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Evaluation of Resistance in Soybean Germplasm to Soybean Mosaic Potyvirus under Field Conditions

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Abstract: Soybean germplasm found resistant to two SMV isolates through mechanical inoculation under screen house conditions were exposed to field conditions. Among 13 soybean varieties, Malakand-96, Bryan and Sherman had no symptoms and no virus was detected by ELISA. Wahab-93, Lugan, Hobbit, Kingsay, Harper, Nare, NARC-V and Clark developed mild mosaic and light veinal chlorosis on a few young leaves. Rincondita and Swat-84 exhibited mosaic and vein clearing on many young leaves. Among soybean lines, GC-81083-63, GC-81084-51, GC-80072-2-6, AGS-253 and AVRDC-13, no visible host reaction was observed. On AVRDC-12 and AVRDC-15 responded mild mosaic symptoms were observed on a few leaves. Malakand-96, Bryan and Sherman and GC-81083-63, GC-81084-51, GC-80072-2-6, AGS-253 and AVDRC-13 lines were re-tested in screen house conditions by inoculating isolate S1 through green peach aphids (*Myzus persicae*). Three soybean varieties and four lines exhibited a mild mosaic on young leaves. A low virus titre was reported in resistant soybean germplasm.

Key words: *Glycine max*, resistance, soybean mosaic potyvirus

Introduction

Soybean mosaic virus (SMV) is one of the most prevalent virus of soybean *Glycine max* (L.) Merrill in the world as well as in Pakistan. It is a species of the genus potyvirus, contain flexuous rods averaging about 750x15-18 nm (Galvez, 1963; Ross, 1967; Soong and Milbrath, 1980). Nucleic acid in SMV virions have single stranded RNA, constituting 5.3 % of the particle mass and having a molecular weight of $3.25x10^6$d (Hill and Benner, 1980a, b).

SMV is transmitted in nature by insect vector belonging to the family Aphididae (Abney *et al.*, 1976). Some 16 aphid species including *Acyrthosiphon pisum*, *Aphis faba* and *Myzus persicae* have been reported to transmit the virus in a non-persistent manner (Bos, 1972). Up to 30 % or more of the seeds of diseased plants are infected depending on cultivar and time of infection before flowering (Bos, 1972). The virus is present in seed coat and embryo and green seeds contain more virus than mature ones (Koshimizu and Iizuka, 1963). Yield losses due to virus infection depends upon virus strains, host genotype and time of infection. However, the virus causes 35-50 % crop loss under natural infections (Ross, 1977) and as high as up to 93 % in experimentally inoculated plants (Sinclair and Backman, 1989). Yield losses maximum up to 45 and 48 % reduction of growth components have been reported by S1 and P1 isolates of SMV in Pakistan (Arif *et al.*, 2002).

SMV produces variable host reaction depending upon the combination of soybean genotype and virus strain (Cho and Goodman, 1979). Most commercial soybean cultivars produce mosaic symptoms when infected with SMV (Bos, 1972; Kwon and Oh, 1980; Lim, 1985). Other susceptible cultivars and lines developed severe mosaic, mottling and necrotic symptoms when inoculated with virulent SMV strains (Cho and Goodman, 1982). However, the host response depends upon genotype, virus strains, time of infection and prevailing climatic conditions. Various sources of SMV resistance have been identified in soybean germplasm elsewhere in the world (Cho and Goodman, 1979, 1982; Goodman *et al.*, 1979; Lim, 1985). Most sources were resistant to some but not all prevalent strains of the virus. Resistance to some SMV strains that produce mosaic symptoms was shown to be conditioned by a single dominant gene (Koshimizu and Iizuka, 1963; Ross, 1977; Kiihl and Hartwig, 1979) whereas resistance to severe isolates, which produced necrotic symptoms on susceptible cultivars, was shown to be conditioned by a single recessive gene (Kwon and Oh, 1980).

SMV has also been reported from various soybean growing areas of NWFP, Pakistan and prevalent virus isolates have been characterized (Arif and Hassan, 2000). Twenty nine soybean varieties and exotic germplasm lines were tested in screen house conditions but none was found immune to two local isolates of SMV. However, 11 commercial varieties and seven lines were either resistant to SMV-isolates, S1 or P1 or to both (Arif *et al.*, 2000).

This study was conducted to evaluate the resistance of soybean germplasm to SMV through its natural insect vector under field and screen house conditions.

