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Effects of seed priming with salicylic acid on chlorophyll *a* fluorescence parameters of basil (*Ocimum basilicum* L.) infested by field dodder (*Cuscuta campestris* Yunk.)

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Abstract
This research was undertaken to assess the ameliorative effect of salicylic acid (0.5 mM) on photosystem II (PS II) in two basil (*Ocimum basilicum* L.) varieties (Iranian and Italian) infested with field dodder (*Cuscuta campestris* Yunk.) in the greenhouse of University of Tabriz, Iran, in 2017. The treatments were arranged as factorial using randomized complete block design with four replications. Results indicated that application of salicylic acid improved the maximum quantum efficiency of photosystem II (F_v/F_m) and performance index (PI) of basil plants infested by field dodder. The time span from F_0 to F_m (T_{FM}) and the energy necessary for the closure of all reaction centers (S_m) were significantly increased and the size of the plastoquinone pool (Area) declined in plants exposed to field dodder infestation. Application of salicylic acid increased the chlorophyll *a* fluorescence parameters studied in both basil varieties, especially in the Italian variety.

Keywords: *Cuscuta campestris*; chlorophyll *a* fluorescence; *Ocimum basilicum*; Salicylic acid, Seed priming.

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Introduction

Sweet basil (*Ocimum basilicum* L.) is an annual medicinal plant from Lamiaceae family that is widely used for food and oral care (Tewari *et al.* 2012). *O. basilicum* is regarded as the common host of field dodder (*Cuscuta campestris* Yunck.), a holostemparasitic weed species, in Iran (Browicz *et al.* 1982). *C. campestris* infestation reduces the growth and productivity of sweet basil plants (Behbahani 2014). This weed obtains its needs entirely from the host plants by the production of absorptive haustoria (Aly 2013).

Salicylic acid (SA) is a signaling hormone that regulates biological processes, such as thermogenesis, flowering or defense against pathogens (Nawrath *et al.* 2005). Application of exogenous SA provides protection against several types of biotic and abiotic stresses (Janda *et al.* 2014). Farooq *et al.* (2008) reported that the seed treatment with SA not only enhanced seedling emergence, root and shoot length, seedling fresh and dry weight, and leaf and root score, but also improved the chilling tolerance in maize mainly by the activation of several antioxidants. Gharib and Hegazi (2010) also suggested that SA could alleviate the harmful effects of cold stress in common bean. According to Bayat *et al.* (2012), SA may ameliorate the adverse effects of salinity on the growth and ornamental traits of *Calendula officinalis* L. Priming with SA can enhance defenses mechanisms of plants in response to parasitism by viruses, bacteria and fungi (Luna *et* *al.* 2012). Priming with SA not only improved seedling growth, but also activated the antioxidant defense system under high temperature and salinity in sweet sorghum (Nimir *et al*. 2015) and under chilling stress in rice seedlings (Pouramir-Dashtmian *et al*. 2014). SA mediated changes in growth, photosynthesis, nitrogen metabolism, antioxidant enzymes and proline in *Cicer arietinum* (Hayat *et al*. 2012). Under stress conditions, SA have shown beneficial impact on plants by accumulation of osmotically active compounds such as proline (Szepesi *et al.* 2005). Janda *et al.* (2000) reported that exogenous SA application decreased net photosynthesis, stomatal conductivity and transpiration prior to cold stress, but had no effect on maximum quantum efficiency of photosystem II (PSII) (Fv/Fm). Munne-Bosch and Penuelas (2003) observed that the drought-induced increases in accumulation of endogenous SA in *Phillyrea angustifolia* plants leaves were associated with decreases in the maximum efficiency of PSII (Fv/Fm). However, Ghassemi-Golezani and Lotfi (2015) found that after SA application in plants under saline and non-saline conditions, the initial fluorescence (F_0) decreased, but F_v/F_m and performance index (PI) increased.

As SA has a vital role in signaling adaptive responses to several type of stresses by regulating the antioxidant system (He and Zhu 2008), the present study aimed to investigate the impact of SA in improving the defense system of sweet basil against field dodder by changing the chlorophyll a fluorescence (ChlF) parameters.

