Selkesa Genaza (Tiblete)	Chickpea	2	1	1
West Gojam	Yelmana Densa	Adet R.C Station	Chickpea	20	1	2
West Gojam	Yilmana Densa	Mosobo	Chickpea	9	0	0
West Gojam	Bahirdar Zuriya	Kenbaba (Enkurte)	Chickpea	5	0	0
West Gojam	Yilmana Densa	Mosobo	Chickpea	4	0	1
West Gojam	Yilmana Densa	Abraham Damot	Chickpea	2	2	1
West Gojam	Gonji Kolela	Turi Abo	Chickpea	4	2	3
West Gojam	Gonji Kolela	Woyzazirt	Chickpea	2	0	0
West Gojam	Gonji Kolela	NA	Chickpea	4	4	1
East Gojam	Mota	Kuntra	Chickpea	4	2	2
East Gojam	Enemay	Bichena S.Station (Adet ARC)	Chickpea	25	18	19

Table 1 (Continued)

East Gojam	Dejen	Teke	Chickpea	9	8	9
			Sub total	**334**	**154(46.1%)**	**170 (50.9%)**
North Shewa	Siyadebere na Wayu	Deneba	Lentil	31	29	31
North Shewa	Moretena Jiru	DbARC, Enewari Station	Lentil	10	9	10
North Shewa	Enewari	Mangudo	Lentil	10	9	6
North Wello	Meket	Ambaye	Lentil	10	0	3
North Gondar	Dabat	Dar Abo	Lentil	32	15	2
North Gondar	Dabat	Gebsoye	Lentil	12	3	10
North Gondar	Dabat	Charneta	Lentil	10	0	0
North Gondar	Dabat	Gedebiya	Lentil	10	10	10
North Gondar	Wogera	Guncoban Derge	Lentil	16	5	5
North Gondar	Wogera	Amaba Giorgis	Lentil	10	10	10
East Gojam	Enemay	Bichena S.Station (Adet ARC)	Lentil	6	6	6
			Sub total	**157**	**96 (61.2%)**	**93 (59.2%)**
South Wello	Tehuledere	Aba Rahemet K.08	Grasspea	9	6	2
North Gondar	Dabat	Dar Abo	Grasspea	10	4	5
North Gondar	Dabat	Gebsoye	Grasspea	27	0	0
North Gondar	Western Dembiya	Efes Bemeda	Grasspea	18	0	0
South Gondar	Libo Kemekem	Selkesa Genaza (Tiblete)	Grasspea	12	0	0
West Gojam	Bahirdar Zuriya	Kinbaba Chigign Tabya	Grasspea	8	0	0
West Gojam	Yilmana Densa	Mosobo	Grasspea	14	2	0
West Gojam	Gonji Kolela	Turi Abo	Grasspea	6	0	0
West Gojam	Gonji Kolela	NA	Grasspea	4	0	0
East Gojam	Enemay	Bichena S.Station (Adet ARC)	Grasspea	8	0	0
East Gojam	Enemay	Mahebere Niway	Grasspea	5	0	0
			Sub total	**121**	**12(9.9%)**	**7(5.8%)**
North Gondar	Dabat	Dar Abo	Fenugreek	10	1	3
North Gondar	Wogera	Guncoban Derge	Fenugreek	8	1	0
North Gondar	Wogera	Amaba Giorgis	Fenugreek	5	0	0
			Sub total	**23**	**2**	**3**
			Total	**635**	**264(41.6%)**	**273 (43%)**

***** CpCSV, Chickpea Chlorotic Stunt Virus; 5G4, Broad Spectrum Monoclonal Antibody; DbARC,, Debre birihan Agricultural Research Centre; NA, not available.

Table 2. Test results of lentil samples for major viruses in central, north and northwest regions of Ethiopia during2019 main cropping season*.

