Acta Sci. Pol. Hortorum Cultus, 16(2) 2017, 57–74

www.acta.media.pl ISSN 1644-0692

ORIGINAL PAPER

Accepted: 13.10.2016

FOLIAR APPLICATION OF SALICYLIC ACID WITH SALINITY STRESS ON PHYSIOLOGICAL AND BIOCHEMICAL ATTRIBUTES OF SUNFLOWER (*Helianthus annuus* L.) CROP

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ABSTRACT

The potential agricultural lands are falling prey to salinity in the world over including Pakistan. The limited water supply is also becoming a serious problem to feed the humans and livestock production. Therefore, research studies were undertaken to enhance the growth and development of sunflower (*Helianthus annuus* L.) on saline soils to increase productively of crop. The treatments consisted of (a) two lines of sunflower (Hysun-33 and LG-56-63), (b) two levels of salinity (0, 120 mM (NaCl)) and (c) two levels of salicylic acid (0, 200 mg L^{-1}) and were arranged in a completely randomized design with four replications. The results showed that biological yield was significantly reduced due to imposition of salinity at the rate of 120 mM (NaCl) on both sunflower lines (Hysun -33, LG 56-63). The stem length was also reduced due to decrease in biological yield in response to salinity. However, the exogenous application of salicylic acid at the rate of 200 mg L^{-1} mitigated the adverse effects of salts and improved the biological yield and stem length under saline and non-saline environments. The quantity of chlorophyll (SPAD) values were impacted negatively in response to salt stress, however, the phenomenon was recovered by foliar spray of salicylic acid. The nutrient concentration of K^+, Cl^- and Na^+ were altered because of presence of excess quantity of NaCl in the substrate. The translocation of K^+ ion was reduced substantially, while higher amount of Na^+ and Cl^- ions were absorbed, thus creating ionic imbalance in the plant system. The foliar spray of salicylic acid (200 mg L^{-1}) enhanced the uptake of K^+ from the soil medium. The salicylic acid proved a potential phytoprotectant to mitigate the adverse effects of salinity and thereby improving the physiological and biochemicals attributes, stem length and also enhanced uptake of K^+ ion while depressing Na^+ and Cl^- ions in plant system.

Key words: Salinity, salicylic acid, biological yield, Na^+, Cl^- and K^+ contents

INTRODUCTION

Currently, the world is experiencing continuous decline in productivity of crops, because of a number of eco-edaphic factors [Hasanuzzaman et al. 2012, 2013 a–c]. Among these, salinity is a major environmental stress. It results in limiting the germination, plant vigor and yield of most of agricultural crops to a significant proportion [Hasanuzzaman et al. 2013 b, c, Shahbaz and Ashraf 2013]. Most affected regions of

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the world are arid and semi- arid regions. Out of the total arable lands, one fifth of the land is hard hit by salinity and more area is also falling prey to this menace [Rasool et al. 2013]. On the other hand, the population of the world has reached to about 7.2 billion and is expected to rise by 9.6 billion by the year 2050. This phenomenal growth in population as well as livestock demands to raise the productivity of crops by more than 70% over the current level to suffice the future needs [Varshney et al. 2013].

Thereby, the galloping efforts are needed to address various environmental stresses to sustain the productivity of crops, particularly staple foods and oilseed crops. The severe salinity stress results in significant changes in plant systems, i.e., water stress, toxicity of ions, nutritional imbalances, oxidative stress and reduction in cell progression and disintegration of membrane structures [Djanaguiraman and Prarad 2013, Shahbaz and Ashraf 2013]. Resultantly, the plant growth and potential productivity of plants for production of economic yield is affected to a significant level under salinity stress [Hessini et al. 2015]. Razzaghi et al. [2015] reported that negative effects of salts could be mitigated by regulating the photosynthetic capacity in crop plants, because the photosynthetic capacity in directly related to stomatal and non-stomatal limitations. The various physiological processes occurring during the period from germination of seed to maturity are greatly impacted by the amount of soil moisture, concentration of salts and their interactive effects and crop species. The photosynthetic capacity of wheat and *Brassica* spp were significantly affected by salinity stress. The salinity stress led to the oxidative stress, because of phenomenal production of reactive oxygen species (ROS) with concurrent reduction in molecular oxygen in various crop plants [Shahbaz and Ashraf 2013].

