Bangladesh J. Bot. **53**(4): 895-902, 2024 (December) DOI: https://doi.org/10.3329/bjb.v53i4.77508

GENOME-WIDE ANALYSIS OF CUCUMBER (*CUCUMIS SATIVUS* L.) LINES TO IDENTIFY ZUCCHINI YELLOW MOSAIC VIRUS RESISTANCE LOCI

ÖMER FARUK COŞKUN*, TARIK GÖKBULUT[1], HAKAN FIDAN[2], AHMET FIKRET FIRAT[3], HASAN PINAR[3] AND OSMAN GÜLŞEN[3]

Department of Horticulture, Faculty of Agriculture,
Hatay Mustafa Kemal University, Hatay, Türkiye

Keywords: Biotic stress, Cucumber, GWAS, MAS, Virus resistance, ZYMV

Abstract

Zucchini yellow mosaic virus (ZYMV) is one of the most important pathogens that cause yield losses in cucumber. The objectives of this study were to investigate the structure of genetic resistance to ZYMV in cucumber for marker-assisted selection (MAS). Forty-eight resistant and 48 susceptible lines were selected from a germplasm collection of 600 cucumber lines. Bulk DNAs of resistant and susceptible lines were screened to find candidate marker(s) using ISSR, RAPD, and a cleaved amplified polymorphism (CAP) primer, which targeted over 5000 cucumber loci. A total of 54 candidate marker loci were identified. In association analyses, using TASSEL software, associated markers were detected. The results explained 78% of the total ZYMV variation. The remaining 22% of the variation was possibly due to the presence of additional genes and environmental effects that caused lower repeatability. The markers developed in this study may allow successful discrimination of resistant and susceptible cucumbers for markers assisted selection in breeding programs.

Introduction

Plant diseases are an important problem that hinders production and export by reducing the yield and quality of vegetables (Hussain *et al*. 2021). Environmental conditions, pathogens, and insects can cause plant diseases. Plant diseases need to be detected early and controlled to ensure sustainable vegetable production. There are many diseases that can affect cucurbits such as fungi, bacteria, viruses, and insects. Crop losses caused by viral diseases are significant in Cucurbitaceae species (Islam *et al*. 2018). As a whole, RNA viruses pose a significant threat to vegetable production (Kone *et al*. 2017). The zucchini yellow mosaic virus (ZYMV) is a member of the family Potyviridae and contains only one RNA sequence (Romay *et al*. 2014a). This virus is an aphid-borne potyvirus and one of the most common pathogens causes major losses worldwide in members of the Cucurbitaceae family (Kamberoglu *et al*. 2016). Cucurbit crops, such as watermelon, cucumber, and squash, are susceptible to the virus. The wild cucurbit can be an effective virus reservoir (Romay *et al*. 2014b). A lack of information, poor management practices, and the use of uncertified seeds also contribute to the spread of viral diseases (Ahsan *et al*. 2020). Genetic resistance is an effective way to limit losses caused by virus diseases (Nagendran *et al*. 2017). Breeding for ZYMV resistance is the best approach. It is possible to breed varieties that are virus-resistant using conventional breeding methods. A single recessive gene (*zym*-FL) in watermelon is responsible for its high level of ZYMV resistance (Guner 2018). Inheritance of this trait has been previously characterized (Cardoso *et al*. 2010). According to these results, cucumber resistance to ZYMV may be inherited, recessively. Thus, introduction of resistance

*Author for Correspondence: <omerfaruk.coskun@mku.edu.tr>. [1]Erciyes University, Faculty of Science, Department of Biology, Kayseri, Türkiye. [2]Akdeniz University, Faculty of Agriculture, Department of Plant Protection, Antalya, Türkiye. [3]Erciyes University, Faculty of Agriculture, Department of Horticulture, Kayseri, Türkiye.

