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DETECTION OF RESISTANCE AGAINST LEAF SPOT, YELLOW MOSAIC AND URDBEAN LEAF CRINKLE VIRUSES IN MASH BEAN (*VIGNA MUNGO*)

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ABSTRACT

Among foliar diseases Leaf spot, yellow mosaic virus (YMV) and Urd bean leaf crinkle virus (ULCV) are destructive diseases of mash bean (*Vigna mungo*) in Pakistan. Incorporation of resistance against biotic stress in plants is traditional strategy of crop improvement through breeding. Effective breeding for biotic stresses entails readily accessible resistant germplasm. 67 genotypes were evaluated for multi disease resistance was tested for two consecutive years (2019 and 2020) at Barani Agricultural Research Institute, Chakwal. Experiment was repeated with an idea to decrease the effect of environment and those material was selected which performed same in both years. Out of 67 genotypes 12 were resistant against leaf spot infection and 19 genotypes were found highly resistant against yellow mosaic virus during both years. In case of ULCV disease did not appear during 2020. However, 19 genotypes showed resistance against ULCV during first year of experiment. Two genotypes namely 3CM-703 and 4CM-720 exhibited resistance response to all foliar diseases under study. Three genotypes viz. 4CM-716, 4CM-720, 5CM-708 demonstrated resistance against YMV as well as leaf spot. 4CM716, 4CM720, 5CM708, 7CM701, 7CM702, 7CM704, 7CM705, 7CM706 expressed resistance against leaf spot and ULCV simultaneously. The data generated is useful for improvement of mash bean through breeding.

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INTRODUCTION

Mash bean or black gram [*Vigna mungo* (L.) Hepper] belongs to family Leguminosae (Verdcourt, 1970). It is cultivated on marginal land where other crops perform poorly. Being leguminous, it requires a lesser amount of nitrogenous fertilizer, and fits well as source of protein (25-32%) (Qayyum *et al.*, 2019). In Pakistan mash bean is cultivated over an area of 27.6 thousand hectares 13.6 thousand tonnes is harvested annually (Ahmed *et al.*, 2012). Mash bean is the major food legume cultivated in Pakistan during the summer season after gram and mung bean occupying 1.5% of total area under pulses contributes 1.4% to total pulses production (GOP, 2017). The crop is mainly grown under un-irrigated conditions having 70 percent share at provincial level from Gujranwala division (GOP, 2019). In Pakistan, among pulses, mash bean is the least researched crop due to which a continuous decline in Area and production has been observed during last three decades regardless of its high nutritive and financial value (Achakzai and Taran, 2011).

Due to pre- and postharvest injuries, biotic stress might become damaging severely. The causal organisms that cause biotic stress in plant deprive their nutrients, that can lead to mortality of plant. In spite of the lacking immunity as it exists in animal kingdom, plants have established means to deal with biotic stresses controlled by plant's genetic codes. Hence there is a prerequisite to evolve varieties of crops possessing genes against biotic stresses so as to confirm food security (Gong *et al.*, 2014) It is against this background, the evolution of resistant plants to biotic stresses is a vital resolution of plant breeding strategies with relevant implications for both farmers and the seed and agrochemical industries. Genomic resistance includes negligible cost once cultivars are developed, and high productivity. The major disadvantage of genetic resistance to biotic stresses is the reality that selection pressure is placed on pathogen populations to develop means of defeating the resistance, thus practically limiting the time of effectiveness. Its consequence is that breeding plants for disease resistance has appeared as a regular process and scientist engage in identifying sources of disease resistance through screening and the genotype that carry resistance against specific disease is used as parent in breeding programme although it may not be high yielding. There becomes prospect that breeder succeeds in developing a line high yielding as well as resistant against disease.

The global yield of mash is very low because of shortage of fitting and high-yielding, disease resistant varieties and fundamental information regarding production technology. Therefore, research on mash improvement with intension to develop high yielding varieties with resistance to diseases is the need of hour.

MATERIALS AND METHODS

Germplasm

The mash bean germplasm consisting of 67 entries including check (Indian Selection) was evaluated for disease resistance for two years 2019 and 2020.

Experiment location

Experiments were conducted with collaboration of Pulses section of Barani Agricultural Research Institute Chakwal (32°55′49″N 72°51′20″E, 525 m asl).