The deleterious effects of ROS could be mitigated by simulating several metabolites, including phytohormones and changes at the level of endogenous macromolecules [Razzaghi et al. 2015]. Thereby, the negative effects of salinity could be averted by adoption of short-term and long-term strategies. On the long-term frontiers, the genetic manipulations to incorporate the traits of tolerance salt stress would be economical and value for money to sustain productivity [Ashraf and Harris 2013]. However, this adaptive strategy requires heavy investment both in capital goods and human resources [Ashraf and Harris 2013]. Furthermore, the abiotic stresses are genetically controlled by multiple genes in plants, in contrast to biotic stresses, which are monogenic ones. The success of genetically modified crop species depends on expression of genes, which are involved in signaling and regulatory pathways in response to environmental stresses. Thereby, the expression of tolerance to abiotic stresses including salinity is unpredictable and compounded by other prevailing limitations [Fayez and Bazaid 2014]. Alternatively, the short term strategy by employing anti-oxidant compounds offers their potential services to alleviate the perils of salinity on productivity of crops [Fayez and Bazaid 2014]. Thereby, the tolerance to salinity stress may be enhanced through certain interventions for greater accumulation of compatible osmolytes in plant tissues [Rivas et al. 2011].

A great variation exists among crop species due to their genetic make-up, severity of stress and degree of simulating and/or accumulation of osmolytes in reaction to external vagaries [Zhang et al. 2014]. Most of crop species cannot produce osmolytes in sufficient amount to face the threat of abnormalities [Shahbaz and Ashraf 2013]. The tolerance against stressful condition could be augmented by foliar application of certain osmoprotectants, (viz, salicylic acid, glycine betaine, ascorbic acid, trehalose) in most of crop plants [Kishor et al. 2014]. Among these phytoprotectants, salicylic acid has been reported to be most cost-effective and efficient in improving the salt tolerance in majority of crop species [Noreen et al. 2016]. Salicylic acid (SA) and its analogue belongs to phenolics and is regarded as endogenous signal molecule and performs the role in defense mechanism [Aldesuquy et al. 2012]. The various studies have been reported about its potential functions in resistance to salt stress in wheat seedlings [Loatfy et al. 2012], heavy metal toxic effects on mustard [Ahmad et al. 2011] and temperature stress on cucumber [Dong et al. 2014]. Application of

0.05 mM salicylic acid reduced damaging effects of oxidative stress in *Catharanthus roses* [Misra et al. 2014]. It is further reported that foliar spray of SA enhanced the relative water content, biological yield, soluble protein, proline concentration and photosynthetic pigments of peanut seedlings [Kong et al. 2012]. Various researchers reported beneficial effects of exogenous application of SA on rapeseed [Parashar et al. 2014], wheat [Kong et al. 2012], soybean and mungbean [Yang et al. 2013], cotton [Noreen et al. 2013] and sunflower [Noreen and Ashraf 2008, Noreen et al. 2012] under stressful environments. The degree of tolerance of plants to vagaries of salinity could be enhanced by improving photosynthetic capacity [Makino 2011]. Therefore, it is imperative to equip the plants to maintain higher rates of photosynthesis in plants [Makino 2011]. The photosynthetic capacity and stomatal conductance were greatly induced by application of salicylic acid [Noreen et al. 2011].

The processes of uptake and transport of ions in plants are altered by exogenous application of SA. The absorption of Na^+ and Cl^- was appreciably reduced by foliar spray of SA at the rate of 1.0 mM (NaCl) [Noreen et al. 2013]. In wheat, the osmotic potential, photosynthetic capacity, chlorophyll pigments, production of shoot and root biomass were enhanced by application of salicylic acid under stress conditions. Moreover, the uptake of K^+ increased with simultaneous reduction in Na^+ and Cl^- contents in leaf tissues [Arfan et al. 2007]. Noreen and Ashraf [2008] and Noreen et al. [2013] found a positive correlation between the progressive growth and salicylic acid applied exogenously on sunflower crop under saline conditions. Furthermore, it was also reported that foliar spray of SA at the rate of 200 mg L^{-1} enhanced the regulatory system of antioxidant enzymes in response to salinity [Noreen et al. 2009].

At present Pakistan is facing an acute shortage of edible oils and an amount of US $ 2.25 billion is spent on import to meet the demand. The sunflower is a short duration, high yielding and very good quality oil crop among other oilseed crops. It is also classified as relatively salt tolerant crop, i.e. upto to EC_e 4.8 dSm^{-1}, and could be grown under irrigated and rainfed production areas [Francois 1996]. There are reports that increase in salinity could cause considerable reduction in head diameter, stem diameter, plant height and reduction in yield. The effect of osmoprotectants including salicylic acid to overcome stressful affects and/or stimulating tolerance to abiotic stresses in plant species has been studied by a number of researchers [Noreen et al. 2009, 2012]. However, the research studies on interactive effects of genotypes × environment × management practices (G × E × M) growth, stage and ionic constituents in the soil medium are of paramount important for raising the tolerance level under different ecological environments [Hasanuzzaman et al. 2015]. Therefore, a research study was undertaken with an objective to quantify the effects of exogenously applied salicylic acid on physiological and biochemical attributes and concentration of K^+, Cl^{-1}, Na^+ ions in sunflower (*Healianthus annuus* L.) crop under salt-stress conditions.

