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Full Length Article

Categorization of Available Cucumber Genotypes against Zucchini Yellow Mosaic Virus and Root-Knot Nematode (Meloidogyne incognita)

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Abstract

In the present study, fifteen cucumber genotypes were screened against *Zucchini yellow mosaic virus* (ZYMV) and root-knot nematode (*Meloidogyne incognita*) individually and in combinations. All the cucumber genotypes behaved differently regarding root and shoot lengths and weights, number of galls, eggmasses and ZYMV infection when inoculated with *M. incognita* and ZYMV alone and in combinations. None of the fifteen cucumber genotypes was found immune, highly or moderately resistant to *M. incognita*. Two genotypes *viz.*, Alpha Prime and Patio were found resistant to the nematode. Contrarily, Max Pack and Beti-alpha were highly susceptible as evident by maximum galls on their roots and reductions in growth variables. Similarly, the genotypes Best PIC, Songrooh, Northern Pack, C-7 and C-5 appeared as moderately susceptible whereas Shaheen, Jakson, C-1, C-2, C-3 and C-9 genotypes showed susceptible reactions to the nematode alone. Similar trend was noticed when the genotypes were inoculated with both the nematode and the virus. Similarly, on the basis of disease rating scale, two genotypes *viz.*, Alpha Prime and Patio were grouped as moderately resistant to ZYMV. On the other hand, eleven genotypes *viz.*, Max Pack, Shaheen, Songrooh, Northern Pack, Jakson, Beti-alpha, C-1, C-2, C-3, C-5 and C-9 were catalogued as highly resistant while two genotypes Best PIC and C-7 were categorized as susceptible against ZYMV alone. Similarly, all the genotypes behaved in the same way when inoculated with *M. incognita*. Two genotypes Alpha Prime and Patio were found resistant against both the pathogens inoculated simultaneously and hence are recommended for cultivation. © 2021 Friends Science Publishers

Keywords: Galls; Resistance; Root-knot nematode; Varietal screening; ZYMV

Introduction

Cucumber (Cucumis sativus L.) is a significant seasonal vegetable which belongs to crop family of Cucurbitaceae. It is a clambering trailing plant that yields tube-shaped fruits which are mostly used in domestic cuisines. The crop is fourth in line for significance after tomatoes, cabbage and onions in Asia, while second to tomato in Europe. China was the first in yielding 60% produce in 2018, chased by Iran, Turkey and Russia. Cucumber is cultivated over 3367 hectares with yearly produce of 68664 tons in Pakistan (FAO 2019). It is cultivated on large scale; however, its production is seriously threatened by mosaic viruses and root-knot nematodes (Ashfaq et al. 2017; Ahsan et al. 2020). Root-knot nematodes (RKN) have extensive host range and infest various seasonal and perennials (Mukhtar and Kayani 2019; Nazir et al. 2019). Approximately 90% of the horticultural crops-producing areas are infested with RKN (Trudgill et al. 2000; Tariq-Khan et al. 2017a; Mukhtar and Kayani 2020). There are more than 100 described species of RKN in which Meloidogyne incognita

and *M. javanica* are commonly prevailing in tropics and subtropical zones of the globe including Pakistan (Trudgill et al. 2000; Mukhtar et al. 2017). A similar dominance of these two species has also been reported for Pakistan, with 52% M. incognita and 31% M. javanica, 8% M. arenaria, 7% M. hapla and 2% other RKN species (Tariq-Khan et al. 2017b; Kayani et al. 2018). For okra grown in the central Punjab province of Pakistan, RKN incidences were 74.7% for M. incognita, 24.0% for M. javanica, 1.6% for M. arenaria and 0.8% for M. hapla (Kayani and Mukhtar 2018; Hussain and Mukhtar 2019), respectively. Similarly, cucumber grown in the Pothowar region of the Punjab province, Pakistan, was found to be infested by 78% by M. incognita, 19% by M. javanica, 2% by M. arenaria and 1% by M. hapla (Kayani et al. 2013; Azeem et al. 2021). From Pakistan, five RKN species have been reported so far from tropical and cooler areas. The first three species have also been found infecting vegetables in the Western Himalavan region of Azad Jammu and Kashmir (Khan et al. 2020). Furthermore, these nematodes develop disease complexes with soil borne fungal and bacterial pathogens causing vascular wilts and

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damping off in many crops and result in huge yield losses. Similarly, root-knot nematodes break resistance in cultivars resistant to wilts (Aslam *et al.* 2019; Asghar *et al.* 2020).