allele into elite lines requires generations of backcrossing and inoculations of progenies with pathogens, extending the time required for developing resistant plants. It is possible to reduce this time by using marker-assisted selection (MAS). A MAS study in plants can be performed by molecular characterization (Tecirli *et al*. 2018, Coskun 2022, Coskun and Gulsen 2024) as well as association mapping (Kumar *et al*. 2022, Coskun 2023). Genetic maps in plants enable the identification of gene regions associated with biotic stress factors in plants. By using this technique, it is possible to identify genes that are associated with disease resistance and toleration to biotic stress. There have been several DNA markers linked to the recessive ZYMV resistance gene (*zym*) previously reported (Park *et al*. 2004). Different ZYMV resistance loci are likely to exist. Therefore, additional investigation linked to the *zym* loci covering highly variable germplasm is needed for marker development. The aim of this study was to determine the genetic structure that underlies cucumber resistance to ZYMV for marker-assisted selection.

Materials and Methods

The study was carried out in the Molecular Biology Laboratory, Faculty of Agriculture, University of Erciyes, Türkiye. A total of 600 accessions were previously tested by ELISA for ZYMV resistance by the classical method. A total of 48 resistant and 48 susceptible lines were selected from this population for further analysis. Total DNA was extracted from 30 mg tissue using a modified CTAB DNA extraction procedure. Equal amounts of DNA from five resistant and 5 susceptible lines were mixed to prepare two bulks, which were later used to screen for molecular marker polymorphism. For marker production, 1160 RAPD and 26 ISSR, and one CAP primers were used to evaluate resistant and susceptible bulks. Those primers producing marker polymorphism between the bulks were applied to 48 resistant and 48 susceptible samples. A CAP marker developed by Amano *et al.* (2013) was also used for analysis. This produced 338 bp PCR fragments, then digested with DraI enzyme producing 313 and 25 bp fragments. Each of 15 µl PCR components consisted of 0.66 mM of each of primers, 200 µM of each dNTPs, 1.5 µl of 10× PCR buffer, 2-2.5 mM of $MgCl_2$, ddH_2O, one unit of Taq polymerase and 20 ng of template DNA. PCR products of four marker systems were separated on 2-3% agarose gel at 110 V for 3-6 hrs and visualized under UV light. Each band was scored as present (1) or absent (0) and data were analyzed with the NTSYS-pc (version 2.1) software package (Rohlf 2000). These similarity matrices were used to construct a dendrogram using the UPGMA to determine genetic relationships among the germplasm studied. Population structure was analyzed using a model-based approach, Bayesian method by software Structure, version 2.3.2 (Pritchard *et al.* 2000). Association analyses between marker loci and phenotypic values were performed using the general linear model (GLM) functions in TASSEL stand-alone version 3.1 (Bradbury *et al*. 2007). Finally, backward regression was used to develop a regression model to predict phenotype based on genotype using SAS software.

Results and Discussion

In this study, 1187 primers were used which included 1160 RAPD, 26 ISSR, and one CAP primer. The primers were applied first to the bulk DNA of 5 susceptible and 5 resistant individuals, followed by all 96 samples. It was amplified more than 5000 loci in *Cucumis sativus*. A total of 1160 RAPD primers were applied to 96 samples, and these samples were scored after visualized in an agarose gel. A total of 13 polymorphic fragments were produced among 96 resistant and susceptible lines from 26 ISSR primers. Moreover, DraI enzyme digestion of one CAP marker produced a codominant polymorphism among the lines. Amano *et al.* (2013) reported a CAP marker (dCAPS-G99A) cosegregating with resistance. This marker was analyzed for the

lines in this study (Fig. 1). High association were observed between this CAP marker and resistance. The samples 54, 71, 72 and 74, however, had resistance alleles despite being susceptible. Similarly, among the resistant samples detected by classical screening, lines 80 and 250 were heterozygous and Lines 272, 91, 125, 147, 148, 379, 341, 345, 188, 203 and 214 had susceptible allele. The CAP marker developed by Amano *et al.* (2013) was also assessed for other known cultivars and lines in this study. This CAP marker was tested on known resistant (e.g., Silyon RZ, TMG-1, Sardis) and susceptible lines (e.g., Maraton and 8 others). It was found that Silyon and TMG-1 had resistant alleles, but Sardis had susceptible alleles. A susceptible allele was present in all susceptible samples. Using cluster analysis, 54 markers were analyzed to identify loci with associations. Three out of four different matrices (correlation, simple matching, average distance, and Dice similarity) clustered the 54 markers into two subclusters (Figs 2 and 3).

