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## Evaluation of Resistance in Soybean Germplasm Against Soybean Mosaic Potyvirus

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**Abstract:** None of the 29 soybean cultivars and 40 germplasm lines tested were found immune to two isolates of soybean mosaic potyvirus (SMV-S1 and SMV-P1). Malakand-96 was the only cultivar found highly resistant to both S1 and P1 isolates. Swat-84, Bryan, Hobbit-87, Kingsay, Lugan, Sherman and Harper-87 were resistant to S1 and P1 whereas Rincondita was resistant to S1 and moderately resistant to P1 isolate. Similarly, Clark, Nare, NARC-V and Wahab-93 were resistant to P1 but not to S1 isolate. Wahab-93, Kharif-93, Ajmeri, Hodgson, Mid-Spray, Full-Walter, NARC-II, NARC-IV, NARC-VI, Clark and Nare were moderately resistant to S1 whereas Kharif93, Ajmeri, Hodgson, Full-Walter, NARC-II, NARC-IV, Rincondita, Mid-Pharoah, NARC-VI and Winchester were moderately resistant to P1 isolate. William, Rawal-I, Mid-Pharoah, Bass, NARC-III, NARC-I, NARC-V and Winchester were susceptible, to SMV-S1 whereas William, Rawal-I, Mid-Spray, Bass, NARC-I and NARC-III were susceptible to P1, Weber-84 was highly susceptible to both isolates of SMV. Among 40 soybean lines, GC-81083-63, GC-81084-51, GC-80072-2-6, AGS-253 and AVRDC-12, AVRDC-13 and AVRDC-15 were highly resistant to both isolates of SMV. Lines GC-81075-44, GC-81090-108, GC-81084-147, GC-81090-10, AGS-85, AGS-249, AVRDC-10, AGS-297, AVRDC-7 were resistant to S1 and P1 isolates whereas L-85-2308, AVRDC-5, AVRDC-14, were resistant to S1 and moderately resistant to P1 isolate. Line L-77-1863 was resistant to P1 but moderately resistant to S1 isolate. Similarly, GC-81080-13, GC-82117-8, AGS-154, L-88-8502, AVRDC-3, AVRDC-4, AVRDC-8 were moderately resistant to S1 and P1 isolates and AVRDC-11, GC-81084-134, GC-8108441, GC-81080-36, GC-81090-94, AVRDC-2, AVRDC-6 were moderately resistant to only P1 isolate. Ten soybean lines were susceptible and 3 highly susceptible to Si whereas 4 lines were susceptible and only one line was highly susceptible to P1 isolate.

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

### Introduction

Soybean mosaic potyvirus (SMV) is one of the most economically destructive viral disease of soybean, *Glycine max* (L.) Merrill in the world as well as in Pakistan. Yield losses due to virus infection depends upon virus strains, host genotype and time of infection. A 35-50% crop loss has been reported under natural infections (Ross, 1977; Goodman and Oard, 1980) and as high as up to 93% in experimentally inoculated plants (Sinclair and Backmann, 1989). Soybean mosaic potyvirus is transmitted in nature by insect vector belonging to the family Aphididae (Abney *et al.*, 1976). Some 16 aphid species including *Acyrtosiphon pisum*, *Aphis faba* and *Myzus persicae* have been reported to transmit the virus in a non-persistent manner (Bos, 1972). Non-vector transmission is through seed and by mechanical means but seed transmission is the most important source of primary infection and disease spread (Bos, 1972; Bowers and Goodman, 1979).

Soybean mosaic potyvirus, a species of the potyvirus genus, contain flexuous rods with a modal length of 750 × 15-18 nm (Galvez, 1963; Ross, 1967; Soong and Milbrath, 1980). Virus particles, ranging from 300 to 900 nm, long have been reported (Soong and Milbrath, 1980); infectivity is highly correlated with particle size, the most infectious particles being over 656 nm long (Sinclair and Backmann, 1989). Nucleic acid in SMV virions have single stranded RNA, constituting 5.3% of the particle mass and having a molecular weight of 3.25 × 10<sup>6</sup>d (Hill and Benner, 1980a, b). Soybean mosaic potyvirus produced variable symptoms 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 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 the host 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 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). This paper reports the work on evaluation of resistance in soybean germplasm to two prevalent isolates of SMV.

