Pakistan Journal of Nematology (2019)37(1):91-101 ISSN 0255-7576 (Print)
ISSN 2313-1942 (Online)
www.pjn.com.pk
http://dx.doi.org/10.18681/pjn.v37.i01.p91-101

Assessment of photosynthetic fluorescence in tomato cultivars infested with root-knot nematode

A. Ghasemzadeh[1], S. Jamali[1†], M. Esfahani[2] and H. Pedramfar[1]

[1]*Plant Protection Department, Faculty of Agricultural Sciences, University of Guilan, Rasht, Iran*
[2]*Department of Agronomy and Plant Breeding, Faculty of Agricultural Sciences, University of Guilan, Rasht, Iran*

†Corresponding author: jamali@guilan.ac.ir, jamali_s2002@yahoo.com

Abstract

Biotic stresses caused by nematodes create restrictions in plant growth. In this article, the physiological effects of root-knot nematode (*Meloidogyne incognita* Race 2) stress was assessed on photosynthetic fluorescence attributes in two tomato cultivars, Falat Y as a susceptible, and Gina VF as a tolerant cultivar. The experiment was done in a completely randomized split plot design under greenhouse conditions with four nematode populations, 0 (as control), 500, 1000 and 2000 second stage juveniles, and four sampling times (20, 40, 60 and 80 days after inoculation). After sampling, purification, identification, and population of root-knot nematode species and race were determined, and after amplification of purified population on the tomato cultivar cv. Rutgers, inoculums were sufficiently obtained. In four-leaf stage of the plant growth, the nematode inoculum levels were introduced, and photosynthetic parameters were evaluated at different times. The results showed that by increasing levels of nematode inoculum, chlorophyll fluorescence parameters were highly affected. In general, the nematode-stressed plants under both light and dark conditions, amount of minimum and maximum fluorescence (F_0 and F_m) and the difference between them (F_V), rate of non-photochemical quenching, photochemical quenching and descriptive parameter of non-chemical quenching increased, while the efficiency rate of photosystem II under both conditions trended to a downward with increasing nematode levels. The correlation between nematode levels and various sampling times had different effects on the measured characteristics. Overall, Falat Y cultivar had relatively greater photosynthetic parameters than Gina VF cultivar.

Keywords: Chlorophyll, fluorescence, *Lycopersicon esculentum*, *Meloidogyne incognita*

Tomato (*Lycopersicon esculentum* Mill.) is one of the major crops grown across the world and is used in the pharmaceutical industry. This plant is a good model for the physiological, cellular, biochemical and molecular genetics studies (Hille *et al*., 1989). Research on tomato has increased our knowledge about some of the growth processes; and this plant, for a long time, has been considered as a model to study the correlation between the organs, relationships, source, destination, and mode of application of assimilate. According to the UN Food and Agriculture Organization (FAO), currently, more than 4,800,000 hectares of land in the world are devoted to the cultivation of tomatoes, and about 182 million tons are produced each year. In 2017, Iran has produced 6,177,290 tons per year, allocated the sixth ranking in the world (FAOSTAT, 2017). Chlorophyll fluorescence measurement is a relatively new parameter and technique which, in recent years, has been used to study the effect of various biotic and abiotic stresses such as

Published by Pakistan Society of Nematologists
Received:04 Jun, 2018
Accepted:20 Nov, 2018

drought, salinity, and temperature on leaf photosynthesis efficiency in field and greenhouse conditions (Brestic & Zivcak, 2013; Baker & Rosenqvist, 2004; Zobayed *et al*., 2005). This technique is based on physiological measurement and measures light absorption mechanism for photosystem II complex optical system for leaf. Using Chlorophyll fluorescence is a reliable and non-destructive method for assessing photosynthesis and judging about the physiological status of a plant. The fluorescence of photosystem II can serve as an important tool to be used in determining the degree of stress in plants (Zushi *et al*., 2012; Tomar & Jajoo, 2013).

The ratio of Fv/Fm shows maximum quantum yield of photosystem II of photochemical reaction and is an important parameter to determine the photosynthetic system status. Environmental stresses that affect the efficiency of photosystem II, reduce the ratio of Fv/Fm (Boureima *et al*., 2012). Evaluation of physiological changes by stress in sensitive and tolerant varieties can be useful in identifying biological and non-biological mechanisms of stress tolerance; one of the physiological changes is the relative amount of chlorophyll whose relation to the measurement of fluorescence can be useful in such studies (Stirbet *et al*., 2018).