As the greenhouse production pattern becomes more popular, the injury instigated by RKN also becomes even more serious (Jinling *et al.* 2003; Mukhtar 2018; Mukhtar *et al.* 2021). Annual yield losses due to plant-parasitic nematodes have been estimated to exceed 173 billion US \$ and RKN share most of the damages to their credit (Elling 2013). However, nematode damage in reality might even be higher, as many growers are unaware of plant-parasitic nematodes or damage is contributed to secondary pathogens neglecting plant-parasitic nematodes being the primary cause (Jones *et al.* 2013).

Cucurbit viral diseases are also posing problems globally. It has been testified that viral segregations via aphids is central warning issues which deliver up to 100% crop losses. Among viruses Zucchini yellow mosaic virus (ZYMV) was the first emerging cucurbits virus which threatens cucurbit survival since 1981. It has been reported from more than 50 countries throughout the world where squashes are cultivated as traditional crop. In Pakistan, ZYMV was reported in 1993 and severe epidemics were encountered in the Punjab and NWFP during 2003-2005, causing 75-100% losses. In combination with Watermelon mosaic virus (WMV) it causes 40-50% losses in yield (Malik et al. 2006). Numerous viral strains have been studied via sap transmission together from cucurbits crops and then their host ranges were specified. The learning exposed that Cucumber mosaic virus (CMV), ZYMV, WMV, Zucchini yellow fleck virus, Squash mosaic virus, and Melon necrotic spot virus are strains which poses great threat to cucurbits.

In addition to disease complexes with soil-borne fungi and bacteria, RKN have also been found associated with viruses in a number of ways. Synergistic or antagonistic effects of RKN with virus infected plants have been reported by some workers (Iheukwumere *et al.* 2008; Youssef *et al.* 2011). Therefore, the present study was planned to catalogue available cucumber genotypes against *Zucchini yellow mosaic virus* and root-knot nematode individually and in combination.

Materials and Methods

The present study was carried out at Plant Virology Laboratory, Department of Plant Pathology, Pir Mehr Ali Shah Arid Agriculture University Rawalpindi (33.5651°N, 73.0169°E), Pakistan. Zucchini yellow mosaic virus (ZYMV) was collected from Plant Virology Laboratory, Department of Plant Pathology while *M. incognita* was taken from Plant Nematology Laboratory, Pir Mehr Ali Shah Arid Agriculture University Rawalpindi, Pakistan. Cucumber germplasm (Shaheen, Best pick, Songrooh, Alpha Prime, Northern Pack, C-1, C-2, C-3, C-5, C-7, C-9, Beti alpha, Patio, Jakson and Max Pack) were collected from Federal Seed Certification Department, Islamabad, Pakistan.

Nematode inoculum

The inoculm of root-knot nematode used in the evaluation of cucumber germplasm was obtained from the already identified culture maintained in the Nematology lab. The nematode was further mass produced on tomato cv. Money Maker in pots in the greenhouse of the Department of Plant Pathology, Pir Mehr Ali Shah Arid Agriculture University, Rawalpindi, Pakistan at $25^{\circ}C \pm 2$. For collection of eggs, *M. incognita* infected roots were removed from pots, washed with tap water, cut into approximately 1–2 cm pieces and vigorously shaken in a bottle containing 0.5% NaOCI for 5 min. The eggs were collected on a 38 mm sieve and washed in a beaker. The egg suspension was poured onto an extraction tray and juveniles were collected. The freshly hatched second stage juveniles were standardized and concentrated.

Maintenance of viral inoculum

For the maintenance of ZYMV, mechanical transmission method was adopted (Dheepa and Paranjothi 2010). Leaves infected with ZYMV were separately used for the sap extraction. Infected leaves were macerated in 0.1 M phosphate buffer (pH 7.0) containing 1.0% sodium sulphite (1:2 W/V). Prepared inoculum was filtered through double layer muslin cloth. Sap was applied on healthy leaves of cucumber (Best Pick) which were already dusted with 600 mesh carborandum powder. Inoculated plants were kept under greenhouse conditions at 25–27°C for 30 days. Plants were regularly inspected for the disease development.