Zone	District	Localition	Average number of pea aphis per 130cm^2	Wilt Incid. (%)	AB Incid	AB seve	VLS incid	VLS Seve	No. of samples tested	No. of samples positive to		
										5G4	CpCSV	PSbMV
E/Shewa	Adea	DZARC	Yes	50	20	15	100	65	10	4	2	0
E/Shewa	Adea	DZARC	Yes	50	20	15	100	65	6	0	0	0
E/Shewa	Adea	DZARC		-	-	-	-	-	8	2	1	0
E/Shewa	Adea	DZARC		-	-	-	-	-	5	1	0	0
E/Shewa	Adea	DZARC		-	-	-	-	-	12	0	2	3
E/Shewa	Adea	DZARC		-	-	-	-	-	9	3	2	0
E/Shewa	Adea	DZARC		-	-	-	-	-	13	1	1	1
E/Shewa	Adea	DZARC		-	-	-	-	-	8	0	1	2
E/Shewa	Adea	DZARC		-	-	-	-	-	5	5	2	0
E/Shewa	Adea	DZARC		-	-	-	-	-	7	0	0	4
E/Shewa	Adea	DZARC		-	-	-	-	-	4	0	0	0
E/Shewa	Ejere	Ejere	0.1	15	100	75	15	10	13	0	0	7
N/Shewa	Minjar-Shenkora	Zewolde	0.2	15	75	50	20	20	7	0	0	3
N/Shewa	Minjar	Arerti (OS)		25	100	85	20	20	10	10	0	2
N/Shewa	Minjar	NA	0.3	5	15	15	75	75	9	2	1	8
E/Shewa	Ejere	Nanawa	0.3	5	30	20	50	50	12	0	0	4
E/Shewa	Lume	Giriti Garbi	0.2	40	55	40	50	20	11	4	0	0
E/Shewa	Lume	Tulu re'e	0.0	10	75	55	25	15	7	0	0	4
E/Shewa	Adea	Ude	0.1	45	35	20	20	10	10	0	0	4
E/Shewa	Adea	Denkaka	0.1	20	15	10	30	10	10	0	0	7
E/Shewa	Adea	Gandagorba	2.1	10	80	45	45	15	10	0	0	6
E/Shewa	Adea	Godino	2.1	15	40	25	60	40	10	2	0	5
E/Shewa	Adea	Kataba	0.0	15	30	15	50	30	10	0	0	6
E/Shewa	Adea	Ambalta	0.2	10	40	20	35	10	10	1	0	0
E/Shewa	Adea	near ambalta	0	30	35	15	30	15	11	0	0	4
E/Shewa	Gimbichu	on station	3.5	5	0	0	45	20	10	4	2	2
E/Shewa	Gimbichu	Bayu	4.5	15	0	0	15	5	10	1	1	0
E/Shewa	Gimbichu	Adadigole	0.0	60	5	2	25	10	10	0	0	0
N/Shewa	Dananba	Xadde	0.1	30	0	0	20	25	10	0	0	0
N/Shewa	Dananba	Wale	0.3	5	0	0	35	30	12	3	5	1
N/Shewa	Dananba	Mangudo	0.0	3	0	0	10	25	10	0	0	0

Table 2. (Continued)

Zone	District	Location	Aphid count	WILT (%)	AB Incid.	AB Seve	VLS incid	VLS Seve	No. of samples tested	No. of samples positive to:		
										5G4	CpCSV	PSbMV
N/Shewa	Dananba	Mangudo	12.1	5	0	0	70	50	10	0	0	0
N/Shewa	Moretinajiru	Mangudo	2.0	15	0	0	80	60	14	2	0	0
N/Shewa	Moretinajiru	Ariselele	4.3	60	0	0	20	15	10	0	0	0
N/Shewa	Moretinajiru	Bolo	0.3	25	0	0	20	40	10	1	1	0
N/Shewa	Moretinajiru	Wera	4.7	20	0	0	30	65	10	0	0	0
N/Shewa	Moretinajiru	Bymot	0.7	20	0	0	20	15	7	1	0	2
N/Shewa	Moretinajiru	Jihur	1.2	3	0	0	5	25	10	3	1	0
N/wollo	Gubalafto	Sakala	3.7	10	5	5	10	15	10	4	2	0
N/wollo	Gaazoo	Koso	1.3	3	0	0	20	25	10	1	0	0
N/wollo	Wadla	Hamust	9.2	1		0	1	1	4	1	0	0
S/Gonder	Lygaent	Nechome	4.2	15	10	2	2	2	8	7	0	0
S/Gonder	Dabat	Debat Zuria	0.4	5	0	0	30	5	10	5	0	0
S/Gonder	Dabat	NA	0.0	15	0	0	5	5	10	3	0	0
S/Gonder	Dabat	Gedebiya	0.0	30	0	0	30	30	8	5	0	0
S/Gonder	Wogera	NA	0.0	2	0	0	2	2	10	8	0	0
East Gojjam	Debrework	Debrework Zuria	-	28	0	0	1.5	1.5	10	5	1	0
			Total						**440**	**89**	**25**	**75**
			Percent							**20.2**	**5.7**	**17**

*AB, Ascochyta blight; DZARC, Debre Zeit Agricultural Research Centre; VLS, virus-like symptoms; 5G4, Broad-spectrum monoclonal antibody; CpCSV, chickpea chlorotic stunt virus; PSbMV, pea seed-borne mosaic virus; incid=incidence; Seve=severity; NA, not available.