In fact, drought stress, by having an adverse effect on carbon assimilation, reduces the capacity and transmission of electron, therefore the system quickly reaches Fm, which reduces fluorescence variable (Fv). However, by increasing light intensity, photosynthetic systems, with a set method for reducing induced excitation energy, loses the excess energy by non-photochemical increasing extinction as the non-radiative process. With this mechanism, while protecting the reaction center, it causes the least damage. Therefore, photochemical efficiency of photosystem II is expressed as Fv/Fm ratio (the ratio of variable fluorescence to maximal fluorescence). Therefore, environmental stress, by affecting on photosystem II, decreases this ratio (Stirbet, 2013; Gautum *et al*., 2014).

In a study, to investigate the influence of cyst nematode *H. sacchari* and drought stress on water needs and growth of rice plants, it was found that under water shortage condition in sandy soil, nematode caused damage in sensitive plants, and their chlorophyll content decreased. The highest reduction in plant growth was observed when the number of nematodes was more (Audebert *et al*., 2000). Ye *et al*., (2011) determined response of photosynthesis to root-knot nematodes *M. incognita* on both resistant and susceptible varieties of cucumber; wherein the nematode infection caused a significant reduction in chlorophyll content of cucumber susceptible cultivars. Reduction in the amount of chlorophyll was lower in the resistant cultivars though net photosynthetic rate in both cultivars, after being infected with the nematodes decreased. The aim of this study was to evaluate effect of root-knot nematode, *Meloidogyne incognita* race 2 on photosynthetic parameters and chlorophyll fluorescence of two tomato cultivars and its effects on the growth of host plant.

Materials and Methods

Preparation of nematode inoculum (*M. incognita*): In the spring of 2013, tomato in greenhouse and agricultural lands in Rasht City were sampled and roots infected with galls and its surrounding soils were collected. Infected roots were packed in plastic bags, transferred to laboratory and kept at 4 °C. Contaminated roots were washed by gentle movements and gentle stream of water. After removing the dirt from the surface, egg sacs were removed and separately placed in a small glass cavity containing distilled water and covered with lid. The isolated egg-masses were not mixed with each other. In greenhouse experiments, the egg-masses were placed in the holes to a depth of 3 to 5 cm, adjacent to the roots of seedlings of tomato varieties Rutgers. Pots contained 1 kg of sterilized soil autoclaved for 30 minutes at 121° C and 1.5 bar atmospheric pressure. Seedlings at 2-4 true leaf stage were grown in good condition

in a greenhouse for 46 to 60 days. Sterilization of pots and seeds of tomato was done using 10% solution of commercial sodium hypochlorite. At harvest time, pieces of tomato root galls and egg-masses were individually selected and transferred to laboratory for identification of nematode species. Perineal pattern slides were prepared as per (Taylor & Netscher, 1974) and identified by using Jepson's key (Jepson, 1987). Population study was done through the differential host test based method (Barker *et al*., 1985). Reactions of tested plants were evaluated 60 days after inoculation. After identifying the desired species (*M. incognita*), several consecutive reproduction periods were done to get the pure population mass on tomato. Then, to extract inoculums, Hussey and Barker's method was used (Hussey & Barker, 1973).

Preparation of tomato seedlings: Disinfected seeds of Gina VF (as a tolerant cultivar) and Falat Y (as a susceptible cultivar) (Gharabadiyan *et al*., 2012) were planted in sterile pots containing sterile soil, sand manure, and peat (1:1:2). In the 4-6 leaves stage, each plant was inoculated with different population levels of nematodes. Both tomato cultivars, Gina VF and Falat Y, were inoculated with the nematode levels of 500, 1000 and 2000 juveniles in water. Control plants were inoculated with sterile water. A total of 128 pots (64 pots for each cultivar), were used; 16 pots as control, 16 pots each for different inoculum levels and for two varieties. They were considered in four intervals timeframe of 20, 40, 60 and 80 days after inoculation for measuring the desired parameters. Seedlings were kept in greenhouse conditions throughout the experiment (16/8 hours in light/dark, temperature of 28/26° C). Measuring chlorophyll fluorescence characteristics was done using MINIPAM machine (Walz, Germany).

Chlorophyll parameters: For measuring chlorophyll fluorescence parameters, a part of the desired leaf was placed in dark for 30 minutes. Using measuring device, MINIPAM, fluorescence was shed onto leaves. When the light intensity is sufficient, fluorescence increases from F_0 to its maximum amount of F_m. This increase reflects the gradual increase of fluorescence and reduction in photochemical reaction rate (Baker & Rosenqvist, 2004; Jedmowski *et al*., 2013). Another important parameter of chlorophyll fluorescence is Fv, which is obtained by F_m- F_0. The ratio of F_v/F_m shows the maximum quantum efficiency of photosystem II to convert the absorbed light into chemical energy.

