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Tri-species bridge crosses (*C. annuum* L. x *C. chinense* Jacq.) x (*C. chinense* Jacq. x *C. frutescens* L.) as an alternative approach for introgression of Cucumber Mosaic Virus (CMV) and Chilli Veinal Mosaic Virus (CVMV) resistance from *C. frutescens* L. into *C. annuum* L.

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ABSTRACT. *Cucumber mosaic virus (CMV) and Chilli Veinal Mosaic Virus (CVMV) are among the most destructive viruses affecting chilli crop in Sri Lanka. Identification of resistant sources and combining them in to cultivated forms is essential in resistance breeding. Capsicum frutescens L. has been reported as a source of variation for many different traits including disease resistance to improve chilli (Capsicum annum L.). However, strong inter-specific hybridization barriers exist between them. In the present study, wide hybridization approach for introgressing C. frutescens L. genes into C. annuum L. was performed through genetic bridging using C. Chinense Jacq. as a bridge species. Diverse collection of 115 accessions from three cultivated species of C. annuum L. (28), C. Chinense Jacq. (63) and C. frutescens L. (24) was screened for CMV and CVMV resistance. Two C. frutescens L. accessions were resistant to both viruses and six C. Chinense Jacq. accessions were resistant to CVMV. In Genetic bridge approach three way hybrids and double crosses were produced among these three species. The double crosses [(C. annuum L. x C.chinense) x (C. Chinense Jacq.x C. frutescens L.)] and [(C. Chinense Jacq.x C. annuum L.) x (C. Chinense Jacq. x C. frutescens L.)] were more successful than the three way crosses when considering the combining of C. frutescens L. traits into C. annuum L. and development of resistance to CMV and CVMV.*

Keyword: *Capsicum, CMV, CVMV, virus resistance, introgression*

INTRODUCTION

Chilli and pepper (*Capsicum annuum* L.) are among the most important commercially-grown vegetable crops in the world. Being a heavy consumer and a producer of chilli, Sri Lanka has a huge potential to increase the production to meet its domestic requirements. However, despite continuous efforts at various levels, productivity and production of chilli have not gained the momentum expected. One of the major problems for improving the yield of chilli is heavy infestations of pests and diseases, particularly the virus diseases (Reddy *et al* 2014). Cucumber mosaic virus (CMV) has been described as one of the five most important viruses infecting vegetable species worldwide. In *Capsicum* spp., infection of CMV can cause severe systemic mosaic symptoms, leaf distortion and fruit lesions, thereby drastically reducing marketable yield (Rashid *et al* 2007). Control of CMV is a challenge as the virus is having a broad host range that includes many weeds species and is transmitted by a large number of aphid species (Ben Chaim *et al*., 2001; Xinqiu *et al*., 2012).

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Although Department of Agriculture has recommended ten chilli varieties since 1962 to date none of the varieties has shown a satisfactory degree of resistance against the insect pests and the viruses. Though, various insecticides have been found effective against insect pests of chilli, repeated use of chemicals leads to secondary pest problems and serious environmental hazards and it has become an uneconomical practice to the farmers. Hence, host plant resistance is in arguably the most important pest control strategy which is environmentally friendly with low running costs (Ashfaq *et al*., 2014). In order to achieve this objective, identification of source of resistance through efficient screening techniques is an indispensable pre-requisite.

A wide range of intraspecies genetic variation would support for a given plant species to adapt them to changing environmental conditions and continuously-emerging threats of pests and diseases. To achieve this, almost all modern varieties of crops have been improved using genetic diversity derived directly from a wild relative. As genetic resources for breeding of improved chilli varieties which were targeted on resistance to diseases, adaptation to abiotic stresses and improvement of nutritional quality and yield, wild and related capsicum species are useful (Shuh and Fonetenot, 1990). With this regard, *C. annuum*, *C. frutescens* L. and *C. chinense* Jacq. are the mostly considered three different species (Subramanya, 1983). These three species have been reported to share the same ancestral gene pool and are sometimes called the '*annuum-chinense-frutescens* complex' (Tanksley *et al* 1984). A certain degree of crossability among these species under field conditions has been reported. Cytogenetic studies has shown aberrant chromosome pairing between *C. chinense* and the other two taxa, and hand crosses have often resulted in viable and fertile hybrids (Egawa and Tanaka, 1986). At present more and more commercial cultivars are being released that have resulted from crosses between these three taxa. Interspecific hybridization has been used to introgression of useful traits from wild and related species into cultivated varieties in many Solanaceous crops, particularly in terms of pest and disease resistance (Yayeh and Bosland 2000;Yoon *et al*., 2006).

Within the last decade or so, pepper breeders have identified various new sources of resistance to CMV or resistance to CMV in several accessions of *C. annuum*, *C. frutescens* and *C. baccatum* (Kang *et al*., 2010). Thus, interspecific hybridization between *C. annuum* and other related species (*C. chinense, C .frutescens etc.)* is currently one of the methods considered for introgression of the resistant genes into cultivated varieties (Manzur *et al*., 2015). However, successful wide hybridization attempts to introgress of disease resistance traits in *C. annuum* have been scarce (Yoon *et al*., 2005; Eggink *et al*., 2014). Postzygotic barriers which avoid fertilization due to pollen-pistil incompatibilities and post-zygotic barriers that leads into embryo/endosperm abortion, hybrid weakness or sterility have been suggested as the main cause of cross compatibility problems between these species (Egawa. 1986; Bermawie, N. and B. Pickersgill. 1992; Yang, 2001).

Genetic bridge which is the based on the use of phylogenetically-closer species to the two species affected by crossability barriers is an alternative approach to overcome the above problem. In this method, the bridge species is used to obtain hybrids with one of the target species, and subsequently these hybrids are crossed to the other target species (Shivanna et al. 2015). Therefore, *C. chinensis* would be an ideal bridge species to perform the wide hybridization between *C. annuum* and *C. baccatum* (Pickersgill, 1988).

Having the above background, the objective of the present study was to identify resistant or tolerant sources of Capsicum spp. for CMV and CVMV and to incorporate those traits into cultivated chilli spp. by using bridge method.

