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Pathological, Chemical and Molecular Analysis of Eggplant Varieties Infected With Root-knot Nematodes (*Meloidogyne* spp.)

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ABSTRACT

Eggplant crop (*Solanum melongena* L.) is one of the top ten vegetable crops grown in the world. It can be cultivated in different seasons during the year. Eggplants are known to be extremely susceptible to root-knot nematodes infection, causing severe damage leads to great losses. In the present study six eggplant varieties i.e., SNO-8-1, 108-3-1, BPCL-1, SCR-2, CLA-11-2 and SWD-13-1 were evaluated for their susceptibility to *Meloidogyne* spp. under greenhouse conditions. Results revealed that all tested varieties were susceptible to *Meloidogyne* spp. Variety (108-3-1) is considered the most susceptible one. Nematode parameters i.e., number of galls, egg masses, females/root system and J_2/250g soil were recorded (594, 381, 421 and 802) respectively. On the other hand, results showed that variety (BPCL-1) was the most tolerant one. In respect to the accumulation of total phenol compounds, HPLC was used to study the difference of chemical analysis between tolerant and susceptible eggplant varieties. Chromatogram analysis revealed that the highest total phenol concentration (36720.10 mAu) was detected in soil of root exudates of most tolerant variety (BPCL-1), in contrary the lowest accumulation of total phenol (32040.70 mAu) was recorded in soil of root exudates of susceptible variety (108-3-1). The genetic variation among the six eggplant varieties was studied too using the SSR technique, conducted with 10 different primers. The results showed genetic polymorphism among the six tested varieties, especially the EEMS15 and EEMS16 primers within the used primers.

Key words: Root-knot nematodes, Eggplants, HPLC, SSR, Resistance

INTRODUCTION

In Egypt, Eggplant varieties cultivated in an area of 117885.7 feddan of eggplants having an annual production of 1341312 tons (FAO, 2020). Root-knot nematodes (*Meloidogyne* spp.) are a major threat to a wide range of crops influencing their health, quality and yield (Sayed *et al.,* 2019;Bakr *et a*l., 2020). Root-knot nematodes cause approximately 5% crop loss globally including in advanced countries and vegetables (Begum *et al.,* 2014). It is necessary to find out the different methods to select resistant eggplant varieties to *Meloidogyne* spp. Ullah *et al.,* (2011), Devi *et al.,* (2015) and Tanimola *et al.,* (2015) found variability in eggplant

varieties for susceptibility toward root-Knot nematode.

The chemical analysis using HPLC was conducted with the most susceptible eggplant variety and the most resistant one. Pegard *et al*., (2005) reported that phenolic compounds, especially chlorogenic acid, may be involved in resistance cultivars compared to susceptible cultivars. Similar results were also observed by Selim *et al*., (2021) and Patel *et al*., (2018). Genetic resources can be evaluated morphologically in the seedling stage at the greenhouse and molecularly characterized by using DNA marker techniques Ainurrachmah Aida *et al*., (2021). (SSR) markers are a popular source of genetic markers owing to their high reproducibility, multi-allelic nature, codominant inheritance, abundance, and wide genome coverage. A high level of polymorphism makes SSR an ideal marker for mapping and diversity studies, fingerprinting and population genetics (Nunome *et al*., 2003).

This study aims to find the host status of six eggplant varieties against *Meloidogyne* spp. infection, Genetic diversity of eggplant germplasm and the morphological responses of eggplant to nematode infection were evaluated.

MATERIALS AND METHODS

1-Preparation of *Meloidogyne* spp. inoculum:

Three months old eggplant roots infested with *Meloidogyne* spp. were used to prepare nematode inoculum as described by Hussey and Barker (1973). Second-stage juveniles were obtained by transferring the extracted eggs into Baermann trays with soft tissue paper to allow egg hatching according to Oostenbrink (1960). After 72 hours from the trays set up the number of second-stage juveniles/ml was estimated by counting 3 samples of 1 ml using a counting dish under a stereomicroscope.

2-Evaluation of six eggplant varieties to *Meloidogyne* spp. under greenhouse conditions.