Chlorophyll fluorescence parameters in leaves adapted to darkness such as F_0 (base fluorescence) and F_m (maximal fluorescence), and to lightness such as F_t (fluorescence intensity) and F_{ms} (maximal fluorescence intensity) were measured. Moreover, for the calculations, other required parameters, including maximum photochemical efficiency of photosystem II (F_v/F_m), arousal capacity of photosystem II ($F1_v/F1_m$), photochemical (qp) and non-photochemical (qNP) turning off, quantum yield of photosystem II (ΦPSII) and electron transport rate (ETR) were measured (Oxborough, 2004; Tuba *et al*., 2010).

Statistical analysis: Factorial experiment in a completely randomized design was done with 4 treatments splitsplit plot with 4 time frames on two varieties and 4 replications. Data analysis was done using SAS software (Ver. 9.3) and data comparison was carried out using Tukey's test.

Results

Chlorophyll fluorescence factors

Minimal fluorescence in bright condition F: Results of data interaction comparison showed a significant difference with control in the first and second sampling time (at $P \leq 0.01$ and $P \leq 0.05$, respectively) in Falat Y plants, treated with 2000 larvae of *M. incognita*. Also, in Gina VF figure, during the first sampling stage, there was a statistically significant difference (at $P \leq 0.01$) among all treatments, and the differences were observed during the second sampling time in 2000 nematodes treatment at $P \leq 0.05$. In the fourth sampling time, there was a significant difference between control and 2000 nematode

larvae treatments in the second stage juveniles (at $P \leq 0.01$). In general, as the nematode population levels increased, the amount of F increased, with maximum increase at the third sampling time. Generally, the Gina VF cultivar had higher F mean (Table 1).

Maximal fluorescence (*M*): The mean comparison results of the interaction between nematode population levels and sampling time showed that, in Falat Y, there was a statistically significant difference between 1000 and 2000 nematode treatments at the first sampling time as well as between all nematode levels-treated plants and their controls during the second and third sampling time ($P \leq 0.01$). During the fourth sampling time, the difference between 1000 and 2000 nematode treatments and the control group was significant at 5% and 1% levels, respectively. While, in Gina VF cultivar, at the first sampling time, a significant difference was observed between all treatments of nematode population levels and control at $P \leq 0.01$. During the second and third sampling, it was significant between the second stage juvenile nematodes of 1000 and 2000 treatments and control (at $P \leq 0.05$ and $P \leq 0.01$, respectively). Also, during the fourth sampling time, there was a significant difference between 500 larvae treatment and control group at 5% level and between 1000 and 2000 nematode treatments and the control group at 1% level. The general results indicated that by increasing nematode population, the amount of M increased; this increase was maximum in Gina VF at all sampling time (Table 1).

Minimal fluorescence in darkness (F_o): The results showed that, in Falat Y, a significant difference was observed in F_o between 1000 and 2000 larvae treated plants at the first and fourth sampling time as compared with the control (at $P \leq 0.01$). Moreover, there was a significant difference between all treatments of nematode levels during the second and third stage of sampling as compared with the control (at $P \leq 0.01$). Also, in Gina VF, the amount of F_o had a statistically significant difference at the first sampling time of 1000 and 2000 nematode treatments in comparison to the control group, at $P \leq 0.05$ and $P \leq 0.01$, respectively. At the second, third and fourth sampling times, the difference in F_o were statistically significant (at $P \leq 0.01$) between all the population level treatments and the control group. The overall results suggest that by increasing the nematode population levels, F_o amount increased as well. These results determine the impacts of the population and sampling times on the factor in two cultivars (Table 1).

Maximal fluorescence in the darkness (F_m): Based on the comparison of interaction of larvae treatments on sampling time, both the tomato cultivars have been placed in two statistically different groups, in which Gina VF had a higher mean in F_m amount. However, as nematode populations levels increased, the amount of F_m also increased. Moreover, the F_m data average in control plants had statistically significant difference with that in all nematode treated ones, thereby; it had been placed in different statistical group. In addition, the amount of F_m in both cultivars treated with 500 larvae was significantly different from those treated with 2000 larvae, and thus, they were statistically placed in two different groups. Based on different sampling time, F_m data averages were placed in three statistically different groups, in which the first sampling time was in one group, the second and third samplings in a same group, and the fourth sampling in the other group. The highest average of F_m was recorded during the third sampling time followed by the second and fourth time, and the lowest was recorded at the first time of sampling. It means that there was an increasing trend in the index by accumulation time of sampling (Table 2).

The fluorescence in darkness Fv: The results of Fv measurement in the two tomato cultivars showed that they were in two statistically different groups, so that the mean of Fv was higher in Gina VF than Falat Y. Also, there was a significant difference in Fv between each nematode population levels and control group.