Screening of cucumber genotypes against ZYMV and *M. incognita*

The cucumber germplasm was screened against ZYMV and RKN alone and conjointly. Sterilized seeds of cucumber germplasm were sown in earthen pots (15 cm diameter) containing sterilized soil. Seven days after emergence, cucumber seedlings were inoculated with 10 mL nematode suspension (2,000 J2) by pouring the suspension in the plant root regions while virus inoculum was applied at 2-4 leaf stage by mechanical inoculation method in triplicate. Rests of the practices were kept same for all the treatments and plants were watered keeping in view the requirements. All the treated and untreated plant pots were kept under insect pest free glasshouse conditions at 25-27°C for symptoms development. Data recording was done seven weeks after inoculations. All the cucumber genotypes were categorized using disease rating scale proposed by Ashfaq et al. (2007). A modification of rating scale based on number of galls proposed by Mukhtar et al. (2013) was used to assess the degree of resistance or susceptibility of cultivars.

Data on viral infection percentage, growth parameters *viz.*, root length, shoot length, root and shoot weight was taken while nematode reproduction parameters like number of galls and eggmasses was also recorded. Reciprocal effect of ZYMV and nematode *M. incognita* infection was also investigated. All the data were subjected to analysis of variance using the GenStat package 2009, (12th edition) version 12.1.0.3278 (www.vsni.co.uk). The means were compared by Duncan's Multiple Range Test at 5%.

Results

Effect of *M. incognita* and ZYMV on growth variables of cucumber genotypes

All the cucumber genotypes behaved differently regarding root and shoot lengths and weights when inoculated with *M. incognita* alone. The maximum root lengths were recorded with Patio, Alpha Prime and C-1 while the root weights were found to be the maximum in case of C-9 and Betialpha. On the other hand, root lengths were found to be the minimum in case of C-9 and Beti-alpha and root weights were the minimum in case of C-1 and Patio as shown in Fig. 1. Similarly, C-1, Patio and Alpha Prime showed the maximum shoot lengths while in case of shoot weights no significant differences were observed among cucumber genotypes as shown in Fig. 2.

Likewise, cucumber genotypes varied significantly regarding root and shoot lengths and weights when inoculated with ZYMV alone. Root lengths and weights were the maximum in Alpha Prime and Patio genotypes while the minimum lengths and weights were recorded in case of Max Pack and Beti-alpha genotypes (Fig. 3). On the other hand, all the cucumber genotypes behaved similarly in case of shoot lengths and weights with few exceptions as shown in Fig. 4. When cucumber genotypes were inoculated with both the pathogens, similar results were obtained regarding root lengths and weights shoot lengths and weights (Fig. 5 and 6).

Effect of *M. incognita* and ZYMV on galls and eggmasses

The maximum galls and eggmasses were produced by *M. incognita* on Beti-alpha followed by Max Pack while galls and eggmasses were the minimum in case of Alpha Prime and Patio. The rest of the genotypes were found intermediate regarding these parameters (Fig. 7). When cucumber genotypes were inoculated with both the pathogens, similar results were obtained regarding number of galls and eggmasses (Fig. 8) as in case of individual inoculation of the pathogens with few exceptions.

Effect of *M. incognita* and ZYMV on virus infection

Maximum ZYMV infection was observed in Beti-alph followed by Jackson, Max Pack and C-2 while the infection



Fig. 1: Effect of root-knot nematode alone on root length and root weight of cucumber genotypes



Fig. 2: Effect of root-knot nematode alone on shoot length and shoot weight of cucumber genotypes



Fig. 3: Effect of ZYMV alone on root length and root weight of cucumber genotypes



Fig. 4: Effect of ZYMV alone on shoot length and shoot weight of cucumber genotypes

was found to be the minimum in case of Alpha Prime and Patio (Fig. 9). When cucumber genotypes were inoculated with both the pathogens, similar results were obtained



Fig. 5: Combined effect of root-knot nematode and ZYMV on root length and root weight of cucumber genotypes



Fig. 6: Combined effect of root-knot nematode and ZYMV on shoot length and shoot weight of cucumber genotypes



Fig. 7: Effect of cucumber genotypes on number of galls and eggmasses by root-knot nematode



Fig. 8: Effect of cucumber genotypes on number of galls and eggmasses by root-knot nematode when inoculated with ZYMV

regarding ZYMV infection as in case of individual inoculation of the pathogens with few exceptions (Fig. 10).