MATERIALS AND METHODS

The research studies were undertaken to quantify the impact of salicylic acid under saline environment on physiological and biochemical attributes of two sunflower lines under green house conditions at Bahauddin Zakariya University, Multan-Pakistan. The treatments were consisted of three factors, (a) two sunflower lines (Hysun-33, LG-56-63), (b) two levels of salinity (0 and 120 mM (NaCl)), and (c) two levels of salicylic acid (0 and 200 mg L^{-1}), and arranged in a completely randomized design with four applications. The lines were selected on the basis of their level of tolerance to salinity, i.e., Hysun-33 (sensitive) and LG-56–63 (tolerant) ones. The seed were surface sterilized with 5.0 g L^{-1} sodium hypochlorite for five minutes and dried at room temperature before dibbling in the experimental pots. Ten kg washed river sand was filled in plastic pots measuring 24.5 × 28.0 cm and having a drainage hole at the bottom. The impurities from the sand were removed by a 10-hour leaching with 10 percent HCl followed by thorough washings with de-ionized water. Ten achenes were dibbled at 5 mm depth in the pots during month of October. Irrigation was carried out using modified half strength Hoagland's solution after

Table 1. Detail of JIP parameters from the fast OJIP fluorescence induction. Adapted after Guha et al. [2013]

Terms and formulae	Illustration
F_O = F50 µs	first reliable fluorescence value after the onset of actinic illumination: used as initial value of the fluorescence
F_L = F150 µs	fluorescence value at 150 µs
F_K = F300 µs	fluorescence value at 300 µs
F_J = F2 ms	fluorescence value at 2 ms (J-level of OJIP)
F_I = F30 ms	fluorescence value at 30 ms (I-level of OJIP)
F_P (= F_M)	fluorescence value at the peak of OJIP curve: maximum value under saturating illumination
T_{FM}	time (ms) to reach the maximal fluorescence value F_M
Area	area between fluorescence induction (OJIP) curve and the line F = F_M
$F_V = (F_M\text{-}F_O)$	variable chlorophyll fluorescence
$M_O = (\Delta V/\Delta t)_O\ 4(F300 - F_O) / (F_M - F_O)$	approximated initial slope (in ms^{-1}) of relative variable chl fluorescence curve Vt (for FO =F50 µs)
$V_J = (F_J - F_O) / (F_M - F_O)$	relative variable fluorescence at phase J of the fluorescence induction curve
$V_I = (F_I - F_O) / (F_M - F_O)$	relative variable fluorescence at phase I of the fluorescence induction curve
S_M = Area / F_V	normalized total complementary area above the OJIP transient (reflecting multiple turnover Q_A reduction events)
$N = S_M\ M_O\ (1 / V_J)$	number of Q_A redox turn overs until FM is reached
K_N	no-photochemical de-excitation rate constant in the excited antennae for non-photochemistry
K_P	photochemical de-excitation rate constant in the excited antennae of energy fluxes for photochemistry
$ABS / RC = M_O\ (_I / V_J)\ (1 / \varphi_{PO})$	absorption flux (for PSII antenna chls) per reaction center (RC)
$TR_O / RC = M_O\ (_1 / V_J)$	trapped energy flux per RC (at t = 0)
$ET_O / RC = M_O\ (1 / Vj)\ \psi_O$	electron transport flux per RC at t = 0)
$DI_O / RC = (ABS / RC) - (TR_O / RC)$	dissipated energy flux per RC (at t = 0)
$RC / CS_m = \psi_O\ (VJ / MO)\ (ABS / CS_m)$	density of reaction centers per excited cross-section (at t = t_{FM})
$ABS / CS_m = F_M$ (at t = t_{FM})	absorption flux per excited cross section, approximated by $_{FM}$
$TR_O / CS_m = \varphi_{PO}\ (ABS / CS_m)$ (at t = t_{FM})	trapped energy flux per excited cross section, approximated by $_{FM}$
$ET_O/CS_m = \varphi_{EO}\ (ABS / CS_m)$ at t = t_{PM})	election transport flux per excited cross section, approximated by $_{FM}$
$Dl_O/CS_m = (ABS / CS_m) - (TR_O / CS_m)$ (at t = t_{FM})	dissipated energy flux per excited cross section, approximated by $_{FM}$
$RC/ABS = [(F2_m - F_O) / 4\ (F300\ \mu s - F_O)]\ (F_V / F_M)$	density of reaction centers per PSII antenna chl
$PI_{(ABS)} = [RC/ABS]\ (\varphi_{PO}/(1 - \varphi_{PO})] \times [\varphi_O / (1 - \varphi_O)]$	performance index on absorption basis
$PI_{(CSm)} = [RC / CS_m]\ (\varphi_{PO} / (1 - \varphi_{PO})] \times [\varphi_O / (1 - \varphi_O)]$	performance index on cross section basis

