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Effect of Different Herbicides and Salicylic Acid Treatment on the Photosynthetic Efficiency of Corn Cultivars Using Chlorophyll a Fluorescence Transient Curve Analysis

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Abstract
Photosynthesis is an essential part of the plant function and metabolism and a number of herbicides inhibit certain photosynthetic activities. The aim of this study was to evaluate the effect of some herbicides including Topik, Titos, Equip, Mister, Lumax, Bromicide and Oltima on the photosynthetic efficiency of three corn cultivars (CC-260, 400, 704) in response to salicylic acid (SA) treatment via chlorophyll a fluorescence (ChlF) transient curve (OJIP) analysis. Result showed that Topik, Titos, Equip, Mister and Oltima herbicides had no effect on the ChlF. In contrast, Bromicide and Lumax changed the shape of the ChlF curve as I phase of the OJIP curve was lost and the fluorescence level in the J step was close to I stape. However, application of SA improved the shape of the OJIP curve similar to normal samples. SA application under Bromicide and Lumax treatments un-blocked the electron transfer between Q_A and Q_B of PSII. Our study clearly indicated that the effect of SA on the I-P part of the ChlF curve was more than other parts. Thus, the effect of SA on the PSI activity was more than that of PSII under these herbicide treatments.

Keywords: Chlorophyll a fluorescence; Corn; Herbicide; JIP analysis; Salicylic acid

Introduction
Corn (*Zea mays* L.) plays an important role in the human diet. The performance of this crop is reduced due to the presence of weed species. Weed control is done with different methods and one of them is the chemical control by herbicide application. Different herbicides with different mechanisms disrupt plant growth cycles. These substances modify the cell cycle and plastid metabolism, or they act as endogenous growth regulators, and some others such as electron transport inhibitors, artificial acceptors and inhibitors of ATP synthase, affect the photosynthetic reactions (Percival and Baker 1991). A major location of photosynthetic electron transport inhibition is the donor side of PSII. The mechanism of inhibition stands on a competitive binding of the herbicide to the Q_B^- site on the D_1 protein in the reaction center of PSII (Vermaas *et al.* 1984) thus preventing Q_A^- to reduce Q_B.

Since photosynthesis is an essential part of the plant function and metabolism, and a number of stresses inhibit certain photosynthetic activities, *in vivo* chlorophyll *a* fluorescence (ChlF) has been applied extensively as a fast and non-destructive method for elucidating various aspects of photosynthetic performance in higher plants (Strasser *et al*. 2000, 2004). ChlF signals and fluorescence transients can be analyzed to provide detailed information about the structure, conformation and function of the photosynthetic

apparatus, especially for PSII (Strasser *et al.* 2004; Shabnam *et al.* 2014).

The OJIP fluorescence induction transient is rich in information and can be used to derive a number of parameters by the so-called JIP-test that quantifies the stepwise flow of energy through PSII at the reaction center (RC) level as well as at the excited leaf cross-section (CS) level (Strasser and Strasser 1995). The OJIP Kautsky curves are defined by the names of its intermediate steps (Figure 1): O- level, fluorescence level at 50 ms; J-level, fluorescence plateau at ~2 ms; I-level, fluorescence plateau at ~30 ms; P-level, the maximum fluorescence (Strasser *et al.* 2002; Kalaji and Guo 2008; Brestic *et al.* 2012). JIP-test involves translating the fluorescence measurements of transients (O–J–I–P) into several phenomenological and biophysical expressions that evaluate PSII function (Strasser *et al.* 2004; Jeremy *et al.* 2012). A normal pattern for OJIP (the fluorescence transient) includes two intermediate inflections J (at about 2 ms) and I (at about 20 ms) between the Fo and Fm levels. Due to the fact that the shape of the OJIP fluorescence transient is sensitive to stresses caused by changes in many environmental conditions, the JIP-test is highly suited for *in vivo* investigations of the behavior of the plant photosynthetic apparatus in the field (van Heerden *et al.* 2003).

