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Differentiation of Bean Common Mosaic Virus (BCMV) and Bean Common Mosaic Necrosis Virus (BCMNV) Strains Infecting Common Bean in Samsun Province

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ABSTRACT

Nine *Bean common mosaic virus* (BCMV) and three *Bean common mosaic necrosis virus* (BCMNV) isolates were obtained from leaf and seed samples collected from common bean (*Phaseolus vulgaris* L.) production areas in Samsun province in 2006. BCMV and BCMNV strains were determined by inoculating these isolates on primary leaves of differential host cultivars individually and verifying a range of cultivar reactions against these isolates. Seven isolates were identified as BCMV NL-6 strain, one isolate induced reaction patterns similar to BCMV NL-4 strain and one isolate was characterized as RU-1 strain of BCMV. According to symptom expression of the differential hosts, all BCMNV isolates were found to be NL-3 strain.

Key words: Bean, BCMV, BCMNV, Strain

INTRODUCTION

Forty four viruses may cause infection in common bean (*Phaseoulus vulgaris* L.) in nature (Morales ve Bos, 1988). *Bean common mosaic virus* (BCMV) and *Bean common mosaic necrosis virus* (BCMNV) belongs to the family *Potyviridae* and are known to be one of the most common and destructive viruses infecting common bean in the world (Florez –Estevez et al., 2003). Until 1992, BCMV and BCMNV strains have been designated as BCMV serotype A and BCMV serotype B, respectively. Later, they have been classified into two distinct species (Vetten et al., 1992; McKern et al., 1992;

Mink et al., 1994). BCMV and BCMNV have limited host range, but they may cause yield reduction up to 80%. Both of them are transmitted by aphid species such as *Acyrthosiphon pisum*, *Aphis craccivora*, *A. fabae* and *Myzus persicae* by non-persistent manner, as well as by mechanical, seed and pollen transmission occur in nature (Silbernagel et al., 2001).

BCMV and BCMNV may cause very similar symptoms on bean including mosaic, mottling, leaf curling, stunting and chlorosis (Morales and Bos, 1988). The other type of symptom is systemic necrosis, which is called top necrosis or black root (Cooper and Jones, 1983). Top necrosis occurs due to vascular necrosis as a result of hypersensitive reaction, and it may kill plants at last. If the bean cultivar has the dominant *I* gene and infected with BCMV-necrotic strains, it may show top necrosis in temperatures at 30°C or above (temperature-sensitive necrosis), but if it is infected with BCMNV, top necrosis occurs in all temperatures (temperature insensitive necrosis) (Kelly, 1997). Therefore, it is possible to differentiate BCMV and BCMNV symptomatically using the plants possessing the dominant gene *I* and raising temperature to 30°C (Gilbertson et al., 2001).

Drijfhout et al. (1978) biologically classified the strains of BCMV and BCMNV. The strains NL-3, NL-5 and NL-8 belong to BCMNV, and NL-1 (US-1), NL-2, NL-4 (US-6), NL-6 (US-4), NL-7, US-2 and US-5 are the strains of BCMV. Strain RU-1 was determined in 1985 by researchers at USDA in the seeds imported from Russia and placed in the pathotype 6 (Silbernagel et al., 2001). BCMV and BCMNV strain evolution is not well understood, but it could be mutation or RNA recombination between different viruses or strains (Larsen et al., 2005). The strains of BCMV and BCMNV are grouped into 7 pathotype groups (PG) in terms of pathogenicity genes that they have, and host reactions. The strains in PG (1), PG (2) and PG (3) have one pathogenicity gene, the strains in PG (4) and PG (5) have two pathogenicity genes, and the strains in PG (6) and PG (7) have three pathogenicity genes. According to this grouping, BCMV strains were put into PG (1), PG (2), PG (4), PG (5), PG (6) and PG (7), whereas BCMNV strains were grouped into PG (3) and PG (6) (Drijfhout et al., 1978; Drijfhout, 1994; Silbernagel et al., 2001).

