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VARIATION IN LENTIL GERMPLASM FOR REACTION TO VIRUS INFECTION

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Abstract

This study was conducted to assess the variation among lentil germplasm for their reaction to natural virus infection and to identify sources of resistance against viruses infecting lentil. Based on field observations and ELISA, a great variation was observed among lentil genotypes for their reaction to natural virus infection. Lentil was infected by pea seed-borne mosaic virus (PSbMV), cucumber mosaic virus (CMV), bean yellow mosaic virus (BYMV), broad bean stain virus (BBSV) and faba bean necrotic yellows virus (FBNYV). PSbMV was the most common virus with 64 % of lentil genotypes infected followed by CMV (35%), BBSV (16%), BYMV (14%) and FBNYV (10%).

Out of 108 lentil germplasm accessions/breeding lines tested, none of the genotypes was found as highly resistant. However, thirty (64%) and 9 (35%) genotypes were found as resistant and moderately resistant respectively to viral diseases infection. Out of 9 moderately resistant genotypes, four were from Pakistan (99CL-002, 97CL-010, 93CL-005, 11222), four from Syria (FLIP 66-542, FLIP 96-582, ILL-7517, ILL-7537) and one from Canada (ILL- 8105). All other lines were susceptible to highly susceptible.

Introduction

Lentil (*Lens culinaris* L.) is an important winter season pulse crop mainly grown under rainfed conditions in Punjab, Pakistan. Several diseases infect lentil in various lentil growing regions of the country, which contribute to the irregular yields obtained from this crop annually. The most important diseases of lentil in Pakistan are foliar blight, rust, root rot wilt complex and viruses (Bashir & Malik, 1988). According to the latest survey conducted in 1997 in Punjab, Pakistan, it was found that seed-borne viruses are becoming more important than others lentil diseases (Bashir *et al.*, 1998).

Worldwide, virus diseases pose a significant limitation to lentil production (Bos *et al.*, 1988). Lentil becomes naturally infected with at least 10 different viruses (Bos *et al.*, 1988; Fletcher, 1993). Five viruses viz., pea seed-borne mosaic virus (PSbMV) (Aftab *et al*., 1992), cucumber mosaic virus (CMV) (Bashir *et al*., 1994), faba bean necrotic yellows virus (FBNYV), beet western yellows virus (BWYV) (Makkouk *et al*., 1998) and chickpea chlorotic dwarf virus (CCDV) (Makkouk *et al*., 1998) have been reported to naturally infect lentil in Pakistan.

The importance of these viruses infecting lentil varies from location to location and country to country. In Iran, bean yellow mosaic (BYMV) and cucumber mosaic (CMV) viruses have caused great reduction in lentil production (Kaiser, 1973). In Italy, Vovlas & Rana (1972) reported that lentil is a natural host for pea enation mosaic virus (PEMV), and this virus is also important in lentil in USA (Aydin *et al*., 1987). In Pakistan, the lentil seed-borne viruses may cause 1.5 million US $ loss annually (Bashir *et al*., 1998). Sap inoculation with BYMV at pre-flowering and flowering stages led to 96 and 34% yield reduction respectively (Kumari *el al*., 1994).

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Keeping in view the significance of host plant resistance in viral disease control, this study was conducted to assess variation among lentil germplasm to natural virus infection and identify genotypes showing resistance to viral infection under field conditions.

Material and Methods

One hundred and eight lentil germplasm accessions/breeding lines were obtained from Barani Agricultural Research Institute (BARI), Chakwal (Punjab) and International Centre for Agricultural Research in Dry Areas (ICARDA), Syria. The genotypes originated from eight countries. The accession number and country of origin of the accessions tested in this study are given in Table 1. The experiment was conducted at BARI, Chakwal during winter season of 2002-03 under field conditions. Each accession was planted in a 4-meter row length with 40 cm row to row distance. After every two test entries, one row of a spreader (lentil susceptible to viruses) was planted. No chemical spray was given to encourage insect vector population. During vegetative growth stage of the crop, virus symptoms were observed and recorded at two week interval on each accession using 0-5 scoring scale: 0: All plants in a plot completely free of virus-like symptoms (0% infection), (Highly Resistant-HR); 1-10% plants virus infected plants (Resistant-R); 11-20% virus-infected plants (Moderately Resistant-MR); 20-30% plants infected (Moderately Susceptible-MS); 31-40% plants infected (Susceptible-S); more than 40% plants infected (Highly Susceptible-HS). The final observations were taken on March 28, 2003. Five diseased plants (depending upon availability) from each accessions showing virus-like symptoms were collected and brought in the laboratory at National Agricultural Research Centre (NARC), Islamabad to test by Direct Antigen Coating Enzyme-linked Immunosorbent Assay (DAC-ELISA) as described by Hobbs *et. al.*, (1987). The samples were tested against six polyclonal antisera obtained from Dr. K.M. Makkouk, Virologist, ICARDA, Syria to the following viruses; PSbMV, CMV, BYMV, broad bean stain virus (BBSV), FBNYV, and pea enation mosaic virus (PEMV).