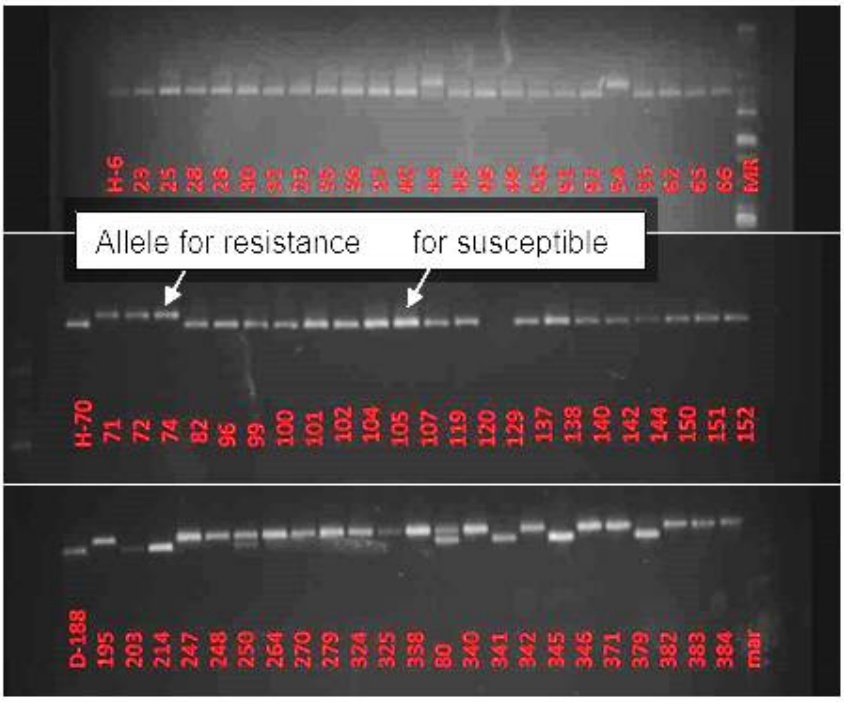

Fig. 1. Gel image obtained with CAP marker of resistant and susceptible individuals detected by classical screening of samples.

TASSEL software used membership coefficients for five subpopulations produced by the STRUCTURE program as covariances. Out of 54 markers, only loosely correlating 38 as detected by NTSYS software were used for substructuring Bayesian analysis. This resulted in five subpopulations among the 96 samples. For estimating candidate markers for ZYMV resistance in cucumber, association analysis was performed using three data files (phenotype, genotype, and substructure) in TASSEL. Based on F-statistics and permutation (1000), candidate markers were detected (Table 1). The CAP marker alone explained 42% of the total variation for ZYMV among the 96 samples, followed by OPM07600 (27%) and OPD07920 (26%).

Table 1. The 54 markers, P values based on F and permutation tests, R^2 values as obtained with TASSEL software.