Fig. 9: Effect of cucumber genotypes on ZYMV infection



Fig. 10: Effect of cucumber genotypes on ZYMV infection when inoculated with *M. incognita*

Reaction of cucumber genotypes to *M. incognita* and ZYMV

None of the fifteen cucumber genotypes was found immune, highly or moderately resistant to *M. incognita*. Two genotypes *viz.*, Alpha Prime and Patio were found resistant to the nematode. Contrarily, Max Pack, Beti-alpha were highly susceptible as evident by maximum galls (> 100) on their roots and reductions in growth variables. Similarly, the genotypes Best PIC, Songrooh, Northern Pack, C-7 and C-5 appeared as moderately susceptible whereas Shaheen, Jakson, C-1, C-2, C-3 and C-9 genotypes showed susceptible reactions to the nematode alone. Similar trend was noticed when the genotypes were inoculated with both the nematode and the virus (Table 1).

Similarly, on the basis of disease rating scale, two genotypes *viz.*, Alpha Prime and Patio were grouped as moderately resistant to ZYMV. On the other hand, eleven genotypes *viz.*, Max Pack, Shaheen, Songrooh, Northern Pack, Jakson, Beti-alpha, C-1, C-2, C-3, C-5 and C-9 were catalogued as highly resistant while two genotypes Best PIC and C-7 were categorized as susceptible against ZYMV alone. Similarly, all the genotypes behaved in the same way when inoculated with M. incognita (Table 2).

Discussion

Cucumber is an important vegetable and one of the most popular members of Cucurbitaceae family. Low yield of cucumber is mainly attributed to viral pathogens and nematodes. The most frequent and economically important pathogens; *Zucchini yellow mosaic virus* and *M. incognita* are highly aggressive to cucumber crop. In the present study

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Number of galls	Reaction	Inoculation of RKN alone	Combined inoculation of RKN and ZYMV
0	Immune	-	-
1-2	Highly Resistant	-	-
3-10	Resistant	Alpha Prime, Patio	Alpha Prime, Patio
11-30	Moderately Resistant	-	-
31-70	Moderately Susceptible	Best PIC, Songrooh, Northern Pack, C-7, C-5	Best PIC, Songrooh, Northern Pack, C-7, C-5
71-100	Susceptible	Shaheen, Jakson, C-1, C-2, C-3, C-9	Shaheen, Jakson, C-1, C-2, C-3, C-9
>100	Highly Susceptible	Max Pack, Beti-alpha	Max Pack, Beti-alpha

Table 2: Response of cucumber genotypes against ZYMV (alone) and combined inoculation of ZYMV and RKN

Description	Reaction	Inoculation of ZYMV alone	Combined inoculation of ZYMV and RKN
0% infection, all plants free o	f Highly Resistant	-	-
symptoms			
1-10% plants infected	Resistant	-	-
> 10-20% plants infected	Moderately Resistant	Alpha Prime, Patio	Alpha Prime, Patio
> 20-30% plants infected	Moderately Susceptible	-	-
>30-40% plants infected	Susceptible	Best PIC, C-7	Best PIC, C-7
More than 40% plants infected	Highly Susceptible	MaxPack, Shaheen, Songrooh, Northern Pack	MaxPack, Shaheen, Songrooh, Northern Pack,
		Jakson, Beti-alpha, C-1, C-2, C-3, C-5, C-9	Jakson, Beti-alpha, C-1, C-2, C-3, C-5, C-9

fifteen cucumber genotypes were screened against ZYMV and root-knot nematode individually and in combinations. All the cucumber genotypes behaved differently regarding root and shoot lengths and weights, number of galls, eggmasses and ZYMV infection when inoculated with *M. incognita* and ZYMV alone and in combinations. The genotypes also showed varying responses to nematode and the virus when inoculated singly and conjointly.