Experimental design

Genotypes were evaluated to study their response against foliar diseases leaf spot, yellow mosaic virus (YMV) and Urdbean crinkle virus (ULCV) under natural infection. The experiment was laid out in a Randomized Complete Block Design (RCBD) at three replicates, with row to row 40 cm and plant to plant 10cm distance so a net plot size of 4 × 1.2 m. Preceding crop, was groundnut during the Kharif season 2018. General agronomic practices were conducted (Qayyum *et al.*, 2019) however no insecticidal spray was applied to encourage the multiplication of insect vectors. Field was examined regularly.

Recording disease incidence data

Disease incidence data on leaf spot, ULCV and YMV were recorded on visual symptoms near maturity. Leaf spot incidence was recorded according 0-9 scale (Mayee and Datar, 1986) whereas incidence of viral diseases was recorded following scale (Ashfaq *et al.*, 2007) and level of resistance and susceptibility were determined. 0-5 Arbitrary Scale used by Bhanu *et al.* (2017) to assess Yellow Mosaic Virus.

Categorization of test material according to response against diseases

As material has been categorized on phenotypic response, material was tested for two consecutive years (2019 and 2020). The disease data of test material that showed same reaction during both years was accepted. Consequently, data generated is more authentic and reliable.

RESULTS AND DISCUSSION

Data presented in Tables is self-explanatory and comprehensible easily. Leaf spot and YMV appeared in high intensity during the second year of experiment and ULCV appeared during 2019 only and no incidence was found during next year. Data on leaf spot, ULCV and YMV were recoded near maturity and presented in the Tables (1, 2 and 3). Data presented in Tables is self-explanatory and comprehensible easily.

Under the present studies although the response of the material against disease was not uniform during both years due to the fact that along with host susceptibility and virulence of pathogen, role of environment is very important. Infected leaf of mash bean by *Cercospora spp.* has semi-round brown lesions with pale-tan to gray centers that are surrounded by dark-brown or reddish slightly depressed margins. A closer look shows white masses of spores growing on the dead tissues. Chemical spray, rotation with non-host crop and cultivation of resistant are suggested as disease management strategy. However not much work has been conducted on search of sources of resistance. In a report of National coordinated pulses programme (NCPP) NARC, Islamabad it was mentioned that screening of Mung and Mash conducted that some accessions of Mungbean exhibited resistance however Mash bean material were found susceptible. (Zahid, 2011).

Table 1: Response of germplasm against leaf spot during 2019 and 2020.

Year	Disease rating (Mayee and Datar, 1986)	Disease Incidence	Response	Genotypes
2019	1	Less than 1%	(HR) Highly Resistant	3CM-702, 3CM-703, 4CM-716, 4CM-720, 4CM-721, 5CM-708,7CM-701,7CM-702, 7CM-703,7CM-704, 7CM-705, 7CM-706, 7CM-709, 8CM-704, 8CM-705, 8CM-708,90CM-048, 90CM-084,90CM-086,90CM-087,94CM-019,95CM-508, 96CM-016, 98CM-001, 98CM-202, 98CM-204, 98CM-519, 98CM-523, 99CM-001, 99CM-005, 99CM-007, 99CM-066, 99CM-201, SPSR (2003)
	3	1-10%	(R) Resistant	3CM-704, 3CM-705, 3CM-706, 3CM-707, 3CM-708, 4CM-716, 4CM-717,4CM-718, 5CM-702, 5CM-706, 5CM-707, 6CM-701, 6CM-702, 6CM-703, 6CM-704, 6CM-705,6CM-706, 6CM-707, 7CM-707,7CM-708,8CM-701, 8CM-702, 8CM-703, 8CM-706,8CM-707, 98CM-201, 99CM-003, 99CM-004, 99CM-008, CH. MASH, INDIAN SELECTION , MASH-88®, MASH-97©
2020	1	Less than 1%	(HR) Highly Resistant	7CM-705, 8CM-708, 90CM-048, 98CM-001, 98CM-523, 99CM-005, 99CM-007
	3	1-10%	(R) Resistant	3CM-702, 3CM-705, 4CM-716, 3CM-706, 4CM-720, 3CM-707, 5CM-708, 3CM-708, 7CM-701, 4CM-716, 7CM-702, 4CM-717, 7CM-703, 4CM-718, 7CM 704,5CM-702, 7CM-706, 5CM-706, 90CM-087, 5CM-707, 94CM-019, 6CM-701, 96CM-016, 6CM-703, 98CM-202, 6CM-707, 98CM-204, 8CM-706, 98CM-519, 8CM-707, 99CM-001, 98CM-201, 99CM-066, 99CM-003, 99CM-201, 99CM-004, SPSR(2003), INDIAN SELECTION, 3CM-704, MASH-97©
	7	26-50%	(MS) Moderately Susceptible	3CM-703, 4CM-721, 7CM-709, 8CM-704, 8CM 705, 90CM-084, 90CM-086, 95CM-508, 6CM-702, 6CM-704, 6CM-705, 6CM-706, 7CM-707, 7CM-708, 8CM-701, 8CM-702, 8CM-703, 99CM-008, CH. MASH, MASH-88®