### Materials and Methods

**Virus isolates:** 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 had characteristic and distinguishable properties (Arif and Hassan, 2000). Both isolates were maintained in soybean cv. Swat-84 and

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Weber-84 under insect proof screen house as already been described (Arif and Hassan, 2000).

**Source of soybean germplasm:** Twenty nine soybean cultivars and 40 exotic soybean lines were screened for resistance to two SMV-isolates. Seeds of Swat-84, Weber-84, Nare, Riconclita, Kingsay were provided by Department of Agronomy, NWFP Agricultural University, Peshawar; Winchester, Harper, 'Logan, Wahab-93, Rabbit were provided by Oil Seed Development Project, Agricultural Research Institute, Tarnab, Peshawar; Malakand-96 by Agricultural Research Station, Mingora, Swat and NARC-I to NARC-VI by Pulses Programme, NARC, Islamabad. Seeds of remaining soybean cultivars were obtained either from Oil Seed Development Project, Agricultural Research Institute, Tarnab, Peshawar or Agricultural Research Station, Mingora, Swat. Seeds of soybean lines were obtained from above sources or gifted by Asian Vegetable Research and Development Centre (AVRDC), Taiwan.

**Plant Culture and Growth Conditions:** Seeds of soybean cultivars/lines were planted in 26 cm diameter clay pots containing autoclaved soil mixture which consisted of field soil, peat, sand and farm yard manure (1:1:1:(v/v)). After germination, 8 plants/ pot were replicated in 5 times (total 40 plants/cultivar) for all soybean cultivars. For control experiments, a population of 30 plants were maintained (8 plants/pots) which was replicated into 4 times. Separate experiments were conducted to test the seed transmission of the virus in 29 soybean cultivars and 40 lines.

**Inoculum preparation and virus inoculation:** 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 wk of virus inoculation) with five volumes (ml/g) of 0.01 M 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 wk of inoculation, plants (particularly 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 performed randomly pooled samples by using Pathoscreen Kit (Agdia, Elkhart, Indiana, USA). Final record on leaf characteristic virus symptoms in soybean cultivars/lines were taken 4-5 wk after inoculation. Host response was assessed according to a modified scale as previously reported by Arif and Hassan (2000).

### Results

**Reactions of soybean cultivars to SMV-S1 and P1 isolates:** No immunity was found in soybean cultivars tested against the two isolates of SMV. Malakand-96 was again highly resistant to both isolates (Table 1). Swat-84, Bryan, Hobbit-87, Kingsay, Lugan and Harper were resistant to both S1 and P1 isolates whereas Rincondita was resistant to SMV-S1 but moderately resistant to P1 isolate (Table 2, 3). Similarly, Clark, Nare, NARC-V and Wahab-93

were resistant to P1 but not to S1 isolate. Wahab-93, Kharif-93, Ajmeri, Hodgson, Mid-Spray, Full-Walter, NARC-II, NARC-IV, NARC-VI, Clark and Nare were moderately resistant to SMV-S1 isolate whereas Kharif-93, Ajmeri, Hodgson, Full-Walter, NARC-II, NARC-IV, Sherman, Rincondita and Winchester were moderately resistant to SMV-P1 isolate (Table 2, 3). Soybean cultivars, such as William, Rawal-I, Mid-Pharaoh, Bass, NARC-III, NARC-V and Winchester, were susceptible to SMV-S1 isolate whereas William, Rawal-I, Mid-Spray, Bass, NARC-I and NARC-III were susceptible to SMV-P1. Weber-84 was highly susceptible to both isolates of SMV (Table 2, 3).