Salicylic acid (SA) or ortho-hydroxy benzoic acid and other salicylates are known to affect various physiological and biochemical activities of plants and may play a key role in regulating their growth and productivity (Hayat 2010). This hormone is synthesized by many plants and is accumulated in the plant tissues as the impact of unfavorable abiotic factors, contributing to the increase of plants resistance to stress (Ding *et al.* 2002). Ghassemi-Golezani and Lotfi (2015) reported that exogenous foliar application of SA decreased initial fluorescence (Fo) and increased photosynthesis relative vitality (PI). Maximum fluorescence (Fm), variable fluorescence (Fv), the activity of the water-splitting complex on the donor side of the PSII (proportional to Fv/Fo) and the average redox state of Q_A in the time span from O to T_{fm} (Sm/T fm) were also enhanced by foliar application of SA. Exogenous application of SA improved maximum quantum efficiency of PSII (Fv/Fm) and performance index (PI) under both saline and non-saline conditions. Nevertheless, the effects of this hormone on fluorescence chlorophyll a (ChlF) parameters' transient curve under application of herbicide in corn plants are unclear. Therefore, the goal of the present study was to assess ChlF transient of three corn cultivars in response to different recommended herbicides for this plant under different concentrations of SA.

Materials and Methods

Plant materials, growth conditions and herbicide treatments

The effects of six herbicides, Topik, Titos, Equip, Mister, Lumax, Bromicide and Oltima, on photosynthetic efficiency of three corn cultivars (CC-260, CC-400, CC-704) in the presence and absence of SA (1 mM) were studied. A pot experiment based on randomized complete block design with three replications was conducted in 2015 in Tabriz, Iran. For the purpose of investigation, 10 seeds of each corn cultivar were

sown at a depth of 1 cm in each plastic pot (20 × 20 cm^2), containing 1.0 kg of perlite. Then, tap water (0.6 dS m^{-1}) was added to achieve 100% field capacity. All pots were kept inside a glass greenhouse under natural light. The minimum and maximum temperatures of the greenhouse were 27 °C and 30 °C, respectively. After the

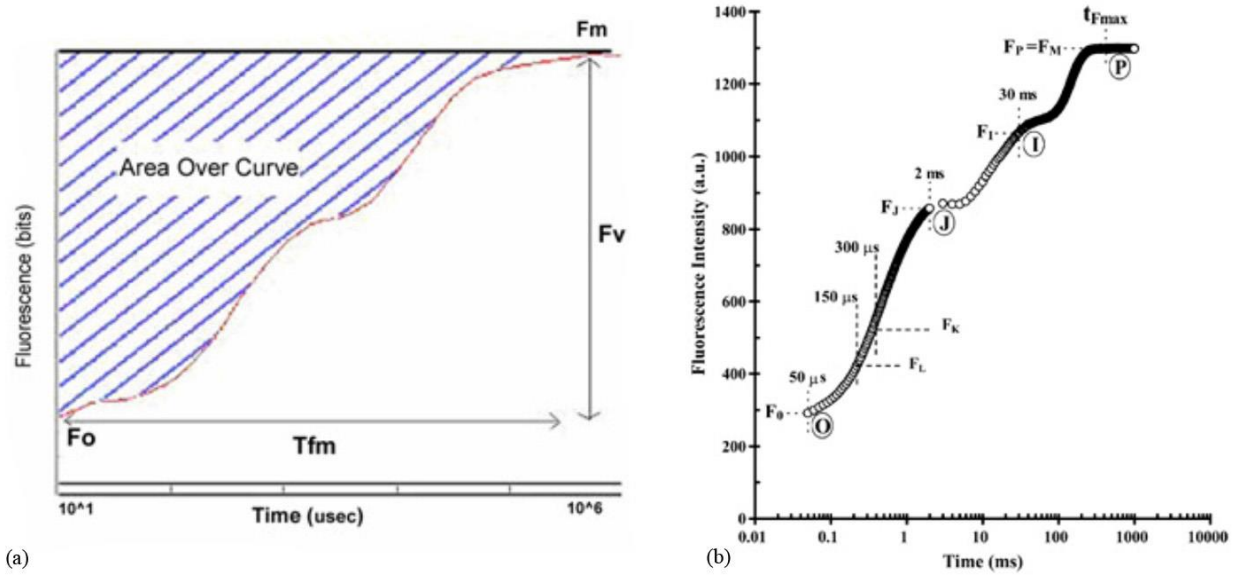

Figure 1. a) Main chlorophyll fluorescence parameters measured by the use of Handy-PEA portable fluorometer (Hansatech Instruments Ltd., UK), b) A typical chlorophyll a polyphasic fluorescence rise OJIP, exhibited by plants. The transients plotted on logarithmic time scale from 50 s to 1 s. The marks refer to the selected time points used by the JIP-test for the calculation of structural and functional parameters. The signals are: the fluorescence intensity Fo (at 50 s), the fluorescence intensities FJ (at 2 ms) and FI (at 30 ms), the maximal fluorescence intensity, FP= FM (at time denoted as tFM) (Strasser *et al.* 2002; Kalaji and Guo 2008; Brestic*et al.* 2012)