So that as the population levels of nematode increased, the amount of Fv increased. Moreover, the amount of Fv was significantly affected by sampling time periods. From the sense, they were located in three different groups (the first time in a group, the second and third periods in a same group, and the fourth time quarter in another group); the Fv obtained through third sampling time had the highest value followed by the second and fourth one, and the first time of sampling had the lowest value (Table 2).

Table 1. Comparison of means M, Fo and F (mV) of two varieties of tomato in four different levels of nematode populations in four different sampling times.

F	Fo	M	Nematode population levels	Time	Cultivar
143.375	203.75	602.107	1	1	Falat Y
181.125$^{n.s}$	262.75$^{n.s}$	689.607$^{n.s}$	2	1	Falat Y
182.125$^{n.s}$	376.50**	727.607**	3	1	Falat Y
238.875**	485.50**	916.357**	4	1	Falat Y
272.678	383.25	1317.608	1	2	Falat Y
291.187$^{n.s}$	512.75**	1407.1085**	2	2	Falat Y
301.937$^{n.s}$	543.75**	1484.358**	3	2	Falat Y
327.437*	545.50**	1510.608**	4	2	Falat Y
291.338	352	1353.564	1	3	Falat Y
293.838$^{n.s}$	489.50**	1568.314**	2	3	Falat Y
295.088$^{n.s}$	477**	1626.314**	3	3	Falat Y
296.388$^{n.s}$	571**	1765.814**	4	3	Falat Y
274.985	359	1121.361	1	4	Falat Y
305.735$^{n.s}$	409$^{n.s}$	1167.861$^{n.s}$	2	4	Falat Y
306.235$^{n.s}$	533.25**	1192.111*	3	4	Falat Y
306.985$^{n.s}$	561.25**	1334.861**	4	4	Falat Y
150.467	612.75	1104.215	1	1	Gina VF
379.717**	672.75$^{n.s}$	1513.465**	2	1	Gina VF
357.967**	686*	1566.715**	3	1	Gina VF
368.492**	740.50**	1565.565**	4	1	Gina VF
303.640	357	1437.342	1	2	Gina VF
308.640$^{n.s}$	522.50**	1487.842$^{n.s}$	2	2	Gina VF
339.640$^{n.s}$	572**	1502.592*	3	2	Gina VF
373.14*	625**	1530.842**	4	2	Gina VF
323.765	487	1433.096	1	3	Gina VF
338.265$^{n.s}$	567.50*	1452.596$^{n.s}$	2	3	Gina VF
353.765$^{n.s}$	623**	1507.596*	3	3	Gina VF
365.265$^{n.s}$	631**	1652.096**	4	3	Gina VF
287.469	409	1239.010	1	4	Gina VF
287.969$^{n.s}$	389.50$^{n.s}$	1314.760*	2	4	Gina VF
321.219$^{n.s}$	462.50$^{n.s}$	1347.510**	3	4	Gina VF
375.969**	605.25**	1428.260**	4	4	Gina VF

Note :*, ** and n.s are significant level at 5%, 1% and non-significant difference, respectively.

Table 2. Comparison of means Fm (mV) and Fv two tomato varieties at four different levels of nematode populations in four different sampling times.

Fv	Fm	Cultivar
1768.20b	2209.81b	Falat Y
2222.64a	2782.84a	Gina VF
		Nematode population level
1788.34b	2183.81c	1
1997.06a	2475.34b	2
2052.88a	2587.13ab	3
2143.41a	2739.03a	4
		Time
1493.66c	1998.72c	1
2236.16a	2743.88a	2
2427.88a	2952.63a	3
1824b	2290/09b	4

Photochemical quenching (QP): Results of PQ measuring showed that there was a significant difference between the first and second times of sampling in Falat Y (at $P \leq 0.01$). It was also observed in Gina VF (at $P \leq 0.05$). Moreover, the difference was also between the first and third sampling times in Falat Y (at $P \leq 0.05$) as well as between the first time of sampling and the third and fourth sampling times in Gina VF (at $P \leq 0.01$). However, the highest recorded PQ was in the second time of sampling for Falat Y, and in the first one for Gina VF (Table 3).

The fluorescent in lighting situations FV': The results of the mean comparison of interaction of the FV' on the time of sampling showed that in Falat Y, there were significant differences between the second, third, and fourth sampling time and first sampling at 1% level; the highest average was at the third time of sampling and the lowest belonged to the first one. Whereas, in Gina VF, there was no significant difference between the second and first time of sampling; but, there were significant differences between the third and fourth sampling time and first time of sampling at 5% and 1% levels; so that, the highest average belonged to the third time of sampling (Table 3).