Marker	*F* test-based *P*-value	Permutation-based *P-value*	R^2
CAP	0.0000000000003	0.001	0.44
OPM07600	0.00000003	0.001	0.29
OPD07920	0.0000002	0.001	0.26
(AG)8YC500	0.000003	0.001	0.23
OPBA16670	0.00001	0.001	0.19
(GT)8YA1000	0.00007	0.004	0.18
OPE16770	0.00009	0.005	0.16
OPU012000	0.00009	0.005	0.16
OPN041000	0.0002	0.014	0.14
OPAL111000	0.0005	0.024	0.13
OPP12550	0.0008	0.037	0.12
OPBA131800	0.0009	0.042	0.12
OPBE121100	0.0008	0.039	0.12
OPAA19900	0.0006	0.032	0.12
OPAA191100	0.002	0.082	0.11
OPT162000	0.001	0.068	0.11
OPAF09650	0.003	0.15	0.09
OPAJ20690	0.004	0.17	0.09
OPT15420	0.004	0.179	0.09
OPAL09700	0.03	0.749	0.05
OPAR12450	0.05	0.87	0.04
OPAU021600	0.06	0.931	0.04
OPAU021500	0.02	0.674	0.05
OPAT05400	0.004	0.166	0.09
OPBF11700	0.04	0.857	0.04
OPBB6530	0.016	0.514	0.06
OPN04600	0.09	0.979	0.03
OPAF091300	0.07	0.964	0.03
OPL15800	0.01	0.401	0.07
OPN05820	0.01	0.432	0.07
OPAX09650	0.05	0.889	0.04
OPBG11320	0.007	0.296	0.07
OPAE18380	0.03	0.688	0.05
OPT05420	0.09	0.979	0.03
OPAA071100	0.004	0.186	0.08
OPAA07750	0.07	0.964	0.03
OPT15520	0.03	0.795	0.05
OPAN011100	0.01	0.395	0.07
OPP121750	0.01	0.536	0.06
OPA09400	0.06	0.945	0.04
OPAR12550	0.04	0.87	0.04

In cucumber, all markers revealed 80% of the variation in ZYMV resistance. Of all the markers, those significantly associated markers based on both F- and permutation-test revealed 78 and 75% of the total variation before and after Bonferroni correction ($P < 0.0001$). Only five

markers remained in the regression model after Bonferroni correction method. Although previous studies reported monogenic control of ZYMV resistance in cucumber (Cardoso *et al*. 2010), the present findings indicated that the markers developed for the resistance locus failed to distinguish resistant and susceptible lines. A large germplasm collection was screened to elucidate the genetic structure of ZYMV resistance among cucumbers including lines and popular cultivars worldwide.

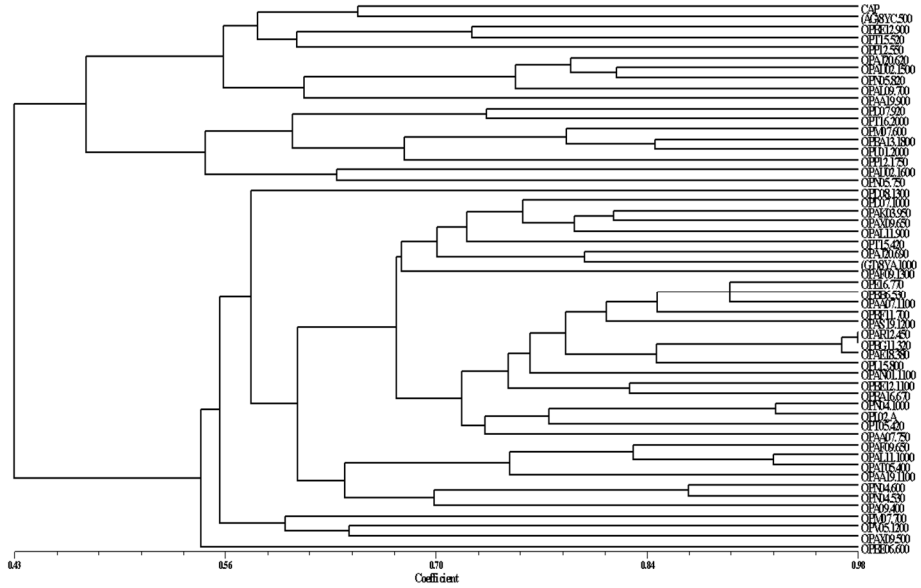

Fig. 2. Dendrogram based on similarity of simple matching.