seedling establishment, the plants were thinned to five plants per pot. During the growth period (the duration of this period), the pots were weighed and the weight loss was made up with Hoagland solution (Electrical conductivity= 1.3 dS m^{-1} and pH= 6.5-7). The perlite in the pots was washed every 20 days in order to prevent a further increase in electrical conductivity due to adding the Hoagland solution. Herbicides were applied by the recommended dose (2 L ha^{-1}).

Chlorophyll a fluorescence measurements

The induction of ChlF (OJIP transient) was monitored with a Handy-PEA portable fluorometer (Hansatech Instruments Ltd., UK). Before measuring the experimental signals, the plants were kept in the dark for at least 30 min and exposed to saturated white light to estimate the initial (Fo) and maximum (F_m) fluorescence. Measurements were carried out on the upper surface of fully developed leaves after four days of treatment application.

All the data were analyzed on the bases of the utilized experimental design, using SAS 11 software. The means were compered by the LSD test at $p \leq 0.05$.

Results

Corn cultivars showed different response to different herbicides in the presence and absence of SA (Table 1). The minimum and maximum fluorescence (Fo and Fm, respectively) of these

cultivars were significantly different after the application of different herbicides, and application of SA affected this variation (Table 2). Maximum reduction in Fo and Fm was observed on CC-260 plants treated with Bromicide and SA (Table 2).

Control (distil water) and Topik

The chlorophyll *a* fluorescence (ChlF) of the control and Topik herbicide samples showed the typical polyphasic rise of the OJIP transient in all of the corn cultivars (Figure 2). Furthermore, the treated plants with salicylic acid and Topik changed the shape of ChlF transient in all of the cultivars, and consequently Fo increased in CC-704 and decreased in CC-400 and 260, but Fm slightly decreased in all cultivars (Table 2 and Figure 2).

Titos, Equip and Mister

The application of these herbicides changed the shape of OJIP transient curves in corn cultivars after four days of treatment when compared to the control. The consumption of these herbicides in CC-400 and CC-704 treated with SA significantly changed OJIP curves due to changing in ChlF parameters such as Fm in comparison with the control (Table 2 and Figure 3). Foliar application of SA in CC-400 increased the value of J-I-P levels and Fm, after treatment with Titos, Mister and Ecoip herbicides (Table 2 and Figure 3). In contrast, application of SA decreased Fm and the value of the J-I-P levels in CC-704 after the application of these herbicides (Table 2 and Figure 3).

Table 1. Analysis of variance for the chlorophyll fluorescence *a* parameters (F0 and Fm) of the corn cultivars in response to salicylic acid and different herbicides application

Source of variation	df	Mean squares	
		F_0	Fm
Block	2	29371.1*	2873073.7*
Treatment	47	29371.1**	96307.4**
Error	94	63.32	4101.2
CV (%)	-	1.49	2.52

*P≤ 0.05; **P≤ 0.01

Table 2. Mean comparison of minimum (Fo) and maximum (Fm) fluorescence of corn cultivars in response to salicylic acid and different herbicide applications

Treatment		Fo			Fm		
		704	400	260	704	400	260
SA0	Control	503	538	549.3	2381	2421.3	2691
	Topic	505.3	504.3	565.3	2545	2680	2583
	Mister	535.3	512.6	534.3	2529	2614.3	2641.3
	Titos	586	502	504.3	2624	2446	2528.3
	Oltima	481	511.3	535.3	2332	2703	2631.3
	Lomax	553	523	501.3	2481	2627	2581.3
	Bromicide	528	496	519.3	2262	2750.6	2662.3
	Equip	503.3	453	515	2470	2208	2461
SA (1mM)	Control	581	506.3	547	2216	2441	2531
	Topic	589.3	465	539.3	2461	2580	2579
	Mister	530.3	547	527.3	2198	2787	2731.3
	Titos	532.3	547.3	512	2300.3	2656.3	2527.3
	Oltima	516	559.3	467.3	2550.3	2695.3	2490
	Lomax	569	520.3	557	2312	2796.3	2842
	Bromicide	555.3	527	721	2261.3	2691.3	2992.3
	Equip	483.3	513.3	487.3	2318	2424.3	2367
LSD (0.05)							

