Effects of Cucumber Mosaic Virus infection and drought tolerance of tomato plants under greenhouse conditions: Preliminary results

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Abstract. In nature, plants are simultaneously exposed to a combination of biotic and abiotic stresses limiting their yield, and thus, it is useful evaluating effects of biotic and abiotic stresses on plant growth and development. Here, a combination effect of drought stress and Cucumber mosaic virus (CMV) infection were investigated on some physiological traits of tomato plants under greenhouse conditions. Two levels of CMV infection (infected and non-infected) and four drought stress (100% Field capacity (FC), 80% FC, 60% FC and 40% FC) were used as treatments to set a factorial experimental design. After two weeks, systemic infection of CMV and some physiological traits including the relative water content (RWC), electrolyte leakage (EL), chlorophyll and carotenoid contents were measured. Results showed that combination of CMV and drought stress delayed appearance of drought symptoms. Both infected and non-infected plants showed the lowest RWC, total chlorophyll, carotenoid and the highest EL observed in 40% FC, which may be related to effectiveness of drought on CMV. Since drought stress ameliorated the sign of CMV infection, it is concluded that there is a correlation between abiotic and biotic stresses improving tolerance level of this tomato variety.

Keywords: CMV, relative water content, chlorophyll

1. Background

Tomato (*Lycopersicum esculentum* L.) is one of the most important cultivated crops in the world. Generally, the crop is vulnerable to drought stress [1]. Under field conditions drought and pathogen stress often occurs simultaneously. Plant viruses are often discovered and studied as pathogenic parasites that cause diseases in agricultural plants and are obligate intracellular symbionts. Viruses use host resources to support their own reproduction and dissemination, so it is widely believed that virus infections are harmful to the host. However, this paradigm represents an incomplete picture of virus–host relationships [2]. Little is known about the biology of plant viruses and their hosts in natural systems. Plants support a large number of positive single-stranded RNA

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viruses that are less common in many other host kingdoms. Moreover, the combination of drought and pathogen stress has been noted to be devastating for growth and yield of crop plants [3]. Several studies in Arabidopsis, bean, and grapevine have shown that drought stress makes the plant vulnerable to pathogen infection [4–7]. Conversely, reports also indicate that drought stress enhances the defense response of plants against pathogen [8, 9]. Pathogen infection has also been shown to alter the response of plants to water-deficit conditions. Drought can have positive effect and reduce disease levels but in many cases it increases the disease susceptibility [10–12]. For instance, it is well-known that rain-fed rice suffering for repeated and intermittent drought heavily suffers from blast disease caused by the fungus *Magnaporthe oryzae* [13]. Recently, it is showed that drought restricts the multiplication of *R. solanacearum* in chickpea, which suggests that combined stress can induce robust defense responses in chickpea. Abiotic stress such as drought and frost induces dehydration, resulting in osmotic stress and associated oxidative stress [14]. One ubiquitous protective mechanism against drought and frost in plants is the accumulation of certain organic metabolites, the osmo-protectants and antioxidants. The primary metabolic changes in the plants caused by virus infection and drought stress were investigated using metabolic profiling [15].

Stress tolerance virus-infected plants can exhibit either increased susceptibility to drought stress as a consequence of weakened basal defense or enhanced drought tolerance as a result of pathogen-induced priming [16]. For example, *Maize dwarf mosaic virus* infected sweet corn plants (*Zea mays* var. saccharata) simultaneously exposed to drought stress showed more reduction in ear weight, leaf area and plant height compared to non-infected plants [17]. *Maize dwarf mosaic virus*-induced yellowing of leaves could be one of the reasons for reduced growth and yield of this virus infected plants under combined stress. Simultaneous exposure of Arabidopsis plants to drought, and *Turnip mosaic virus* resulted in higher reduction in plant weight and leaf number under combined stresses compared to individual stress [18]. Early infection of these pathogens causes chlorotic local lesions, mosaic and mottling. Consistently photosynthetic capacity is reduced to shield from subsequent drought stress induced ROS damage.