sowing at every two days interval [Hoagland and Arnon 1950, Epstein 1972]. Evaporated water was replenished with distilled water at other times. The seedlings were thinned to four per pot at day five after complete germination.

The salt stress was imposed by using neutral full strength Hoagland's nutrient solution at day 15 after sowing. The salt levels were raised progressively with an increment of 50 mM (NaCl) at two days interval, as to avoid any osmotic shock to the seedlings. Salicylic acid (2-hydroxybenzoic acid) was dissolved in a few drops of dimethyl sulfoxide and finally pH adjusted 5.5 with KOH (1.0 N) solution. Salicylic acid was applied in combination with 0.1 percent (v/v) Tween 80 (polyoxyethylene sorbiton monooleate) as surfactant to maximize penetration into the leaf tissues. The plants were foliated with salicylic acid solution at day 30 (15 days after imposition of salt stress) after sowing; while untreated plants also received spray of distilled water, adjusted at pH 5.5. A constant volume of 10 mL salicylic acid and/or distilled water was sprayed per plant to ensure full coverage.

The measurements on chlorophyll (SPAD) values were recorded at day 45 after sowing (15 days after foliar spray of salicylic acid) at 9:30 to 10:00 hours by using portable chlorophyll Meter (Minolta Camera, Co.). Thereafter, seedlings were uprooted, rinsed with distilled water and blotted. The plants were divided into shoot and root organs and quantified fresh biomass production. Plant material was oven-dried at 70°C for 48 hours for biological yield and chemical assay. The total soluble proteins and free amino acids were determined by methods of Bradford [1976] and Hamilton and Van Slyke [1943], respectively. The concentrations of K^+, Cl^- and Na^+ were determined according to Allen et al. [1986].

The chlorophyll 'a' fluorescence measurements were recorded by employing portable Flour Pen (FP-100) Instrument between 09:00 to 10:00 hours. Data were recorded on the recently fully expanded leaves, i.e. using the same leaves used for determining of chlorophyll (SPAD) values by following methods [Guha et al. 2013, Noreen et al. 2016]. Firstly, the leaves were acclimatized to dark environment for 30 minutes by fixing leaf clips, as to ensure that all PSII were in dark adapted state with open reaction centers. Then, chlorophyll 'a' fluorescence transients were obtained by illuminating the leaves with a beam of saturating light at 650 nm peak wave length, which were obtained from six light-emitting diodes focused on the leaf surface through the clips on a spot of 5 mm diameter circle. Measurements were replicated for four times and a single leaf per plant constituted each replicate. The original OJIP transients were double normalized between the two fluorescence extreme O(Fo) and $P(F_M)$ phases and the variable fluorescence between OP expressed as Vok were detemined according to Strasser et al. [2004]. The various parameters obtained from OJIP fluorescence induction were analyzed with those of Guha et al. [2013]. Detail of terms and formulae used for various OJIP fluorescence inductions are as follows. The data were analyzed according to Steel and Torrie [2000].

RESULTS

Data for shoot and root fresh and dry weights differed significantly in response to different levels of salinity and salicylic acid in both lines of sunflower. Averaged across salicylic acid levels, shoot and root fresh and dry weights of two lines of sunflower decreased significantly at $p < 0.01$ and $p < 0.001$ levels, respectively due to differential salinity regimes. While, the exogenous application of salicylic acid at the rate of 200 mg L^{-1} enhanced significantly the shoot and root fresh and dry weights of sunflower at $p < 0.001$ and $p < 0.01$ levels, respectively compared to untreated check. The line "LG-56-63" produced greater amount of shoot and root fresh and dry mass as compared to line "Hysun-33" under both saline and non-saline conditions. Averaged across salicylic acid treatments, the salts stress also markedly decreased the root fresh and dry weight in both lines of sunflower. The two lines differed significantly ($p < 0.05$) in response to salinity. Data further showed that exogenous application of salicylic acid significantly ($p < 0.01$) improved the fresh and dry biomass of both lines of sunflower as compared to untreated plants under both saline and non-saline conditions. The line "LG-56-63" produced greater qu-

