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Genetic Resistance to Viruses in Hot Pepper Landraces of Sudan

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Abstract

Thirty three hot pepper (*Capsicum annuum*) accessions prefixed (HSD), collected from different regions of Sudan were obtained from the Agricultural Research Corporation (Sudan) gene bank. The accessions were tested for resistance to six viruses TMV P (0) and P (1,2); PVY P (0) and PVY P (1,2); PVMV; TEV; PepMoV and CMV. Hundred seedlings from each accession were mechanically inoculated. Each time the susceptible plants were cut off and the numbers of the resistant plants were determined as percentage. The doubled antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) was performed, to verify the presence or absence of the virus in the resistant plants. All tests were done at the pepper laboratory at Montfavet (INRA-France). For TMV p(0) seven accessions showed resistance in the range of 10% to 67% of the plants while for TMV p(1,2) only four accessions showed resistance in the range of 11% to 50%. Four accessions showed resistance to PVY p(0) in the range of 8% and 9% while for PVY p(1,2) only two accessions HSD 1008 and 1040 showed 2% of resistant plants. For PepMov only two accessions HSD 533 and HSD 1201 showed resistance of 10% of the plants. For CMV installation two accessions HSD 1055 and HSD 1032 showed resistance in the range of 24% and 17% respectively. None of the plants resistant to CMV installation were found resistant to CMV migration None of the accessions tested for TEV and PVMV were resistant. Screening this collection of the hot pepper accessions of the gene bank for viruses resistance and their fixation will be useful for breeding resistant hot pepper cultivars.

Keywords: *Capsicum annuum*; Landraces; Virus Resistance Sudan

Abbreviations: TMV: Tobacco Mosaic Virus; PVY: Potato Virus Y; PVMV: Pepper Veinal Mottle Virus; TEV: Tobacco Etch Virus; PepMoV: Pepper Mottle Virus; CMV: Cucumber Mosaic Virus; ARC: Agricultural Research Corporation.

Introduction

Parasites and pests are known to attack pepper in the various environmental conditions. Damage caused by parasites is recognized as the most limiting constraint worldwide. Yoon JY, et al. [1] reported that virus complex rated the highest incidence (92%) in a survey involving twenty nine countries from Asia, Africa, Europe and America, taking into accounts both the occurrence and incidence of pathogens in the surveyed countries. In the Sudan, viral diseases are among the most important diseases that limits pepper production. Of this cucumber mosaic virus (CMV) and tobacco mosaic virus (TMV) are the most serious diseases [2,3]. Also potyviruses, Potato virus Y (PVY) and tobacco etch virus (TEV) were reported but at the moment are not very important economically. Genetic resistances are currently used against most of these diseases. For TMV the genetic

resistance conferred by the allelic series at the L-locus may be defeated in marginal conditions and none of them confers resistance to the critical strain of the virus above 30°C. In such conditions, the infection leads to mosaic symptoms above 30°C and plant necrosis when the temperature drops to 22–25°C [4-6] . In the Chinese cultivars 'Zao-Feng' and 'Ben–Xi' two major dominant genes (Ht) independent from the L locus stabilize the resistance of the L1 allele at the high temperatures. Other polygenic systems stabilizing the expression of L1 resistance at high temperatures were found in tropical varieties 'Perennial', 'PM 687' and 'SC 81'. This suggests that plant genotypes that are adapted to hot climates maintain an efficient resistance when the L alleles are introduced.

With regard to potyviruses, many pepper accessions showed resistance to the common strains of PVY, and 125 accessions among 500 accessions in the INRA (France) germplasm were shown to be resistant [7]. Some of these resistance sources were further analyzed and showed the diversity of the resistance systems in the pepper to face this diversified group of viruses. Several loci for monogenic recessive or dominant resistance are known and many polygenic and oligogenic resistance systems were revealed. Cook AA, et al. [8] identified the first PVY resistance locus pvr2. It consists of two alleles: pvr21 from the cultivar 'Yolo Y' that confers resistance to PVY p (0) Gebre-Selassie K, et al. [9] and pvr22 from 'Florida VR2' that confers resistance to PVY p (0,1) and common strains of TEV (TEV-C) [9-11]. The pvr2 resistance is monogenic recessive. It has been used for a long time by breeders [12]. Gene pvr2 is localized on the chromosome "Jaune" Pochard E, et al. [13] and it is localized in the pepper molecular map [14].