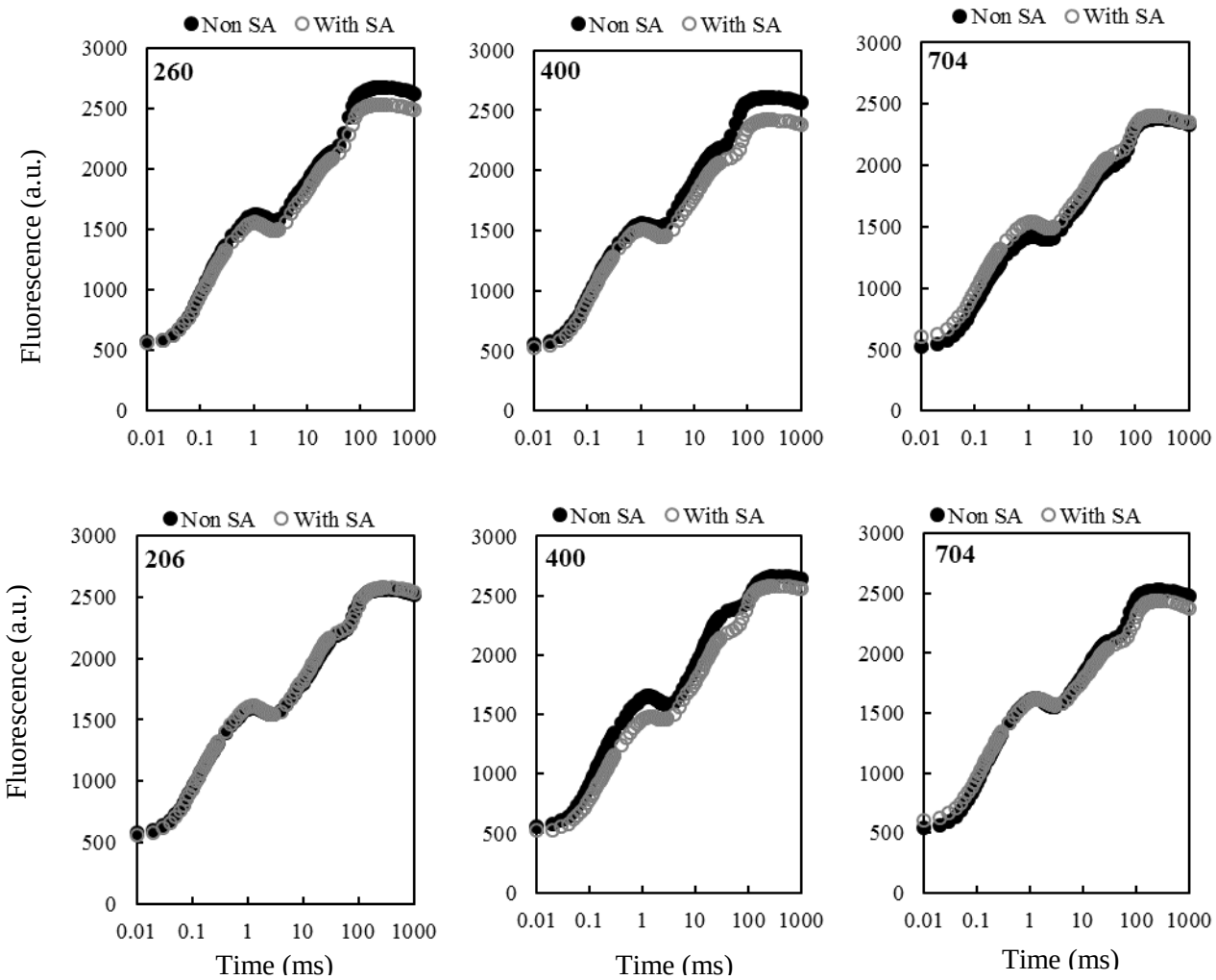

Figure 2. Chlorophyll *a* fluorescence transients plotted on a logarithmic time scale and measured on three corn cultivars in response to salicylic acid under control (up) and application of Topik herbicide (down)

Lumax, Bromicide and Oltima

The CC-260 under Lumax and Oltima had normal shape of ChlF curve, but application of Bromicide on this cultivar changed the shape of OJIP transient (Figure 4). The I- step of the OJIP curve was lost and the fluorescence level in the J step was close to the I step. Application of SA caused CC-260 plants under Bromicide herbicide to have normal shape (Figure 4). SA application on this cultivar under Bromicide and Oltima decreased the level of J-I-P phase, but SA increased the level of these phases under Lumax (Figure 4).