Table 2. Shoots and roots weight of sunflower lines as affected salt stress and salicylic acid levels (*n* = 4)

Treatments			Shot fresh weight (g platn^{-1})	Shoot dry weight (g plant^{-1})	Root fresh weight (g plant^{-1})	Root dry weight (g plant^{-1})	Stem length (cm)
Line	Salt stress (mM)	Salicylic acid (mg L^{-1})					
Hysun-33	0	0	7.21	1.34	1.21	0.17	48.3
		200	13.0	1.61	1.22	0.19	51.2
	120	0	6.3	0.83	1.12	0.17	44.3
		200	7.56	1.4	1.22	0.20	47.8
LG-56-63	0	0	13.8	2.7	2.66	0.55	57.1
		200	14.8	2.24	3.47	0.44	60.1
	120	0	11.2	1.62	1.83	0.30	46.0
		200	16.8	2.26	4.56	0.52	50.8
Line (L)			2.31**	0.21*	0.17*	0.9*	2.1**
Salt stress (SS)			0.36*	0.19**	0.18**	0.8*	2.2*
Salicylic acid (SA)			0.86*	0.15*	0.14*	0.7*	1.7**
L × SS			0.46*	0.26*	0.23*	0.8*	1.4*
L × SA			0.59*	0.19*	0.21*	0.8*	1.1*
SS × SA			0.38*	0.15*	0.16*	0.7*	0.9*
L × SS × SA			ns	ns	ns	ns	ns

Table 3. Chlorophyll (SPAD), total free amino acids and total soluble protein contents of sunflower liens as affected by salt stress and salicylic acid levels (*n* = 4)

Treatments			Chlorophyll (SPAD) values	Total soluble proteins (mg g^{-1})	Total free amino acids (mg g^{-1})
Line	Salt stress (mM)	Salicylic acid (mg L^{-1})			
Hysun-33	0	0	35.92	5.3	1.59
		200	38.72	4.26	1.72
	120	0	30.03	8.85	1.88
		200	40.6	9.21	2.26
LG-56-63	0	0	32.98	2.58	1.49
		200	38.95	3.81	1.51
	120	0	31.57	11.39	2.02
		200	41.57	11.59	2.38
Line (L)			0.9**	0.06*	0.05*
Salt stress (SS)			0.8*	0.05**	0.04**
Salicylic acid (SA)			1.3*	0.04**	0.03**
L × SS			0.8*	0.03*	0.03*
L × SA			0.7*	0.03*	0.02*
SS × SA			0.9*	0.04*	0.03*
L × SS × SA			ns	ns	ns

Table 4. Concentration of K^+, Na^+ and Cl^- ions in shoot and root organs of sunflower lines as affected by salt stress and salicylic acid ($n = 4$)

Treatments								
Line	Salt stress (mM)	Salicylic acid (mg L^{-1})	Shoot K^+ (mg g^{-1})	Root K^+ (mg g^{-1})	Shoot Na^+ (mg g^{-1})	Root Na^+ (mg g^{-1})	Shoot Cl^- (mg g^{-1})	Root Cl^- (mg g^{-1})
Hysun-33	0	0	39.1	41.2	5.4	0.1	12.1	9.9
		200	45.6	43.8	6.8	24.2	10.0	7.9
	120	0	17.4	21.8	17.4	44.1	28.1	18.9
		200	21.9	25.2	15.2	23.4	25.1	16.8
LG-56-63	0	0	38.0	39.7	6.9	12.6	10.3	10.3
		200	44.4	42.4	7.8	10.1	9.0	8.4
	120	0	15.6	20.0	15.1	38.1	29.8	24.4
		200	18.4	23.4	17.1	33.2	24.8	21.4
Line (L)			0.8*	0.4**	0.3**	0.4**	0.5*	0.5**
Salt stress (SS)			0.7**	0.5*	0.2*	0.5*	0.5**	0.5**
Salicylic acid (SA)			0.6**	0.3*	0.3*	0.3*	0.4**	0.3*
L × SS			0.4*	0.2*	0.2*	0.2*	0.3*	0.2*
L × SA			0.3*	0.3*	0.2*	0.2*	0.2*	0.2*
SS × SA			0.3*	0.2*	0.2*	0.2*	0.2*	0.2*
L × SS × SA			ns	ns	ns	ns	ns	ns

antity of biological yield compared line "Hysun-33". The stem length was significantly reduced due to imposition of 120 mM (NaCl) salinity stress. However, the stem length was improved appreciably by spraying of crop with salicylic acid at the rate of 200 mg L^{-1} solution (tab. 2).