Common bean is an important crop in the Black Sea Region, and Samsun province supply about 20% of fresh and 6% of snap bean production of Turkey (Anonymous, 2002). In the earlier studies, BCMV (Açıkgöz, 1984; Fidan and Yorgancı, 1990; Lisa et al., 1994; Güzel and Arlı-Sökmen, 2003), BCMNV (Güzel and Arlı-Sökmen, 2003), *Bean yellow mosaic virus* (BYMV) (Açıkgöz, 1984; Lisa et al., 1994), *Cowpea aphid borne mosaic virus* (CABMV) (Yılmaz and Özaslan, 1987), *Cucumber mosaic virus* (CMV) (Güzel and Arlı-Sökmen, 2003), *Tobacco black ring virus* (TRRV) (Gümüş et al., 2001) and *Alfalfa mosaic virus* (AMV) (Güzel and Arlı-Sökmen, 2003) were determined in bean growing areas in Turkey.

Reactions of differential hosts help to basically clarify the most strains of both BCMV and BCMNV (Drijfhout et al., 1978; Tu, 1986;Omunyin et al. 1995; Saiz et al., 1995). But, some strains have been further characterized by sequencing viral genes and 3'untranslated region of viral RNA (Khan et. al., 1993) or by serologically (Naderpour et al., 2010) as well as differential host reactions. Comparisons of aminoacid sequences of the coat protein region of BCMV and BCMNV isolates identified these isolates in only pathogroup level (Xu and Hampton, 1998; Flores-Estevez et al., 2003), however, it was emphasized in the study that extensive biological assays on differential bean cultivars need to be carried out to confirm the results (Flores-Estevez et al., 2003). Phylogenetic analysis of partial coat protein aminoacid sequences of the isolates infecting lima bean in Peru were used to show whether the isolates are new strains or the strains of BCMV (Malgarejo et al., 2007).

Characterization of BCMV and BCMNV strains is important to be aware of the possible existence of novel virus pathotypes. These novel pathotypes have possibility to be used for identification of new resistance genes in bean cultivars. Also, knowing the virus strains present in bean production regions is valuable for improving bean cultivars using available resistance genes (Saiz et al., 1995). To our knowledge, there is no study related to BCMV and BCMNV strain discrimination in Turkey. This paper presents a preliminary report on BCMNV and BCMV strains infecting common bean in Samsun province.

MATERIALS AND METHODS

Surveys and Sample Collection

Samplings were carried out at different locations of Bafra, Carsamba, Central, Kavak, Ladik, Ondokuzmayıs, Tekkeköy and Terme districts of Samsun province in 2006. Leaves of plants showing viral diseases symptoms and seeds were collected from bean fields and growers, respectively, and maintained at -18 °C or + 4 °C until tested.

Virus Detection

Seed and leaf samples were tested by Double antibody sandwich-enzyme linked immunosorbent assay (DAS-ELISA) according to Clark and Adams (1977) using anti-BCMV and BCMNV polyclonal antisera (BIOREBA, Switzerland). Then, the samples found to be positive were also tested for *Bean yellow mosaic virus* (BYMV), *Alfalfa mosaic virus* (AMV), *Cucumber mosaic virus* (CMV) and *Soybean mosaic virus* (SMV) to determine and exclude the mixed-infected samples. Absorbance values at 405 nm were recorded using the Tecan Spectra II Microplate Reader (Austria). Samples having absorbance values of at least two-fold higher than the mean absorbance values of the healthy controls were considered as positives (Strausbaugh, 2003b). Then, the samples found to be infected with only BCMV or BCMNV were selected for further analysis.

Differential Bean Cultivars

Virus-free seeds of differential bean cultivars (Table 1) (Drijfhout et al., 1978; Lillian and Msuku 2001; Silbernagel et al., 2001) were supplied by the North Central Regional Plant Introduction Station, USA and used to determine the strains of BCMV and BCMNV.

Inoculum Preparation and Virus Inoculation

BCMV and BCMNV isolates that were positive in ELISA were propagated and maintained in *Phaseolus vulgaris* L cv. Dubbele Witte or Sutter Pink. Bean plants were rub-inoculated with carborandum powder (400-500 mesh) at primary leaf stage when the size of leaf reached to ½ - ¾ of whole size (Drijfhout et al., 1978). The seeds of differential bean cultivars were sown in plastic pots. The infected leaves of 2-6 week old plants were harvested and homogenized in a chilled sterilized mortar and pestle using phosphate buffer (1% K_2HPO_4 containing 0.1% Na_2SO_3, pH: 7.5) (Sengooba et al., 1997). Each isolate was inoculated on to leaves of common bean cultivars, and then the inoculated plants were kept in controlled room conditions at 22 °C ±1 and 12 hours of photoperiod. In a parallel study, the plants containing the dominant *I* gene were maintained at 30 °C ±1 after inoculation to differentiate BCMV-necrotic strains.