Incidence of YMV was high during 2020 as compared to 2019. Mungbean yellow mosaic caused by Mungbean yellow mosaic virus is the most destructive viral disease of mungbean and mash bean in South Asia and adjacent areas (Bakar, 1981; Jayasakera and Ariyarantne, 1988; Malik, 1991). The virus is widely distributed throughout the country and infects other pulse crops such as cowpea, moth and common beans in addition to mungbean and mash beans. MYMV is more serious problem of mash bean than mungbean as the resistant cultivars of mungbean have been evolved (Bashir *et al.*, 2006). The local land races of mash bean are grown by small farmers are highly susceptible to the MYMV, and may impose heavy losses (Bashir and Malik, 1988).

Table 2: Response of germplasm against YMV during 2019-2020.

Year	Disease Severity Index	Disease Reaction	Genotype
2019	1	HR	3CM-702, 7CM-709, 3CM-703, 8CM-703, 3CM-704, 8CM-706, 3CM-705, 90CM-048, 3CM-706, 90CM-086, 4CM-716, 94CM-019, 4CM-717, 95CM-508, 4CM-718, 98CM-202, 4CM-720, 98CM-204, 5CM-702, 98CM-519, 5CM-707, 98CM-523, 5CM-708, 99CM-003, 6CM-702, 99CM-005, 6CM-703,99CM-007, 6CM-704, 99CM-008, 6CM-705, 99CM-066, 6CM-706, 99CM-201, 6CM-707, MASH-88®, 7CM-703, MASH-97©, 7CM-706, SPSR(2003)
	2	Resistant	3CM-707, 3CM-708, 4CM-716, 4CM-721, 5CM-706, 6CM-701, 7CM-702, 7CM-704, 7CM-705, 7CM-707, 7CM-708, 8CM-701, 8CM-702, 8CM-704, 8CM-705, 8CM-707, 90CM-084, 90CM-087, 98CM-001, CH. MASH
	3	Moderately Susceptible	7CM-701, 96CM-016, 98CM-201, 99CM-004
	5	Highly Susceptible	8CM-708, 99CM-001, INDIAN SELECTION
2020	1	Highly Resistant	3CM-705, 3CM-708, 4CM-721, 98CM-201, 98CM-202, 99CM-003, 99CM-004 3CM-702, 4CM-717, 4CM-720, 8CM-703, 8CM-706, 98CM-204, MASH-88(c)
	2	Resistant	3CM-703, 4CM-716, 4CM-718, 5CM-702, 5CM-704, 5CM-708, 6CM-704, 7CM-701, 7CM-702, 7CM-705, 8CM-701, 8CM-707, 8CM-708, 95CM-508, 98CM-523, 99CM-008, 99CM-066
	3	Moderately Susceptible	3CM-706, 4CM-715, 5CM-704, 6CM-701, 6CM-702, 6CM-706, 7CM-706, 7CM-707, 8CM-702, 8CM-704, 8CM-705, 90CM-086, 90CM-087, 96CM-016, 98CM-519, 99CM-005 ,CH-MASH(c), S.P.S(2003)
	4	Susceptible	3CM-707,6CM-705,6CM-707,7CM-708,7CM-709,90CM-048, 98CM-001, 99CM-001,99CM-007, 99CM-201, MASH-97(c)
	5	Highly Susceptible	3CM-704, 6CM-703, 7CM-703, 7CM-704, 90CM-084, 94CM-019, INDIAN-SEL