Investigation of pathogen-induced drought tolerance on *N. benthamiana* plants infected with *Brome mosaic virus*, *Cucumber mosaic virus* and *Turnip mosaic virus* showed delayed appearance of leaf wilting and stem dehydration under combined virus and drought stresses compared to only drought stressed plants [19]. *Brome mosaic virus* and *Cucumber mosaic virus* -infected plants showed increased accumulation of osmo-protectants like glucose, fructose and sucrose. In addition, virus infected plants also showed lower transpiration rate due to partial stomatal closure resulting in better water retention in leaf tissues. Conceivably, the metabolic and physiological changes due to virus infection combated drought stress effects and thereby imparted combined stress tolerance. In plants exposed to a combination of virus, heat and drought stresses, this triple stress combination suppressed the R-gene-mediated defense response and increased the endoplasmic reticulum bound unfolded protein response (UPR) pathway, which were not observed under individual stresses. Reanalysis of the transcriptase data from virus and drought stress experiments using Bio Conductor package in R [20] revealed that the number of genes differentially expressed under individual drought stress and virus infection was 434 and 539, respectively, but when both stresses were applied simultaneously 1370 genes were differentially expressed. The aim of this study was investigation of interaction between virus infection and drought stress on some physiological characterizations of tomato plants under greenhouse condition.

2. Material and methods

2.1. Plant material and growth condition

Tomato plugs, Super Majjar variety were planted and grown in 2L pots containing an air-dried loamy soil, sterilized with hot air (Table 1) under greenhouse condition (Table 2). Irrigation was done with fresh water (Table 3) based on field capacity (FC) until full establishment.

Table 1
Soil characteristics used in this experiment

Variable	Rate	Variable	Rate
ECe	0.93 dS m^{-1}	Mn^{2+}	1.96 mg kg^{-1}
pH	7.12	Fe^{2+}	2.65 mg kg^{-1}
Total N	0.08%	Na$^+$	4 meq l^{-1}
P	8 mg kg^{-1}	Mg^{2+}	3.14 meq l^{-1}
K$^+$	210 mg kg^{-1}	Ca^{2+}	2.6 meq l^{-1}
Zn^{2+}	0.63 mg kg^{-1}	Cl$^-$	0.5 meq l^{-1}
Cu^{2+}	0.25 mg kg^{-1}	HCO$_3^-$	0.3 meq l^{-1}

Table 2
Some climatic characteristics of greenhouse

Night Temperature (°C)	Day Temperature (°C)	Relative humidity (%)	CO$_2$ concentration (ppm)	Light intensity (mmol m^{-2}s^{-1})
16	24	50 ± 5	280 ± 55	18

2.2. Inoculation, disease symptoms analysis and drought stress

Tomato seedlings in 3 leafy stages were used for inoculation with Cucumber Mosaic Virus isolate under greenhouse condition. Extracts were prepared by grinding the inoculum in 1% (w/v) solution of K$_2$HPO$_4$ at pH 7.5 containing 0.01% Na$_2$SO$_3$, 2% polyvinylpyrrolidone (PVP) and 0.05% ethylene diamine tetra acetic acid (EDTA). After inoculation, the plants were examined regularly and the symptoms were recorded. In 6–8 leafy stage, and after be assured about inoculation, plants were prepared for drought stress treatments based on field capacity (FC) as followed: 100% FC (control-no stress), 80% FC, 60% FC and 40% FC (severely stressed). FC value of soil used in the experiment was calculated based on oven method [21] and pressure plates [22]. Drought stress treatments was conducted during 10 days to prevent any sever shock occurred on plants. Thus, for first-three days all plants received 100% FC, and then, the other treatments were used.