Materials and Methods

Experimental conditions

This research was conducted to determine the effect of seed priming with salicylic acid on chlorophyll *a* fluorescence of Italian (Italian Large Leaf) and Iranian (Mobarake) sweet basil (*O. basilicum*) varieties infested by field dodder (*C. campestris*). The experiment was conducted in 2018 in a greenhouse of the Faculty of Agriculture, University of Tabriz, Iran. The treatments were arranged as factorial on the basis of randomized complete block design with four replications. Seeds of basil varieties were obtained from Eden Brothers and Pakan Bazr Company and seeds of field dodder were obtained from the margins of the research field of the Faculty of Agriculture, University of Tabriz, Iran. The field dodder seeds were sanded down with small sandpaper to break their dormancy. Both basil and field dodder seeds were first disinfected with Benomyl at a rate of 2 g/kg, then washed with distilled water several times. The disinfected basil seeds were pretreated with 0.5 mM of salicylic acid for 24 h at 25°C. Then seeds from each one of the basil and field dodder species were sown in pots (height 23 cm; diameter 7 cm), filled with a silty loam soil. Physical and chemical properties of the experimental soil are shown in Table 1. A parallel setup, but without using SA and field dodder seed, served as the control. All pots kept in the greenhouse under following conditions: natural light 14 h, minimum and maximum temperature 25 and 30 °C, respectively, relative humidity 35–40%. The plants were harvested at flowering stage, about 60 days after planting. Basil plants were thinned to 6 per pot after emergence and before infestation with field dodder. To keep the soil water content near field capacity during the growing period, the

Table 1. Some physicochemical characteristics of the used soil in pots.

Soil	
Texture	Silty loam
pH	8
EC (dsm^{-1})	1.23
Organic carbon ($g\ kg^{-1}$)	14.1
Total N (%)	0.05
P ($mg\ kg^{-1}$)	33
K ($mg\ kg^{-1}$)	165
CEC ($cmol\ kg^{-1}$)	17.4

plants were watered with the same amount of water every 1 or 2 days.

Chlorophyll *a* fluorescence

Chlorophyll a fluorescence (ChlF) parameters were measured by Handy-PEA portable fluorimeter. Fluorescence emission was monitored from the upper surface of the leaves. The initial fluorescence (F_0) was estimated after dark-adaptation of the leaves for 30 min and maximum fluorescence (F_m) was obtained after exposure to the saturated white light. The following parameters were also measured: variable fluorescence (Fv), photosynthetic performance index (PI), the time needed to reach the maximum fluorescence (T_{FM}), the energy necessary for the closure of all reaction centers (Sm) and the area above the fluorescence induction OJIP curve between O and P (Area). The maximum efficiency of PSII (F_v / F_m) and S_m were determined by the following formulae (Kalaji *et al*. 2011):

$$F_v / F_m = (F_m - F_0) / F_m$$

$$S_m = Area / (F_m - F_0)$$

Statistical analysis

Data analysis was performed by SAS 9.1.3, and the figures were drawn by Excel 2013 software. After analysis of variance, the means for each trait were compared by Duncan's Multiple Range test at 0.05 level of significance.

Results

Basil varieties showed different response to application of salicylic acid (SA) and field dodder infestation (Table 2). The stress of field dodder imposed on basil plants led to reductions of the parameters F_v/F_m, PI and Area (Figure 1 and Table 3). Application of SA significantly improved maximum efficiency of PSII (F_v/F_m) in basil plants infested by field dodder. Differences between SA-treated and untreated plants for F_v/F_m under control conditions were not significant (Figure 1a). PI of plants exposed to field dodder infestation decreased, but application of SA increased PI of plants significantly under both infested conditions. The highest PI was recorded for SA-treated plants under non-infested condition. However, in non-infested condition, there was no significant

Table 2. Analysis of variance for the chlorophyll fluorescence *a* parameters of the basil varieties in response to field dodder infestation and salicylic acid application.

Source of variation	df	Mean squares				
		F_v/F_m	PI	T_{FM}	Area	S_m
Block	3	0.048	0.0001	4111	306684**	0.39
Basil varieties (A)	1	0.001**	0.0001*	13152**	652744**	34.51**
Field dodder (B)	1	0.0001*	0.001	35622**	7011256*	0.78
Salicylic acid (C)	1	0.009**	0.001*	689**	326665	12.67
A × B	1	0.066*	0.0001**	4854*	2954211*	3.30*
A × C	1	0.001	0.0001**	7458**	294757	9.49
C × B	1	0.001	0.001	6805	312106	7.54
A × B × C	1	0.0001*	0.0001*	3255	104788	4.08
Error	21	0.007	0.0004	1035	112989	12.34
CV (%)		9.45	12.68	8.08	9.28	14.09

*p≤ 0.05; **p≤ 0.01; Fv /Fm: ; PI: photosynthetic performance index; T_{FM}: time needed to reach the maximum fluorescence intensity; Area: the area above the fluorescence rise between F_0 and F_m; S_m: energy needed to close of all reaction centers.