Table 3. Test results of chickpea samples for major viruses in central, north and northwest regions of Ethiopia during 2019 main cropping season*.

Zone	District	Kebele	Altit.	WILT (%)	AB Inci	AB Sev	VLS inci	VLS Seve	No. of samples tested	No. of samples positive to		
										5G4	CpCSV	PSbMV
E/Shewa	Adea	DZARC	1824	15	30	20	20	15	10	9	1	0
E/Shewa	Lume	Tulu re'e	2006	10	2	2	3	2	10	8	7	0
E/Shewa	Adea	Gandagorba	1882	25	3	1	15	10	9	4	4	0
E/Shewa	Adea	Kataba	2221	5	0	0	2	20	10	8	8	0
E/Shewa	Gimbichu	Chafee D. on station	2427	15	45	30	2	2	7	1	1	0
N/Shewa	Minar-Shenkora	Arerti Zuria	1778	25	65	50	5	15	6	3	2	0
N/Shewa	Minjar	Arerti (sation)	1779	15	100	80	10	10	6	1	1	0
N/Shewa	Tewolederi	Jari	1585	30	30	15	5	20	10	5	4	0
N/Shewa	Marsa	Tigoamba	1719	3	0	0	3	2	3	2	0	0
N/wollo	Habru	Kebele 6	1871	2	80	40	2	2	10	9	0	0
S/Gonder	Lygaent	Chechew	2762	0	0	0	2	2	8	8	7	0
S/Gonder	Fogora	Amoragadal	2075	5	0	0	2	2	10	8	0	0
S/Gonder	Fogora	NA	1884	5	0	0	0	0	6	5	1	0
S/Gonder	Fogora	Fogora Zuria	1861	3.3	0	0	2	3	10	8	7	0
S/Gonder	Dabat	Maskel Yesus	2552	5	0	0	2	2	7	5	2	0
S/Gonder	Dabat	Benkal	2691	5	0	0	2	2	10	10	7	0
Gonder Z.	Wogera	Sabiya	2379	3	0	0	2	2	10	9	8	0
Gonder Z.	Wogera	Minziro	1977	20	0	0	1.5	1	10	5	7	0
CZ- Gonder	Gonder Zuria	Minziro	1915	7	3	5	1	1	10	8	6	0
West Gojjam	Yilmanadensa	Wencet	2238	20	0	0	0	0	-	-	-	-
West Gojjam	Yilmanadensa	Sheba	2269	15	0	0	1	1	4	1	0	0
West Gojjam	Gonjikolola	Asterewos	2276	7	0	0	0	0	-	-	-	-
West Gojjam	Gonjikolola	Werzazirt	1882	20	5	5	2	2	5	3	0	0
West Gojjam	Gonjikolola	Agereselam	1802	11.5	0	0	1	1	9	7	4	0
West Gojjam	Gafat	gafat Zuria	1754	3	0	0	2	2	2	2	0	0

Table 3. (Continued)

Zone	District	Kebele	Altit.	WILT (%)	AB Inci	AB Sev	VLS inci	VLS Seve	No. of samples tested	No. of samples positive to		
										5G4	CpCSV	PSbMV
East Gojjam	Mota	Mota Zuria	2205	5	2	1.5	1	1	11	7	3	0
East Gojjam	Debrework	Ajaje	2572	1	0	0	0	0	-	-	-	-
East Gojjam	Debrework	Diyatiba	2528	0	0	0	0	0	-	-	-	-
East Gojjam	Debrework	Minci	2565	30	0	0	2	2	10	10	6	0
East Gojjam	Debrework	Debrework Zuria	2542	28	0	0	1.5	1.5	8	7	4	0
North Shewa(O)	Warra Jarso	Go'astion	2463	3	0	0	1	1	16	16	16	0
North Shewa (O)	Warra Jarso	Go'astion	2463	20	0	0	1	1	2	0	0	0
North Shewa (O)	fiche Zuria	Dire Doyu	2722	30	0	0	1	1	7	4	3	0
North Shewa (O)	Girar Jarso	Warxu	2673	5	0	0	1	1	8	0	0	0
Total									**244**	**173**	**109**	**0**
Percent										**70.9**	**44.7**	**0**