### Reactions of soybean lines to SMV-S1 and P1 isolates:

Table 4 shows the response of soybean lines against SMV-S1 and SMV-P1 isolates. No immunity was found in 40 soybean lines tested against any of two isolates of SMV. Soybean lines such as GC-81083-63, GC-81084-51, GC-80072-2-6, AGS-253, AVRDC-12, AVRDC-13, and AVRDC-15 were highly resistant to both isolates of SMV (Table 5, 6). Lines GC-81075-44, GC-81090-108, GC-81084147, GC-81090-10, AGS-85, AGS-249, AVRDC-10, AGS-297, AVRDC-7 were resistant to both isolates SMV-S1 and P1 whereas L-85-2308, AVRDC-5, AVRDC-14, were resistant to SMV-S1 and moderately resistant to SMV-P1 (Table 5, 6). Soybean line L-77-1863 was resistant to SMV-P1 but moderately resistant to SMV-S1. Similarly, GC-81080-13, GC-821 17-8, AGS-154, L-88-8502, AVRDC-3, AVRDC-4, AVRDC-8 were moderately resistant to SMV-S1 and P1 isolates and AVRDC-11, GC-81084-134, GC-81084-41, GC-81080-36, GC-81090-94, AVRDC-2, AVRDC-6 were moderately resistant to only SMV-P1 isolate. (Table 5, 6). A list of 10 soybean lines were susceptible and 3 highly susceptible to S1 isolate whereas 4 lines were susceptible and one line was highly susceptible to P1 isolates of SMV (Table 5, 6).

### Discussion

Soybean mosaic potyvirus is one of the most economically important virus disease of soybean. The work reported previously (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 elsewhere (Abney *et al.*, 1976; Bos, 1972) and possibly in this area as well (M. Arif, unpublished) but transmission through seed plays an important role in the epidemiology and ecology of this virus. It is 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 cultivars. 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 particular strain which are not prevalent in this country. No work has been done on the screening of soybean germplasm against SMV in Pakistan. The present studies were carried out to screen and evaluate commercially grown soybean cultivars and exotic soybean lines for resistance against two isolates of SMV.

Among 29 soybean cultivars and 40 Vines, none of the cultivar/line found to be immune to SMV-S1 and SMV-P1

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Table 1: Host response of soybean cultivars to soybean mosaic potyvirus isolates, SMV-S1 and SMV-P1

Soybean germplasm	SMV-S1		Control	SMV-P1		Control
	F <sup>a</sup>	Disease Index <sup>b</sup>	F <sup>a</sup>	F <sup>a</sup>	Disease Index <sup>b</sup>	F <sup>a</sup>
Wahah-93	8/40	3	0/30	10/40	2	0/30
Kharif-93	32/40	3	0/30	40/40	3	0/30
William	40/40	4	0/30	40/40	4	0/30
Ajmeri	38/40	3	0/30	35/40	3	0/30
Rawal-I	40/40	4	0/30	40/40	4	0/3
Hodgson	35/40	3	0/30	35/40	3	0/30
Malakand-96	03/40	1	0/30	2/40	1	0/30
Mid-Pharoah	35/40	4	0/30	30/40	3	0/30
Mid-Spray	40/40	3	0/30	38/40	4	0/30
Full-Walter	40/40	3	0/30	40/40	3	0/30
Swat-84	22/40	2	0/30	30/40	2	0/30
Bass	40/40	4	0/30	40/40	4	0/30
Bryan	8/40	2	0/30	6/40	2	0/30
NARC-I	40/40	4	0/30	40/40	4	0/30
NARC-II	30/40	3	0/30	35/40	3	0/30
NARC-III	34/40	4	0/30	40/40	4	0/30
NARC-IV	30/40	3	0/30	30/40	3	0/30
NARC-V	35/40	4	0/30	35/40	2	0/30
NARC-VI	32/40	3	0/30	40/40	3	0/30
Sherman	5/40	2	0/30	8/40	2	0/30
Hobbit-87	20/40	2	0/30	18/40	2	0/30
Rincondita	30/40	2	0/30	35/40	3	0/30
Kingsay	26/40	2	0/30	30/40	2	0/30
Logan	14/40	2	0/30	15/30	2	0/30
Winchester	35/40	2	0/30	20/40	2	0/30
Clark	32/40	3	0/30	26/40	2	0/30
Nara	25/40	3	0/30	27/40	2	0/30
Weber-84	40/40	5	0/30	40/40	5	0/30

<sup>a</sup>Frequency of virus infection = number of plant infected/number of plant tested

<sup>b</sup>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

Table 2: Reaction of soybean cultivars to SMV-S1

Immune	Highly resistant	Resistant	Moderately resistant	Susceptible	Highly susceptible
Nil	Malakand-96	Sherman Bryan Swat-84 Lugan Hobbit Rincondita Kingsay Harper-87	Wahab-93 Kharif-93 NARC-II NARC-IV NARC-VI Clark Nare Ajmeri Hodgson Full-Walter Mid-Spray	William Rawal-I Mid-Pharoah Bass NARC-I NARC-III NARC-V Winchester	Weber-84