Strain Differentiation

Strains were determined by inoculating BCMV and BCMNV isolates on primary leaves of differential host cultivars with two replicates, and verifying a range of cultivar reactions against these isolates. Reactions to each isolate were scored visually 21 days after inoculation. Non-inoculated leaves of the plants with no visible symptom were tested by DAS-ELISA to reveal the presence of latent infection. Plants with systemic symptoms or plants positive in ELISA were evaluated as susceptible (S), otherwise resistant (R). If a plant has systemic necrosis at 30 °C or at 23 °C, this condition was named temperature-sensitive necrosis (TSN) or temperature-insensitive necrosis (TIN), respectively. Evaluations were made by considering the phenotypic appearance of the currently known BCMV and BCMNV strains on differential bean cultivars which were shown in Table 1 (Drijfhout et al., 1978; Drijfhout, 1994; Silbernagel et al., 2001).

Table 1. Differential Bean Cultivars and Pathogen-host Interactions Used in Strain Identification

Bean Cultivars and Their Resistance Genes	BCMV and BCMNV Strains										
	NL1	**NL7**	**NL8**	**US5**	**NL6**	**US2**	**NL2**	**NL3**	**NL5**	**RU1**	**NL4**
1.Dubbele Witte (*i*)	S	S	S	S	S	S	S	S	S	S	S
2. Redlands Greenleaf C (*bc-u,bc-1*)	R	S	R	S	S	S	S	S	S	S	S
3. Redlands Greenlaef B (*bc-u,bc-1*^{2})	R	R	R	S	S	R	R	S	S	S	S
4. Sanilac (*bc-u,bc-2*)	R	R	S	R	R	S	S	S	S	S	R
5. U1 114 (*bc-u,bc-1,bc-2*)	R	R	R	R	R	S	S	S	S	S	R
6. Monroe (*bc-u, bc-1*^{2}*,bc-2*^{2})	R	R	R	R	R	R	R	R	R	R	S
7. IVT7214 (*bc-u,bc-2, bc-3*)	R	R	R	R	R	R	R	R	R	R	R
8. Widusa, BTS-1 (*I*)	R	R	S*	R	S**	R	S**	S*	S*	S**	R
9. I.T.40031, Top Crop (*I-bc-1*)	R	R	R*	R	S**	R	S**	S*	S*	R	R
10. Amanda (*I, bc-1*^{2})	R	R	R*	R	S**	R	R	S**	S*	R	R
11. IVT 7233 (*I, bc-u,bc-1*^{2}*,bc-2*^{2})	R	R	R*	R	R	R	R	R*	R*	R	R

R: Resistant, S: Susceptible, S*: susceptible, all plants usually show vein necrosis and systemic necrosis at temperatures < 30^{o}C (temperature-insensitive necrosis: TIN), S**: Susceptible, the number of plants with top necrosis increases at temperatures > 30^{o}C (temperature-sensitive necrosis: TSN), R*: Resistant, no systemic necrosis, pin-point necrotic local lesion.

RESULTS AND DISCUSSION

Identification of BCMV and BCMNV Strains

Eighty three leaf and 48 seed samples (131 samples in total) were collected in Bafra, Çarsamba, Central, Kavak, Ladik, Ondokuzmayıs, Tekkeköy and Terme districts of Samsun province (Table 2). The percentages of BCMV and BCMNV-infected samples were 34.4% and 7.6%, respectively. The only 9 BCMV and 3 BCMNV isolates were propagated and maintained for strain identification tests (Table 3) because some positive-samples had low virus content or mixed infection of other viruses. None of the positive samples collected in Ondokuzmayıs and Tekkeköy districts could be propagated and reached to sufficient inoculum level. All BCMNV isolates were obtained from leaf samples collected in Çarşamba and Bafra districts (Table 3) whereas BCMV isolates were acquired from both seed and leaf samples taken from Çarşamba, Central, Terme, Ladik and Bafra districts of Samsun. None of the positive samples collected in Ondokuzmayıs and Tekkeköy districts could be propagated and reached to sufficient inoculum level.