Six varieties of eggplant namely SNO-8-1, 108-3-1, BPCL-1, SCR-2, CLA-11-2 and SWD-13-1 were tested for susceptibility to *Meloidogyne* spp. One seedling (2 months old) from each variety was chosen and cultivated individually in 15 cm clay pots filled with a non-sterilized mixture of sand and clay loamy soil (2:1, v/v). Each seedling was inoculated with a suspension of 3000 eggs and larvae of *Meloidogyne* spp. by pipetting into three holes made around the root system. Each treatment was replicated three times, while others left free of nematode served as control treatment. Pots were daily watered with tap water and weekly fertilized with a nutrient solution of 5 ml of 2 g/l (N:P: K, 20:20:20. All pots were arranged in a complete randomized block design in a greenhouse as described by Duncan (1955). Sixty days after nematode inoculation, plants were uprooted; roots were rinsed with tap water to remove adhering soil particles. Nematode and

plant growth parameters were recorded. Nematode parameters recorded are the number of galls, egg masses, females/root system, J_2/250 g soil, final population (PF) and reproduction factor (RF) were recorded. Reproduction factor was calculated according to the equation RF= PF/ PI as the PI (initial population) (Norton, 1978). Egg masses were assessed according to Daykin and Hussey (1985). Females were collected according to Mahdy (2002). Soil nematode population was enumerated as described by Barker (1985). The root-knot galling index was assessed on a scale of 0-10 according to Bridge and page (1980).

Plant parameters.

1-Plant growth parameters i.e., shoot and root lengths, root and shoot fresh weights were estimated at 60 days from nematode inoculation.

2-Physiological and biochemical analysis.

2-1- Membrane leakage (ML) was determined as described by Sun *et al*. (2006).

2-2-Antioxidant enzymes activity:

-Peroxidase was measured according to the method described by Fehrman and Dimond (1967).

-Polyphenol oxidase (PPO) was measured according to the method described by Broesh (1954).

3- HPLC ANALYSIS

Chromatogram analysis of root exudates on six eggplant varieties was performed using HPLC according to the modified methods of Selim *et al*. (2014). For conducting HPLC analysis, 1.5 g of fresh shoots and roots were collected from three replicates of *Solanum melongena* CVs. SNO-8-1, 108-3-1, BPCL-1, SCR-2, CLA-11-2 and SWD-13-1. Fresh shoots and roots were immediately freeze-dried using liquid nitrogen. The freeze-dried materials were mixed thoroughly individually with 15 ml of ethyl acetate in plastic test tubes for 5 min. After separation, the suspension was filtrated into new tubes through two cotton layers and evaporated under a vacuum to completion. The extracted compounds were then dissolved in 150 µl absolute methanol and 50 µl of each chemical extraction was injected into HPLC. Spectral analysis was conducted to compare the detected peaks with similar retention times in all extractions.

4- DNA isolation

Extraction and purification of genomic DNA

DNA was extracted from the eggplant samples according to the DNeasy Mini Kit (Qiagen Santa Clarita, CA).

Simple Sequence Repeat "SSR "

Ten SSR primers were used in the detection of polymorphism Table (1) according to Stàgel *et al*., (2008). SSR-PCR Reactions, Thermocycling Profile PCR and Detection of the PCR Products were carried out according to Ibrahim *et al*., (2019).

Table (1): primer sequences revealing Allelic variation in 10 **SSR** loci.

Primer	Sequence Forward	Sequence Reverse
EEMS06	TCATGCGAAGATTAATTAAATGTGA	GAGTGGATGATCAAGAATGGC
EEMS07	CCATGCCAGAATGGAAACTT	AACGAAAACACGATCAACCC
EEMS10	TCAAGCAGAACGAAGATGGA	GTAGGGGACGTGGATTCAGA
EEMS12	CGGGCAACTCTTCACATTTT	ATTGGTTTGCTATCGAATTTCT
EEMS13	TGAGATACGCGTACAATGACTTC	GGGGTTTTGCTGCTGTTATC
EEMS14	GGAATGGACCAAACCCCTAA	AGAGCTTCGTTGCTTGGTGT
EEMS15	GGGACAAATCTGACCTTTGG	CTGGTGGCAAATTCTTCGAT
EEMS16	CAATTTTTCGGTTCACTAATCAAG	CTTCAAGGAAAAAGGAGGCC
EEMS17	TGACATGTAGCTGGGCAGAG	TGGAGTGTGCATCCCAAATA
EEMS18	GGAGAAACTGAAAAATTTGTAGAGAG	GAGGAGTTTCCGACATGAGC

Data analysis

SSR analysis were carried out according to Hammer *et al*., (2001).

Statistical analysis

All measurement data for all experiments in this study were analyzed according to the methods described by Snedecor and Cochran (1989).