Resistance to PVY was also reported in *C. annuum* line 'CM 334' [15,16]. Two independent genes that are different from pvr2 are implicated in PVY resistance. Gene Pvr 4, confers a dominant resistance to all known pathotypes p (0, 1 and 1, 2) and to PepMoV. Pvr 5 is a recessive gene conferring only resistance to PVY pathotype (0). Resistance is not affected by temperature. Allelism test showed that Pvr4 and pvr5 are independent from pvr21 and can be recombined [16]. However, recent results showed that pvr5 is genetically linked to pvr2 [17].

Multipotyvirus polygenic resistance was reported in the line 'Perennial' [18-20]. This resistance resulted from several QTLs with additive and interaction effects. These QTLs showed different levels of specificity regarding PVY pathotypes or other potyviruses as do major potyvirus resistance genes [21]. One of the QTLs from 'Perennial' was also shown to have a major effect against all the PVY pathotypes and was located in the same locus as pvr2. This increased the scientific interest in this locus that may bear both specific alleles (pvr21 and pvr22) and a major QTL. Other polygenic resistances to potyviruses are also known in *C annuum* 'SC81' and 'PM 949' [22]. Partial resistance of pepper to PVMV was reported [23]. The only complete resistance was reported in a doubled haploid (HD) line HDA 801 obtained from F1 between two susceptible *C. annuum* lines in INRA (Montfavet, France) Gebre-Selassie K, et al. [24], Palloix A [25] suggested that the absolute resistance in HDA 801, resulted from complementation between two recessive genes: one from 'Perennial' and the other was pvr22 from 'Florida VR2'. This was further confirmed [26]. Also it was confirmed with the mapping of this complementary gene, in the pepper genetic map [21]. The complementary gene was named pvr6. Resistances to CVMV have been recently identified in Perennial and CM 334 [18,27]. It seemed to be controlled by dominant genes in perennial, but further analysis is required. An important variability of the modes of action of these genes was reported: pvr1 and pvr22 control a complete inhibition of the virus accumulation in infected cells [28]. The resistance mechanism of pvr21 controls restriction of the viral short-distance cell-to-cell movement required for the systemic spread of the virus Arroyo R, et al. [29], while pvr3 slows long distance movement [30]. Pvr4 and pvr5 are under study but the former was hypothesized to control a hypersensitive type of resistance i.e. migration from cell to cell [16]. A new source of dominant potyvirus resistance is reported in a selection in *C. chinense* PI 159235 [31]. It was tentatively named Pvr7 and it confers a hypersensitive-type of resistance to both PVY and PepMoV. Pvr7 was shown to be linked to but distinct from Pvr4 that controls the same viruses. The spectrum of action of these resistance loci or QTLs is also highly variable: some alleles confer resistance to one pathotype of PVY, others to several pathotypes, even to several distinct potyviruses. This diversity of resistance systems offers large possibilities to the breeders for the genetic control of this virus group in pepper.

Variability in *Capsicum spp*. Still offers great choice of gene combination to construct durable resistances [25]. Most hot pepper cultivars grown in the Sudan are indigenous types that are showing great variability. Ahmed AH [2] collected and characterized some of the local types and now this material is maintained in the Agricultural Research Corporation (ARC) genebank. Still heterogeneity exists between and within these characterized accessions. The objective of this study is to screen for resistance to six viruses TMV P (0) and P (1,2); PVY P(0) and P(1,2); PVMV; TEV; PepMoV and CMV in this collection.

Materials and Methods

Thirty three Hot pepper (*Capsicum annuum*) accessions prefixed (HSD), obtained from the Agricultural Research Corporation (Sudan) gene bank were used in this study

(Table 1). Tests were done for six viruses TMV P (0) and P (1,2); PVY P(0) and P(1,2); PVMV; TEV; PepMoV and CMV. All tests were done at the pepper laboratory at Montfavet (INRA-France).

Serial No	Accession No	Serial No	Accession No
1	533	18	1053
2	640	19	1055
3	720	20	1060
4	965	21	1061
5	998	22	1061
6	1001	23	1070
7	1008	24	1072
8	1023	25	1085
9	1029	26	1102
10	1032	27	1107
11	1033	28	1113
12	1036	29	1124
13	1040	30	1125
14	1043	31	1125
15	1045	32	1201
16	1048	33	1209
17	1052		

Table 1: Hot Pepper Accessions Tested for Viruses Resistance.