Table 3: Reaction of soybean cultivars to SMV-P1

Immune	Highly resistant	Resistant	Moderately resistant	Susceptible	Highly susceptible
Nil	Malakand-96	Nare Bryan Swat-84 NARC-V Hobbit Wahab-93 Kingsay Harper-87 Lugan Clark Sherman	Rincondita Kharif-93 NARC-II NARC-IV NARC-VI Winchester Ajmeri Hodgson Full-Walter Mid-Pharoah	William Rawal-I Bass NARC-I NARC-III Mid-Spray	Weber-84

isolates. This may be due to high virulence of the virus or susceptibility of host genotype or both. Immunity 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 our case, Malakand-96 in cultivars and GC-81083-63, GC-81084-51, GC-80072-2-6, AGS-253, AVRDC-12, AVRDC-13 and AVRDC-15 in lines, were found to possess a high degree of resistance to 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 (Hunst and Tolin, 1982; Ross, 1969).

Soybean germplasm tested elsewhere in the world has been found 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

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Table 4: Host response of soybean lines to soybean mosaic potyvirus isolates, SMV-S1 and SMV-P1

Soybean lines	SMV-S1		Control		SMV-P1		Control	
	F <sup>a</sup>	Disease Index <sup>b</sup>	F <sup>a</sup>	Disease Index <sup>b</sup>	F <sup>a</sup>	Disease Index <sup>b</sup>	F <sup>a</sup>	Disease Index <sup>b</sup>
GC-81080-13	25/40	3	0/30	0/30	20/40	3	0/30	0/30
GC-81084-134	38/40	4	0/30	0/30	32/40	3	0/30	0/30
GC-81084-41	35/40	4	0/30	0/30	32/40	3	0/30	0/30
GC-81075-44	12/40	2	0/30	0/30	05/40	2	0/30	0/30
GC-81090-108	15/40	2	0/30	0/30	10/40	2	0/30	0/30
00-81084-118	40/40	5	0/30	0/30	38/40	5	0/30	0/30
GC-81083-63	03/40	1	0/30	0/30	04/40	1	0/30	0/30
GC-82117-8	25/40	3	0/30	0/30	28/40	3	0/30	0/30
GC-81084-147	15/40	2	0/30	0/30	17/40	2	0/30	0/30
GC-81084-51	04/40	1	0/30	0/30	03/40	1	0/30	0/30
GC-00181-99-6	34/40	5	0/30	0/30	36/40	4	0/30	0/30
GC-80072-2-6	04/40	1	0/30	0/30	03/40	1	0/30	0/30
GC-81090-16	35/40	4	0/30	0/30	30/40	4	0/30	0/30
GC-81080-36	20/40	4	0/30	0/30	19/40	3	0/30	0/30
00-81090-94	32/40	4	0/30	0/30	28/40	3	0/30	0/30
GC-81090-10	10/40	2	0/30	0/30	05/40	2	0/30	0/30
AGS-85	20/40	2	0/30	0/30	21/40	2	0/30	0/30
AGS-253	04/40	1	0/30	0/30	04/40	1	0/30	0/30
AGS-249	05/40	2	0/30	0/30	07/40	2	0/30	0/30
AGS-154	28/40	3	0/30	0/30	32/40	3	0/30	0/30
L-88-8502	30/40	3	0/30	0/30	35/40	3	0/30	0/30
1-77-1863	26/40	3	0/30	0/30	26/40	2	0/30	0/30
L-85-2308	20/40	2	0/30	0/30	21/40	3	0/30	0/30
AGS-297	21/40	2	0/30	0/30	27/40	2	0/30	0/30
00-81084-37	40/40	5	0/30	0/30	40/40	4	0/30	0/30
AVRDC-1	40/40	4	0/30	0/30	38/40	4	0/30	0/30
AVRDC-2	35/40	4	0/30	0/30	30/40	3	0/30	0/30
AVRDC-3	25/40	3	0/30	0/30	26/40	2	0/30	0/30
AVRDC-4	30/40	3	0/30	0/30	31/40	3	0/30	0/30
AVRDC-5	25/40	2	0/30	0/30	20/40	3	0/30	0/30
AVRDC-6	32/40	4	0/30	0/30	28/40	3	0/30	0/30
AVRDC-7	20/40	2	0/30	0/30	25/40	2	0/30	0/30
AVRDC-8	40/40	3	0/30	0/30	40/40	3	0/30	0/30
AVRDC-9	38/40	4	0/30	0/30	35/40	2	0/30	0/30
AVRDC-10	24/40	2	0/30	0/30	25/40	2	0/30	0/30
AVRDC-11	38/40	4	0/30	0/30	40/40	3	0/30	0/30
AVRDC-12	10/40	1	0/30	0/30	15/40	1	0/30	0/30
AVRDC-13	04/40	1	0/30	0/30	04/40	1	0/30	0/30
AVRDC-14	24/40	2	0/30	0/30	32/40	3	0/30	0/30
AVRDC-15	20/40	1	0/30	0/30	18/40	1	0/30	0/30