2.3. Identification of systemic infection of Cucumber Mosaic Virus

In order to test the systemic infection of Cucumber Mosaic Virus on tomato, the inoculated leaves (positive control), the internode between the Cucumber Mosaic Virus -inoculated leaves and the non inoculated upper leaves were precisely harvested. Then, the total RNA was extracted and used for future studies. Five μg of total RNA were used for reverse transcriptase polymerase chain reaction (RT-PCR) to amplified Cucumber Mosaic Virus RNA [23]. The amplified fragment was gel extracted and analyzed by sequence analysis.

2.4. Relative water content (RWC)

Leaf samples were weighed to determine the fresh mass (FM), soaked in distilled water at 25°C for 4 h to determine the turgid mass (TM), then oven-dried at 80°C for 24 h to determine the dry mass (DM). Finally, RWC was calculated based on method of Barrs and Weatherley [24].

Table 3
Water characteristics used in this experiment

EC (dS m^{-1})	pH	Ca	Mg	K	Na	Cl	HCO$_3^-$
				(meq L^{-1})			
1.05	7.54	2.10	5.80	0.20	8.50	13.00	3.90

2.5. Electrolyte leakage (EL)

Leaf segments were cut out at random, washed 3 times with distilled water in order to remove surface contaminants, and then placed individually in stoppered vials containing 10 ml of distilled water. Consequently, they were incubated at room temperature (25°C) on a shaker ($100 \times g$) for 24 h to measure EC of the solution (EC1). Then the same vials with leaf samples were placed in an autoclave at 120°C for 20 min and the second measurement of conductivity (EC2) was done after cooling the solution to room temperature. The ion leakage was calculated using method of Lutts et al. [25].

2.6. Chlorophyll (Chl) and carotenoid (car) contents

Acetone (80%) was used for assessment of Chl content (mg g^{-1} FM). Precisely, 0.25 g leaf disk was placed in 10 mL acetone (80%) for extraction, then centrifuged at 8000 g for 10 min and supernatant separated precisely for future experiment, and homogenization of leaf tissue with the buffer extraction was continued until colorless. The collected supernatants were made to a final volume of 50 ml. Absorbance of the extract was read at 645 and 663 nm for chlorophyll and at 470 nm for carotenoid with a spectophotometer (Shimadzo AA–670, Japan). Acetone 80% was used as blank. Then, chlorophylls a, b and total content were calculated based on method of Roades [22]. Total carotenoids content was calculated following the method of Lichtenthaler [26].

2.7. Experimental design

The experiment was set up as factorial (two factors including inoculation and drought stress), based on completely randomized design, with 8 treatments and 3 replications, each replication consisted of 3 pots. Statistical analysis of data was carried out using analysis of variance (*ANOVA*) procedure on GenStat program (12th edition). The means were separated with LSD at 5% level of confidence.

3. Results and discussion

Systemic symptoms observed in infected tomato plants with Cucumber Mosaic Virus (CMV), which had an amplified fragment by specific primers in RT-PCR. The appearance of drought stress symptoms delayed 2–5 days, compared with mock-inoculated plants. These results indicated that CMV infection may improve drought tolerance in many CMV- host plants. There is a report on the combination effects of drought and infection with some viruses including *Brome Mosaic Virus*, *Cucumber Mosaic Virus* and Turnip Mosaic Virus on N. benthamiana plants, which showed delayed appearance of leaf wilting and stem dehydration compared to only drought stressed plants. Moreover, accumulation of osmo-protectants like glucose, fructose and sucrose increased with virus infection. Transpiration rate reduced, because of partial stomatal closure resulting in better water retention in leaves [27].