Maximal quantum efficiency of photosystem II ($F_{V'}/F_{M'}$):The mean comparison results of the interaction of nematode population levels on the time of sampling showed that in Falat Y, in the third time of sampling, the difference was just significant in 2000 second stage juveniles treated nematodes with control at 5% level. Also, in Gina VF, at the first time sampling, there was a significant difference between the nematode treatment of 500 and 1,000 larvae and control at $P \leq 0.01$; while no significant difference was observed between the second stage nematode larvae in 2000 treatments and control. The overall results showed that by increasing the nematode population levels, the FV'/ FM' level decreased; the highest amount of the decrease was observed during the fourth time sampling (Table 4).

Quantum efficiency of photosystem II (F_v/F_m): The mean comparison results of interaction of the nematode population levels on sampling time showed that, at the first sampling time, there was remarkable difference (at $P \leq 0.05$) in between the ratio of F_v/F_m in Falat Y plants treated with 500 second nematode larvae and control plants, as well as it was found (at $P \leq 0.01$) between 1000 and 2000 larvae treated plants and control ones. In the second time of sampling, a significant difference (at $P \leq 0.01$) was observed between all nematode treatment levels and control.

During the third time of sampling, there was only a significant difference in the ratio of F_v/F_m between the second nematode of 1000 larvae treated plants and control (at $P \leq 0.05$). In the fourth sampling time, it was also observed between treatments of 2000 larvae and control group. In addition, in Gina VF, during the first and the second sampling time, a significant difference in F_v/F_m was observed between all larvae treatments and control group (at $P \leq 0.01$). In the third sampling time, it was significant (at $P \leq 0.05$) between 1000 and 2000 nematodes treatments and control. Also, in the fourth time of sampling, it was between 500 larvae treatment and control group (at $P \leq 0.01$). The overall results indicate that by increasing nematode population levels, the amount of F_v/F_m decreased, however, in the third time of sampling, it had the highest value. Vice versa, in the first sampling time, it possessed the lowest value (Table 4).

Non-photochemical quenching (*QN*): The results of QN measuring showed that, in Falat Y, at the first sampling time, there was statistically a difference between its values in 1000 and 2000 of larvae-treated plants and non-larvae treated ones at $P \leq 0.01$. In addition, in the second and third time of sampling, there was a significant difference between all treatments of nematode populations and control group (at $P \leq 0.01$). During the fourth time of sampling, the QN values were significantly different between the 500 larvae-treated plants and control group ($P \leq 0.05$) as well as 1000 and 2000 larvae-treated plants and control ($P \leq 0.01$).

Moreover, in Gina VF, the value of QN was statistically different between all nematode treatments and control group at the first time of sampling (at $P \leq 0.01$); while in the second time of sampling, the difference was observed between 500 and 1,000 larvae treatments and control group. As compared to the control, in treatment of 2000 larvae in the second sampling time, a significant difference was observed (at $P \leq 0.01$) in QN value of Gina VF plants. During the third and fourth sampling time, the difference was between treatments of 1000 and 2000 larvae of and control group. Overall, the results indicated that by increasing nematode populations, the levels of QN also increased. However, the highest QN value was recorded during the third sampling period (Table 4).

Table 3. **Comparison of means QP and Fv 'two tomato varieties at four different levels of nematode populations in four different sampling times.**

Fv'	QP	Time	Cultivar
546.29	893.08	1	Falat Y
1131.60^{**}	982.09^{**}	2	Falat Y
1284.35^{**}	930.460^{*}	3	Falat Y
905.56^{**}	$899.92^{n.s}$	4	Falat Y
1123.32	994.70	1	Gina VF
$1158.38^{n.s}$	928.741^{*}	2	Gina VF
1166.08^{*}	897.61^{**}	3	Gina VF
1014.22^{**}	884.75^{**}	4	Gina VF

Table 4. Comparison of means Fv / Fm, QN and Fv '/ Fm' two tomato varieties at four different levels of nematode populations in four different sampling times.