A lot of research work has been conducted due to the significance of the problem (Ahmad and Harwood, 1973; Bashir and Malik, 1988; Iqbal *et al.*, 1990; Malik, 1991; Haq, 1991; Bashir *et al.*, 1996; Ghafoor *et al.*, 1996; Bashir and Zubair, 2002; Bashir *et al.*, 2006). The virus is capable to infect a wide host range(Munawwar *et al.*, 2014). Transmission of virus is through *Bemisia tabaci* Genn. and has not been reported through any other source (Ahmad and Harwood, 1973; Nene, 1972). Vector multiplication is favored by high temperature from June to August providing ample opportunity for the spread of disease resulting severe damage to this crop (Shakoor *et al.*, 1977). The leaves and other parts become completely yellow in severe cases. The losses may be as high as 100 percent (Malik, 1991). The large seeded material originated from AVRDC Taiwan gave highly susceptible response and failed to survive during summer months when tested under our conditions (Ahmad, 1975; Malik *et al.*, 1988). The best means of the control of diseases in plants is sowing of resistant cultivars (Agrios, 2005). Scientists have made difficult task to identify resistance against the disease under our conditions and have achieved success. Efforts of identification of sources of resistance dates back to 1975 when Ahmad screened 157 local and exotic material of mungbean against the disease, but failed to identify any variety as a source of resistance. During 1983 to 1986 NIAB introduced some mungbean varieties developed through mutation breeding for commercial cultivation (Malik, 1991; Sarwar and Rajput, 1999). In 2002 Bashir and Zubair selected 53 entries showing resistance against MYMV under field conditions (Bashir and Zubair, 2002). Later on Bashir *et al.* (2006) conducted a study to identify new sources of resistance against MYMV from local material of mungbean and mash beans during 2003. Germplasm consisted of 110 entries of mungbean and134 mash bean entries. Out of material tested 43 entries of mash bean showed highly resistant response and 28 entries were found resistant against the disease under field conditions. A number of mash bean genotypes have been identified as source of resistance to disease as a result of work conducted during this period. (Ali and Akram, 1999; Iqbal *et al.*, 1990; Munawwar *et al.*, 2014; Qayyum *et al.*, 2019).

Table 3: Response of germplasm against ULCV during 2019.

Year	Disease Rating	Disease Reaction/ Incidence	Genotypes
2019	1	Highly Resistant All plants free of symptoms	8CM-702, 8CM-703, 99CM-005
	2	Resistant 1-10% plants infected showing mild crinkling at the top, pods normal	3CM-703, 6CM-701, 3CM-704, 6CM-702, 3CM-705, 6CM-703,3CM-707, 6CM-704,3CM-708, 6CM-705, 4CM-716, 6CM-706, 4CM-716, 7CM-701, 4CM-717, 7CM-702, 4CM-718, 7CM-704, 4CM-720, 7CM-705, 5CM-702, 7CM-706, 5CM-706, 7CM-708, 5CM-707, 5CM-708
	5	Susceptible 31-40% plants infected showing all the typical disease symptoms	97CM-054, 98CM-001, 98CM-202, 98CM-519, 99CM-001, 99CM-004, 99CM-008, 99CM-066, 99CM-201, CH.MASH, INDIAN SELECTION , MASH-88®, MASH-97©, SPSR(2003)
	6	Highly Susceptible More than 40% plants infected showing all the plants with severe symptoms, few pods containing few seeds	3CM-702, 3CM-706, 4CM-721, 7CM-709, 90CM-048, 94CM-019, 95CM-508, 96CM-01697CM-518, 98CM-201, 98CM-523

ULCV infection was observed during the first year and next year the material remained free from disease probably due to unfavorable environmental conditions. However, among the material tested 42 percent material showed highly resistant and resistant response against the disease (Tables 6). ULCV is problem of mash bean cultivation in south Asia and could cause reduction in yield up to 80% when infection occurs at early stage of plant growth (Bashir *et al.*, 1991). Aftab *et al.* (1993) reported the yield losses due to disease varies from 32.6% to 88.3% depending on the genotypes infected. The Urd bean leaf crinkle virus is unclassified virus and more destructive than viruses causing infection in legumes (Ashfaq *et al.*, 2007). ULCV is an important disease of Urdbean in Pakistan because it causes huge losses in production of Urdbean. The susceptible germplasm and favorable environmental conditions also contribute towards the wide spread outbreak of viral diseases. ULCV is characterized by leaf crinkling, rugosity, and distortion of leaves. Colour of leaves become abnormally dark green and flowers may also be sterilized. (Bashir *et al.*, 2000). In addition to Urdbean, the virus host ranges cowpea, mungbean, pigeonpea and tepary bean (Beniwal *et al.*, 1980). Transmission is through mechanical means, by beetles (Bashir *et al.*, 2000). Seed transmission of virus has also been reported varying from 2.7 to 46 percent (Kolte and Nene, 1972; Riaz *et al.*, 1994; Ahmad *et al.*, 1997).