In the present study, all the treated genotypes showed decreases in plant growth parameters as compared to untreated control. This might be due to penetration of second stage juveniles in the roots and their migration to vascular bundles. The nematode induced severe root galling which affected the utilization efficiency of water and nutrients and the partitioning of photosynthetic products (Williamson and Hussey 1996). Reduced supply of nutrients to the roots as a result of viral and nematode infections has also been reported (Varshney et al. 2005). Another study showed that combined infection of nematode and virus significantly reduced numbers of galls and eggmasses in Trifolium repens by combined infection (McLaughlin et al. 1993) probably due to changes induced by the pathogens in the plant physiology that resulted in to suppressed nematode development (Goswami et al. 1994). The suppressive effects of ZYMV, CMV and other viruses on the nematode in cucumber and other crops have also been confirmed by many workers (Iheukwumere et al. 2008; Youssef et al. 2011). Patel and Patel (1995) reported that combined infection of TMV and root-knot nematodes caused accelerated reduction of protein nitrogen, total nitrogen and nicotine contents and a greater improvement in total sugar content over singly infected plants. Simultaneous infection with virus and nematode had a more pronounced effect on these chemical constituents suggesting that quality of bidi tobacco would be greatly impaired. McLaughlin and Windhan (1996) studied the effects of peanut stunt virus, M. incognita, and drought on growth and persistence of white clover and reported that drought stress, M. incognita, and peanut stunt virus acted independently in reducing forage productivity and persistence. In a previous study, Walker and Wallace (1975) evaluated the influence of tobacco ringspot virus and *M. javanica*, alone and in combination on the growth and mineral content of French beans. Plants infected with tobacco ringspot virus were clearly intolerant to infection as indicated by their stunted growth and their marked difference in content of phosphorus, chlorine, copper, manganese and zinc from uninfected plants. In contrast, plants infected with M. javanica were tolerant to infection by this parasite; no difference was detected between nematode-infected plants and the uninfected controls. Koulagi et al. (2020) worked on host-delivered RNA interference in tomato for mediating resistance against M. incognita and Tomato leaf curl virus and provided an evidence for generating resistance through RNAi against multiple biotic stresses. Previously, Tobacco rattle virus has also been reported to mediate gene silencing in a plant parasitic root-knot nematode (Dubreuil et al. 2009).

Application of RKN to cucumber genotypes formed galls and eggmasses on the roots. The galls and eggmasses were greater in plants infected with the nematode only as compared to those infected with both the nematode and virus. This might be due to reduced supply of nutrients to the roots as a result of virus attack (Varshney et al. 2005). Present findings are in line with those of Ahmed et al. (2007) who reported similar results. RKN interaction with fungi, bacteria and viruses have been listed and documented and can synergistically provoke certain diseases in many crop plants. These pathogens especially viruses occur in complexes and cause colossal losses through reduction in growth and yield of the crop. M. incognita is not a vector of plant viruses but under field conditions, it was observed that M. incognita occurred concomitantly with viruses in the same plant, as is the case with cucumber. The negative interaction exists between the nematode and virus. The virus may have stressed the plant thus reduced multiplication and infectivity of nematode (Goswami et al. 1994).

It has been often observed that a particular cultivar resistant to some bacterial pathogens becomes susceptible in the presence of root-knot nematodes. For example, wilt causing bacteria produce some toxins in host tissue which induce the wilt symptoms. In resistant cultivars, the mechanism of production of the toxins is inhibited or it is detoxified by the natural defense of the plant, hence, wilting does not take place. However, the nematode infections induced certain changes in host physiology, leading to failure of detoxification mechanism of the resistant cultivars, and the plants become susceptible. An eggplant cultivar, Pusa purple cluster, highly resistant to Pseudomonas solanaecarum becomes susceptible in the presence of M. incognita. Similarly, field resistance in potato to P. solanaecarum was broken when the plants were infected with *M. incognita*. Wilt fungus resistant cultivars also become susceptible in the presence of root-knot nematodes. Wilt resistant cotton cultivars succumbed to Fusarium in the presence of M. incognita. Tomato cv. Chesapeake resistant to Fusarium wilted in the presence of the same nematodes' species. The wilt severity, however, varied with the nematode species, presence of M. hapla caused 60% wilting, whereas 100% wilting occurred with M. incognita (Khan 2008).

Conclusion

It is concluded from the present study that combined infection of root-knot nematode and ZYMV caused reductions in growth variables of fifteen cucumber genotypes. Two genotypes Alpha Prime and Patio were found resistant against both the pathogens inoculated simultaneously and hence are recommended for cultivation. Furthermore, these cultivars could be used in breeding programs to develop new cultivars resistant to both the nematode and the virus.

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Author Contributions

HMA and MA designed the study, executed experimental work, analyzed the data and prepared the manuscript. TM designed the study, supervised the experimental work and edited the manuscript. All the authors edited the manuscript.

Conflict of Interest

The authors declare that there is no conflict of interest of any type *i.e.*, among authors, with the institution of authors, with previous publication *etc*.

Data Availability

The authors keep all the data which will be available on request.

Ethics Approval

The study does not involve animals or humans and therefore, does not involve any ethical approval.

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