Treated CC-400 plants with Lumax, Bromicide and Oltima changed the shape of ChlF curve in comparison with the control (Figure 4). These herbicides slightly changed Fo and Fm in CC-400 plants treated with SA in comparison with SA0 (Table 2). So, SA application had no effect on the shape and the value of ChlF transient in CC-400. Application of Lumax on CC-704 changed the shape of the ChlF curve, but application of SA on these plants caused plants to have normal shape of the ChlF transient (Figure 4). SA on CC-704 treated with Lomax decreased Fm (Table 2). CC-704 plants under Bromicide

and Oltima had normal OJIP curves. Application of SA in CC-704 under the use of Oltima increased the value of I-P phase due to increasing the Fm significantly as compared to the non-treated plants (Table 2 and Figure 4). Bromicide on CC-260, significantly increased Fo and Fm (Figure 4 and Table 2).

Discussion

In this research different herbicides with different modes of action were applied on several corn cultivars with or without salicylic acid. Some of these herbicides directly affected the photosynthetic apparatus. Damaging effects of one herbicide on plants depend on the particular location where a physiological reaction was inhibited in a plant cell and its compartments. In the chloroplast, there are two main target sites of herbicide action. One target site is represented by the electron transport chain (ETC) with its electron carriers and enzymes, which are involved in phosphorylation and NADP photoreduction. Another main target site is the biosynthesis of chlorophylls and carotenoids that are present in the light-harvesting complex (LHC) and the antennae of the photosynthetic reaction centers (Dayan and Zaccaro 2012).

The OJIP transient recorded in corn cultivars treated with Topik (Figure 2), Titos, Equip, Mister (Figure 3) and Oltima (Figure 4) herbicides were similar to the control treatment without changing the shape of ChlF curve. However, application of Lumax on CC-704 and Bromicide on CC-260 affected the shape of the OJIP curves greatly (Figure 4). But, application of SA with both herbicides on both cultivars changed the shape of the ChlF curve similar to normal samples (Figure 4). In other words, the mode of action of Bromicide and Lumax treatments was through the blocking of electron transfer between the primary and secondary quinones (Q_A and Q_B) of PSII by binding to the Q_B-binding site and accepting electrons from Q_A in the chloroplasts (Govindjee *et al*. 1997; Hess 2000; Ikeda *et al.* 2003). The photosynthetic electron transport chain was interrupted, leading to the concomitant inhibition of ATP production and carbon fixation (Hess 2000; Kohno *et al.* 2000). Plant parts are supposed to act as redox-cycling electron acceptors in the chloroplasts, where they mediate in the transfer of electrons from ferredoxin in PSI to molecular O_2, thereby blocking photosynthetic electron transfer and the formation of NADPH (Rabinowitch and Fridovich 1983). Each step of the Kautsky curve represents those photochemical events associated with PSII and a straight line (in our study between J-I steps) represents a loss of the OJIP steps, indicating serious damage to the electron transfer chain in photosynthesis. The final I–P part of the fast ChlF transient reflects the rate of reduction of ferredoxin (Schansker *et al.* 2003; Schansker *et al.* 2005) and is taken as a measure of the relative abundance of PSI with respect to PSII (Desotgiu *et al.* 2010).

Our study clearly indicated that the effect of SA on the I-P part of the curve was higher than other parts. The response of the cultivars to SA under various herbicides was different. The CC-260 was not affected by the application of Titos, Equip and Mister. However, the I-P phase of ChlF in CC-400 increased but in CC-704 decreased as the result of those herbicides (Figure 3).