Results and Discussion

The viral diseases reactions of each accession as recorded on 0-5 scoring scale are given in Table 2. The viral diseases symptoms on exotic type accessions were more severe than on local types. Based on symptoms observed and disease severity recorded on 0-5 scale, the tested genotypes were divided into five groups. The first group consisted of 30 genotypes (28%), which were rated as “1” showing resistant reaction (R) to viral infection under field conditions. No virus was detected from these genotypes when samples were tested by ELISA against six polyclonal antisera. Out of 30 resistant genotypes, only one line (FLIP 95-12L) was of exotic origin whereas all others were of local types. The second group consisted of 9 (8%) accessions/lines which were rated as “2” showing moderately resistant (MR) reaction to viral infection. Out of 9 accessions, four (99CL-002, 97CL-010, 93CL-005, 11222) were of local origin, four (FLIP 96-542, FLIP 96-582, ILL-7517, ILL-7537) from ICARDA, Syria and one (ILL-8105) from Canada. The third group consisted of 15 genotypes (14%) and were rated as “3” showing moderately resistant reaction. Six from this group were of local types whereas the others were exotic. The fifth and sixth groups consisted of 19 and 35 genotypes showing susceptible (S) to highly susceptible (HS) reaction to viral infection respectively. Majority of the susceptible lines were of exotic in origin (Table 2).

Table 1. Lentil genotypes evaluated for virus infection and their country of origin.

Genotypes	Source of Seed	Country of Origin	Genotypes	Source of Seed	Country of Origin
9CL-002, 99CL-003, 99CL-006, 99CL-001, 8CL-004, 98-CL-008, 98CL-012, 97CL-02, 97CL-006, 97CL-007, 97, CL-010, 7CL-011, 96CL-003, 96CL-009, 95CL-008, 5CL-009, M-93, 93-CL 001, 93CL-005, S-1, -1, AKM-351, AKM-397, 78-2603, E14-1, 14-2, E14-3, E14-4, E14-5, E14-6, 314-7, 14-8, E14-9, E14-10, X 99-3124, X 99-125, X 99-3126, X 99-3127, X 99-3108, X 9-3110, X 99-3111,	BARI, Chakwal	Pakistan	FLIP 66-542, FLIP 96-582, FLIP 96-81, FLIP 96-6L, FLIP 96-2L, FLIP 95-22L, FLIP 95-12L, FLIP 87-9L, ILL-23131, ILL-323, FLIP 96-9L, FLIP 96-13L, FLIP 97-1L, FLIP 97-4L, FLIP 97-7L, FLIP 97-10L, Natalia Inta, LC-460053, FLIP-2002-4l, FLIP 2002 5L, FLIP 2002-6L, ILL-358, ILL-4401, ILL-5597, ILL-5684,	ICARDA	Syria
99-3112, X 99-3116, X 99-3121, X 99-125,			ILL-5714, ILL-5715, ILL-5725, ILL-5755, ILL-5871, ILL-6258, ILL-6465, ILL-7127,		
99-27a, X99-3191, X 99-3196, X 99-3198,			ILL-7177, ILL-7180, ILL-7193, ILL-7199, ILL-7200, ILL-7207, ILL-7213, ILL-5717,		
99-3199, X 99-3200, X 99-3202, (53)			ILL-7537 (43)		
1218, 11120, 112222, M-85 (4)	AARI, Faisalabad	Pakistan	ILL-2439 (1)	ICARDA	Ethopia
L-5480 (1)	ICARDA	Czechoslov-akia	ILL-5244 (1)	ICARDA	Jordan
L-5604 (1)	ICARDA	Turkey	ILL-8105, ILL-8106 (2)	ICARDA	Canada
L-2580 (1)	ICARDA	India	Local Check (Small seeded) (1)	NARC	Pakistan

Table 2. Reaction group of lentil genotypes based on visual scoring scale (0-5).