Materials and Methods

Soybean germplasm and virus isolates: Soybean varieties and exotic lines were obtained from either local sources or gifted by Asian Vegetable Research and Development Centre (AVRDC), Taiwan in 1999. Seeds of Swat-84, Weber-84, Nare, Ricondita, Kingsay were obtained from Department of Agronomy, NWFP Agricultural University, Peshawar. Harper, Lugan, Wahab-93, Hobbit-87 were obtained from Oil Seed Development Project, Agricultural Research Institute, Tarnab, Peshawar in 1999. Malakand-96 from Agricultural Research Station, Mingora, Swat and NARC-V by Pulses Programme, NARC, Islamabad.

Soybean mosaic virus isolate (SMV-S1) was isolated from an infected soybean plant from Swat and another isolate, SMV-P1 was isolated from an infected soybean plant in Peshawar. Both isolates with characteristic and distinguishable properties (Arif and Hassan, 2000) were maintained in soybean cv. Swat-84 and Weber-84 under insect proof screen house conditions as described by Arif and Hassan (2000).

Soybean germplasm culture under field conditions: Seed of soybean varieties and lines tested were obtained from healthy plants identified through screening experiments conducted under screen house conditions (Arif *et al.*, 2000). A lot of 10 seed of each soybean varieties/lines used in the experiment was tested by DAS-ELISA (Lister, 1978) to check the seed-borne infection of SMV. Virus-free seed of selected soybean varieties/lines were sown in field (three rows/cultivar or line) at NWFP Agricultural University, Experimental Farm at Malakandher.

After germination, thinning and roughing was made and a population of 40 seedlings/germplasm were maintained. At 2-3 leaf stage, seedlings were tested by DAS-ELISA (Arif and Hassan, 2000) to determine seed borne infection if any. Six to eight weeks after germination, natural infection of individual plants of each cultivar/line was assessed by a modified scale (Arif *et al.*, 2000) and DAS-ELISA (Arif and Hassan, 2000).

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Inoculum preparation, inoculation and assessment of virus replication: The virus inoculum was prepared by homogenizing leaves of Swat-84, Weber-84 mechanically inoculated with SMV isolates and having well developed mosaic symptoms (preferably harvested after 3 weeks of virus inoculation) with five volumes (ml/g) of 0.01M sodium phosphate (pH 7.0) in pestle and mortar or a waring blender. The inoculum was squeezed through a double layer of muslin cloth and was applied on carborundum (600 mesh) dusted primary leaves by rubbing leaves after dipping forefingers in inoculum or inoculation was made by rubbing leaves with cotton swab that had been dipped into the inoculum. Plants were kept for symptom development in insect proof screen house. After 3 weeks of inoculation, symptoms less plants were back indexed on *Phaseolus vulgaris* cv. top crop (Milbrath and Soong, 1976) and Weber-84 (Arif and Hassan, 2000). DAS-ELISA was also done randomly by pooling the samples using Patho-screen Kit (Agdia, Elkhart, Indiana, USA). Final record on characteristic virus symptoms was taken 4-5 weeks after inoculation. Virus incidence was determined as percentage of infected to total and disease severity was assessed according to a modified scale as previously reported by Arif and Hassan (2000).

Establishment of aviruliferous aphid culture: A population of green peach aphid, *Myzus persicae* was collected from young shoot of peach and rose plants (non-host for SMV). The identity of aphid species was confirmed with the help of Dr. Anyatullah, (Associate Professor, Department of Entomology, NWFP Agricultural University, Peshawar). A colony of selected aphids were pass through *P. vulgaris* cv. top crop and reared on vigorously grown *Brassica juncea* cv. tendergreen mustard 25 ± 2°C at 70-80 % relative humidity. The aphids were sub-cultured on fresh host plants after 15 days. Before using the aphid culture as virus vector, a pool sample of 10 aphids per lot were tested by DAS-ELISA to ensure that they were virus free.

Transmission of SMV in soybean resistant germplasm through aphid vector: Healthy seed of eight resistant varieties/lines and a susceptible control was sown in 36 cm diameter pots in screen house. After germination, a population of 10 seedlings/pot/cultivar or line were maintained. At 2-3 leaf stage, 4 pots of each cultivar/line were kept in a cage. A pot with 5-6 well-grown source plants of Weber-84 infected with SMV-P1 isolate and heavily infested with *Myzus perciae* (50-100 aphids/plant) was kept in the centre of four pots. A time of 48-72 h was given for movement, feeding and transmission of virus from source to healthy plants of each variety/lines. Aphids were killed by insecticide and plants were kept for symptom development. Different level of resistance was assessed by visual assessment (symptomatology) and DAS-ELISA.