*AB, Ascochyta blight; DZARC, Debre Zeit Agricultural Research Centre; VLS, virus-like symptoms; 5G4, Broad-spectrum monoclonal antibody; CpCSV, chickpea chlorotic stunt virus; PSbMV, pea seed-borne mosaic virus; incid=incidence; Seve=severity; NA, not available.

In chickpea, out of 334 samples tested, CpCSV was detected in 170 (50.9%) samples, whereas unspecified luteoviruses and poleroviruses detected by 5G4 monoclonal antibody were in 154 (46.1%) samples. In lentil, out of 157 samples, 96 (61.2%) were positive for either luteoviruses or poleroviruses or both when tested by 5G4 broad spectrum legume virus monoclonal antibody, while CpCSV was identified from 93 (59.2%) samples. Small number of grass pea and fenugreek samples were tested and the viruses tested for were rarely recovered (Table 1).

During the 2019 main cropping season, TBIA test results for 440 lentil samples collected from 47 fields showed that luteoviruses and poleroviruses were the dominant viruses identified from 89 (20.2%) samples, followed by PSbMV and CpCSV identified from75 (17%) and 25 (5.7%) samples, respectively (Fig. 3; Table 2). On the other hand, out of 236 chickpea samples collected from 34 fields, luteoviruses and poleroviruses were again the dominant and widely distributed over the study locations and detected in 173 (73.3%) samples, followed by CpCSV which was recovered from 109 (46.2%) samples, while PSbMV was not detected in all the chickpea samples tested (Fig. 3; Table 3).

Occurrence and distribution of lentil and chickpea viruses across altitude ranges

Among lentil fields sampled, high proportion (46.8%) of PSbMV was recorded between altitudes ranging from 2001-2300 masl, followed by from 1700-2000 masl(Table 4). The proportion of PSbMV showed a decreasing trend with the increase in altitudes from 2301-2600 to 2601-2900 masl, and none of the samples detected positive when collected from 2901-3300 masl. The proportion of luteoviruses and poleroviruses were not consistent and higher proportions were recorded at both altitude extremes. Regarding viruses infecting chickpea,luteoviruses and poleroviruses had higher proportion and comparably distributed across the locations surveyed except at altitudes ranging between 1500 – 1800 masl where low proportions were recorded. Whereas, PSbMV was not recovered from chickpea fields of all the surveyed locations (Table 4).

Discussion

Lentil is known to be infected by many pathogens of fungal, viral and nematode origins as well as abiotic factors (Beniwal *et al*., 1993).Several surveys conducted during the last three decades have identified and documented six viruses in lentil that are widespread and cause yellowing/stunting/necrosis, and about ten viruses that cause mosaic or mottling symptoms worldwide (Makkouk *et al.*, 2003; Kumari *et al.*, 2009). Most of these viruses have also been identified in Ethiopia (Tadesse *et al.*, 1999; Abraham and Makkouk, 2002; Bekele *et al*., 2005; Abraham *et al.*, 2006). In the present study, viruses

namely PSbMV, CpCSV and other luteoviruses and poleroviruses reacting with the broad-spectrum monoclonal antibody (5G4) were detected, with the

Table 4. Effect of altitude on the occurrence and distribution of lentil and chickpea viruses, 2019 main season