Data for chlorophyll (SPAD) values differed considerably in both lines of sunflower in response to salinity stress and foliar spray of salicylic acid. The imposition of salt stress (120 mM, NaCl) caused marked reduction in SPAD values, whereas, the adverse effects were mitigated by spraying of salicylic acid at the rate of 200 mg L^{-1} solution. Data for total soluble proteins and total free amino acids revealed that salinity caused significant increase in the quantum of total soluble proteins and total free amino acids contents. The amount of total soluble proteins and total free amino acids were significantly ($p < 0.001$) increased in response to salinity stress compared to non-saline condition. The exogenous application of salicylic acid enhanced total protein and amino acid contents under saline and non-saline conditions (tab. 3).

The concentration of K^+ in shoot and root tissues was significantly ($p < 0.05$ and $p < 0.001$), respectively reduced by imposition of 120 mM (NaCl) salt stress. The response of salinity to both lines of sunflowers was dissimilar in maintaining the quantities of K^+ in their plant parts. The exogenous application of salicylic acid at the rate of 200 mgL^{-1} caused increase in the amount of K^+ in shoot and roots tissues across the different salinity levels in both lines of sunflower. The salinity stress of 120 mM (NaCl) resulted in significant ($p < 0.001$) increase in the content of Na^+ ion in shoot and root tissues. The concentration of Na^+ ion in shoots and root tissues was significantly ($p < 0.05$ and $p < 0.01$), respectively reduced by foliar spray of salicylic acid in both sunflower lines under saline environment. Furthermore, the salt stress at 120 mM (NaCl) caused significant

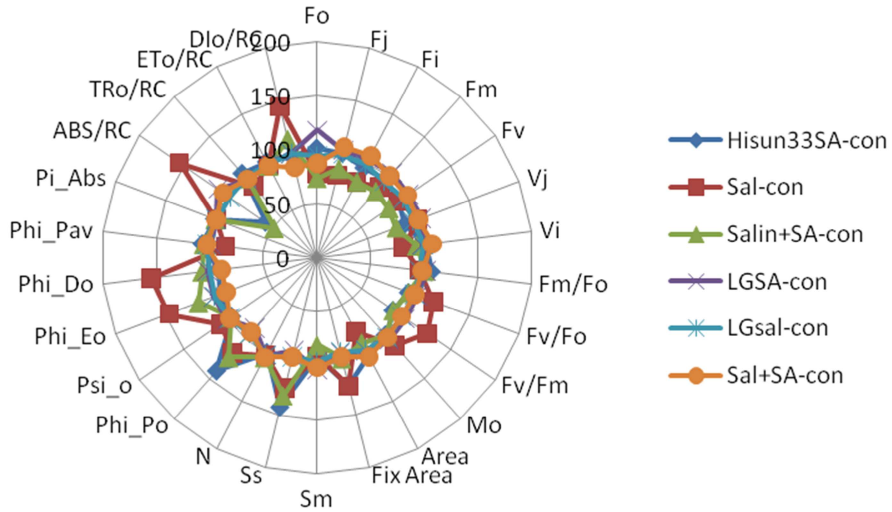

Fig. 1. Radar plot showing the changes in chlorophyll 'a' florescence transients in dark adapted sunflower lines under varying conditions of salinity and salicylic acid

increase in rising the amount of Cl^- in shoot and root tissues. However, the adverse effects of salinity were mitigated by exogenous application of 200 mg L^{-1} salicylic acid (tab. 4).

The changes induces by various treatments in the biophysical parameters and derived from the transient curves JIP are illustrated at Radar Plot (fig. 1). The JIP parameters, Fo, Fj, Fi, Fm, Fv were significantly affected by varietal differences, however, salinity and/or interaction of variety and salinity produced a little affect on these parameters, except Fi parameter. The parameters namely, Vj, Vi, Fm/Fo, Fv/Fo, Fv/Fm were non-significantly affected by salinity and interaction between variety and salinity. However, values of Vj and Vi were significantly affected by varietal differences. The parameters related to Mo, Area, Fix Area, Sm and Ss were also non-significantly affected by salinity and its interaction with variety, except the values for Mo and Fix Area were significantly influenced by varieties. Furthermore, the parameters related to Ni, Phi-Po, Ps-o, Phi-Eo, Phi-Do were non-significantly affected by variety, salinity and their interaction. However, values of Psi-o and Phi-Eo were significantly affected by varietal differences. The other parameters, Phi-pav, Phi-abs, ABS/RC, TRO/RC, ETo/RC and DIo/RC were also non significantly affected by salinity. However, Phi-pave, ABS/RC, TRo/Rc, ETo/RC and DIo/RC ere significantly affected by varietal differences.