RWC is considered as an important criterion of plant water status. Results indicated the lowest value of this variable in both infected and non-infected plants under 40% FC. Moreover, decrease in water stress from 40 to

Table 4

Interaction between drought stress and CMV infection on RWC, EL, Chl and Car

CMV	*Drought	RWC	EL	Total Chl	Car	Chl a	Chl b
	(%)			(mg g–1 F.W.)			
Infected	40	39.6c	100.00a	8.66d	1.18c	4.80d	3.86b
	60	60.0b	98.61a	11.47d	1.22c	6.55d	4.91b
	80	97.0a	9.38b	23.51b	4.84a	20.46a	3.06b
	100	98.3a	12.46b	28.97a	5.58a	23.63a	5.33b
Non-infected	40	41.9c	98.56a	10.50d	0.62c	6.00d	4.50b
	60	47.8b	97.72a	16.17c	1.22c	7.23d	8.94a
	80	59.0b	12.27b	23.07b	1.03c	10.25c	12.82a
	100	96.5a	8.09c	20.47b	2.49b	15.04b	5.44b
	SE	18.10	4.40	7.55	0.99	1.65	2.31

SE means standard error. *Drought stress was done based on field capacity (FC); RWC: relative water content; EL: electrolyte leakage; Chl: Chlorophyll; Car: carotenoid. Mean values (9 sampling) in each column followed by the same letter are not significantly different by the LSD ($P < 0.05$).

Table 5

Effect of drought stress on RWC, EL, Chl and Car

Drought	RWC	EL	Total Chl	Car	Chl a	Chl b
	(%)		(mg g–1 F.W.)			
40	40.8d	99.28a	9.58c	0.90b	5.40c	4.18b
60	53.9c	98.16a	13.82b	1.22b	6.89c	6.92a
80	78.0b	10.82b	23.29a	1.64b	15.35b	7.94a
100	97.4a	10.28b	24.72a	4.04a	19.34a	5.38a
SE	5.91	1.01	1.46	0.70	1.17	1.63

SE means standard error. *Drought stress was done based on field capacity (FC); RWC: relative water content; EL: electrolyte leakage; Chl: Chlorophyll; Car: carotenoid. Mean values (9 sampling) in each column followed by the same letter are not significantly different by the LSD ($P < 0.05$).

100% FC led to significant RWC increase in both infected and non-infected plants ($p < 0.05$). In addition, there is no significant difference between both groups under 100% FC and virus-infected plants under 80% FC. An approximately 39% reduction of harmful effects of drought on RWC obtained in virus-infection under 80% FC (Table 4). RWC significantly reduced ($p < 0.05$) under drought stress that was in agreement with Wang et al. (2012) and Sharma and Sharma (2008). The highest and lowest RWC obtained in 100% and 40% FC, respectively (Table 5), and a positive linear correlation ($R^2 = 0.988$) observed between increase in RWC and drought stress. CMV infection created a significant change in this variable (approximately 17% increase) compared with non-infected plants (Table 6). The leaves of infected plants had more water compared with mock-inoculated plants, indicating better water retention, which may be related to reduction of stomatal opening and low transpiration rate [28].

The highest EL value obtained in both infected and non-infected plants under 40 and 60% FC. The lowest value observed in non-infected plants under 100% FC. CMV infection increased this variable with approximation of 35% under 100% FC, compared with non-infected plants (Table 4). The EL increase observed as drought stress increased that was in agreement with another resesrch [15]. In addition, a negative correlation ($R^2 = 0.807$) observed between EL increase and water stress severity and approximately 89% increase obtained (Table 5). Simple effect of virus infection was not significant on this variable (Table 6). EL increase is accompanied with

Table 6
Effect of CMV on RWC, EL, Chl and Car

CMV	RWC	EL	Total Chl	Car	Chl a	Chl b
	(%)		(mg g–1 F.W.)			
Infected	73.7a	55.11a	18.15a	2.55a	13.86a	4.29b
Non-infected	61.3b	54.16a	17.55a	1.34b	9.63b	7.92a
SE	4.18	0.71	1.03	0.49	0.82	1.15

SE means standard error. CMV: cucumber mosaic virus; RWC: relative water content; EL: electrolyte leakage; Chl: Chlorophyll; Car: carotenoid. Mean values (9 sampling) in each column followed by the same letter are not significantly different by the LSD ($P < 0.05$).

the increase of cell permeability; thus, an important strategy for the development of drought resistance should be involved in the maintenance of cell membrane integrity.