Altitude Range	Number of samples (%) detected +ve in TBIA				
Lentil	**No. of fields**	**No. of samples**	**5G4**	**CpCSV**	**PSbMV**
1700-2000	15	127	26 (20.5%)	11 (8.7%)	29 (22.8%)
2001-2300	8	79	8 (10.1%)	1 (1.3%)	37 (46.8%)
2301-2600	7	71	11 (15.5%)	4 (5.6%)	6 (8.5%)
2601-2900	13	131	31 (23.7%)	7 (5.3%)	3 (2.3%)
2901-3300	4	32	13 (40.6%)	2 (6.3%)	0
Total	**47**	**440**	**89 (20.2%)**	**25 (5.7%)**	**75 (17%)**
Chickpea					
1500-1800	5	46	13 (28.3%)	7 (15.2%)	0
1801-2100	11	86	74 (86.1%)	37 (43.0%)	0
2101 -2400	6	34	25 (73.5%)	19 (76.0%)	0
2401-2700	10	63	49 (77.8%)	36 (57.1%)	0
2701-2900	2	15	12 (80.0%)	10 (66.7%)	0
Total	**34**	**244**	**173 (70.9%)**	**109 (44.7%)**	**0**

unspecified luteoviruses and polerovirus recovered at higher proportion, followed by PSbMV and CpCSV. Due to lack of monoclonal antibodies, no further attempt was made to identify the individual luteoviruses and poleroviruses (e.g. BWYV, BLRV, and SbDV) affecting legumes in samples that gave a positive reaction to the broad-spectrum McAb '5G4', although one or more of these viruses are likely to exist based on the present test results and previous reports (Tadesse *et al*., 1999; Bekele *et al*., 2005). BWYV, BLRV and SbDV along with CpCSV (Abraham *et al*., 2006) are reported among viruses of regional or global importance infecting lentil (Bos *et al*., 1988; Makkouk *et al*., 2003) causing yellowing, stunting, reddening, shortened internode and leaf rolling symptoms (Fig. 4a & b) and known to have marked effect on lentil yield (Kumari et al., 2009). The decline in lentil production and productivity in lentil in Ethiopia could be due to the high proportion of luteoviruses and poleroviruses reported in this study. Severe lentil damage observed at DZARC during the 2019 main cropping season with symptoms is suggestive of BLRV and BWYV (Fig. 4a & b; Table 2, line 1) for which appropriate management options should be sought. Of viruses causing mosaic/mottling viz., BYMV, Broad

bean stain virus (BBSV), Cucumber mosaic virus (CMV), Pea enation mosaic virus-1 (PEMV-1) and PSbMV (Tadesse *et al*., 1999), AMV and BBMV (Bekele *et al*., 2005), and SbDV (Makkouk *et al.*, 1997) were earlier reported to occur and infect lentils in Ethiopia. Except PSbMV, which had wider distribution and higher incidence (Bekele *et al*., 2005and Table 2 of this study), all the other viruses had limited distribution and low incidence. A study showed that on susceptible varieties, PSbMV causes severe mosaic, chlorotic lesions on leaves, stunting of plants with shortened internodes and a reduction in flower and pod formation (Aftab *et al*., 1992). According to this study, the reduction in plant height, number of pods, number of seeds and yield per plant due to infection by PSbMV were, respectively, 51, 58, 66 and 73%. Another study revealed that PSbMV caused yield losses of 28, 27 and 23% in lentil when infection occurred at pre-flowering, flowering and post-flowering stages, respectively. Depending on the cultivar, virus strain and environmental effects, PSbMV symptoms may vary and also include narrowed leaves, downward leaf rolling, mottling or chlorosis of the leaves with shoot tip necrosis and reduced seed size. Most of these symptoms such as mosaic, narrowed leaves, reddening, chlorosis with shoot tip necrosis and reduced seed size were symptoms observed in some lentil experimental fields infected with what is called an unknown disorder during the 2019 main season at Debre Zeit Agricultural Research Centre (Fig. 4a & c).

The occurrence of PSbMV in lentil appear to have an association with altitude, where the highest proportion of the virus was recorded at altitudes ranging from 1700 – 2000 masl and 2001-2300 masl, and then steadily decreased towards higher altitudes and detected from none of the samples tested between altitudes ranging from 2901-2300masl (Table 4). The decreasing trend of PSbMV with increasing altitudes is possibly associated with decreased activity and population of aphid vectors with increasing altitude. On the other hand, CpCSV and unspecified luteoviruses and poleroviruses detected by broad spectrum monoclonal antibody (5G4) did not consistently show a decreasing or an increasing trend along altitudinal gradient but rather relatively higher proportions were recorded at both altitude extremes. This can be explained by the fact that aphids transmitting CpCSV and other viruses in the families *Solemoviridae* and *Tombusviridae* may do well at wider altitudinal ranges. Moreover, there may be hosts other than lentil serving as initial inoculum sources contributing to a higher prevalences of the viruses coupled with wider ecological niches for the vectors.