The OJIP transients related to chlorophyll 'a' fluorescence in response to varying levels of salt and salicylic acid levels revealed that Vop showed a significant positive peak in response to spray of salicylic acid under untreated check, while it was depressed under saline conditions (fig. 2) The K-Band and L-band were lowered down to a greater extent, however, the situation was averted by spray of salicylic acid in both lines. The fluorescence data normalized between 50 μs and 1s expressed as Voi differed among the periodic time interval. The kinetic differences were resulted as a negative deviation under all treatments for Voi (fig. 3), Voj (fig. 4), Vok (fig. 5) and Vip (fig. 6). The salt stress decreased Fo, Fj, Fi, Fm, Fv, Vi, Vj, Fm/Fo, Area, SM = area/Fv, Pi (ABS), TRO/CSm, and To/RC. The salicylic acid as endogenous substance enhanced the capacity of these parameters and reaction centers, thereby deleterious effects of salts were reduced. Under salt stress, OP-Band and OI Band were reduced in both lines, while K-band and L-band were reduced in line Hysun-33.

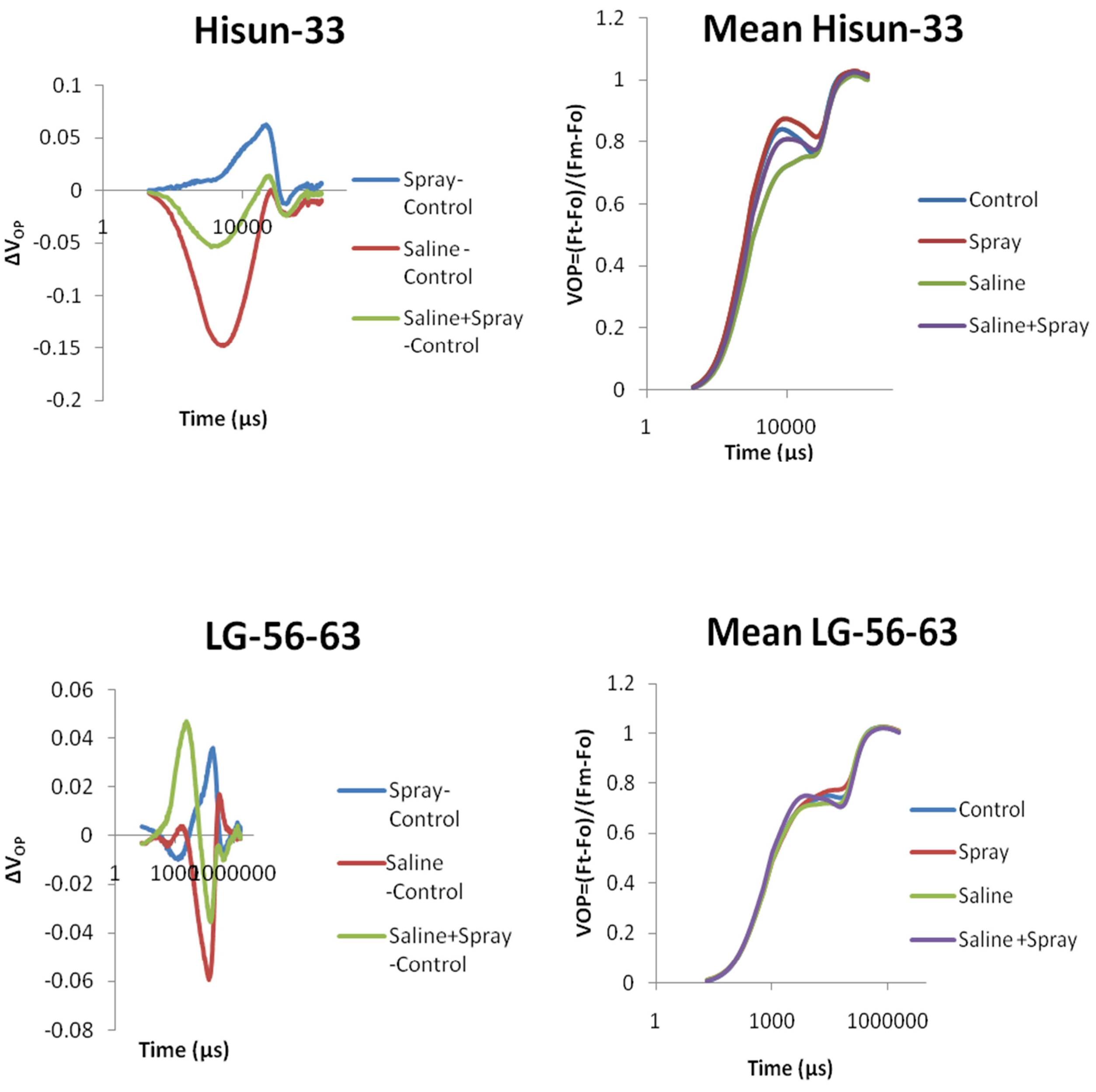

Fig. 2. Kinetic differences of Vop [ΔVop = (Ft – Fo) / (Fp – Fo)] depicting the OP-band deduced from OJIP chl a fluorescence transients