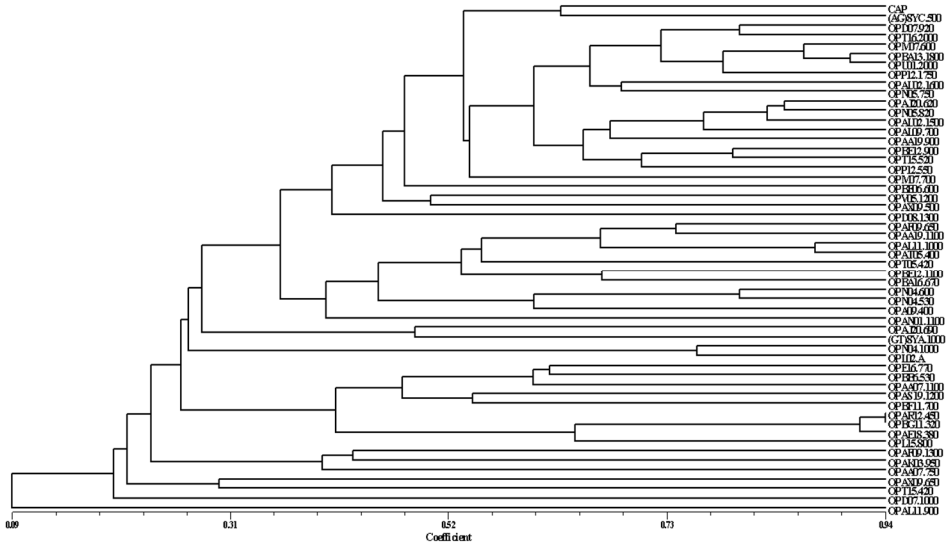

Fig. 3. Dendrogram based on Dice genetic similarity matrix.

The data from classical screening in a previous study and molecular screening in this study were combined to estimate the genetic structure of ZYMV resistance in cucumber. ELISA tests were previously conducted in the Antalya province of Türkiye under greenhouse conditions. This study was repeated twice and each repeatation had 10 replications. In the present study, molecular data were obtained. A total of 1187 primers (1160 RAPD, 26 ISSR and one CAP primer) targeting approximately more than 5000 loci were investigated in this study. Of these primers, 110 primers were applied to all 96 samples and produced 54 polymorphic markers. These efforts aimed at a whole genome survey for ZYMV resistance in cucumber. Based on 54 markers polymorphic between the bulks and among 48 susceptible and 48 resistant cucumbers, cluster analysis was performed to detect associations among the marker loci. Three of four different matrices (correlation, simple matching, average distance and Dice similarity) clustered the 54 markers into two subclusters (Figs 2 and 3). This pointed to polygenic control of ZYMV resistance in cucumbers since closely linked markers were clustered in proximity.

In some studies, genetic loci associated with resistance to ZYMV have been identified in cucurbit species where SRAP, SSR, AFLP, REMAP, ISSR, and SNP markers were used (Abdollahi-Mandoulakani *et al*. 2015, Sigva *et al*. 2015, Shrestha *et al*. 2021). Primer types used in this study were RAPD, ISSR, and CAP. In some studies carried out on cucurbits, the identification of QTL and SNP associated with ZYMV resistance has been performed. In a high resolution mapping study performed on cucumbers, however, ZYMV resistance could not be associated with a specific DNA polymorphism. In a cucumber study, 170 SRAP worked with 586 SSR and 308 AFLP primer combinations. It was determined that an AFLP marker in the E-ACA/MCA primer combination could be an associated marker (Sigva *et al.* 2015). In a study conducted on melon, it was determined that 7 REMAP and 4 ISSR markers were associated with ZYMV resistant populations (Abdollahi-Mandoulakani *et al*. 2015). The number of markers examined in this study is higher than previous studies (Abdollahi-Mandoulakani *et al.* 2015; Sigva *et al.* 2015). The CAP marker was developed by Amano *et al*. (2013) revealed only 42% of the ZYMV resistance variation. This level increased to 75% as a result of the markers developed in this study. The literature review discussed above indicated a single locus for ZYMV resistance. This study indicated, though, possible other loci based on both cluster analyses of 54 markers and regression analyses. The reason behind this contradiction is probably due to the size of the germplasm used in this study. The genome-wide association study included a larger gene pool of cucumbers. The large gene pool in this germplasm including more than 600 cucumber lines may likely present a larger number of loci related to ZYMV resistance.