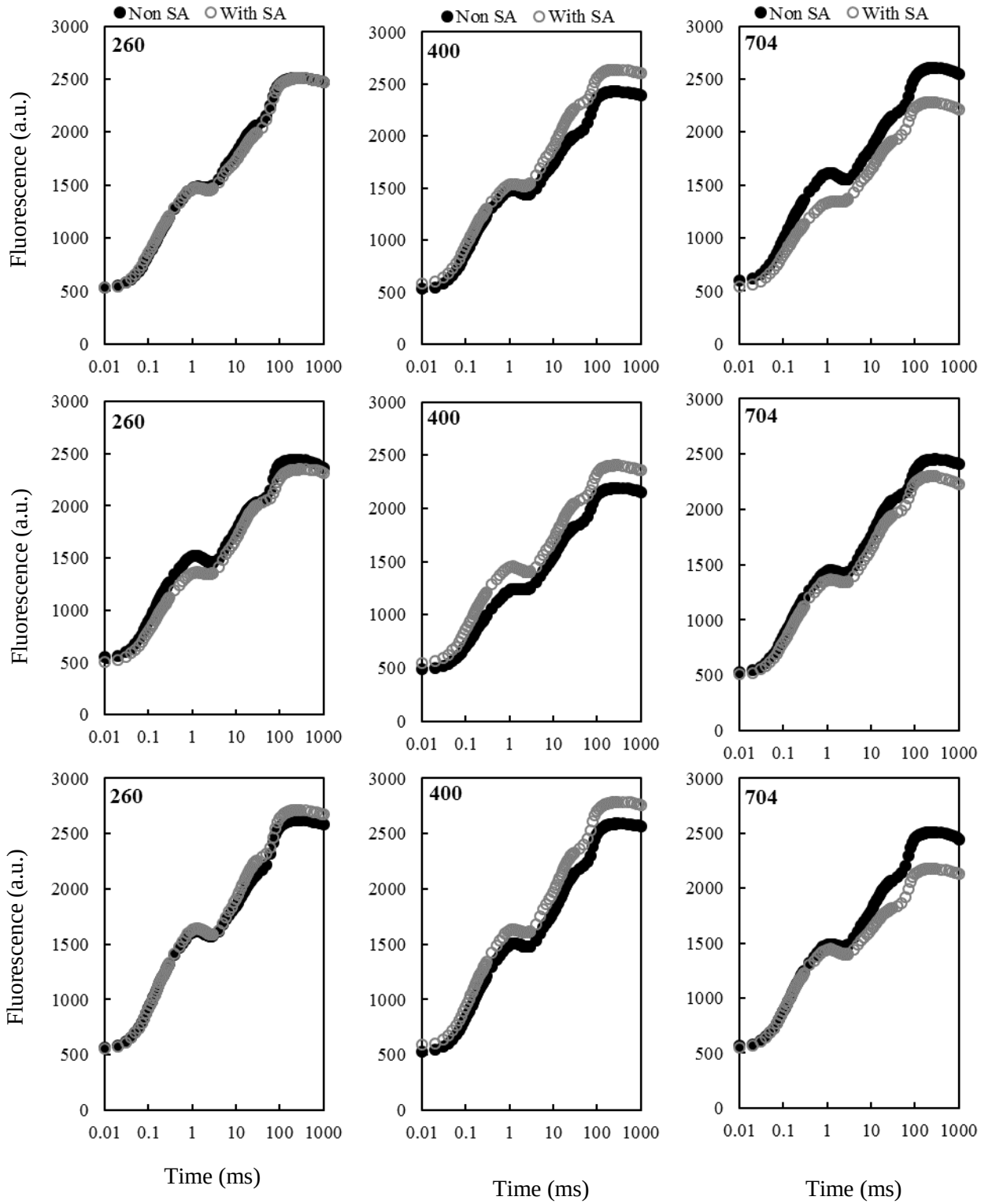

Figure 3. Chlorophyll *a* fluorescence transients plotted on a logarithmic time scale and measured on three corn cultivars in response to salicylic acid under application of Titos, Equip and Mister herbicides