Results

Reaction of selected soybean germplasm under field conditions: Soybean germplasm (varieties/lines) found resistant or resistant to SMV prevalent isolates in screen house conditions (Table 1) were tested for resistance in field conditions. Among 13 soybean varieties, (which were previously reported as highly resistant or resistant to SMV prevalent isolates), Malakand-96, Bryan and Sherman developed no visible symptoms in field conditions and also no virus was detected by DAS-ELISA (Table 1). Wahab-93, Lugan, Hobbit, Kingsay, Harper, Nare, NARC-V and Clark exhibited a mild mosaic and light veinal chlorosis on a few young leaves of selected plants (Table 1). Rincondita and Swat-84 exhibited mosaic and vein clearing on many young leaves. Weber-84, a highly susceptible cultivar used as control, developed severe mosaic and mild mottling, vein clearing on young leaves. Among soybean lines, GC-81083-63, GC-81084-51, GC-80072-2-6, AGS-253 and AVRDC-13, no visible host reaction due to SMV was observed. AVRD-12 and AVRDC-15 developed mild mosaic on a few leaves.

Table 1: Reaction of selected soybean germplasm to soybean mosaic potyvirus in field conditions

Soybean germplasm	F[a] Symptomatology	Disease[b] index	F[a] DAS-ELISA	A_{405}nm[c]
Malakand-96	00/40	0	00/40	0.230
Bryan	00/40	0	00/40	0.251
Sherman	00/40	0	00/40	0.258
Wahab-93	08/40	1	04/40	0.264
Lugan	06/40	1	08/40	0.295
Hobbit-8	09/40	1	05/40	0.266
Rincondita	16/40	2	12/40	0.497
Kingsay	15/40	1	12/40	0.310
Harper-84	10/40	1	11/40	0.396
Swat-84	15/40	2	13/40	0.360
Nare	13/40	1	10/40	0.325
NARC-V	16/40	1	11/40	0.418
Clark	12/40	1	10/40	0.513
Weber-84	22/40	3	25/40	0.986
GC-81083-63	00/40	0	00/40	0.247
GC-81084-51	00/40	0	00/40	0.251
GC-80072-2-6	00/40	0	00/40	0.249
AGS-253	00/40	0	00/40	0.247
AVRDC-12	06/40	1	00/40	0.256
AVRDC-13	00/40	0	00/40	0.259
AVRDC-15	08/40	1	04/40	0.561
GC-81084-118	25/40	3	20/40	1.328

c = Mean obtained from three replicated wells; A_{405} nm after 16 h incubation of substrate at 4°C; $C^+ = 1.982$, $C^- = 0.130$

Table 2: Assessment of level of resistance in soybean resistant germplasm against soybean mosaic potyvirus

Soybean germplasm	F[a] Symptomatology	Disease[b] index	F[a] DAS-ELISA[c]	A_{405}nm[d]
Malakand-96	05/40	1	+	0.311
Bryan	11/40	1	+	0.366
Sherman	07/40	1	+	0.310
GC-81083-63	03/40	1	+	0.296
GC-81084-51	02/40	1	+	0.390
GC-80072-2-6	03/40	1	+	0.416
AGS-253	05/40	1	+	0.503
AVRDC-13	08/40	1	+	0.480
Weber-84 (control)	32/40	3	++	1.211

a = Frequency of virus infection = number of plant infected/number of plant tested

b = Host response index:

0 = no visible symptoms, plants apparently healthy

1 = very mild mosaic (mild mosaic on few leaves/plants)

2 = moderate mosaic (mosaic on many leaves/plant and vein clearing)

3 = severe mosaic (severe mosaic and mild mottling)

4 = severe mosaic (severe mosaic and severe mottling)

5 = severe mosaic plus severe mottling plus necrosis and occasionally death of plants.

c = DAS-ELISA of pooled sample of 10 plants; + = positive; ++ = strong positive; $C^+ = 1.982$, $C^- = 0.130$.

d = A_{405} nm obtained after 16 h of incubation of substrate at 4°C.

GC-81084-118, a highly susceptible line had severe mosaic, mottling and veinal chlorosis on young leaves.

No virus was detected in Malakand-96, Bryan, Sherman and GC-81083-63, GC-81084-51, GC-80072-2-6, AGS-253 and AVRDC-13 by ELISA after 6-8 weeks of planting and was detected in remaining 10 varieties and two lines (Table 1).

Reaction of selected soybean germplasm to SMV through GPA in screen house conditions: The level of resistance in soybean varieties such as Malakand-96, Bryan and Sherman and soybean lines GC-81083-63, GC-81084-51, GC-80072-2-6, AGS-253 and AVDRC-13 was investigated in screen house conditions by inoculation through *Myzus persicae*. After 6-8 weeks of inoculation, all three soybean varieties and four lines developed mild chlorotic mosaic on young leaves (Table 2). Weber-84 developed severe mosaic, mild mottling and veinal chlorosis on younger leaves. DAS-ELISA results confirmed that limited replication of SMV has been occurred in resistant germplasm tested (Table 2).