Regarding chickpea, it is interesting to note that PSbMV was not detected in any of the samples tested across the study areas. Earlier studies also reported similar findings that PSbMV was detected from none of the chickpea

samples tested (Tadesse *et al*., 1999) or very rarely recovered (Bekele *et al*., 2005). Pea seed-borne mosaic virus (PSbMV) is transmitted bythe pea aphid (*A. pisum),* and assessments made by Tadesse *et al*. (1999) and this study revealed that pea aphid was not recorded in any of the chickpea fields, while commonly recorded in lentil fields. This observation substantiates that chickpea is the less inhabited or less preferred host of the pea aphid. This, therefore, strongly support the present finding that PSbMV is widely recovered from lentil fields and in none of chickpea fields assessed. As in the case of lentil, however, CpCSV and other viruses in the families *Solemoviridae* and *Tombusviridae* that could be detectable by broad spectrum monoclonal antibody (5G4) were well distributed across altitudinal ranges at most chickpea growing locations sampled showing that aphids, other than pea aphids, vectoring these viruses might have contributed for their wide distribution and better performance at wider ecological niches. Additionally, it is possible to speculate that other cool-season legumes such as faba bean, field pea and grass pea are growing at these altitude ranges and may serve as inoculum sources as most legume viruses cross-infect species in the family Leguminosae, and hence immensely contributing to their wide distribution.

Several viral diseases having local, regional and global importance have also been reported to attack chickpea, some of which are known to cause considerable damage to the crop and economic losses to the farmers (Bos *et al*., 1988; Najar *et al*., 2000; Latham *et al*., 2004; Chen *et al*., 2011). Regionally including Ethiopia, the most economically important viruses with notable damage to the crop belong to the families *Solemoviridae* and *Tombusviridae* such as BWYV, BLRV, and CpCSV (Tadesse *et al*., 1999; Bekele *et al*., 2005; Abraham *et al*., 2006; Makkouk *et al*., 2014). The present results substantiated the previous findings that viruses in the families *Solemoviridae* and *Tombusviridae* are dominantly identified at higher proportions in most of the major chickpea growing areas (Tables 1 and 3). In addition to PSbMV, BLRV & BWYV, pea aphid is reported to vector CpCSV (Asaad*et al*., 2009), and its wide occurrence along other vectors might have contributed to the prevalence of luteoviruses and poleroviruses in Ethiopia. This, therefore, calls for particular research attention. Berhanu et al. (2005) reported that the major viruses infecting chickpea in Ethiopia were luteoviruses and poleroviruses when tested by broad-spectrum legumes McAb (5G4). When 248 chickpea samples reacted positively to 5G4 monoclonal antibody were retested by monoclonal Ab specific to BWYV, BLRV and SbDV, only 30 (12%) samples were positive to BWYV, and none to BLRV and SbDV, while 218 (87.9%) were negative to BWYV, BLRV and SbDV, suggesting the presence of luteovirus or poleroviruse or both unidentified/undetected by the antibodies used and most were likely

CpCSV. This result is in agreement with findings in the present study that out of 173 chickpea, positive samples to 5G4 McAb, 109 (63%) reacted to CpCSV specific McAb, and the remaining 64 5G4 positive samples could be due to other luteoviruses and poleroviruses. This implies that CpCSV is the major virus threatening chickpea production and productivity and the main cause of chickpea stunt disease.

Conclusion and Recommendation

The study revealed that luteoviruses and poleroviruses including CpCSV, and pea seed-borne mosaic potyvirus (either alone or in combination) coupled with pathogens of fungal origin (particularly aschochyta blight and wilt) are seriously threatening lentil and chickpea production in Ethiopia, an evidence supported by declining production and productivity. If chickpea and lentil crops are infected early in the season, these pathogens can bring about total crop failure. Managing diseases of chickpeas and lintels, in particular, diseases caused by viruses is a big challenge to the country considering that lentils and chickpeas are mainly produced by small-scale farmers who do not have enough land and the capacity to implement scheduled rotation, use certified seeds to manage soil- and seed-born diseases, lack technical capacity and support to identify and monitor diseases and apply possible management practices. Thus, affordable, locally possible and safer disease management options should be considered with due emphasis on developing resistant/tolerant varieties to respective diseases and integrated with other disease management options including cultural practices and chemical control targeting virus vectors.

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