TMV Test

Ten accessions HSD 1060, 1102, 640, 1125,1048, 1008, 1040, 1061,1201 and 1072 were tested for resistance to TMV P(0) and TMV P(1,2). Seeds were sown in sterilized peat moss in the growth chamber (22°C and 12h light per day with an intensity of 8000 lux). Inoculum preparation and inoculation were done as described by Chaine-Dogimont C [32] abrasive carborundum 400 mesh (75mg/ml) was added to the thawed viral solution. Inoculation was done with small piece of foam plastic or by hand. Hundred seedlings were inoculated at the seedling stage on the well-expanded cotyledons, before the emergence of the first true leaf. One week after inoculation, and when the susceptible check cv. 'Lamu' showed a clear mosaic, the plants were evaluated. The resistant plants showed necrotic local lesions on the inoculated cotyledons followed by abscission of the cotyledons, the evaluation was continued for another two weeks. Each time the susceptible plants were cut off and the number of the resistant plants was determined. The doubled antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) Clark MF, et al. [33] was performed, to verify the presence or absence of the virus in the resistant plants. The Station of plant Pathology INRA (Montfavet, France) provided the antiserum.

Potyviruses (PVY, PVMV, TEV and PepMoV) Test

The accessions tested for resistance to each virus check are shown in Table 2. Inoculum and inoculation procedure were done as described by Caranta C, et al. [18]. Hundred seedlings from each accession were mechanically inoculated at the first leaf stage and transferred into a growth chamber (22°C and 12h light per day with an intensity of 8000 lux). Evaluation started two weeks after inoculation, when the susceptible check showed clear symptoms, then continued on weekly basis, for five weeks. Each time susceptible plants were cut off and finally the number of resistant plants was determined. DAS-ELISA was performed, to verify the presence or absence of the virus in the resistant plants. The Station of plant Pathology INRA (Montfavet, France) provided the antiserum.

Accessions Tested for	Accessions Tested for	Accessions Tested for	Accessions Tested for	Accessions Tested for
PVY P(0)	PVY P(1,2)	PVMV	TEV	PepMoV
1048	1125	998	1070	1033
720	1001	1053	1107	1008
1113	1124	1124	1052	640
965	1008	1023	1043	1125
1125	1040	1045	1033	1048
1029	720	1052	998	1085
1061				533
640				1061
1209				1201
				1040

Table 2: Accessions Tested for (PVY, PVMV, TEV and PepMoV).

CMV Installation Test

Ten accessions (HSD 1023, 1036, 998, 1055, 1032, 1060,640, 1070, 1045, 1033) were tested for CMV Installation. Inoculum preparation and inoculation were done as described by Caranta C [34] plants were inoculated mechanically with CMV (Fulton strain) on the third leaf at the 5-6 extended leaf stage. Then the plants were transferred to the growth chamber (constant temperature at 22°C and 12h light). Four days after inoculation, the number of the local lesions induced were counted, a scale of 1-5 is used, where 1= 0-5 lesions; 2= 6-20 lesions; 3=21-50 lesions; 4 = > 51 lesions. The plants that scored 1 were considered as resistant.

Decapitation Test

This test was used to evaluate resistance to CMV migration. Plants scored as resistant to CMV installation from the accessions HSD 1055 and HSD 1032 were subjected to decapitation test. The test was done as described by Pochard E [35] 40-50 days old plants at the five leaf stage were decapitated just above the fourth leaf. Four days after decapitation the third leaf was inoculated with the CMV (Messiaen strain). The decapitation initiated emergence of branches from the axillary buds. Three weeks later, the inoculated plants were scored on the two branches at the inoculated leaf and at the fourth leaf. The scale used was 0,1,2 (1=the axillary branch at the inoculated leaf was necrotic and was showing mosaic symptoms and the branch at the fourth leaf remained symptomless, 2= when the two axillary branches are necrotic and showing mosaic symptoms,0=the two branches did not infected). Then the scoring was continued on a weekly basis for four weeks then the plants remained non-necrotic on the two branches were considered resistant to CMV migration within the plant.

Results and Discussion

For TMV p(0) seven accessions showed resistance in the range of 10 to 67 percent of the plants while for TMV p(1,2) only four accessions showed resistance in the range of 11 to 50 percent (Table 3). For TMV the genetic resistance conferred by the allelic series at the L-locus may be defeated in marginal conditions and none of them confers resistance to the critical strain of the virus above 30°C. In such conditions, the infection leads to mosaic symptoms above 30°C and plant necrosis when the temperature drops to 22–25°C [4-6]. Other polygenic systems stabilizing the expression of L1 resistance at high temperatures were found in tropical varieties 'Perennial', 'PM 687' and 'SC 81'. This suggests that plant genotypes that are adapted to hot climates maintain an efficient resistance. Thus, this collection is from hot climate regions of Sudan and the resistance expected to be stable at high temperature.