coring scale (0-5)	Reaction group	Lentil genotypes falling under each category of reaction group
0	Highly Resistant (HR) (0%)	-
1	Resistant (R) (28%)	99CL-001, 98CL-004, 98CL-008, 97CL-002, 97CL-006, 97CL-007, 97CL-011, 96CL-003, 96CL-009, 95CL-008, 95CL-009, M-93, 93CL-001, AKM-397, E 14-1, E 14-2, E 14-3, E 14-4, E 14-15, E 14-6, E 14-8, E 14-19, E 14-10, X 99-3124, X 99-3125, X 99-3126, X 99-3127, 11120, M-85, FLIP 95-12L. (30)
2	Moderately Resistant (MR) (8%)	99CL-002, 97CL-010, 93CL-005, 111222, FLIP- 96-542, FLIP 96-582, ILL-7517, ILL-7537, ILL-8105 (9)
3	Moderately Susceptible (MS) (14%)	99CL-003, 99CL-006, 98CL-012, S-1, E 14-7, 11218, ILL-4401, ILL-5714, ILL-5871, ILL-6465, ILL-7127, ILL-7179, ILL-7200, ILL-2580, local Check (Medium seed size) (15)
4	Susceptible (S) (18%)	AKM-397, X 99-3108, X 99-3110, X 99-3111, X 99-3112, ILL-23131, FLIP 96-9L, FLIP 97-1L, ILL-358, ILL-5480, ILL-5597, ILL-5604, ILL-5684, ILL-5715, ILL-5725, ILL-6258, ILL-7177, ILL-7193, ILL-8106 (19)
5	Highly Susceptible (HS) (32%)	99CL-012, L-1, 78-26003, X 99-3116, X 99-3121, X 99-3125, X 99-3127, X 99-3191, X 99-3196, X 99-3198, X 99-3199, X 99-3200, X 99-3202, FLIP 96-8L, FLIP 96-6L, FLIP 96-2L, FLIP 95-22L, FLIP 87-9L, ILL-323, FLIP 96-13L, FLIP 97-3L, FLIP 97-4L, FLIP 97-7L, FLIP 97-10L, Natalia inta, lC-460053, FLIP 2002-4L, FLIP 2002-5L, FLIP 2002-6L, ILL-358, ILL-5244, ILL-5755, ILL-7180, ILL-7207, ILL-7213 (35)

Based on ELISA results, the following five viruses viz., BBSV, BYMV, CMV, FBNYV, and PSbMV were detected and identified from the diseased samples collected from plants showing virus-like symptoms under natural infection conditions (Table 3). About 32% samples reacted positively when tested by ELISA, whereas majority (68%) of the samples did not react with any antiserum used in ELISA. About 64% of the symptomatic samples indicated the presence of PSbMV. PSbMV was the most common virus detected from the test samples. CMV was detected from 35% samples followed by BBSV (16%), BYMV (14%) and FBNYV (10%) (Table 3). About 44% genotypes indicated the presence of two or more viruses (mixed infection) under same infection conditions. The samples which did not react to any antiserum used in ELISA may be due to other viruses against which we could not use antiserum, or due to symptoms caused by nutrient deficiency and confusing with virus-like symptoms or may be physiological disorder. Such observations have been previously reported by Makoouk *et al*., (2001).

Field observations (symptoms recorded) and ELISA results indicated that there was a great variation among lentil genotypes to virus infection under field conditions. The lentil genotypes were naturally infected with PSbMV, CMV, BBSV, BYMV and FBNYV. Five viruses were detected infecting lentil crop. Although, we used polyclonal antiserum to PEMV, but none of the samples reacted positively with this antiserum, indicating the absence of PEMV in lentil crop. Previously, PSbMV and CMV have been reported as the most common viruses infecting lentil in Pakistan (Makkouk *et al*., 2001). Makkouk *et al*., (2001) also reported the natural occurrence of chickpea chlorotic dwarf virus (CCDV), beet western yellows virus (BWYV) and chickpea luteovirus (CPLV) in lentil. The possibility of the natural infection of these three viruses (CCDV, BWYV, CPLV) infecting lentil cannot be excluded as we could not use the antisera to these viruses in this study as these antisera were not available with us. It is possible that these three viruses may be infecting lentil in this experiment as majority of the symptomatic samples (68%) did not react with the antisera used in ELISA. It is interesting that Makkouk *et al*., (2001) could not detect BYMV from lentil samples when tested by ELISA, whereas in this study 14% samples indicated the presence of BYMV. In this study, although majority of the samples were found with mixed infection of two or more viruses, but BYMV was also detected in single infection. Narrowing and twisting of the leaflets, stunting and yellowing of plants was commonly observed in lentil infected with BYMV. Such symptoms on lentil plants infected by BYMV were also reported by Kaiser (1973). Although, lentil is reported to be naturally infected by BYMV in Syria (Kumari *et al*., 1994) and Iran (Kaiser, 1973), but natural infection of lentil by BYMV seems to be first report in Pakistan.

The preliminary screening of more than 100 lentil genotypes indicated that PSbMV followed by CMV are the most common viruses of lentil in Punjab, Pakistan. Therefore, more attention should be given to these two viruses to identify sources of resistance. Both the viruses are seed-transmitted (Kumari *et al*., 1994, Ahmad *et al*., 1997) and the distribution of virus-free lentil seed to farmers will reduce the incidence of PSbMV and CMV.