MATERIALS AND METHODS

Plant material and growing conditions

A total of 115 accessions from three cultivated species, namely *C. annuum* (28 accessions), *C. chinense* (63 accessions) and *C. frutescens* (24 accessions) were used in the study. This collection encompassed a comprehensive range of different agro-ecological zones and fruit morphological traits (Annex 1). Seeds were extracted from the collected pods and sown in nursery trays prepared with sterilized nursery mixture of top soil, sand and compost in 1:1:1, respectively. All the germinated accessions (72 out of 115) were transplanted at the five-leaf stage (about 28-30 days after sowing) in polythene pots (36 cm in width and 38 cm in height). The mixture of top soil 2: sand 1: and compost 1 was used as the potting medium Twenty healthy seedlings (two plants per pot) from each accession in two replicates were maintained to screen the response of the accessions against CMV and CVMV. The plants with pots were kept in the open field. More than two hundred of both CMV and CVMV infected plants were maintained at the surrounding of the open field with the testing entries throughout the testing period. It was assumed that these plants would behave as a source of natural infection. All the agronomic practices were done according to the recommendations for chilli by the Department of Agriculture, Sri Lanka except pests and disease management. The visual observations on CMV and CVMV symptoms were recorded and symptomless plants were subjected to the Enzyme Linked Immunosorbent Assay (ELISA) tests. In parallel, seed multiplication was done from the plants showing resistance to CMV and CVMV.

Inter specific Hybridization technique

A total of 40 well grown healthy seedlings from each selected parent materials, namely three accessions of *C. annuum*, two accessions of *C. chinense* and two accessions of *C. frutescens* were raised in pots and used top soil 2: sand 1: compost 1 mixture as the potting medium. Pots with plants were transferred to a planthouse at the flowering stage to prevent from windy and rainy conditions during the hybridization. Prior to hybridization, female flowers were emasculated and pollen was extracted from male flowers and released carefully on the stigma. After the hybridization, the female flowers were covered with oil paper bags (5 cm x 5 cm) to prevent uncontrolled pollination,. Each cross was tagged with the genotypes involved in the hybridization and the date at which it was performed. All the possible cross combinations for interspecific crosses were conducted(Table 1).

Table 1. Combinations of interspecific crosses used in the study.

Male Parent / Female Parent	CA1 (PC-1)	CA2 (WAR)	CA3 (WP-2)	CC 1 (HNM-8)	CC2 (MNM-1)	CF1 (INK-3)	CF2 (MMK-1)
CA1 (PC-1)	x	x	x	C	C	C	C
CA 2(WAR)	x	x	x	C	C	C	C
CA 3(WP-2)	x	x	x	C	C	C	C
CC1 (HNM-8)	C	C	C	x	x	C	C
CC2 (MNM-1)	C	C	C	x	x	C	C
CF1 (INK-3)	C	C	C	C	C	x	x

CF2 (MMK-1)	C	C	C	C	C	x	x

Note: CA1 - CA3 = *Capsicum annuum accessions*; CC 1 – CC2 = *C. chinense accessions*; CF1 – CF2 = *C. Frutescens accessions.*

PC-1=Chilli variety PC-1; WAR= Chilli variety MI Waraniya 1; WP-2 = Chilli accession Watareka purple 2; HNM-8=Chilli accession Homagama Nai Miris 8; HNM-1= Chilli accession Homagama Nai Miris 1; INK-3=Chilli accession Ingiriya Kochchi 3; MMK-1=Chilli accession Meemure Kochchi 1.

The crosses conducted and did not conducted were denoted as “C” and “x”, respectively.

Overall, more than 6,000 hybridizations were performed in this study. Data were collected on number of crosses and their successors in each cross combinations. Seeds were extracted from fully red ripened pods of successful crosses.

Evaluation and Screening of inter-specific crosses for CMV and CVMV

Interspecific crosses and their parents were raised in the field as progeny lines according to a randomized complete block design (RCBD) with three replicates. Forty seedlings per plot in two rows with two seedlings per hill were maintained. Similar to previous occasions CMV and CVMV susceptible plants were maintained within the evaluation fields to create better environment with natural source of infection needed for screening. Data were collected on germination percentage of seeds, observations on CMV and CVMV incidences as well as the plant characters; pod characters; fruiting habit (upright, incline and pendant) fruit size (fruit length and fruit width). Visual observations were recorded on virus like symptoms i.e., mosaic, mottling, leaf curling, upward curling, yellowing, smalling of leaves and necrosis. ELISA tests were conducted for symptomless plants to confirm the resistance reaction to CMV and CVMV.

Genetic Bridge (GB) approach

In the genetic bridge strategy, *C. annuum* and *C. frutescens* lines were combined using the *C. chinense* lines as the bridging parents. The possible combinations of crosses among parents of *C. annuum* and *C. frutescens* species and F1 inter-specific hybrids were conducted. F1 interspecific hybrid materials which have showed resistance to CMV and CVMV were further used for completing the bridge crosses (i.e., crossing to *C. annuum* if *C. frutescens* had been used to obtain the F1, and *vice versa*). Two alternative strategies, as described below were performed to achieve bridge crosses. In alternative 1, firstly the crossing of *C. annuum* was done with the bridge species and later the obtained hybrids were crossed with *C. frutescens*; and in alternative 2, firstly conducted the crossing of *C. frutescens* with the bridge species and later the obtained hybrids with *C. annuum*.

Three way crosses and double crosses of inter-specific hybrids were performed (Annex 2). Two hundred crosses were performed in each combination. Successful crosses were tagged with their pedigree and fully ripened pods were collected and seeds were extracted. Seeds of the successful crosses were raised in the nursery and seedlings of the germinated double and three way crosses were established in the open field as described earlier, according to RCBD with three replicates at the Regional Agriculture Research & Development Centre, Makandura. Data were collected on germination percentage of seeds one month after sowing, observations on CMV and CVMV incidences as well as the pod characters; fruiting habit (upright, incline and pendant), fruit shape and fruit size (fruit length and fruit width). ELISA tests were conducted to confirm the resistance reaction for CMV and CVMV.