Fv'/Fm'	QN	Fv/Fm	Nematode population level	Time	Cultivar
0.7606	28.505	0.8035	1	1	Falat Y
0.7371$^{n.s}$	29.255$^{n.s}$	0.7812*	2	1	Falat Y
0.7462$^{n.s}$	31.255**	0.6820**	3	1	Falat Y
0.7381**	33.755**	0.6908**	4	1	Falat Y
0.7928	19.732	0.8413	1	2	Falat Y
0.7929$^{n.s}$	25.232**	0.7934**	2	2	Falat Y
0.7964$^{n.s}$	29.232**	0.7969**	3	2	Falat Y
0.7826$^{n.s}$	30.232**	0.7989**	4	2	Falat Y
0.7847	23.495	0.8140	1	3	Falat Y
0.8130$^{n.s}$	33.995**	0.8288$^{n.s}$	2	3	Falat Y
0.8183$^{n.s}$	39.495**	0.8378*	3	3	Falat Y
0.8315*	38.495**	0.8115$^{n.s}$	4	3	Falat Y
0.7540	24.495	0.8014	1	4	Falat Y
0.7362$^{n.s}$	28.495*	0.7989$^{n.s}$	2	4	Falat Y
0.7428$^{n.s}$	29.998**	0.7853$^{n.s}$	3	4	Falat Y
0.7665$^{n.s}$	50.498**	0.7762*	4	4	Falat Y
0.8639	22.739	0.7171	1	1	Gina VF
0.7494**	28.300**	0.7626**	2	1	Gina VF
0.7709**	32.739**	0.7588**	3	1	Gina VF
0.7634*	51.089**	0.7488**	4	1	Gina VF
0.7881	28.740	0.8716	1	2	Gina VF
0.7914$^{n.s}$	30.240$^{n.s}$	0.8219**	2	2	Gina VF
0.7742$^{n.s}$	31.740$^{n.s}$	0.8056**	3	2	Gina VF
0.7564$^{n.s}$	36.740**	0.7896**	4	2	Gina VF
0.7741	18.978	0.8349	1	3	Gina VF
0.7658$^{n.s}$	21.478$^{n.s}$	0.8192$^{n.s}$	2	3	Gina VF
0.7651$^{n.s}$	41.978**	0.8047*	3	3	Gina VF
0.7788$^{n.s}$	45.478**	0.8104*	4	3	Gina VF
0.7674	22.454	0.7938	1	4	Gina VF
0.7805$^{n.s}$	23.954$^{n.s}$	0.8271**	2	4	Gina VF
0.7613$^{n.s}$	32.454**	0.8013$^{n.s}$	3	4	Gina VF
0.7357$^{n.s}$	33.954**	0.7800$^{n.s}$	4	4	Gina VF

Non-photochemical quenching (*NPQ*): The results of NPQ measuring revealed that both tomato cultivars were statistically placed in two different groups. In all, Gina VF had a higher mean of NPQ. Also, the amounts of NPQ were placed in three statistically different groups based on treatment of nematode population levels, and the control treatment received the least ratings. However, both tomato cultivars treated with 2000 larvae treatment had the highest average of NPQ and was placed in a group; treatments of 500 and 1000 larvae were in a statistical group. Generally, by increasing nematode population levels, NPQ amount increased (Table 5). It has been also reported in Arabidopsis plant that the amount of its NPQ increased after nematode inoculation (Berger *et al*., 2007).

Table 5. Comparison of means NPQ of two tomato varieties at four different levels of nematode populations in four different sampling times.

NPQ	Cultivar
0.04626b	Falat Y
0.05071a	Gina VF
	Nematode population level
0.04171c	1
0.04568b	2
0.04884b	3
0.05771a	4

Discussion

Studying various physiological changes caused by tensions in sensitive and tolerant varieties can be useful in identifying biological and abiotic stress tolerance mechanisms (Mathur *et al*., 2014). Therefore, we have monitored the changes in one of the main physiological process related to the photosynthetic apparatus, different fluorescence parameters, to understand some mechanisms by which *M. incognita* could influence host plants. In the present study, by increasing nematode population levels, chlorophyll fluorescence parameters were fundamentally altered. Since the amount of chlorophyll fluorescence is directly linked to the activity of chlorophyll in photosystem reaction center, any disturbance, such as change in the structure of the pigments of photosystem II, lead to the maximum reduction in photosystem quantum yield in light conditions compatible with darkness (Yang *et al*., 2009; Guanter *et al*., 2014).

Present results showed that by increasing the nematode population levels, the F_V/ F_M level decreased. The highest amount of the reduction was observed during the fourth time sampling. It was revealed that, photosystem II plays an important role in photosynthetic response to environmental factors in higher plants. At recent decades, chlorophyll fluorescence techniques have been used as rapid, sensitive, and non-destructive methods in plant physiological studies (Baker & Rosenqvist, 2004; Zivcak *et al*., 2014).

These results also indicated that by increasing nematode populations, the amount of maximal fluorescence (M) increased. In fact, the amount of chlorophyll fluorescence shows the safety of thylakoid membrane and the relative efficiency of electron transport from photosystem II to photosystem I. When Quinon molecule (first electron receptor Quinon of photosystem II) is in the fully oxidized state (open state of the reaction center of photosystem II), the system has minimal fluorescence (F_o) which, by increasing the survival of molecules, these fluorescence gradually increases. This process continues until it completely procedure its molecules. In such a case, the center of photosystem is in complete restoration and has the maximum fluorescence (F_m). The ratio of F_v/F_m shows maximum quantum yield of photosystem II of photochemical reaction and is an important parameter to determine the photosynthetic system status. It has been reported that environmental stresses that affect the efficiency of photosystem II reduce the ratio of F_v/F_m (Berna´t *et al*., 2012; Gottardini *et al*., 2014). Measuring these parameters which associated with the acquisition and absorption of photosystem II complex of light leaves optical system, is a trustworthy and non-destructive method for displaying plant photosynthesis and judgments about its physiological status. Overall, in our study, by implementing of nematode stress, both tomato cultivars exhibited an increase in their photosynthetic-related parameters such as minimum and maximum fluorescence and the difference between them,