The crop is more susceptible to leaf crinkle disease as compared to other pulses (Kadian, 1980; Rishi, 1990). Investigators have addressed the disease to identify genetic sources of resistance against the problem and reported that there exists a genetic ratio involved in conditioning resistance and susceptibility which need to be investigated (Haq, 1991; Iqbal *et al.*, 1991; Bashir *et al.*, 1996; Binyamin *et al.*, 2011). According to some workers study of environmental genotype interaction in essential for durable resistance against the disease as some genotypes found resistant at one location show susceptible response at another site (Iqbal *et al.*, 1991; Bashir, 2005). In Table 4 & Table 5 achievement of trial conduced has been summarized. A germplasm tested for multiple diseases resistance 17.9 percent genotypes demonstrated resistance against leaf spot and 28.35 resistance against the viral diseases (Table 4).

Table 4: Genotypes which reacted resistant during 2019 and 2020.

Disease	Genotypes
Leaf Spot	3CM-702, 4CM-716, 4CM720, 4CM708, 7CM701, 7CM702, 7CM703, 7CM704, 7CM705, 7CM706, 8CM708, 90CM048
Yellow Mosaic Virus	3CM702, 3CM703, 3CM705, 4CM716, 4CM717, 4CM718, 4CM720, 5CM702, 5CM708, 6CM704, 8CM-703, 8CM-706, 98CM-202, 98CM-204, 98CM-523, 99CM-003, 99CM-008, 99CM-066, MASH-88
Urd bean Leaf Crinkle Virus	3CM-703, 3CM-704, 3CM-705, 3CM-707, 3CM-708, 4CM-716, 4CM-717, 4CM-718, 4CM-720, 5CM-702, 5CM-706, 5CM-707, 5CM-708, 6CM-701, 6CM-702, 6CM-703,6CM-704, 6CM-705, 6CM-706,

Table 5: Genotypes which reacted resistant response against two and more diseases during both years (2019 & 2020).

Diseases	Genotypes
Leaf Spot, YMV, & ULCV	3CM-703, 4CM-720
Leaf spot & YMV	4CM-716, 4CM-720, 5CM-708
YMV& ULCV	3CM-703, 3CM-705, 4CM-716, 4CM-717, 4CM-718, 4CM-720, 5CM-702, 5CM-708, 6CM-704
Leaf spot & ULCV	4CM716,4CM720,5CM708,7CM701,7CM702,7CM704,7CM705, 7CM706

Most of the problems facing agriculture relate to the growing world population. The almost doubled population will require a more than proportional increase in food production. During the last decade, world grain yield increased around 9.06%, where as the population grew 12% in the same period (FAO, 2016). The key mission for agricultural scientists is to improve yields of food commodities by hook or by crook. The problems of this target are: (i) the restricted options of increasing the area under cultivation; (ii) the ecological regulation which bounds the application of pesticides for management of plant diseases; (iii) global warming and deterioration attributed to abiotic and biotic stresses; (iv) the rare source of valuable characters from wild relatives of crops. Host plant resistance plays an important role in crop protection against diseases. The identification of new disease resistance sources provides breeders with avenues to breed for resistance to diseases. The variability primary gene-pool available with the breeders could serve an important source for various traits including disease resistance. Generally, many valuable genes that confer resistance to disease can be found in the wild species and/or non-domesticated crop relatives. Extensive screening studies with an objective to explore sources of resistance against diseases is a useful tool of crop improvement through breeding. The findings of the work reveal, that there is ample space for improvement of the crop. The investigation is committed to additional research work aimed at procurement more genotypes, and taking into consideration developing new better-quality varieties, goal can be accomplished with the recognition of resistant sources against diseases.

CONCLUSION

The present work demonstrates, that there is abundant space for upgrading of the mash bean crop. In past decades three varieties (Mash 88, Mash 97 & Arooj 11) were released at the platform of Pulses research institute, Faisalabad and Mash Chakwal was released at Barani Agricultural Research Institute, Chakwal. The research on mash bean is committed to further breeding effort aimed at obtaining more genotypes, and in view of releasing new improved varieties, target can be achieved with the identification of tolerant genetic sources against stress situation. A wide range of pathogens affect the yield of cultivated crop species. Resistance of the host plant is the capacity to restrict the pathogen from causing disease by the genetic components of host plant. Breeding has elevated yields intensely in schemes high-input production; nevertheless, selection under ideal growing circumstances is generally accepted to decrease the adaptive potential of varieties to suboptimal situations. This provides enough scope for significant improvement of plant traits through selection. Plant breeding for disease resistance is most effective disease management strategy. (i) It causes increased production by decreasing damages attributed to diseases. (ii) Expenses on application of pesticides are saved.

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CONFLICT OF INTEREST

The authors have not declared any conflict of interests.

AUTHORS CONTRIBUTIONS

All the authors contributed equally to this work.