Compatibility of Inter Specific crosses

In both strategies the compatibility of each cross was conducted at three levels; namely the number of fruit set per number of hybridizations performed; percentage of germinated seeds and plant phenotypic characters such as fruit shape, fruiting habit and fruit size to confirm the hybrid nature of the new materials. Data analysis was done according to the Probit Procedure using SAS.

RESULTS AND DISCUSSION

Resistance responses against CMV and CVMV

Based on visual observations, 24 accessions/genotypes out of the 115 accessions used in the study, did not show virus-like symptoms typical to CMV and/or CVMV (Table 2). However, ELISA tests conducted confirmed the presence of both viruses in majority of the symptomless samples (Table 2). The results revealed that two *C. frutescens* genotypes were resistant to both viruses and six *C. chinense* genotypes were resistant to CVMV disease under naturally-infected conditions. None of the *C. annuum* types used in the study showed any degree of resistance against CMV and/or CVMV diseases (Table 2).

Table 2. Reaction of Capsicum genotypes against CMV and CVMV under field condition.

Accession /genotype Code	Type of symptoms observed	ELISA reading for CMV	Reaction for CMV	ELISA reading for CVMV	Reaction for CMV
Capsicum annuum L. Accessions/Genotypes					
WAR	NS	0.244	(+)	0.106	(+/-)
WAP	NS	0.212	(+)	0.098	(+/-)
PC-1	NS	0.856	(++)	0.106	(+/-)
WP-1	NS	0.924	(++)	0.088	(+/-)
***Capsicum chinense* Jacq Accession/Genotypes**					
INM-1	NS	0.320	(+)	0.085	(+/-)
INM-2	NS	0.286	(+)	0.022	(-)
INM-6	NS	0.644	(++)	0.060	(+/-)
HNM-1	NS	0.188	(+)	0.020	(-)
HNM-3	NS	0.654	(++)	0.062	(+/-)
MNM-10	NS	0.248	(+)	0.078	(+/-)
MNM-11	NS	0.756	(++)	0.060	(+/-)
MNM-14	NS	0.198	(+)	0.070	(+/-)
***Capsicum frutescens* L. Accession/Genotypes**					
HMK-1	NS	0.240	(+)	0.020	(-)
HMK-2	NS	0.246	(+)	0.050	(+/-)

HMK-3	NS	0.222	(+)	0.028	(-)
INK-1	NS	-0.008	(-)	0.012	(-)
INK-2	NS	0.198	(+)	0.082	(+/-)
KLK-2	NS	0.308	(+)	0.068	(+/-)
KLK-3	NS	0.252	(+)	0.062	(+/-)
MMK-1	NS	-0.008	(-)	0.014	(-)
MMK-2	NS	0.228	(+)	0.054	(+/-)
MMK-3	NS	0.188	(+)	0.048	(+/-)
MMK-6	NS	0.190	(+)	0.064	(+/-)
MMK-7	NS	0.208	(+)	0.078	(+/-)
Negative Control		-0.006	(-)	0.005	(-)
Positive Control		0.185	(+)	0.009	(+)

Note: ARU= Arunalu; KA-2= KA2; PC-1= Chilli variety PC-1; WAR= Chilli variety MI Waraniya 1; WP-2 = Chilli accession Watareka purple 2; HNM-8= Chilli accession Homagama Nai Miris 8; HNM-1= Chilli accession Homagama Nai Miris 1; INK-3= Chilli accession Ingiriya Kochchi 3; MMK-1= Chilli accession Meemure Kochchi 1.
PNM= Padukka NaiMiris; HMK= Homagama Kochchi; INK=Ingiriya Kochchi; KMK= Meemure Kochchi.
M= Mosaic, LC= Leaf curling, UC= Upward curling, Y=Yellowing, SL= Smalling of leaves and
NS= No symptoms, NT = Not tested

Reaction to CMV& CVMV: (+) = Positive, (-) = Negative, (+/-) = Border line.

Inter-specific Hybridization and Genetic Bridging

The success rate of the inter-specific crosses was differed with the parent used and it was higher when *C. annuum* and *C. chinense* parents were used as the female parent. Contrary, it was zero when *C. frutescens* was used as the female parent. Inter specific crosses between *C. annuum* and *C. chinense* were successful in both direct and reciprocal crosses. However, it was completely failed in the crosses between *C. annuum* and *C. frutescens*. *C. chinense* and *C. frutscens* crosses were successful when *C. chinense* was used as the female parent (Table 3). The negative reactions for CMV and CVMV indicated that hybrid combinations were resistant to both virus diseases (Table 3). The hybrids produced with CA2 and CA3 were positive to both viruses and also the pod colour of the CA2 based hybrids was purple and was not a preferred quality parameter for green chilli. Therefore, CA2 and CA3 were not used as parents in the proceeding crosses. Phenotypic characters of the pods were useful in conformation of inter-specific hybrids (Table 4). Based on the phenotypic characters, all new materials were confirmed for their hybridity.

Table 3. Crossing Ability and Germination % of Inter-specific crosses According to parental combinations.

Parent species	As a Male Parent		As a Female Parent	
	Pod set (per/200 crosses)	Germination (%)	Pod set (per/200 crosses)	Germination (%)
CA1	16.50	24.00	28.00	18.00
CA2	6.50	7.30	12.00	11.00
CA3	5.50	9.00	10.00	11.00
CC1	14.80	12.60	18.00	23.30
CC2	12.40	11.00	13.30	20.60
CF1	0.00	0.00	6.80	5.70
CF2	0.00	0.00	4.50	7.00

Table 4. Descriptive Results of the Inter specific hybrids among *C. annuum, C. chinense* and *C.frutescens* and reaction to CMV and CVMV.