Accession NO	TMV p(0) Percent Résistant Plants	Accession NO	TMV P(1,2) Percent Resistant Plants
1060	0	1060	0
1102	0	1102	0
640	60	640	50
1125	50	1125	0
1048	10	1048	33
1008	56	1008	0
1040	10	1040	11
1061	67	1061	20
1201	30	1201	0
1072	0	1072	0
Resistant check cv. YW	100	Susceptible check YW	0
Susceptible check cv. lamu	0	Resistant check Novi 3	100

Table 3: Accessions Tested for TMV p(0) and P(1,2) and the Percentage of Resistant Plants.

For potyviruses as shown in Table 4 only four accessions showed resistance to PVY p(0) in the range of 8% and 9% while for PVY p (1,2) only two accessions HSD 1008 and 1040 showed 2% of resistant plants and none of the accessions tested for PVMV and TEV shoed resistance while for PepMov only two accessions HSD 533 and HSD

1201 showed resistance of 10% of the plants. Seven pvr loci were involved in potyvirues resistance as shown in the Figure 1 below and most of them were from tropical sources indicating the importance of testing the collection of the Gene Bank. None of the accessions tested for TEV and PVMV were resistant. Today, secondary centers of diversity for C. annuum exist in south and central Europe, Africa, Asia and the old world tropics [36,37]. Thus, screening this collection for resistances and their characterization could be useful for breeding resistant hot pepper cultivars. TEV isolate (CAU4) from Cuba used in this study is reported to overcome all the known resistance sources Depestre T, et al. [38] polygenic resistance to this isolate, probably resulting from genes interaction, was reported Palloix A [22] Complete resistance to PVMV was reported only in a doubled haploid line 'HDA 801') [24,26].

Accession No	PVY P(0) % Resistant	Accession No	PVY P(1,2) % Resistant	Accession No	PVMV % Resistant	Accession No	TEV % Resistant	Accession No	PepMoV % Resistant
1048	8	1125	0	998	0	1070	0	1033	0
720	0	1001	0	1053	0	1107	0	1008	0
1113	0	1124	0	1124	0	1052	0	640	0
965	0	1008	2	1023	0	1043	0	1125	0
1125	8	1040	2	1045	0	1033	0	1048	0
1029	0	720	0	1052	0	998	0	1085	0
1061	9	Resistant check var. Sc 81	100	Susceptible check var. Yolo Y	0	YW	0	533	10
640	8	Susceptible check var. Florida VR2	0	YW	0	Resistant check var. Florida VR2	100	1061	0
1209	0	Susceptible check var. Yolo Wonder	0	Resistant check line HD 801	100	Susceptible chec var. Avelar	0	1201	10
Resistant check var. Florida VR2	100							1040	0
Susceptible check var. Yolo Wonder	0							YW	0
								Resistant var.Avelar	100

Table 4: Accessions Tested for Potyviruses and the Percentage of Resistant Plants.

For CMV installation only two accessions HSD 1055 and HSD 1032 Table 5 showed resistance to CMV installation resistance 24% and 17% respectively. None of the plants resistant to CMV installation were found resistant to CMV migration within the plant. Resistance to CMV installation by itself has low effect and it has strong effect when combined with other resistance mechanisms such as resistance to migration and multiplication [39].

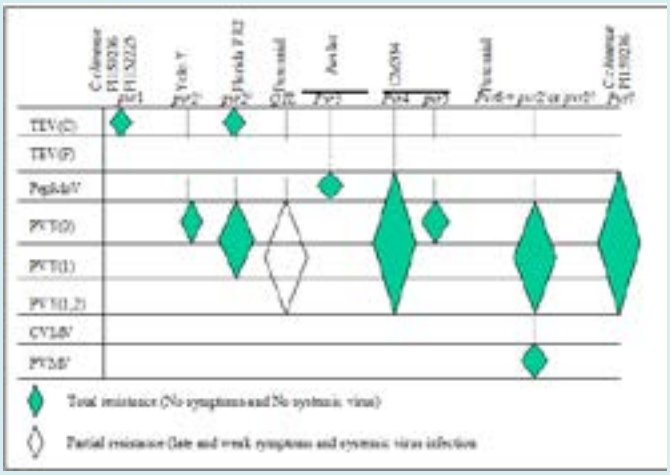

Figure 1: Variability of Potyviruses Infecting Pepper and Analysis of Resistance Sources [22].

Accession No	CMV Installation Percent Resistant
1023	0
1036	0
998	9
1055	24
1032	17
1060	0
640	0
1070	0
1045	9
1033	0
Susceptible check var. Yolo W	0
Resistant check Perennial	100
Resistant check vr.Rami	100

Table 5: Accessions Tested for CMV Installation and Percentage of Resistant Plants.

Fixation of these resistance sources to study their allelism with the reported resistance loci to is important so as to be utilized in breeding programs since. The use of cultivar resistance against viruses infecting pepper might be an effective method to control these viral diseases [40].

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