Results

Evaluation of some eggplant varieties to *Meloidogyne* spp. under greenhouse conditions.

Six eggplant varieties were tested for their susceptibility to the infection with the root-knot nematodes *Meloidogyne* spp. under greenhouse conditions. Results presented in Table (2) indicated that the tested varieties differed greatly to *Meloidogyne* spp. infection. Nematode developing and multiplying stages on all tested varieties were occurred. Two months later, the degrees of root-knot damage were measured using gall index. The eggplant 108-3-1 variety showed the highest gall index (8.67). The lowest (GI) occurred on variety SNO-8-1 (6.33). The highest No. of galls, egg masses, females/root system and J_2/250 g soil were recorded with 108-3-1 variety by (594, 381, 421 and 802) respectively, whereas, the lowest one was recorded too with BPCL-1 variety by (322, 211, 261 and 471) respectively, the highest No. of final nematode population occurred with 108-3-1 variety by (2198), whereas the lowest one was recorded with BPCL-1 variety by (1265). The highest rate of reproduction factor was recorded with 108-3-1 variety by (0.73) among all the eggplant accessions screened and this was significantly higher than rates of reproduction factor from other accessions. The lowest one

was recorded with BPCL-1 variety by (0.42) as shown in Table (2).

Plant growth parameters and chemical constituents of six eggplant varieties as affected by *Meloidogyne* spp. infection

Results in table (3) showed that all plant growth parameters i.e., stem and root lengths, fresh shoot and root weights, as well as chemical constituents of all six eggplant varieties i.e., SNO-8-1, 108-3-1, BPCL-1, SCR-2, CLA-11-2 and SWD-13-1 were affected by root-knot nematode infection. Variety BPCL-1 showed the highest growth parameters and chemical constituents compared with the other varieties. Whereas, the lowest growth parameters and chemical constituents' values were recorded with the 108-3-1 variety. However, no significant difference between BPCL-1 and SWD-13-1 in stem length and fresh root parameters was noticed.

The susceptible variety (108-3-1) showed high membrane leakage and low values in antioxidant enzyme activities i.e., peroxidase and phenol oxidase as shown in table (3).

HPLC analysis

Chemical analysis results showed marked differences between tolerant and susceptible eggplant varieties in respect to the accumulation of total phenolic compounds. The highest accumulation of total phenolic (36720.10mAu) was detected within variety (BPCL-1), followed by SWD-13-1 variety (36556.67mAu). On the other hand, results evaluated that the lowest concentrations of total phenolic (32040.70 mAu) were recorded on variety (108-3-1) as shown in figure (1).

Table (2): Evaluation of six eggplant varieties to *Meloidogyne* spp. under greenhouse conditions.

Eggplant varieties	Nematode parameters/root system				J_2/250 g soil	Pf	Rf *
	Galling index	No. of galls	No. of egg masses	No. of females			
SNO-8-1	6.33^{c}	509^{b}	321^{c}	381^{c}	632^{b}	1843^{b}	0.61^{b}
108-3-1	8.67^{a}	594^{a}	381^{a}	421^{a}	802^{a}	2198^{a}	0.73^{a}
BPCL-1	7.33^{bc}	322^{f}	211^{f}	261^{f}	471^{f}	1265^{f}	0.42^{f}
SCR-2	6.67^{bc}	423^{d}	302^{d}	370^{d}	550^{d}	1645^{d}	0.55^{d}
CLA-11-2	7.67^{ab}	472^{c}	335^{b}	399^{b}	581^{c}	1788^{c}	0.60^{c}
SWD-13-1	6.67^{bc}	361^{e}	241^{e}	285^{e}	503^{e}	1390^{e}	0.46^{e}

-Reproduction factor (Rf)= (Pf/Pi). Pf = Final population Pi =Initial population

-Columns followed by different litters are significantly different according to Duncan's Multiple Test (P≤0.05).