"nt" denotes –not tested

Pedigree of the cross	Number of pods set (per 200 crosses)	Germination (%)	ELISA reading for CMV	Reaction for CMV	ELISA reading for CVMV	Reaction for CMV	Fruiting Habit/Colour	Phenotypic characters of pods	
								Pod length (cm)	Pod width (cm)
CA-1 x CC-1	32	40	0.244	(+)	0.084	(+/-)	Pendant/ Green	5.5	1.7
CA-1 x CC-2	28	32	0.286	(+)	0.086	(+/-)	Pendant/ Green	6.2	1.6
CA-1 x CF-1	6	0	nt	nt	nt	nt			
CA-1 x CF-2	0	0	nt	nt	nt	nt			
CA-2 x CC-1	10	10	0.974	(++)	1.474	(++++)	Pendant/ Purple	8.6	1.6
CA-2 x CC-2	12	12	0.820	(++)	1.522	(++++)	Pendant/ Purple	8.4	1.8
CA-3 x CC-1	12	20	0.654	(++)	1.472	(++++)	Pendant/ Light Green	10.56	1.32
CA-3 x CC-2	10	18	0.801	(++)	1.601	(++++)	Pendant/ Light Green	10.4	1.4
CA-3 x CF-1	0	0	+	nt	nt	nt			
CA-3 x CF-2	0	0	nt	nt	nt	nt			
CC-1 x CA-1	22	18	0.280	(+)	0.080	(+/-)	Incline/ Green	5.6	1.7
CC-1 x CA-2	12	12	0.264	(+)	0.094	(+/-)	Incline/ Purple	4.8	1.8
CC-1 x CA-3	10	10	0.782	(++)	1.782	(++++)	Incline/ Light Green	12.4	2.1
CC-1 x CF-1	12	15	-0.015	(-)	0.055	(-)	Upright/ Light Green	3.4	1.2
CC-1 x CF-2	18	8	-0.010	(-)	0.056	(-)	Upright/ Light Green	4.6	1.4
CC-2 x CA-1	20	18	0.302	(+)	0.092	(+/-)	Incline/ Green	4.8	2.2
CC-2 x CA-2	12	10	0.588	(++)	1.588	(++++)	Incline/ Purple	6.4	2.2
CC-2 x CA-3	10	12	0.188	(+)	1.688	(++++)	Incline/ Light Green	8.6	1.8
CC-2 x CF-1	12	8	-0.020	(-)	0.040	(-)	Incline/ Light Green	5.2	2.2
CC-2 x CF-2	18	7	-0.031	(-)	0.050	(-)	Incline/ Light Green	5.6	1.8
CA-1	0	0	0.882	(++)	1.552	(++++)	Pendant/ Green	4.5	2.0
CA-2	0	0	1.082	(+++)	1.520	(++++)	Pendant/ Purple	5.2	2.2
CA-3	0	0	1.086	(+++)	1.482	(+++)	Pendant/ Yellowish Green	14.5	2.0
CC-1	0	0	0.118	(+/-)	0.108	(+/-)	Pendant/ Light Green	4.3	2.4
CC-2	0	0	0.208	(+)	0.088	(+/-)	Pendant/ Light Green	4.2	2.2
CF-1	0	0	-0.008	(-)	0.058	(-)	Upright/ Green	2.0	0.70
CF-2	0	0	-0.010	(-)	0.044	(-)	Upright/ Yellowish Green	3	1.2

Reaction to CMV& CVMV: (+) = positive, (-) =negative, (+/-)=border line.

Both strategies successfully achieved the wide hybridization between *C. annuum* and *C. frutescens*, by obtaining F_1 hybrids, three-way-hybrids, and double cross generations using the genetic bridge (GB). The GB approach was successful to achieve, three way hybrids using *C. chinense* as the bridge species for both cross alternatives (i.e. crosses between *C. annuum* and *C. chinense* and between *C. chinense* and *C. frutescens*). Moreover, the double crosses [(*C.annuum* x *C.chinense*) x (*C. chinense* x *C. frutescens*)] and [(*C. chinense* x *C. annuum*) x (*C. chinense* x *C.frutescens*)] gave successful results in terms of pod setting and germination. In general, the double cross combinations were more successful than the three way crosses. However, a range of results in terms of crossability barriers was found in three way hybrids and double crosses depending on the directions and the genotypes involved in the crosses (Tables 5 and 6).

Table 5. Crossability and Germination % of three way crosses and Double crosses according to parental combinations.

Hybrid	As a Male Parent		As a Female Parent	
	Pod set (per/200 crosses)	Germination (%)	Pod set (per/200 crosses)	Germination (%)
CC_1 x CF_1	31.30	40.00	16.60	13.00
CC_1 x CF_2	33.33	41.30	16.60	12.00
CC_2 x CF_1	29.00	38.00	18.60	13.30
CC_2 x CF_2	29.00	39.00	24.00	15.00
CA_1 x CC_1	13.30	13.70	27.20	23.00
CA_1 x CC_2	13.80	13.00	28.20	26.00
CF_1	9.0	3.00	0.00	0.00
CF_2	10.00	3.50	0.00	0.00
CA_1	8.00	0.00	18.50	49.00

Three way and double crosses consisted with CA1, CC1 and CF1 parents showed resistant to both CMV and CVMV. Therefore, the findings of the present study revealed that that performances of double crosses in terms of pod setting and germination percentages were better than the three way crosses due to reduction of incompatibility between *C. annuum* and *C. frutescens* by the bridging parent. Similar findings have been reported by Pickersgill (1997) and Nacionusi and Pickersgill (2004) on the success of introgression of TMV resistance from *C. chinense* or *C. charcoense* into *C. annuum*, where interspecific hybridization has seldom been successful. Potential use of *C. chinense* and *C. frutescens* as bridging species in wide hybridization between *C. annuum* and *C. baccatum* has been suggested by Pickersgill (1988).