non-photochemical subsides, photochemical subsides, and non-chemical remission, inversely, they had a reduction in the efficiency of photosystem II in both light and darkness as nematode population increased. However, more investigations are required to decipher the physiological processes by which root-knot nematode (*Meloidogyne incognita*) could affect its host plants.

References

Audebert, A., Coyne, D. L., Dingkuhn, M. & Plowright, R. A. (2000). The influence of cyst nematodes (*Heterodera sacchari*) and drought on water relation and growth of upland rice in Coted Ivoire. *Plant Soil*, 235-242. DOI: 10.1023/A:1004734415254

Baker, N. & Rosenqvist, E. (2004). Application of chlorophyll fluorescence can improve crop production strategies: An examination of future possibilities. *Journal of Experimental Botany*, 55, 1607-1621. DOI: 10.1093/jxb/erh196.

Barker, K. R., Carter, C. C. & Sasser, J. N. (1985). An advanced treatise on *Meloidogyne*. *Plant Pathology*, 4, 212- 233.

Berger, S., Benediktyova, Z., Matous, K., Bonfig, K., Muler, M. J., Nedbal, L. & Roitsch, T. (2007). Visualization of dynamics of plant-pathogen interaction by novel combination of chlorophyll fluorescence imaging and statistical analysisi: differential effects of virulent and avirulent strains of *P. syringae* and of oxylipins on *A. thaliana*. *Journal of Experimental Botany*, 58, 797-806. DOI: 10.1093/jxb/erl208

Berna´t, G., Schreiber, U., Sendtko, E., Stadnichuk, I. N., Rexroth, S., Rogner, M. & Koenig, F. (2012). Unique properties vs. common themes: the atypical cyanobacterium *Gloeobacter violaceus* PCC 7421 is capable of state transitions and blue-light-induced fluorescence quenching. *Plant Cell Physiology*, 53, 528-542. 542. DOI: 10.1093/pcp/pcs009

Boureima, S., Oukarroum, A., Diouf, M., Cisse, N. & Van Damme, P. (2012). Screening for drought tolerance in mutant germplasm of sesame (*Sesamum indicum*) probing by chlorophyll a fluorescence. *Environmental and Experimental Botany*, 81, 37-43.

Brestic, M, & Zivcak M. (2013). PSII fluorescence techniques for measurement of drought and high temperature stress signal in plants: protocols and applications. In: *Molecular stress physiology of plants*. (Edt. by) Rout, G. R. & Das, A. B., Springer Dordrecht pp. 87-131. DOI: 10.1007/978-81-322-0807-5.

FAOSTAT. FAO.org/site/default.aspx.

Gautum, A., Agrawal, D., SaiPrasad, S. V. & Jajoo, A. (2014). A quick method to screen high and low yielding wheat cultivars exposed to high temperature. *Physiology and Molecular Biology of Plants*, 20, 533-537. doi: 10.1007/s12298-014- 0252-4

Gharabadiyan, F., Jamali, S., Ahmadiyanyazdi, A. & Eskandari, A. (2012). Source of resistance to root-knot nematode (*Meloidogyne javanica*) in tomato cultivars. *Journal of Agricultural Technology*, 8, 2011-2012.

Gottardini, E., Cristofori, A., Cristofolini, F., Nali, C., Pellegrini, E., Bussotti, F. & Ferretti, M. (2014). Chlorophyll-related indicators are linked to visible ozone symptoms: evidence from a field study on native Viburnum lantana L. plants in northern Italy, *Ecological Indicators*, 39, 65-74. DOI: 10.1016/j.ecolind.2013.11.021.

Guantem , L., Zhang, Y., Jung, M., Joiner, J., Voigt, M., Berry, J. A., Frankenberg, C., Huete, A. R., Zarco-Tejada, P. & Lee, J. E. (2014). Global and time resolved monitoring of crop photosynthesis with chlorophyll fluorescence. *Proceedings of the National Academy of Science of the United States of America*, 14, 1327-1333. Doi: 10.1073/pnas.1320008111.

Hartman, K. M. & Sasser, J. N. (1985). Identification of *Meloidogyne* species on the basis of differential host test and perineal pattern morphology. In: *An Advanced Treatise on Meloidogyne. Vol. 2. Methodology*. (Edt. by) Barker, K. R., Carter, C. C. & Sasser, J. N. North Carolina State University Graphics, Raleigh, USA, 69-77 pp.