It is also important to discuss the efficiency of classical screening studies. In addition to plant age, temperature, pathogen concentration, and other uncontrollable conditions, results may be significantly influenced. Classical screening was repeated twice and for some samples, screening was repeated more. Despite these efforts, escape is likely to occur. Therefore, strongly believe that increasing the efficiency of classical screening will also increase the level of variation explained by molecular markers developed in this study. Although a single locus for ZYMV resistance in cucumber was reported, along with several major loci, modifying gene(s) partially affecting disease progress in cucumber tissues is/are possible. As reported by Park *et al* (2004), ZYMV markers failed to distinguish samples in this study from samples used in their study for ZYMV resistance. The marker was reported by Amano *et al.* (2013), on the other hand, revealed 42% of the ZYMV resistance variation. The CAP marker was also tested on the known resistant (Silyon RZ, TMG-1, Sardis) and susceptible (Maraton and 8 other known) lines. TMG-1 and Silyon had resistant CAP markers, but Sardis had susceptible ones. The susceptible allele was present in all susceptible samples. The results of this study support the conclusion that ZYMV is polygenic.

The markers developed in this study may allow successful discrimination of resistant and susceptible cucumbers for markers assisted selection in breeding programs. The following regression model can be used to predict ZYMV resistance in cucumber breeding programs. Phenotype ZYMV = 0.37720 + CAP (0.41901) - OPD07920 (-0.35448) - OPBE121100 (-0.15010) - OPU012000 (-0.37963) + AG8YC500 (0.21707).

Acknowledgement

This study was financially supported by the Scientific Research Unit of Erciyes University (FBA-12-3883). ZYMV isolate was provided by the Virology Laboratory of the University of Akdeniz. A few of the cucumber lines for CAP marker analysis were provided by Manier Seed Company, Adana, Türkiye.

References

Abdollahi-Mandoulakani B, Rahmanpour S, Shaaf S, Gholamzadeh-Khoei S, Rastgou M and Rafezi R 2015. Towards the identification of retrotransposon-based and ISSR molecular markers associated with populations resistant to ZYMV in melon. S. Afr. J. Bot. **100**: 141-147.

Ahsan M, Ashfaq M, Mukhtar T and Abbasi NA 2020. Current status and genetic variability of cucumber mosaic cucumovirus (CMV) isolates infecting major cucurbits and solanaceous vegetables in Pothwar region of Pakistan. Pak. J. Agric. Sci. **57(5)**: 1353-1361.

Amano M, Mochizuki A, Kawagoe Y, Iwahori K, Niwa K, Svoboda J, Maeda T and Imura Y 2013. High-resolution mapping of *zym*, a recessive gene for zucchini yellow mosaic virus resistance in cucumber. Theor. Appl. Genet. **126**: 2983-2993.

Bradbury PJ, Zhang Z, Kroon DE, Casstevens TM, Ramdos Y and Bucklar ES 2007. TASSEL: software for association mapping of complex traits in diverse samples. Bioinform. **23**: 2633-2635.

Cardoso AII, Pavan MA and Sakate RK 2010. Inheritance of cucumber tolerance to zucchini yellow mosaic virus. J. Plant Pathol. **92**: 245-248.

Coskun OF 2022. Determination of genetic diversity in some pumpkin genotypes using SSR marker technique. Erzincan Uni. J. Sci. Technol. **15(3)**: 942-952.

Coskun OF 2023. Molecular characterization, population structure analysis and association mapping of turkish parsley genotypes using iPBS markers. Hortic. **9-33**: 61-12. DOI: 10.3390/horticulturae9030336

Coskun OF and Gulsen O 2024. Molecular, morphological and phytochemical characterization of some watermelon (*Citrullus lanatus* L.) genotypes. Hort. Sci. (Prague), **51(3)**: 189-201

Guner N 2018. Inheritance of resistance to zucchini yellow mosaic virus in watermelon. Hortsci. **53(8)**: 1115-1118.