As CMV and CVMV are major viruses having a broad host range it is difficult to control them. Though the conventional methods such as cross protection, eradication of infected plants, crop rotation, use of virus free planting materials and use of chemicals against vectors have been practiced over a long period of time, management of plant virus diseases is not effective. However, use of resistant varieties is considered as an economical and durable method for controlling viral diseases has always been focused on control of insect-vector and use of resistant varieties (Ashfac *et al.*, 2014). The findings of the present study would provide a wealth of information to chilli breeders worldwide

Table 6. Descriptive Results of the Inter specific three way and double crosses among *C. annuum, C. chinense* and *C.frutescens*

Pedigree of the cross	Number of pods set (/200 crosses)	Germination (%)	ELISA reading for CMV	Reaction for CMV	ELISA reading for CVMV	Reaction for CMV	Fruiting Habit/Fruit Colour	Phenotypic characters of pods	
								Pod length (cm)	Pod width (cm)
CA-1 x(CC-1 x CF-1)	22	45	0.020	(-)	0.060	(-)	Pendant/Green	6.2	1.8
CA-1 x(CC-1 x CF-2)	18	52	0.140	(+/-)	0.180	(-)	Pendant/Green	6.4	1.7
CA-1 x(CC-2 x CF-1)	16	48	0.162	(+/-)	0.168	(+/-)	Pendant/Green	6.0	1.6
CA-1 x(CC-2 x CF-2)	18	50	0.202	(+/-)	0.242	(+/-)	Pendant/Green	5.8	1.6
CF-1 x (CA-1 x CC-1)	0	0	nt	nt	nt	nt			
CF-1 x (CA-1 x CC-2)	0	0	nt	nt	nt	nt			
CF-2 x (CA-1 x CC-1)	0	0	nt	nt	nt	nt			
CF-2 x (CA-1 x CC-2)	0	0	nt	nt	nt	nt			
(CA-1 x CC-1) x CF-1	8	2	nt	nt	nt	nt			
(CA-1 x CC-1) x CF-2	12	4	nt	nt	nt	nt			
(CA-1 x CC-1) x (CC-1 x CF-1)	36	36	0.018	(-)	0.048	(-)	Pendant/Green	4.4	1.4
(CA-1 x CC-1) x (CC-1 x CF-2)	40	34	0.232	(+/-)	0.052	(-)	Pendant/Green	4.6	1.4
(CA-1 x CC-1) x (CC-2 x CF-1)	35	30	0.281	(+/-)	0.202	(+/-)	Pendant/Green	4.4	1.5
(CA-1 x CC-1) x (CC-2 x CF-2)	32	32	0.302	(+/-)	0.244	(+/-)	Pendant/Green	4.8	1.5
(CA-1 x CC-2) x CF-1	10	4	nt	nt	nt	nt			
(CA-1 x CC-2) x CF-2	8	3	nt	nt	nt	nt			
(CA-1 x CC-2) x (CC-1 x CF-1)	36	40	0.222	(+/-)	0.188	(+/-)	Pendant/Green	6.2	1.4
(CA-1 x CC-2) x (CC-1 x CF-2)	42	38	0.208	(+/-)	0.194	(+/-)	Pendant/Green	6.4	1.4
(CA-1 x CC-2) x (CC-2 x CF-1)	35	35	0.222	(+/-)	0.214	(+/-)	Pendant/Green	6.2	1.3
(CA-1 x CC-2) x (CC-2 x CF-2)	38	36	0.322	(+/-)	0.222	(+/-)	Pendant/Green	6.0	1.3
(CC-1 x CF-1) x CA-1	12	0	nt	nt	nt	nt			
(CC-1 x CF-1) x (CA-1 x CC-1)	18	20	0.022	(-)	0.064	(-)	Incline/Light Green	4.2	1.4
(CC-1 x CF-1) x (CA-1 x CC-2)	20	18	0.182	(+/-)	0.196	(+/-)	Incline/Light Green	4.0	1.3
(CC-1 x CF-2) x CA-1	16	0	nt	nt	nt	nt			

(CC-1 x CF-2) x (CA-1 x CC-1)	16	16	0.180	(+/-)	0.058	(-)	Incline/Light Green	4.4	1.4
(CC-1 x CF-1) x (CA-1 x CC-2)	18	20	0.248	(+/-)	0.194	(+/-)	Incline/Light Green	4.4	1.3
(CC-2 x CF-1) x CA-1	16	0	nt	nt	nt	nt			
(CC-2 x CF-1) x (CA-1 x CC-1)	22	22	0.234	(+/-)	0.214	(+/-)	Incline/Light Green	4.4	1.4
(CC-2 x CF-1) x (CA-1 x CC-2)	18	18	0.224	(+/-)	0.242	(+/-)	Incline/Light Green	4.2	1.3
(CC-2 x CF-2) x CA-1	20	0	nt	nt	nt	nt			
(CC-2 x CF-2) x (CA-1 x CC-1)	24	24	0.242	(+/-)	0.240	(+/-)	Incline/Light Green	3.8	1.4
(CC-2 x CF-2) x (CA-1 x CC-2)	27	22	0.198	(+/-)	0.234	(+/-)	Incline/Light Green	4.0	1.5
CA-1	0	0	1.012	(++)	1.740	(++++)	Pendant/ Green	4.6	2.0
CA-2	0	0	1.422	(+++)	1.802	(++++)	Pendant/ Purple	5.2	2.2
CA-3	0	0	1.860	(+++)	1.968	(++++)	Pendant/ Yellowish Green	14.2	2.0
CC-1	0	0	0.202	(+/-)	0.214	(+/-)	Pendant/ Light Green	4.4	2.2
CC-2	0	0	0.306	(+)	0.288	(+/-)	Pendant/ Light Green	4.0	2.1
CF-1	0	0	0.012	(-)	0.064	(-)	Upright/ Green	2.1	0.8
CF-2	0	0	0.014	(-)	0.055	(-)	Upright/ Yellowish Green	3.2	1.2
Negative Control			0.008	(-)	0.050	(-)			
Positive Control			0.328	(+)	0.302	(+)			

CONCLUSIONS

Findings of the present study identified chilli genotypes showing resistance to CMV and CVMV and revealed the possibility of wide hybridization between *C. annuum* and *C. frutescens* using the genetic bridge approach, although the degree of success is highly dependent on the genotypes used to obtain hybrids and subsequent crossings. The genotypes with best performance in these studies (accession PC-1 of *C. annuum*, accession Homagama Nai Miris 8 of *C. chinense* and accession Ingiriya Kochchi 3 of *C. frutescens*) are good candidates for introgression breeding from *C. frutescens* to *C. annuum*. These results provide breeders with relevant information on wide hybridization approaches and an appropriate genetic material to be used for successful incorporation of *C. frutescens* gene pool as a source of variation for introgression of CMV and CVMV resistant genes in *C. annuum* breeding programs.

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ANNEXURE

Annex 1. Morphological traits of the germplasm/accessions collected.