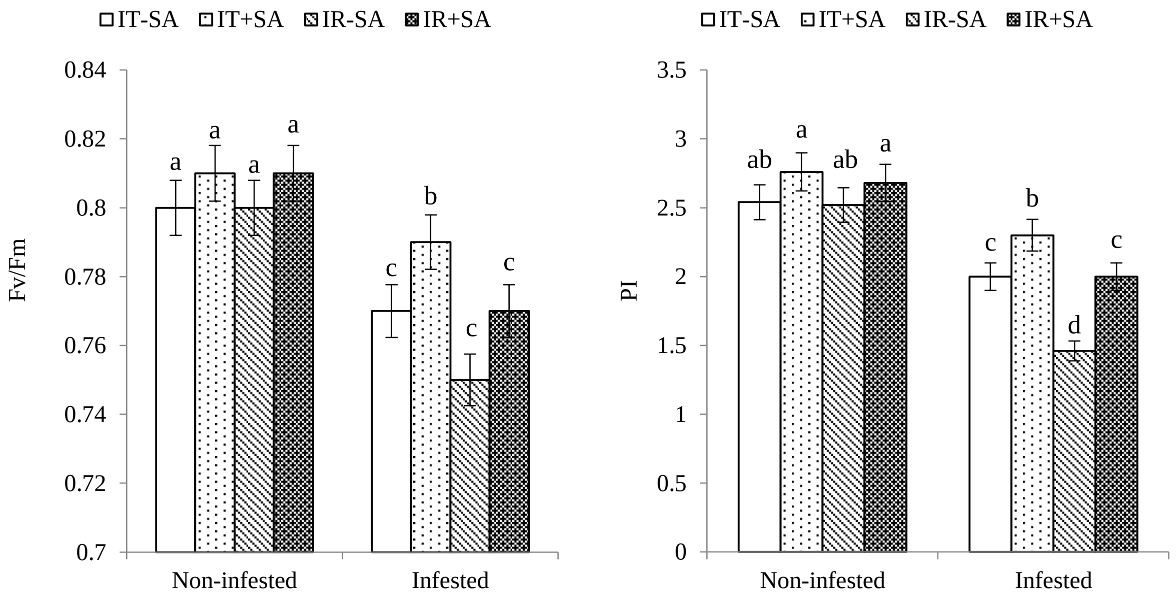

Figure 1. Response of basil varieties to salicylic acid under field dodder infestation; (a): F_v/F_m; (b): PI; each value is the mean four replicates; bars represent standard error of the mean; means with different letters within each characteristic show significant difference, based on Duncan's Multiple Range Test at $p \leq 0.05$); IT: Italian variety; IR: Iranian variety; SA: salicylic acid**.**

difference in PI between SA-treated and untreated plants (Figure 1b).

T_{FM} and S_m increased, but the size of the plastoquinine pool in PSII (Area) decreased significantly in basil plants infested by field dodder (Table 3). T_{FM} was also significantly affected by application of SA. SA-treated plants had lower T_{FM} than the plants under non-SA treatment (Figure 3).

As shown in Figures 1 and 2 and Table 3, Iranian cultivar was more sensitive to field dodder infestation in terms of F_v/F_m, PI and T_{FM}. In addition, the favorable effects of salicylic acid on F_v/F_m, PI and T_{FM} were significantly higher in Italian cultivars under field dodder infestation conditions.

Table 3. Means of T_{FM}, S_m and Area of basil cultivars under field dodder infestation.

Field dodder	T_{FM} (ms)		S_m		Area	
	Italian variety	Iranian variety	Italian variety	Iranian variety	Italian variety	Iranian variety
Not infested	321 c	309 d	22.54 c	22.32 c	67000 a	67000 a
Infested	480 b	493 a	27.08 b	30.54 a	54000 b	51000 c

Different letters within each characteristic indicate significant difference at $p \leq 0.05$, based on Duncan's Multiple Range Test; T_{FM}: time needed to reach the maximum fluorescence intensity; S_m: energy needed to close of all reaction centers; Area: the area above the fluorescence rise between F_0 and F_m.

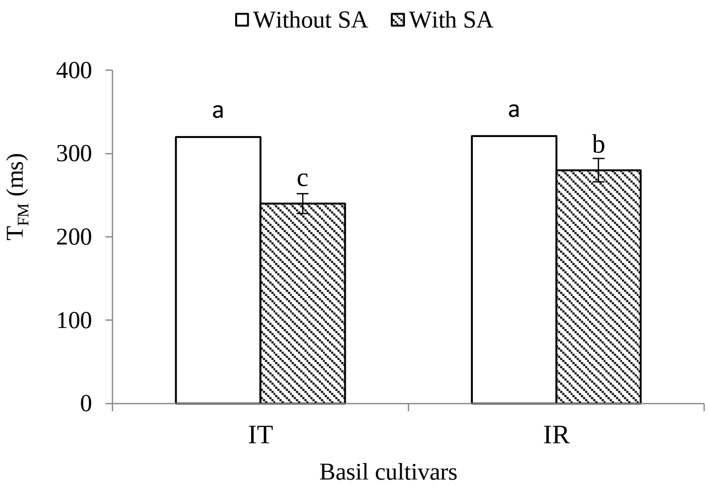

Figure 2. Changes in T_{FM} of basil varieties (left in response to salicylic acid application; each value is the mean of four replicates; means with different letters show significant difference, based on Duncan's Multiple Range Test at $p \leq 0.05$); IT: Italian variety; IR: Iranian variety; SA: salicylic acid**.**