Table 2. The number of samples collected and found to be infected in the districts of Samsun province

Districts	Number of Fields Surveyed	Number of Samples		Number of Infected Samples		
		Leaf	Seed	BCMV	BCMNV	Mixed
Central	7	-	7	3	0	0
Çarşamba	43	38	19	11	2	1
Bafra	9	22	2	8	1	5
Terme	17	13	11	9	0	0
Ladik	2	-	2	1	0	0
Ondokuzmayıs	3	7	-	3	0	1
Kavak	2	-	2	0	0	0
Tekkeköy	7	3	5	3	0	0
Total	88	83	48	38	3	7

Pathogenicity phenotype of 12 isolates was determined by inoculation to the differential bean cultivars carrying different resistance gene combinations (Table 1). According to symptom expression of the differential host groups (HGs), seven isolates (BTür-5, ÇKur-1, SMAlb-1, TSak-1, LMer-2, SMAsr-6, TÇrd-1) were identified as NL-6 strain, one isolate (TMer-3) induced reaction patterns similar to NL-4. One isolate (TÇrd-6) was similar to RU-1 strain of BCMV. All BCMNV isolates (BTür-2, ÇAhu-2, ÇAhu-3) were found to be NL-3 strain (Table 4).

Symptom expressions of differentials made strain evaluation possible in most cases visually, for instance the inoculated leaves of cv. Monroe (HG 6) showed very distinct symptoms with RU-1 and NL-6 strains in the current study (Fig. 1).

Table 3. Origins of BCMV and BCMNV Isolates Used in Strain Identification

Districts	**Village**	**Isolate (Source)**		**Virus**
Çarşamba	Kurtuluş	ÇKur-1	(Seed)	BCMV
		ÇAhu-2	(Leaf)	BCMNV
	Ahubaba	ÇAhu-3	(Leaf)	BCMNV
Terme		TÇrd-1	(Seed)	BCMV
	Çardak	TÇrd-6	(Seed)	BCMV
	Sakarlı	TSak-1	(Seed)	BCMV
	Merkez	TMer-3	(Leaf)	BCMV
Central	Alibeyli	SMAlb-1	(Seed)	BCMV
	Asarağaç	SMAsr-6	(Seed)	BCMV
Ladik	Merkez	LMer-2	(Seed)	BCMV
Bafra		BTür-2	(Leaf)	BCMNV
	Türbe	BTür-5	(Leaf)	BCMV

In our study, TÇrd-6 isolate were determined to be similar to RU-1 strain. Because, symptom expression of this isolate was temperature-sensitive top necrosis in

Black Turtle Soup-1 (HG 8) and Top Crop (HG 9) cultivars (data not shown), and it differed from the results of Lillian and Msuku (2001) in which the only HG 8 exhibited temperature-sensitive systemic necrosis when infected with RU-1 strain. Result of the current study showed that our RU-1 strain was slightly different than the standard RU-1 (Table 4).

Table 4. Reactions of BCMV and BCMNV isolates obtained in this study and standard strains

Isolates and Standard Strains	Differential Cultivars											Pathogroup
	1.Dubbele Witte	2.R.G.C	3.R.G.B	4.Sanilac	5.U1 114	6.Monroe	7.IVT 7214	8.Widusa	9.I..T.40031	10.Amanda	11.IVT 7233	
TÇrd-6	S	S	S	S	S	R	R	S**	S**	R	R	6
BCMV RU-1[a]	S	S	S	S	S	R	R	S**	R	R	R	6
BTür-5	S	S	S	R	R	R	R	S**	S**	R	R	4
ÇKur-1	S	S	S	R	R	R	R	S**	S**	R	R	4
SMAlb-1	S	S	S	R	R	R	R	S**	S**	R	R	4
TSak-1	S	S	S	R	R	R	R	S**	S**	R	R	4
LMer-2	S	S	S	R	R	R	R	S**	S**	R	R	4
SMAsr-6	S	S	S	R	R	R	R	S**	S**	R	R	4
TÇrd-1	S	S	S	R	R	R	R	S**	S**	R	R	4
BCMV NL6 [a]	S	S	S	R	R	R	R	S**	S**	S**	R	4
TMer-3	S	S	S	R	R	S	R	R	R	R	R	7
BCMV NL4 [a]	S	S	S	R	R	S	R	R	R	R	R	7
BTür-2	S	S	S	S	S	R	R	S*	S*	S**	R*	6
ÇAhu-2	S	S	S	S	S	R	R	S*	S*	S**	R*	6
ÇAhu-3	S	S	S	S	S	R	R	S*	S*	S**	R*	6
BCMNV NL-3 [a]	S	S	S	S	S	R	R	S*	S*	S**	R*	6

[a] Standard strain, R: Resistant, S: Susceptible, S*: susceptible, all plants usually show vein necrosis and systemic necrosis at temperatures < 30°C (temperature-insensitive necrosis: TIN), S**: Susceptible, the number of plants with top necrosis increases at temperatures > 30°C (temperature-sensitive necrosis: TSN), R*: Resistant, no systemic necrosis, pin-point necrotic local lesion.