Hille, J., Koornneef, M., Ramanna, M. S. & Zabel, P. (1989). Tomato: a crop species amenable to improvement by cellular and molecular methods, *Euphytica*, 42, 1-23.

Hussey, R. S. & Barker, K. R. (1973). A comparison of methods of collecting inocula of *Meloidogyne* spp. including a new technique, *Plant Disease Reporter*, 57, 1025-1028.

Jedmowski, C., Ashoub, A. & Bru¨ggemann, W. (2013). Reactions of Egyptian landraces of *Hordeum vulgare* and *Sorghum bicolor* to drought stress, evaluated by the OJIP fluorescence transient analysis, *Acta Physiologiae Plantarum*, 35, 345-354. DOI: 10.1007/s11738-012-1077-9.

Jepson, S. B. (1987). *Identification of root-knot nematodes*. Cambrian News Ltd, 265 p.

Mathur, S., Agrawal, D. & Jajoo, A. (2014). Photosynthesis: limitations in response to high temperature stress. *Journal of Photochemistry and Photobiology*, B: Biology, 137, 116-126. DOI: 10.1016/j.jphotobiol.2014.01.010.

Oxborough, K. (2004). Imaging of chlorophyll a fluorescence: theoretical and practical aspect of an emerging technique for the monitoring of photosynthetic performance. *Journal of Experimental Botany*, 55, 1195-1205. DOI:10.1093/jxb/erh145.

Stirbet, A. & Govindjee (2012). Chlorophyll a fluorescence induction: a personal perspective of the thermal phase, the J-I-P rise. *Photosynthesis Research*, 113, 15-61. Doi: 10.1007/s11120-012-9754-5.

Stirbet, A. (2013). Excitonic connectivity between photosystem II units: what is it, and how to measure it? *Photosynthesis Research*, 116, 189-214. DOI: 10.1007/s11120-013-9863-9

Stirbet, A., Lazar, D., Kromdijk, J. & Govindjee (2018). Chlorophyll a fluorescence induction: Can just a one-second measurement be used to quantify abiotic stress responses? *Photosynthetica*, 56, 86-104. Doi: 10.1007/s11099-018-0770-3.

Taylor, D. P. & Netscher, C. (1974). An improved technique for preparing perinneal pattern of *Meloidogyne* spp. *Nematologica*, 20, 268-269 pp.

Tomar, R. S. & Jajoo, A. (2013). A quick investigation of the detrimental effects of environmental pollutant polycyclic aromatic hydrocarbon fluoranthene on the photosynthetic efficiency of wheat (*Triticum aestivum*). *Ecotoxicology*, 22, 1313-1318. DOI: 10.1007/s10646-013-1118-1.

Tuba, Z., Saxena, D. K., Srivastava, K., Singh, S., Czebol, S. & Kalaji, M. H. (2010). Chlorophyll a fluorescence measurements for validating the tolerant bryophytes for heavy metal (Pb) biomapping. *Current Science*, 98, 1505-1508.

Yang, J., Kong, Q. & Xiang, C. (2009). Effects of low night temperature on pigments, chl a fluorescence and energy allocation in two bitter gourd (*Momordica charantia* L.) genotypes, *Acta Physiologiae Plantarum Acta*, 31, 285-293.DOI: 10.1007/s11738-008-0231-x

Ye, D. Y., Qianchum, T. & Chen, J. F. (2011). Photosynthetic response to the root-knot nematode *Meloidogyne incognita* in resistant cultivar sour cucumber (*Cucumis hysteria* Chaker.). *Chinese Journal of Agriculture Science*, 44, 4248-4257.

Zivcak, M., Kalaji, M. H., Shao, H. B., Olsovska, K. & Brestic, M. (2014). Photosynthetic proton and electron transport in wheat leaves under prolonged moderate drought stress. *Journal of Photochemistry and Photobiology*, B Biology, 137, 107-115. DOI: 10.1016/j.jphotobiol.2014.01.007l

Zobayed, S., Afreen, F. & Kozai, T. (2005). Temperature stress can alter the photosynthetic efficiency and secondary metabolite concentrations in St. Johns Wort. *Plant Physiology and Biochemistry*, 43, 977-984. DOI: 10.1016/j.plaphy.2005.07.01

Zushi, K., Kajiwara, S. & Matsuzoe, N. (2012). Chlorophyll a fluorescence OJIP transient as a tool to characterize and evaluate response to heat and chilling stress in tomato leaf and fruit. *Scientia Horticulturae*, 148, 39-46. DOI*:* 10.1016/j.scienta.2012.09.022