It was observed that majority of the lentil genotypes of exotic in origin were susceptible to viral disease infection, whereas the genotypes and breeding lines developed in Pakistan were resistant to virus infection. Our previous data (un-published) also support this observation. As most of the viruses infecting lentil are seed-borne in nature, therefore, the import of exotic lentil germplasm without proper quarantine measures should be restricted to avoid the introduction of new viruses through seed (Bashir, *et al*., 1995). The local and exotic genotypes found resistant to virus infection in this study could be utilized in breeding programme to develop virus resistant lentil cultivars.

Table 3. Viruses infecting different lentil genotypes identified by DAC-ELISA.

Infecting viruses	Lentil genotypes found infected
Pea Seed-borne mosaic virus (PSbMV) (64%)	93CL-005, 99CL-002, 99CL-003, 97 CL-010, 99CL-012, S-1, L-1, AKM-351, 78-26033. E-14-7, X 99-3108, X 99-3110, X 99-3111, X 99-3112, X 99-3116, X 99-3121, X 99-3125. X 99-27a, X99-3191, X 99-3196, X 99-3198, X 99-3199, X 99-3200, X 99-3202, 11222. FLIP 66-542, FLIP 96-582, FLIP 96-81, FLIP 96-6L, FLIP 96-2L, FLIP 95-22L, FLIP 87-9L, FLIP 96-9L, FLIP 96-13L, FLIP 97-1L, FLIP 97-4L, FLIP 97-7L, FLIP 97-10L, FLIP-2002-4l, FLIP 2002 5L, FLIP 2002-6L, Natalia Inta, LC-460053, ILL-323, ILL-358, ILL-2439, ILL-23131, ILL-4401, ILL-5244, ILL-5480, ILL-5604, ILL-5597, ILL-5684, ILL-5714, ILL-5715, ILL-5717, ILL-5725, ILL-5755, ILL-5871, ILL-6258, ILL-6465, ILL-7127, ILL-7177, ILL-7180, ILL-7193, ILL-7199, ILL-7200, ILL-7207, ILL-7213,
Cucumber mosaic virus (CMV) (35%)	99CL-012, S-1, L-1, AKM-351, 78-26033, X-99-3108, X-99-3110, X-99-3116, X 99-3121. X 99-3125, X 99-27a, X99-3191, X 99-3196, X 99-3198, X 99-3199, X 99-3200, FLIP 96-2L, FLIP-96-6L, FLIP-8L, FLIP 87-9L, FLIP 95-22L, FLIP 96-9L, FLIP 96-13L, FLIP 97-1L, FLIP 97-4L, FLIP 97-7L, FLIP 97-10L, FLIP-2002-4L, FLIP 2002 5L, FLIP 2002-6L. Natalia Inta, LC-460053, ILL-5244, ILL-5725, ILL-7180, ILL-7207, ILL-7213.
Bean yellow mosaic virus (BYMV) (14%)	FLIP 66-542, FLIP 96-582, FLIP 96-81, FLIP 97-1L, FLIP 97-4L, FLIP 97-7L, FLIP 97-10L, FLIP-2002-4L, FLIP 2002 5L, FLIP 2002-6L, Natalia Inta, ILL-7199, ILL-7200, ILL-7207, ILL-7213,
Broadbean stain virus (BBSV) (16%)	99CL- 012, AKM 351, FLIP 96-81, FLIP 96-6L, FLIP 96-2L, FLIP 95-22L, FLIP 87-9L. FLIP 96-9L, FLIP 96-13L, ILL-5755, ILL-5871, ILL-6258, ILL-6465, ILL-7127, ILL-7177, ILL-7180, ILL-7193.
Fababean necrotic yellow virus (FBNYV) (10%)	X99-3191, X 99-3196, X 99-3198, X 99-3199, X 99-3200, X 99-3202, FLIP 97-4L, FLIP 97-7L, FLIP 97-10L, ILL-7207, ILL-7213,
Lentil genotypes infected with two or more viruses (mixed infection) (44.%)	99CL-012, S-1, L-1, AKM-351, 78-26033, X 99-3108, X 99-3110, X 99-3116, X 99-3121. X 99-3125, X 99-27a, X 99-3191, X 99-3196, X 99-3198, X 99-3199, X 99-3200, X 99-3202, FLIP 66-542, FLIP 96-582, FLIP 96-8L, FLIP 96-6L, FLIP 96-2L, FLIP 95-22L. FLIP 87-9L, FLIP 96-13L, FLIP 97-1L, FLIP 97-4L, FLIP 97-7L, FLIP 97-10L, FLIP 2002-4L, FLIP 2002-5L, FLIP 2002-6L, Natalia Inta, IC-460053, ILL-323, ILL-358, ILL-5244, ILL-5755, ILL-5871, ILL-6258, ILL-6465, ILL-7127, ILL-7177, ILL-7180, ILL-7200, ILL-7207, ILL-7213.

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