Index no.	Accession/ germplasm	Code	Location/ Agro-ecological zone	Mature pod colour	Pod shape	Fruiting habit
***Capsicum annuum* L.Genotypes**						
01	MI-2	MI-2	MahaIlluppallama (DL1)	Dark green	Elongated Blunt	Pendent
02	KA-2	KA-2	MahaIlluppallama (DL1)	Dark green	Elongated pointed	Pendent
03	Arunalu	ARU	MahaIlluppallama (DL1)	Green	Elongated pointed	Upward
04	MI-hot	MI-H	MahaIlluppallama (DL1)	Green	Elongated Blunt	Pendent
05	MI-Green	MI-G	MahaIlluppallama (DL1)	Dark green	Elongated pointed	Pendent
06	MICH-3	MI-3	MahaIlluppallama (DL1)	Dark green	Elongated pointed	Pendent
07	Galkiriyagama selection	GKS	MahaIlluppallama (DL1)	Green	Elongated pointed	Pendent
08	MI Waraniya 1	WAR	MahaIlluppallama (DL1)	Yellowish green	Elongated pointed	Pendent
09	Batalu an miris	BAT	Gampaha (WL1)	Light green	Elongated pointed	Pendent
10	Waraniya purple	WAP	Colombo (WL1)	Purple	Elongated pointed	Pendent
11	ICPN selection	ICP	MahaIlluppallama (DL1)	Yellow	Elongated pointed	Pendent
12	LGM selection	LGM	MahaIlluppallama (DL1)	Light green	Elongated pointed	Pendent
13	Hot Beauty selection	HBS	MahaIlluppallama (DL1)	Dark green	Elongated pointed	Pendent
14	CAS 218 selection	CAS	MahaIlluppallama (DL1)	Green	Elongated pointed	Pendent
15	B.L.9853	BL3	MahaIlluppallama (DL1)	Green	Elongated pointed	Pendent
16	Henemiris	HNM	Nochchiyagama (DL1)	Light green	Elongated pointed	Pendent
17	PC-1 selection	PC-1	Batticaloa (DL1)	Light green	Conical Blunt	Pendent
18	Kaithady selection	KDS	MahaIlluppallama (DL1)	Green	Elongated pointed	Pendent
19	Ruhunumiris 1	RM-1	Angunakolapellessa (DL1)	Green	Conical Blunt	Pendent
20	Ruhunumiris 2	RM-2	Angunakolapellessa (DL1)	Green	Conical Blunt	Pendent
21	Watareka purple -1	WP-1	Homagama (WL1)	Purple	Elongated pointed	Pendent
22	Watareka purple -2	WP-2	Homagama (WL1)	Dark purple	Conical Blunt	Upward
23	Watareka Round	WTR	Homagama (WL1)	Dark purple	Round	Upward
24	Cluster chilli	CLC	MahaIlluppallama (DL1)	Light green	Elongated pointed	Upward cluster
25	Chinese chilli 1	CC-1	China	Dark green	Elongated pointed	Pendent
26	Chinese chilli 2	CC-2	China	Green	Elongated pointed	Pendent
27	Chinese chilli 3	CC-3	China	Green	Elongated	Pendent

					pointed	
28	Chinese cluster	CHC	China	Light green	Elongated pointed	Upward Cluster
***Capsicum chinense* Jacq.Genotypes**						
29	IngiriyaNaiMiris 1	INM-1	Ingiriya (WL1)	Light green	Irregular conical	Pendent
30	IngiriyaNaiMiris 2	INM-2	Ingiriya (WL1)	Light green	Irregular conical	Pendent
31	IngiriyaNaiMiris 3	INM-3	Ingiriya (WL1)	Light green	Irregular conical	Pendent
32	IngiriyaNaiMiris 4	INM-4	Ingiriya (WL1)	Light green	Irregular conical	Pendent
33	IngiriyaNaiMiris 5	INM-5	Ingiriya (WL1)	Light green	Irregular conical	Pendent
34	IngiriyaNaiMiris 6	INM-6	Ingiriya (WL1)	Light green	Irregular conical	Pendent
35	IngiriyaNaiMiris 7	INM-7	Ingiriya (WL1)	Dark green	Irregular conical	Pendent
36	IngiriyaNaiMiris 8	INM-8	Ingiriya (WL1)	Dark green	Irregular round	Pendent
37	IngiriyaNaiMiris 9	INM-9	Ingiriya (WL1)	Light Yellow	Irregular long	Pendent
38	IngiriyaNaiMiris 10	INM-10	Ingiriya (WL1)	Light purple	Irregular conical	Pendent
39	IngiriyaNaiMiris 11	INM-11	Ingiriya (WL1)	Light purple	Irregular conical	Pendent
40	IngiriyaNaiMiris 12	INM-12	Ingiriya (WL1)	Light purple	Irregular conical	Pendentcl uster
41	HomagamaNaiMir is 1	HNM-1	Homagama (WL1)	Light green	Irregular conical	Pendent
42	HomagamaNaiMir is 2	HNM-2	Homagama (WL1)	Light green	Irregular conical	Pendent
43	HomagamaNaiMir is 3	HNM-3	Homagama (WL1)	Light green	Irregular conical	Pendent
44	HomagamaNaiMir is 4	HNM-4	Homagama (WL1)	Light green	Irregular conical	Pendent
45	HomagamaNaiMir is 5	HNM-5	Homagama (WL1)	Dark green	Irregular conical	Pendent
46	HomagamaNaiMir is 6	HNM-6	Homagama (WL1)	Dark green	Irregular round	Pendent
47	HomagamaNaiMir is 7	HNM-7	Homagama (WL1)	Light Yellow	Irregular conical	Pendent
48	HomagamaNaiMir is 8	HNM-8	Homagama (WL1)	Light Yellow	Irregular conical	Pendent
49	HomagamaNaiMir is 9	HNM-9	Homagama (WL1)	Light purple	Irregular conical	Pendent
50	HomagamaNaiMir is 10	HNM-10	Homagama (WL1)	Light purple	Irregular conical	Pendent
51	KalawanaNaiMiris 1	KNM-1	Kalawana (WL1)	Light green	Irregular conical	Pendent
52	KalawanaNaiMiris 2	KNM-2	Kalawana (WL1)	Light green	Irregular conical	Pendent
53	KalawanaNaiMiris 4	KNM-3	Kalawana (WL1)	Light green	Irregular conical	Pendent
54	KalawanaNaiMiris 5	KNM-4	Kalawana (WL1)	Light green	Irregular conical	Pendent
55	KalawanaNaiMiris 6	KNM-5	Kalawana (WL1)	Light green	Irregular conical	Pendent