Table (3): Plant growth parameters and chemical constituents of six eggplant varieties as affected by *Meloidogyne* spp. infection

Eggplant varieties	Stem length (cm)	Root length (cm)	Fresh shoot weight (g)	Fresh root weight (g)	Peroxidase (O.D.g^{-1} fr.wt.after 2min)	Phenoloxidase (O.D.g^{-1} fr.wt.after 2min)	Membrane leakage (%)
SNO-8-1	13.00^{cd}	14.33^{d}	8.07^{d}	6.96^{c}	0.59^{e}	0.70^{e}	19.53^{b}
108-3-1	11.33^{d}	10.67^{e}	5.32^{e}	5.78^{c}	0.52^{f}	0.62^{f}	25.25^{a}
BPCL-1	20.33^{a}	26.33^{a}	19.64^{a}	19.95^{a}	1.07^{a}	1.28^{a}	7.70^{d}
SCR-2	16.00^{b}	19.33^{c}	12.40^{c}	12.38^{b}	0.74^{c}	0.87^{c}	16.47^{b}
CLA-11-2	14.67^{bc}	15.33^{d}	9.55^{d}	9.11^{bc}	0.68^{d}	0.79^{d}	18.27^{b}
SWD-13-1	19.00^{a}	22.33^{b}	16.61^{b}	17.13^{a}	0.87^{b}	1.17^{b}	11.73^{c}

Columns followed by different litters are significantly different according to Duncan's Multiple Test (P≤0.05).

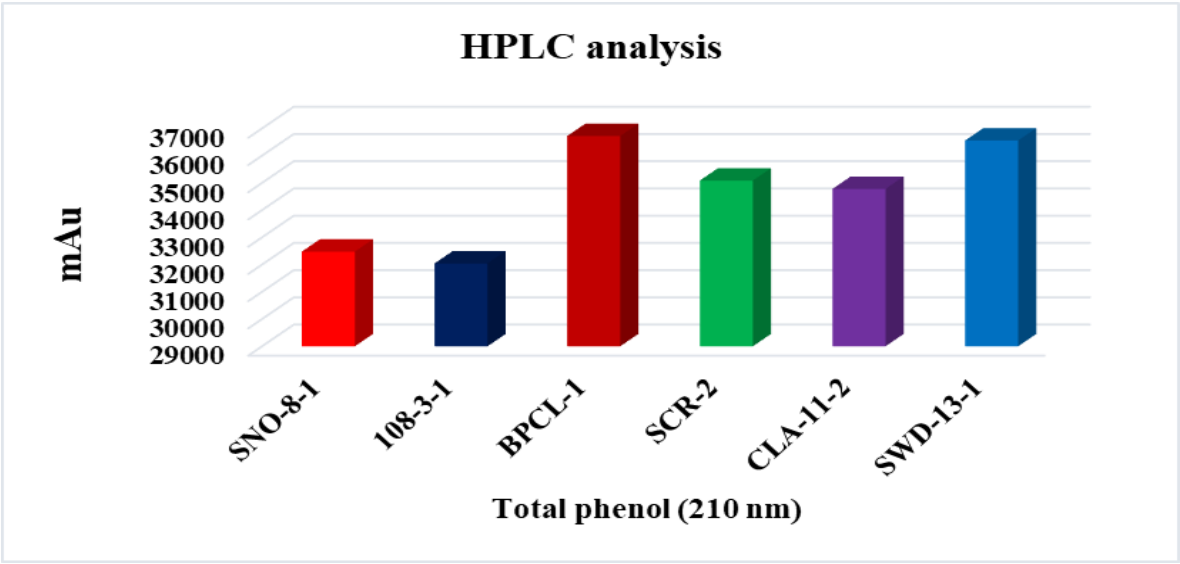

Figure (1): Total phenolic compounds accumulated in root exudates of six eggplant varieties detected at 210 nm on HPLC.

DNA polymorphism

Genetic screening of tested six eggplant varieties using ten different simple sequence repeats (SSR) primers revealed that, the tested varieties are genetically varied. Amboise polymorphism was detected within EEMS15 and EEMS16 as shown in tables (4 and 5) and figures (2 and 3) less polymorphism among tested eggplant varieties was recorded with the other used 8 SSR primers (EEMS06, EEMS07, EEMS10, EEMS12, EEMS13, EEMS14, EEMS17 and EEMS18).