Temperature-sensitive top necrosis occurred in plants possessing the dominant *I* gene such as HG 8 (Black Turtle Soup- 1) and HG 9 (Top Crop) when inoculated with the isolates, BTür-5, ÇKur-1, SMAlb-1, LMer-2, SMAsr-6, TÇrd-1 and TSak-1 whereas it did not occur in HG 10 (cv. Amanda) plants. Similar results were obtained with an isolate in HG 10 (cv. Amanda) by Lillian and Msuku (2001) who characterized this isolate as NL-6 strain. Contrary to these results, Drijfhout et al. (1978) demonstrated that NL-6 strain in their study developed temperature sensitive necrosis in cv. Amanda. BTür-5, ÇKur-1, SMAlb-1, LMer-2, SMAsr-6, TÇrd-1 and TSak-1 isolates in the current study were designated as BCMV NL-6 strain. On strain characterization, the results at 23°C rather than 30°C are considered because some isolates of BCMV and BCMNV may not exhibit symptoms expected at higher temperatures, and the results at conditions lower than 30°C are more reliable (R. Larsen, *personal communication*).

The results with TMer-3 isolate showed consistency with the results of Drijfhout et al. (1978), Drijfhout, 1994 and Silbernagel et al. (2001) in terms of systemic mosaic reactions in HG 1, HG 2, HG 3 and HG 6 (Fig. 2), and this isolate was identified as NL-4 in our study.

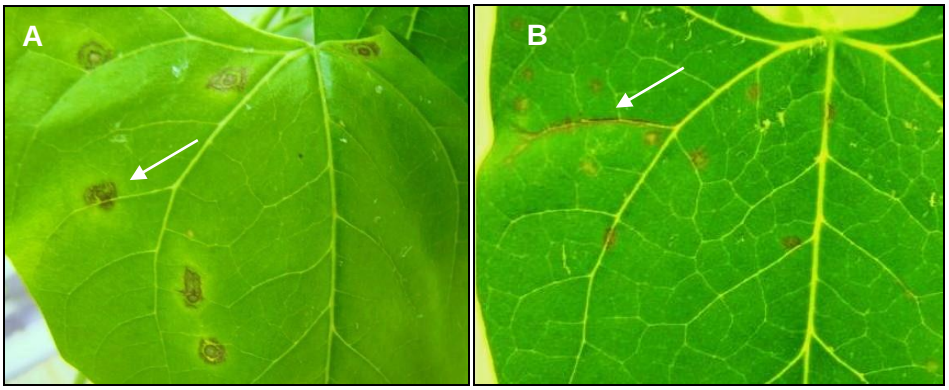

Figure 1. Large necrotic lesions (A) and vein necrosis and necrotic lesions (B) on inoculated leaves of cv. Monroe with RU-1(A) and NL-6 (B) strains. These reactions limit the virus spread outside inoculated leaf and confer virus resistance.

Phenotypic appearance of BCMNV isolates (BTür-2, ÇAhu-2 and ÇAhu-3) on differentials revealed that all isolates belonged to NL-3 strain. The plants in HG 1 (cv. Dubbele Witte), HG 2 (cv.Redlands Greenleaf C), HG 3 (cv. Redlands Greenleaf B), HG 4 (cv. Sanilac) and HG 5 (cv. UI-114) showed systemic mosaic, while the plants in HG 10 (cv. Amanda) and HG 11 (cv. IVT 7233) produced temperature sensitive systemic necrosis (Table 4) and pin-point local lesions (Fig. 3) on non-inoculated leaves, respectively. Response of cv. Widusa (HG 8) to all BCMNV isolates also systemic necrosis at normal conditions (temperature-insensitive necrosis). Parallel

results were obtained by Drijfhout et al. (1978), Guzman et al. (1997) and Silbernagel et al. (2001). However, reactions of some individual plants in differential HG 9 (Top Crop and I.T.40031) were variable to BCMNV NL-3 strain in our study with either temperature insensitive top necrosis (Fig. 3) or vein necrosis (data not shown) on non-inoculated leaves. Similarly, the results with Jubila (HG 9) and Top Crop (HG 9) cultivars differed in some individual plants against to NL-3 strain in the previous study (Strausbaugh et al., 2003a).