Hussain A, Noman A, Arif M, Farooq S, Khan MI, Cheng P, Qari SH, Anwar M, Hashem M, Ashraf MF, Alamri S, Adnan M, Khalofah A, Al-zoubi OM, Ansari MJ, Khan KA and Sun Y 2021. A basic helix-loop-helix transcription factor CabHLH113 positively regulate pepper immunity against *Ralstonia solanacearum*. Microb. Pathog. **156**: 104909.

Islam W, Akutse KS, Qasim M, Khan KA, Ghramh HA, Idrees A and Latif S 2018. *Bemisia tabaci* mediated facilitation in diversity of begomoviruses: evidence from recent molecular studies. Microb. Pathog. **123**: 162-168.

Kamberoglu MA, Caliskan AF and Desbiez C 2016. Current status of some cucurbit viruses in Cukurova region (Adana and Mersin provinces) of Turkey and molecular characterization of zucchini yellow mosaic virus isolates. Rom. Biotechnol. Lett. **21**: 11709-11719.

Kone N, Asare-Bediako E, Koita O, Kone D and Winter S 2017. Seasonal and spatial variation in the prevalence of viral diseases and associated aphid-borne viruses in cucurbits in Cote d'Ivoire. Ann. Agric Sci. **62(2)**: 227-234.

Kumar R, Das MA, Behera TK, Jat GS, Choudhary H, Talukdar A, Dash P and Singh D 2022. Association mapping, trait variation, interaction and population structure analysis in cucumber (*Cucumis sativus* L.). Genet. Resour. Crop. Evol. **69**: 1901-1917.

Nagendran K, Mohankumar S, Aravintharaj R, Balaji CG, Manoranjitham SK, Singh AK, Rai AB, Singh B and Karthikeyan G 2017. The occurrence and distribution of major viruses infecting cucurbits in Tamil Nadu state, India. Crop Prot. **99**: 10-16.

Park Y, Katzir N, Brotman Y, King J, Bertrand F and Havey M 2004. Comparative mapping of ZYMV resistances in cucumber (*Cucumis sativus* L.) and melon (*Cucumis melo* L.). Theor. Appl. Genet. **109**: 707-712.

Pritchard JK, Stephens M, Rosenberg NA and Donnely P 2000. Association mapping in structured populations. Am. J. Hum. Genet. **67**: 170-181.

Rohlf FJ 2000. NTSYS-pc, Numerical Taxonomy and Multivariate Analysis System. Version 2.11. New York, Exeter, Setauket.

Romay G, Lecoq H and Desbiez C 2014a. Cucurbit crops and their viral diseases in Latin America and the Caribbean islands: A review. J. Plant Pathol. **96**: 227-242.

Romay G, Lecoq H, Geraud-Pouey F, Chirinos DT and Desbiez C 2014b. Current status of cucurbit viruses in Venezuela and characterization of Venezuelan isolates of zucchini yellow mosaic virus. Plant Pathol. **63(1)**: 78-87.

Shrestha S, Michael VN, Fu Y and Meru G 2021. Genetic loci associated with resistance to zucchini yellow mosaic virus in squash. Plants **10(9)**: 1935.

Sigva HO, Firat AF, Hazarhun G and Ipek A 2015. Development of AFLP markers associated with zucchini yellow mosaic virus resistance in cucumber (*Cucumis sativus* L.). Turk. J. Bot. **39**: 982-987.

Tecirli T, Dalda-Sekerci A, Coskun OF and Gulsen O. 2018. Morphological and molecular diversity among *Heliotropium greuteri* samples. Erciyes Univ. J. Inst. Sci. Technol. **34**: 1-7.

(Manuscript received on 30 September, 2024; revised on 05 November, 2024)