56	KalawanaNaiMiris 7	KNM-6	Kalawana (WL1)	Light purple	Irregular conical	Pendent
57	KalawanaNaiMiris 8	KNM-7	Kalawana (WL1)	Light purple	Irregular conical	Pendent
58	KalawanaNaiMiris 9	KNM-8	Kalawana (WL1)	Light purple	Irregular conical	Pendent
59	KalawanaNaiMiris 10	KNM-9	Kalawana (WL1)	Light purple	Irregular conical	Pendent
60	KalawanaNaiMiris 11	KNM-10	Kalawana (WL1)	Light green	Irregular conical	Pendent
61	KalawanaNaiMiris 12	KNM-11	Kalawana (WL1)	Light green	Irregular conical	Pendent
62	KalawanaNaiMiris 13	KNM-12	Kalawana (WL1)	Light green	Irregular conical	Pendent
63	KalawanaNaiMiris 14	KNM-13	Kalawana (WL1)	Light green	Irregular conical	Pendent
64	KalawanaNaiMiris 15	KNM-14	Kalawana (WL1)	Light green	Irregular conical	Pendent
65	KalawanaNaiMiris 16	KNM-15	Kalawana (WL1)	Light green	Irregular conical	Pendent
66	KalawanaNaiMiris 17	KNM-16	Kalawana (WL1)	Light green	Irregular conical	Pendent
67	Kalawana Nai Miris 18	KNM-17	Kalawana (WL1)	Light green	Irregular round	Pendent
68	Horana Nai Miris 1	HRN-1	Kalawana (WL1)	Light green	Irregular conical	Pendent
69	Horana Nai Miris 2	HRN-2	Kalawana (WL1)	Light green	Irregular conical	Pendent
70	Horana Nai Miris 3	HRN-3	Kalawana (WL1)	Light green	Irregular conical	Pendent
71	Horana Nai Miris 4	HRN-4	Kalawana (WL1)	Light purple	Irregular conical	Pendent
72	Horana Nai Miris 5	HRN-5	Kalawana (WL1)	Light green	Irregular conical	Pendent
73	Horana Nai Miris 6	HRN-6	Kalawana (WL1)	Light green	Irregular conical	Pendent
74	Horana Nai Miris 7	HRN-7	Kalawana (WL1)	Light green	Irregular round	Pendent
75	Meemure Nai Miris 1	MNM-1	Kalawana (WL1)	Light green	Irregular conical	Pendent
76	Meemure Nai Miris 2	MNM-2	Kalawana (WL1)	Light green	Irregular conical	Pendent
77	Meemure Nai Miris 3	MNM-3	Kalawana (WL1)	Light green	Irregular conical	Pendent
78	Meemure Nai Miris 4	MNM-4	Kalawana (WL1)	Light green	Irregular conical	Pendent
79	Meemure Nai Miris 5	MNM-5	Kalawana (WL1)	Light green	Irregular conical	Pendent
80	Meemure Nai Miris 6	MNM-6	Kalawana (WL1)	Light green	Irregular conical	Pendent
81	Meemure Nai Miris 7	MNM-7	Kalawana (WL1)	Light green	Irregular conical	Pendent
82	Meemure Nai Miris 8	MNM-8	Kalawana (WL1)	Light purple	Irregular conical	Pendent
83	Meemure Nai Miris 9	MNN-9	Kalawana (WL1)	Light purple	Irregular conical	Pendent
84	Meemure Nai Miris 10	MNM-10	Kalawana (WL1)	Light green	Irregular conical	Pendent
85	Meemure Nai	MNM-11	Kalawana (WL1)	Light green	Irregular	Pendent

	Miris 11				conical	
86	Meemure Nai Miris 12	MNM-12	Kalawana (WL1)	Light green	Irregular conical	Pendent
87	Meemure Nai Miris 13	MNM-13	Kalawana (WL1)	Light green	Irregular conical	Pendent
88	Meemure Nai Miris 14	MNM-14	Kalawana (WL1)	Light green	Irregular conical	Pendent
89	Padukka Nai Miris 1	PNM-1	Padukka (WL 1)	Light green	Irregular conical	Pendent
90	Padukka Nai Miris 2	PNM-2	Padukka	Light green	Irregular conical	Pendent
91	Padukka Nai Miris 3	PNM-3	(WL 1)	Light purple	Irregular conical	Pendent
***Capsicum frutescens* L. Genotypes**						
92	Homagama Kochchi 1	HMK-1	Homagama (WL 1)	Green	Elongated Pointed	Upward
93	Homagama Kochchi 2	HMK-2	Homagama (WL 1)	Green	Elongated Pointed	Upward
94	Homagama Kochchi 3	HMK-3	Homagama (WL 1)	Green	Elongated Pointed	Upward
95	Homagama Kochchi 4	HMK-4	Homagama (WL 1)	Yellow	Elongated Pointed	Upward
96	Homagama Kochchi 5	HMK-5	Homagama (WL 1)	Light yellow	Elongated Pointed	Upward
97	Homagama Kochchi 6	HMK-6	Homagama (WL 1)	Light yellow	Elongated Pointed	Upward cluster
98	Ingiriya Kochchi 1	INK-1	Ingiriya (WL1)	Green	Elongated Pointed	Upward
99	Ingiriya Kochchi 2	INK-2	Homagama (WL 1)	Green	Elongated Pointed	Upward
100	Ingiriya Kochchi 3	INK-3	Homagama (WL 1)	Green	Elongated Pointed	Upward
101	Ingiriya Kochchi 4	INK-4	Homagama (WL 1)	Green	Elongated Pointed	Upward
102	Ingiriya Kochchi 5	INK-5	Homagama (WL 1)	Light Green	Elongated Pointed	Upward
103	Kalawana Kochchi 1	KLK-1	Kalawana (WL1)	Green	Elongated Pointed	Upward
104	Kalawana Kochchi 2	KLK-2	Kalawana (WL1)	Green	Elongated Pointed	Upward
105	Kalawana Kochchi 3	KLK-3	Kalawana (WL1)	Green	Elongated Pointed	Upward
106	Kalawana Kochchi 4	KLK-4	Kalawana (WL1)	Green	Elongated Pointed	Upward
107	Kalawana Kochchi 5	KLK-5	Kalawana (WL1)	Green	Elongated Pointed	Upward
108	Kalawana Kochchi 6	KLK-6	Kalawana (WL1)	Green	Elongated Pointed	Upward
109	Meemure Kochchi 1	MMK-1	Meemure (WL1)	Green	Elongated Pointed	Upward
110	Meemure Kochchi 2	MMK-2	Meemure (WL1)	Green	Elongated Pointed	Upward
111	Meemure Kochchi 3	MMK-3	Meemure (WL1)	Green	Elongated Pointed	Upward
112	Meemure Kochchi 4	MMK-4	Meemure (WL1)	Green	Elongated Pointed	Upward
113	Meemure Kochchi 5	MMK-5	Meemure (WL1)	Light Green	Elongated Pointed	Upward