Discussion

Our analysis of data showed that field dodder severely hampered PSII activity in basil leaves and declined F_v/F_m (Figure 1a). This finding shows the destruction of PSII reaction centers in basil plants parasitized by field dodder. A significant decline in F_v/F_m also indicated an increase in energy dissipation as heat and the destruction of photosynthetic apparatus (Lotfi *et al*. 2005). These results are in agreement with those of Saric-Krsmanovic *et al.* (2018) who reported lower values of this parameter in plants infested by the field dodder.

Seed priming with SA increased chlorophyll *a* fluorescence by increasing F_v/F_m under field dodder infestation condition (Figure 1a). Gornik and Lahuta (2017) also reported that SA pretreatment caused an increase in F_v/F_m. Seed priming with SA has stimulatory effects on pigments content, Rubisco activity and chlorophyll *a* fluorescence (Janda *et al*. 1998). According to Saric-Krsmanovic *et al.* (2018), changes in F_0 value in plants infested by field dodder increase the probability of energy trapping by PSII centers. Seed priming with SA may affect photosynthetic apparatus in mature basil plants by declining the number of inactive PSII reaction centers where electrons can be transferred out of decreased QA, which leads to increase in F_0 and Fv/Fm (Lotfi *et al*. 2005) (Figure 1a).

Seed priming with SA significantly improved the photosynthetic performance index (PI) of basil plants infested by field dodder (Figure 1b). SA may have improved PI by affecting the maximum quantum yield of primary photochemistry, the quantum yield of electron transport and number of reaction centers (Lotfi *et al.* 2015).

This research showed that parasitism with field dodder significantly increased T_{FM} and S_m, and decreased the Area in sweet basil plants (Table 3). According to Mehta *et al.* (2010), reduction in the Area parameter is due to the blockage of electron transfer from reaction centers. The increase in T_{FM} and S_m may be attributed to increasing energy necessary for the closure of all reaction centers (S_m), and consequently increasing the time to reach F_m (T_{FM}). However, seed treatment with SA reduced T_{FM} (Figure 2) and increased Fv/Fm in mature basil plants, which increased the average redox state of Q_A from 0 to T_{FM} (Lotfi *et al.* 2015). Cameron *et al.* (2008) also reported the photosynthesis suppression of *Phleum bertolinii* and *Plantago lanceolate* by the parasitic *Rhinanthus minor.*

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اثر پرایمینگ بذر با اسید سالیسیلیک روی پارامترهای فلورسانس کلروفیل a در ریحان آلوده به سس (*Cuscuta campestris* Yunk.)

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چکیده

این تحقیق به منظور ارزیابی اثر بهبود دهنده اسید سالیسیلیک (۰/۵ میلی مولار) بر فتوسیستم II (PS II) در دو رقم ریحان (*Ocimum basilicum* L.) (ایرانی و ایتالیایی) آلوده به علف هرز سس مزرعه (*Cuscuta campestris* Yunk.) در شرایط گلخانه در دانشگاه تبریز در سال ۱۳۹۶ انجام شد. آزمایش در قالب فاکتوریل بر پایه طرح بلوک‌های کامل تصادفی در چهار تکرار پیاده شد. نتایج نشان داد که کار برد اسید سالیسیلیک، حداکثر کارایی کوانتومی فتوسیستم II (F_v/F_m) و شاخص عملکرد فتوسنتزی (PI) را در ریحان آلوده به سس بهبود داد. در گیاهان آلوده به سس، مدت زمان لازم بین فلورسانس حداقل و حداکثر (T_{FM}) و انرژی لازم برای بسته شدن مراکز واکنش (S_m) به طور معنی‌داری افزایش و میزان پلاستوکوئینون موجود (Area) کاهش یافت. استفاده از اسید سالیسیلیک پارامترهای مورد مطالعه فلورسانس کلروفیل *a* را در هر دو رقم ریحان افزایش داد، ولی این افزایش در رقم ایتالیایی بیشتر بود.

واژه‌های کلیدی: اسید سالیسیلیک؛ پرایمینگ بذر؛ ریحان ؛ سس؛ فلورسانس کلروفیل *a*.