Discussion

Soybean mosaic potyvirus is one of the most economically destructive virus of soybean in all over the world. The work reported previously by Arif and Hassan (2000) revealed that at least two of the isolates of SMV are widely prevalent in soybean growing areas of the NWFP. Although, the virus is transmitted by more than 16 species of aphids in non-persistent manner (Abney *et al.*, 1976; Bos, 1972) and possibly in this area as well (Arif *et al.*, 2002) but transmission through seed plays an important role in the epidemiology and ecology of this virus. However, per cent infection and severity of the disease depends upon time of infection, virus strain, prevalent climatic conditions and host genotype (Goodman *et al.*, 1979; Irwin and Goodman, 1981). It is a well known fact that if a virus is transmitted by aphid vector as well as through seed, its management and control in the crop would be highly difficult. The best approach is then only the cultivation of resistant varieties. Various sources of resistance have been identified in soybean germplasm to SMV elsewhere in the world (Cho and Goodman, 1979, 1982; Lim, 1985) but resistant material developed in other parts of the world may be against a particular strain of the virus which may or may not be prevalent in Pakistan, because research on prevalence and distribution of SMV strains has not been conducted so far in Pakistan before completion of these studies. Fortunately, detailed studies under this project research have now been carried out and sufficient information have now been available on SMV and its prevalent isolates\strains to two main soybean growing localities of the NWFP which are main soybean growing areas in Pakistan. However, similar work may also be extended to other provinces of Pakistan to assess the incidence and to determine the variability of the pathogen if any.

Previously reported that among 29 soybean varieties and 40 lines, screened and tested both in screen house and field conditions under great inoculum potential, none of the cultivar/line found to be immune to SMV-S1 and SMV-P1 isolates. This may be due to high virulence of the virus or susceptibility of host genotype or both. Immunity to SMV in soybean germplasm is rarely be available in elsewhere in the world. Cho and Goodman (1982) reported high degree of resistance (apparent immunity) in 5 lines to 7 SMV strains. In these studies, Malakand-96 and lines GC-81083-63, GC-81084-51, GC-80072-2-6, AGS-253, AVRDC-12, AVRDC-13 and AVRDC-15, were found to possess a high degree of resistance to the two prevalent SMV isolates. Other soybean germplasm showed variable reaction ranging from resistant to highly susceptible to both isolates. Pathogenic variation among SMV isolates and various levels of reactions of germplasm have been reported by Hunst and Tolin (1982) and Ross (1969).

Field studies conducted for testing resistant materials in natural conditions further confirmed that no immunity was found against SMV prevalent isolates. Malakand-96, Bryan and Sherman and exotic soybean lines, GC-81083-63, GC-81084-51, GC-80072-2-6, AGS-253, AVRDC-13 remained virus free under field conditions. But when the same material was exposed to high inoculum pressure in screen house through vector inoculation, a mild mosaic was observed on young leaves and virus was detected in low concentration by ELISA. This indicated that the soybean germplasm reported above contained high level of resistance (if not the immunity) to SMV which can be incorporated through conventional breeding procedures for the development of resistant varieties.

Soybean germplasm tested elsewhere in the world has been found to resistant to some but not all strains/isolates of SMV. Reactions of soybean germplasm to SMV strains or isolates that produce mosaic symptoms was shown to be conditioned by a single dominant gene (Kiihl and Hartwig, 1979; Koshimizu and Iizuka, 1963; Ross, 1977) whereas resistance to severe isolates which produce necrotic symptoms on susceptible varieties was shown to be conditioned by single recessive gene (Kwon and Oh, 1980). However, in this study it will be premature to assess that the resistance against SMV-S1 and SMV-P1 is based on either a single dominant or a single recessive gene, further detail studies are needed to elucidate the mechanism of gene operation in varieties expressing mosaic and necrotic type of symptoms through detailed hybridization studies. With out going in to the discussion and details of the genotypic background of the varieties/lines, the breeders can select and breed SMV resistant soybean varieties, even on the basis of this information. Varieties, Malakand-96, Sherman, Bryan, Swat-84, Lugan, Hobbit, Recondita, Kingsay, Harper or soybean lines which has shown resistance to available isolates can be safely recommended to growers for general cultivation if other agronomic characters of these varieties\lines are desirable. The findings of this research would be highly useful and beneficial to plant breeders, agronomists, plant protectionists, extenionists and also to the soybean growers. This work will certainly serve as a base line to exploit soybean breeding research in Pakistan and elsewhere in the world.

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