114	Meemure Kochchi 6	MMK-6	Meemure (WL1)	Light Green	Elongated Blunt	Upward cluster
115	Meemure Kochchi 7	MMK-7	Meemure (WL1)	Yellow green	Elongated Pointed	Upward

Annex 2. Three way and Double inter specific crosses made to bridge *Capsicum annuum* L. with *C. frutescens* L.

Female Parent	Male Parent	Inter-Specific Cross (Three way or Double Cross)
CA x *CC* (PC-1 x HNM-8)	*CF* (INK-3)	(*CA* x *CC*) x *CF* (PC-1 x HNM-8)x(INK-3)
CA x *CC* (PC-1 x MNM-1)	*CF* (INK-3)	(*CA* x *CC*) x *CF* (PC-1 x MNM-1) x (INK-3)
CA x *CC* (PC-1 x HNM-8)	*CF* (MMK-1)	(*CA* x *CC*) x *CF* (PC-1 x HNM-8) x (MMK-1)
CA x *CC* (PC-1 x MNM-1)	*CF* (MMK-1)	(*CA* x *CC*) x *CF* (PC-1 x MNM-1) x (MMK-1)
CA (PC-1)	*CC* x *CF* (HNM-8x INK-3)	*CA* x (*CC* x *CC*) (PC-1) x (HNM-8x INK-3)
CA (PC-1)	*CC* x *CF* (HNM-8x MMK-1)	*CA* x (*CC* x *CC*) (PC-1) x (HNM-8x MMK-1)
CA (PC-1)	*CC* x *CF* (MNM-1 x INK-3)	*CA* x (*CC* x *CC*) (PC-1) x (MNM-1 x INK-3)
CA (PC-1)	*CC* x *CF* (MNM-1 x MMK-1)	*CA* x (*CC* x *CC*) (PC-1) x (MNM-1 x MMK-1)
CC x *CF* (HNM-8x INK-3)	*CA* (PC-1)	(*CC* x *CC*) *x CA* (HNM-8x INK-3) x (PC-1)
CC x *CF* (HNM-8x MMK-1)	*CA* (PC-1)	(*CC* x *CC*) *x CA* (HNM-8x MMK-1) x (PC-1)
CC x *CF* (MNM-1 x INK-3)	*CA* (PC-1)	(*CC* x *CC*) *x CA* (MNM-1 x INK-3) x (PC-1)
CC x *CF* (MNM-1 x MMK-1)	*CA* (PC-1)	(*CC* x *CC*) *x CA* (MNM-1 x MMK-1) x (PC-1)
CF (INK-3)	*CA* x *CC* (PC-1 x HNM-8)	*CF* x (*CA* x *CC*) (INK-3) x (PC-1 x HNM-8)
CF (INK-3)	*CA* x *CC* (PC-1 x MNM-1)	*CF* x (*CA* x *CC*) (INK-3) x (PC-1 x MNM-1)
CF (MMK-1)	*CA* x *CC* (PC-1 x HNM-8)	*CF* x (*CA* x *CC*) (MMK-1) x (PC-1 x HNM-8)
CF (MMK-1)	*CA* x *CC* (PC-1 x MNM-1)	*CF* x (*CA* x *CC*) (MMK-1) x (PC-1 x MNM-1)
(*CA* x *CC*) **(PC-1 x HNM-8)**	**(*CC x CF*)** **(HNM-8x INK-3)**	**(*CA* x *CC*) x (*CC* x *CF*)** **(PC-1 x HNM-8) x (HNM-8x INK-3)**
(*CA* x *CC*) **(PC-1 x MNM-1)**	**(*CC x CF*)** **(HNM-8x MMK-1)**	**(*CA* x *CC*) x (*CC* x *CF*)** **(PC-1 x MNM-1) x (HNM-8x MMK-1)**
(*CA* x *CC*) **(PC-1 x HNM-8)**	**(*CC x CF*)** **(MNM-1 x INK-3)**	**(*CA* x *CC*) x (*CC* x *CF*)** **(PC-1 x HNM-8) x (MNM-1 x INK-3)**
(*CA* x *CC*) **(PC-1 x MNM-1)**	**(*CC x CF*)** **(MNM-1 x MMK-1)**	**(*CA* x *CC*) x (*CC* x *CF*)** **(PC-1 x MNM-1) x (MNM-1 x MMK-1)**

Note: CA=*Capsicum annuum*; CC=*C. chinense*; CF=*C. frutescens.*
PC-1=Chilli variety PC-1; WAR= Chilli variety MI Waraniya 1; WP-2 = Chilli accession Watareka purple 2; HNM-8=Chilli accession Homagama Nai Miris 8; HNM-1= Chilli accession Homagama Nai Miris 1; INK-3=Chilli accession Ingiriya Kochchi 3; MMK-1=Chilli accession Meemure Kochchi 1.
The bold crosses are double crosses whereas all others are three way crosses.