The lowest total chlorophyll (Chl) observed in both groups of plants under 40% FC and also in infected plants under 60% FC. The highest value indicated in CMV infected plants under 100% FC, compared with others (Table 5). An approximately 29% differences obtained between infected and non-infected plants under 100% FC. Moreover, infected plants under 40% and 100% FC showed 70% difference. The highest total Chl observed in 100% FC (Table 5), approximately 61% more than 40% FC, and a negative correlation observed between drought stress level and this variable ($R^2 = 0.933$). Such retardation in the content of photosynthetic pigment in response to drought stress was attributed to the ultra-structural deformation of plastids including the protein membranes forming the thylakoids which in turn causes untying of photosystem II, which captures photons, so its efficiency declined, thus causing declines in electron transfer, ATP and NADPH production and eventually CO_2 fixation process [29, 30].

On the other hand, no significant effect of CMV infection observed on this variable (Table 6).

Carotenoid (Car) content increased in CMV infected tomatoes under 80% and 100% FC. The lowest value observed in both groups plants under 60% and 40% FC. It is clearly seen that CMV infection encouraged the tolerance mechanism to some extent (Table 4). Difference between infected and non-infected plants under 80% FC and 100% FC were about 78% and 55%, respectively. The highest level of this variable observed in non-stressed plants (100% FC) (Table 5) and a negative correlation ($R^2 = 0.79$) observed between Car content and drought stress severity that was in agreement with the research on African eggplants [31]. Carotenoids might have a protective role and protect chlorophyll from photo oxidation. CMV infection increased the Car in infected plants, approximately 55% higher compared with non-infected plants (Table 6).

Interactive effects of drought stress × CMV infection led to the highest Chl a under both 80 and 100% FC. This means that tolerance obtained to some extent, inhibiting Chl a loss under stress. The lowest rate of this variable showed in both groups of plants under 40 and 60% FC. Differences between infected and non-infected plants under 100% FC was about 36%, meaning that infection led to higher Chl a compared with non-infected plants (Table 4). Simple effect of water stress showed a negative correlation ($R^2 = 0.939$) with Chl a increase, and the highest and the lowest values observed in 100 and 40% FC, respectively (Table 5). In the other words, a 72% reduction indicated in this variable as stress intensified. CMV infection also improved this variable approximately 30% compared with non-infected plants (Table 6).

The highest Chl b obtained in non-infected plants under 60 and 80% FC and this difference was significant, compared with others. Difference between infected and non-infected plants under 60% FC was about 45%, meaning that Chl b may be break downed under stressed condition in infected plants (Table 4). A polynomial correlation ($R^2 = 0.979$) observed between severity of drought stress and Chl b, however, the lowest value indicated in 40% FC. CMV infection also resulted to significant effect on this variable and the lowest value observed in CMV infected plants, by approximately 49% reduction (Table 6). Our results were in agreement with several reports of decrease contents of chlorophylls and carotenoids under drought [32].

4. Conclusions

In the present study, we evaluated the interactive effect of drought stress and CMV infection in tomato plants variety Super Majjar. Data showed that drought stress significantly influence some physiological aspects of tomato growth and development and led to reduction of RWC, total Chl, Chl a, Chl b and carotenoid and increment of electrolyte leakage. On the other hand, CMV infection ameliorated the carotenoid, Chl a, total chlorophyll and RWC to some extent. Plant response to stress combination is affected by the type of abiotic stress and the pathogen involved. Both susceptibility and tolerance were observed in plants simultaneously exposed to drought and virus. However, it is not clear why some interactions resulted in tolerance while others lead to susceptibility. Finally, it is concluded that there is a correlation between abiotic and biotic stresses in improving tolerance of this variety of tomato.

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