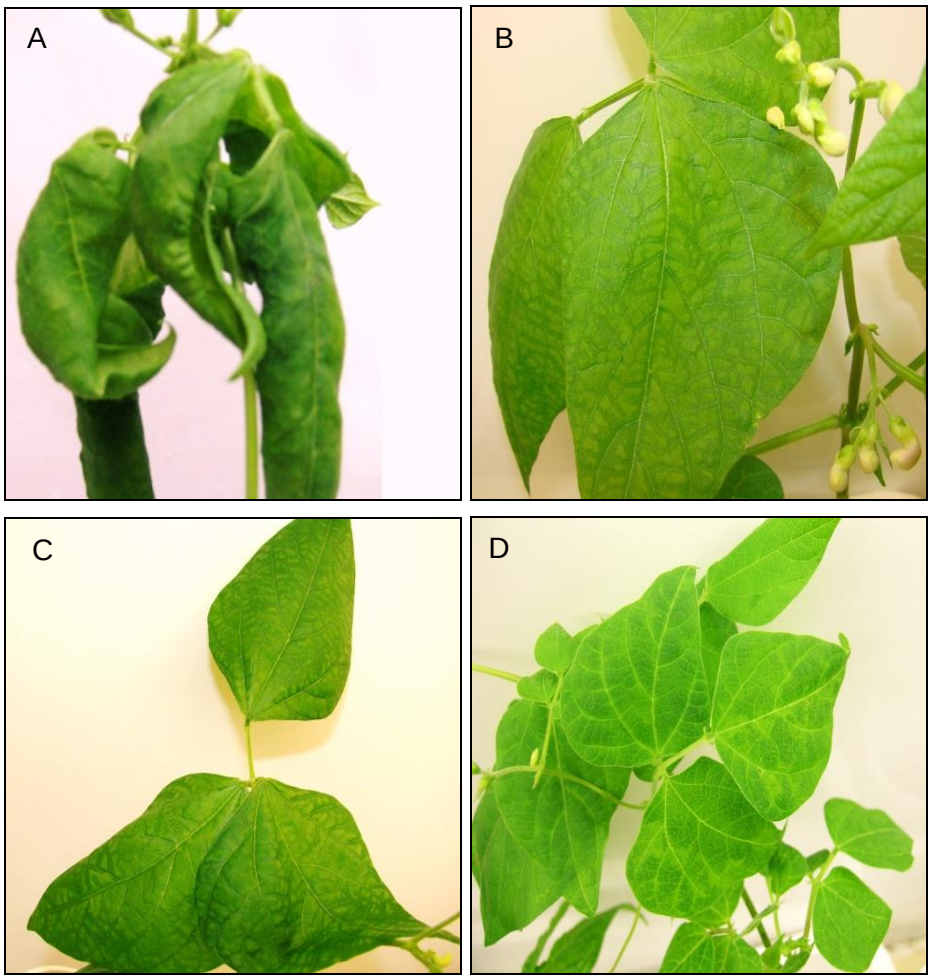

Figure 2. Systemic mosaic reactions of TMer-3 isolate (BCMV NL-4) in HG1 (Dubble Witte) (A), HG2 (Redlands Greenleaf C (B), HG3 (Redlands Greenleaf B) and HG6 (Monroe) (D).