Table (4): Restriction analysis of **SSR** regions using EEMS15 primer

EEMS15							
Band No.	**M. W. bp**	**Varieties**					
		1	**2**	**3**	**4**	**5**	**6**
1	**800**	-	+	-	-	-	-
2	**270**	+	+	+	+	+	+
3	**230**	-	-	+	-	+	-
4	**180**	+	-	-	-	-	-
5	**120**	-	-	-	-	+	-
6	**100**	+	-	+	-	-	-

Table (5): Restriction analysis of **SSR** regions using EEMS16 primer

EEMS16							
Band No.	**M. W. bp**	**Varieties**					
		1	**2**	**3**	**4**	**5**	**6**
1	**1100**	-	-	-	-	+	+
2	**830**	-	-	+	-	+	+
3	**620**	+	-	-	-	+	-
4	**440**	-	-	-	-	+	-
5	**270**	+	-	-	-	+	-
6	**190**	-	+	-	-	+	-
7	**120**	+	+	+	+	+	+
8	**90**	+	+	-	-	-	+

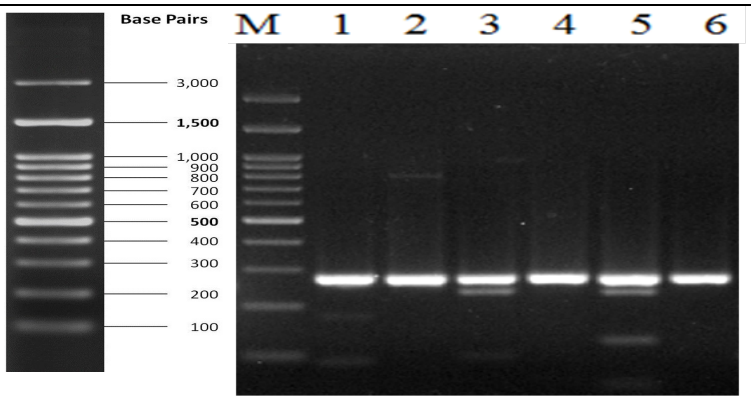

Fig. (2): SSR profiles of the six eggplant varieties using the SSR primer (EEMS15). M: 100bp DNA ladder (Fermentas, Germany). Lanes 1 to 6 represent (SWD-13-1; SNO-8-1; CLA-11-2; 108-3-1; BPCL-1 and SCR-2), respectively.

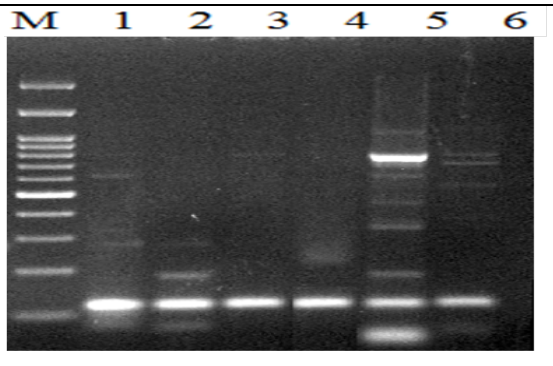

Fig. (3): SSR profiles of the six eggplant varieties using the SSR primer (EEMS16). M: 100bp DNA ladder (Fermentas, Germany). Lanes 1 to 6 represent (SWD-13-1; SNO-8-1; CLA-11-2; 108-3-1; BPCL-1 and SCR-2), respectively.

DISCUSSION

All eggplant varieties showed variability in growth and nematode population in their response to *Meloidogyne* spp. Results revealed that variety BPCL-1 was the most tolerant variety, whereas variety 108-3-1 was the most susceptible one.

The variability in pathogenicity across varieties might be due to the variation in the genetic structure of the tested varieties which encoding for specific substances that play a crucial role in controlling root-knot nematode as reported by Ullah *et al.,* (2011) and Devi *et al.,* (2015). This could also mean that they might have different genetic constituents that conferred different phenotypic traits. The tolerant eggplant cultivar might have failed to produce functional feeding sites in the host after invasion due to hypersensitive responses facilitated by resistant genes that might have led to failure in nematode development. Once feeding sites are not produced in the host plant, to *Meloidogyne* spp. will not be able to access nutrients and as such will have their development and reproduction impaired as mentioned by Colak-Ates *et al.,* (2018).

The tolerance might be a result of post-infection resistance in which the nematodes penetrated the roots, but failed to develop adequately and this is linked to the early hypersensitive reaction that might have led to the death of cells in root tissues around the nematodes. This is due to the presence of toxic or antagonistic chemicals in the roots of eggplant as mentioned by (Tanimola *et al*., (2015). In the resistant roots, catalase activity is decreased as a result of root-knot nematodes attack. There is a possible role of alkaloids or phenolics that may inhibit the synthesis of these enzymes and act as an elicitor of resistance in plants attacked by *Meloidogyne* species. The final population was directly proportional to the rate of reproduction. The nematode multiplication was the maximum at the initial inoculum level and then started decreasing at the highest inoculum levels. It might be due to the intraspecific competition among nematodes for food. *Meloidogyne* spp. suppressed the brinjal (*Solanum melongena* L.) growth with the increase in inoculum level and a corresponding reduction in the growth(Al-Hendy *et al*., 2021; Bakr *et al*., 2022). In such situations, the degree of damage depends on the susceptibility and tolerance of the host plant as reported by Begum *et al.,* (2014).