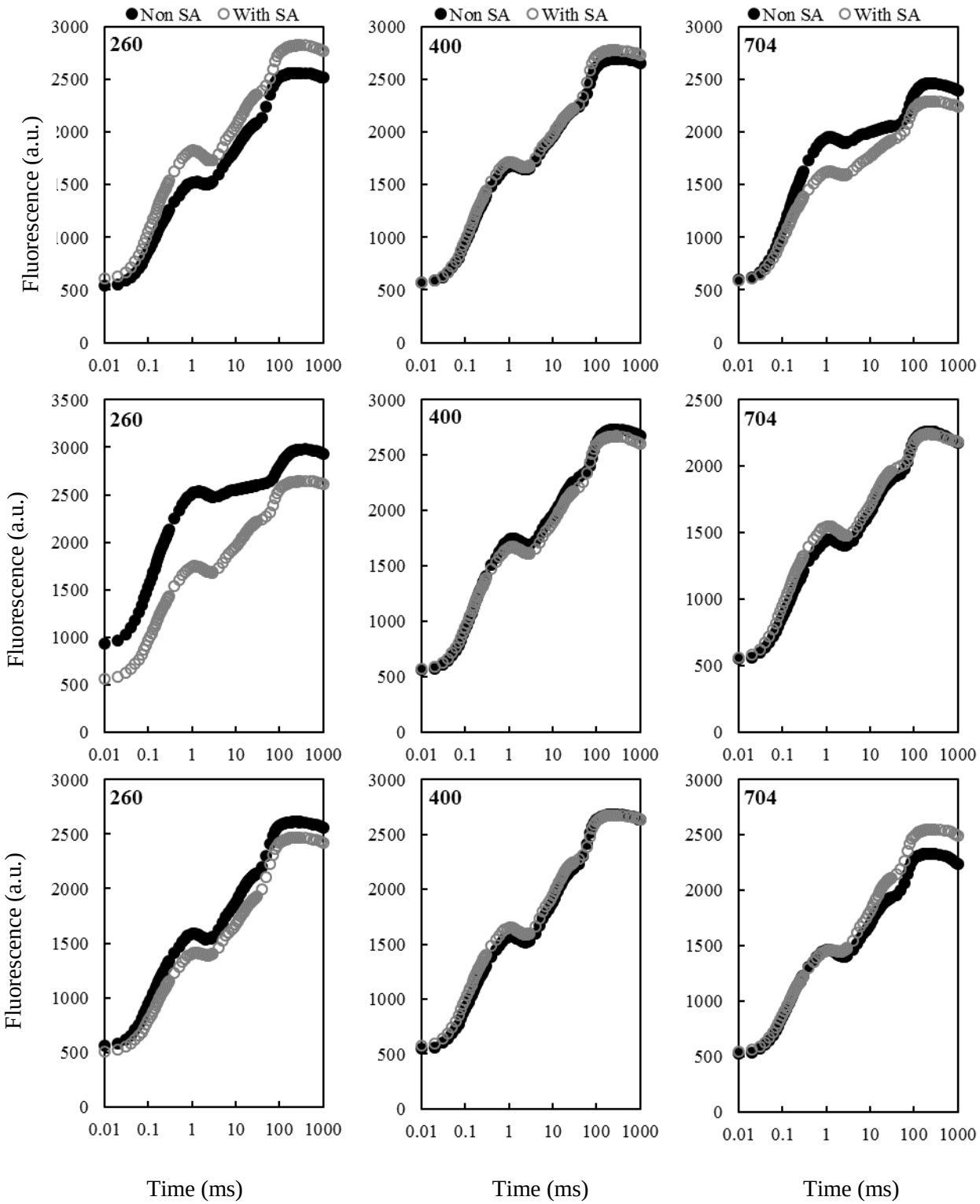

Figure 4. Chlorophyll *a* fluorescence transients plotted on a logarithmic time scale and measured on three corn cultivars in response to salicylic acid under application of Lumax, Bromicide and Oltima herbicides

Application of SA on plants under Lumax increased Fm and the value of the J-I-P phases in CC-260 but decreased Fm and these phases in CC-704 (Table 2 and Figure 4). All of the ChlF transient curves with application of SA in CC-260 decreased under Bromicide; on the other hand, SA

significantly reduced Fo and Fm, and changed OJIP curve (Table 2 and Figure 4). According to our study the effect of SA on the PSI was more than that of PSII under herbicide treatments. An enhancement in Fm when SA is applied may be caused by the inducing of electron transport at the donor side of PSII, which decreases Tfm at most of the measuring times.

It seems that electron leakage in the thylakoid membrane and oxidative damage to the thylakoid phospholipid membrane of the chloroplasts interrupts the electron transport chain (Z scheme) from PSII to PSI. This interruption of the Z scheme will change the shape of the Kautsky curves (Dayan and Zaccaro 2012). PSI inhibitor herbicides require light in order to generate superoxide radicals. This, in turn, generates other reactive radicals, including the highly toxic hydroxyl radicals, and ultimately leads to the peroxidation of lipid bilayers (Dayan and Watson 2011).

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