<sup>a</sup>Frequency of virus infection = number of plant infected/ number of plant tested

<sup>b</sup>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

Table 5: Reaction of soybean lines to SMV-S1

Immune	Highly resistant	Resistant	Moderately resistant	Susceptible	Highly susceptible
Nil	GC-81083-63	GC-81075-44	GC-81080-13	GC-81084-134	GC-81084-118
	GC-81084-51	GC-81090-108	GC-82117-8	GC-81084-41	GC-00181-99-6
	GC-80072-2-6	GC-81084-147	AGS-154	GC-81090-16	GC-81084-37
	AGS-253	GC-81090-10	L-88-8502	GC-81080-36	
	AVRDC-12A	GS-85	L-77-1863	GC-81090-94	
	AVRDC-13	AGS-249	AVRDC-3	AVRDC-1	
	AVRDC-15	L-85-2308	AVRDC-4	AVRDC-2	
		AGS-297	AVRDC-8	AVRDC-6	
		AVRDC-5		AVRDC-9	
		AVRDC-7		AVRDC-11	
		AVRDC-10			
		AVRDC-14			

Table 6: Reaction of soybean lines to SMV-P1

Immune	Highly resistant	Resistant	Moderately resistant	Susceptible	Highly susceptible
Nil	GC-81083-63	GC-81075-44	GC-81080-13	GC-00181-99-6	GC-81084-118
	GC-81084-51	GC-81090-108	GC-82117-8	GC-81084-37	
	GC-80072-2-6	GC-81084-147	AGS-164	GC-81090-16	
	AGS-253	GC-81090-10	L-88-8502	AVRDC-1	
	AVRDC-12	AGS-85	L-85-2308		
	AVM:C-13	AGS-249	AVRDC-5		
	AVRDC-15	L-77-1863	AVRDC-4		
		AGS-297	AVRDC-8		
		AVRDC-3	AVFIDC-11		
		AVRDC-9 AVRDC-14			
		AVRDC-10	GC-81084-134		
		AVRDC-7	GC-81084-41		
			GC-81080-36		
			GC-81090-94		
			AVRDC-2		
			AVRDC-6		

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conditioned by single dominant gene (Kiihl and Hartwig, 1979; Koshimizu and Iizuka, 1963; Ross, 1977) whereas resistance to severe isolates which produce necrotic symptoms on susceptible cultivars, was shown to be conditioned by single recessive gene (Kwon and Oh, 1980). However, in present studies it will be premature to assess that the resistance against SMV-S1 and SMV-P1 is based on either single dominant or a single recessive gene, further detail studies are needed to elucidate the mechanism of gene operation in cultivars expressing mosaic and necrotic type of symptoms. With out going in to the discussion and details of the genotypic background of the cultivars/lines, the breeders can select and breed new soybean cultivars, even on the basis of these preliminary investigations for the development of SMV resistant cultivars. Meanwhile soybean cultivars such as Malakand-96, Sherman, Bryan, Swat-84, Luga, Hobbit, Recondite, Kingsay, Harper or soybean lines which has shown resistance to available isolates can be recommended to growers for general cultivation if other agronomic characters of these cultivars/lines are desirable.

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