Selim *et al.,* (2014) reported that the increase in resistance of the tomato plants to nematode stress seemed to be related to the increased activity of peroxidase and phenol oxidase in plant tissues infected with nematodes. The chemical analysis using HPLC revealed that both the biotic and abiotic elicitors increased the accumulation of different specific compounds in the plants which

showed the greatest reduction in the number of galls and egg masses/root systems. Chemical analyses showed elevated expression of defense enzymes (peroxidase and polyphenol oxidase) and higher accumulation of phenolic compounds due to the induction of systemic resistance as mentioned by Lingaraju and Mallesh (2010). Pegard *et al*., (2005) reported that HPLC analysis of non-infected and infected pepper roots showed an association between phenolics and resistance to root-knot nematodes. Phenolic compounds were concentrated in a small number of cells and the intercellular spaces of root tissue around J2 when observed under UV light. They identified chlorogenic acid as the major phenolic compound in CM334 pepper roots penetrated by nematodes. Chlorogenic acid is known to affect nematode coordination. They revealed that also the oxidized forms of phenolic compounds, which also occurred in high concentrations in the roots of resistant tomato plants, might contribute to the *M. incognita* resistance by creating a toxic environment for nematode penetration and multiplication. Phenolic compounds may be useful for identifying resistant cultivars. The chemical analysis using HPLC was conducted with the most susceptible and resistant tomato cultivar. Results revealed that the roots and shoots of the most resistant cultivars tested recorded more total phenol concentrations rather than the susceptible one. Selim *et al*., (2021) reported that the chemical analysis using HPLC revealed that the accumulation of chemical compounds in roots and shoots of the most tolerant tomato cultivars are a mixture of different chemically active compounds, i.e., acetone-DNPH, acrolein- DNPH, 2,5–dimethyl benzaldehyde - DNPH, formaldehyde - DNPH, isovaleraldehyde DNPH and propionaldehyde-DNPH.

The 24 eggplant varieties were genetically diverse and there were 68% of the total genetic diversity within the population, and 32% among the population as well as the study of genetic diversity makes it possible to select genetically different parents to obtain the desired recombinant in eggplant segregation generation as mentioned by Ainurrachmah Aida *et al*., (2021). Simple sequence repeat markers were used to study the genetic diversity and population structure among 60 varieties of eggplant. Out of 20 SSR markers, 15 were found to be polymorphic. The polymorphic SSR markers generated 46 alleles with an average of 3.06 alleles per locus as reported by Nandi *et al*., (2020). The simple sequence repeat (SSR) markers of the eggplant using a recent high-quality sequence of its whole genome. The SSRs were classified according to their number of repeats and overall length, and were assigned to their linkage group. They found 2,449 of the perfect SSRs in 2,086 genes, across the gene space; 3,524 imperfect SSRs were present in 2,924 genes. Putative functions were assigned via ontology to genes containing at least one SSR

as mentioned by Portis *et al*., (2018). Stàgel *et al*., (2008) reported that have stressed the limitations surrounding the application of SSR markers for diversity studies, emphasizing the possibility of homoplasy and allele size differences can also be generated by indel events, as well as by variation in the SSR repeat number. However, the genetic relationships between the accessions of the full genotype panel as displayed by genetic similarity at the SSR level were in good agreement with prior taxonomic classification based on both genomic and plastidial markers. Using SSR led to considerable diversity within each of the cultivar groups and cultivar groups were separated from each other in different branches. where, the principal coordinates analysis (PCoA) confirmed that each of the cultivar groups is genetically diverse as mentioned by Vilanova *et al*., (2014).

Our study of bioassay revealed that the six eggplant varieties were genetically different. These results are consistent with HPLC results.

Author Contributions:

Conceptualization, MHS, MES, MEM and EMM; data curation, MHS, MES, MEM and EMM; formal analysis, MHS and MES; investigation MHS, MES and MEM; methodology, MHS, MES, MEM and EMM; MHS, writing—original draft, and writing and editing, MHS, MES, MEM and EMM. All authors have read and agreed to the published version of the manuscript.

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