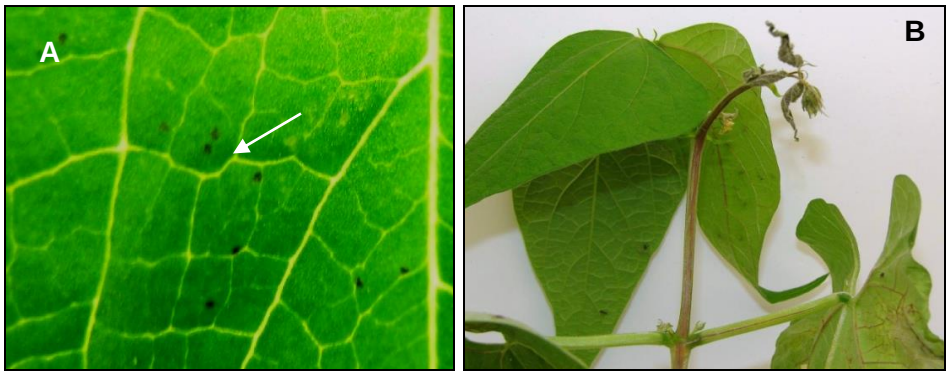

Figure 3. Pinpoint local lesions (A) on inoculated leaf of IVT 7233 cultivar (HG 11) and temperature insensitive top necrosis (B) on I.T. 40031 bean cultivar (HG 9) inoculated with BCMNV NL-3 strain.

Phenotypic appearance of BCMNV isolates (BTür-2, ÇAhu-2 and ÇAhu-3) on differentials revealed that all isolates belonged to NL-3 strain. The plants in HG 1 (cv. Dubbele Witte), HG 2 (cv.Redlands Greenleaf C), HG 3 (cv. Redlands Greenleaf B), HG 4 (cv. Sanilac) and HG 5 (cv. UI-114) showed systemic mosaic, while the plants in HG 10 (cv. Amanda) and HG 11 (cv. IVT 7233) produced temperature sensitive systemic necrosis (Table 4) and pin-point local lesions (Fig. 3) on non-inoculated leaves, respectively. Response of cv. Widusa (HG 8) to all BCMNV isolates also systemic necrosis at normal conditions (temperature-insensitive necrosis). Parallel results were obtained by Drijfhout et al. (1978), Guzman et al. (1997) and Silbernagel et al. (2001). However, reactions of some individual plants in differential HG 9 (Top Crop and I.T.40031) were variable to BCMNV NL-3 strain in our study with either temperature insensitive top necrosis (Fig. 3) or vein necrosis (data not shown) on non-inoculated leaves. Similarly, the results with Jubila (HG 9) and Top Crop (HG 9) cultivars differed in some individual plants against to NL-3 strain in the previous study (Strausbaugh et al., 2003a).

NL-6 strain of BCMV was the most prevalent strain and detected in all districts. NL-4 and RU-1 strains were determined in the samples collected from Çarşamba and Terme districts, respectively, whereas NL-3 strain (BCMNV) was present in only Bafra and Çarşamba districts. This study indicated that necrotic and non-necrotic strains of BCMV and NL-3 strain of BCMNV are naturally infecting bean crop in Samsun province. Seed transfer from one region to another is very common and there is no effective bean certification program in Turkey. So, it is very important to develop and use virus-resistant varieties in bean production. Strain information reported in this study would be valuable for breeders to incorporate resistance genes into bean cultivars where these viral species are problem. We are also currently studying on distinction of virus strains in other bean growing areas of Turkey in more detail and screening bean

genotypes for genetic resistance resources. The results of phenotypic characterization of BCMV and BCMNV strains, especially those of the ones with distinct biological properties in our study need to be combined with the results of nucleotide sequence analysis in the future.

ÖZET

SAMSUN İLİNDE FASULYE ÜRETİM ALANLARINDA ENFEKSİYON OLUŞTURAN BEAN COMMON MOSAIC VIRUS (BCMV) VE BEAN COMMON MOSAIC NECROSIS VIRUS (BCMNV)'ÜN IRKLARININ BELİRLENMESİ

Samsun ilinde 2006 yılında fasulye (*Phaseolus vulgaris* L.) yetiştiriciliğinin yapıldığı alanlardan alınan ve *Bean common mosaic virus* (BCMV) ve *Bean common mosaic necrosis virus* (BCMNV) ile enfekteli olduğu belirlenen örneklere ait toplam 9 BCMV ve 3 BCMNV izolatı elde edilmiştir. İzolatlar 11 farklı fasulye çeşidinden oluşan ırk ayrım setindeki bitkilere ayrı ayrı inokule edilmiş ve çeşitlerin verdiği reaksiyonlara göre BCMV ve BCMNV'nin ırkları belirlenmiştir. BCMV izolatlarının yedi tanesinin BCMV NL-6, bir tanesinin NL-4, bir tanesinin ise RU-1 ırkı olduğu saptanmıştır. BCMNV izolatlarının tamamının NL-3 ırkı olduğu tespit edilmiştir.

Anahtar Sözcükler: Fasulye, BCMV, BCMNV, Streyn

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