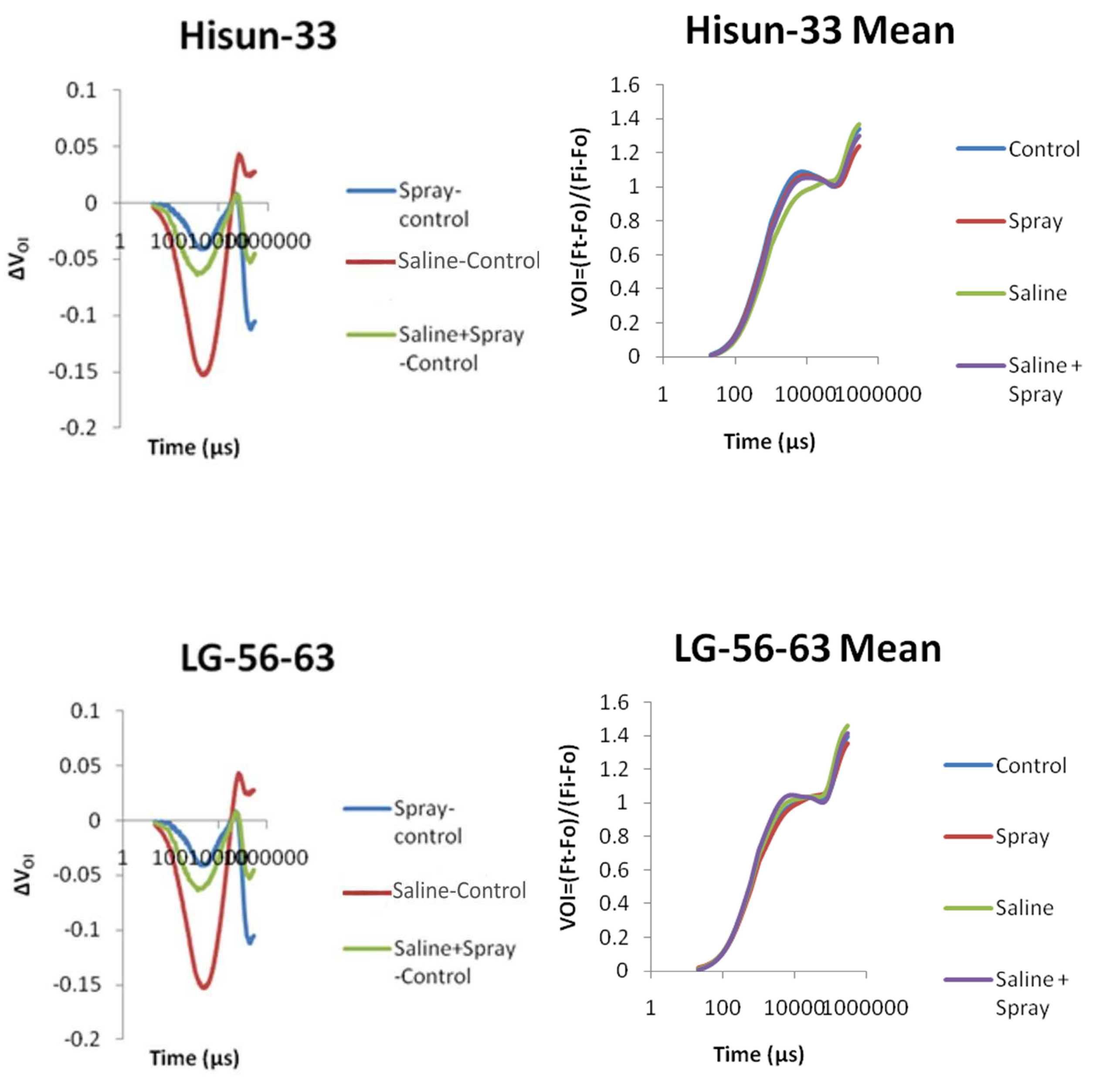

Fig. 3. Kinetic differences of Voi [ΔVoi = (Ft – Fo) / (Fi – Fo)] depicting the OI-band deduced from OJIP chl a fluorescence transients

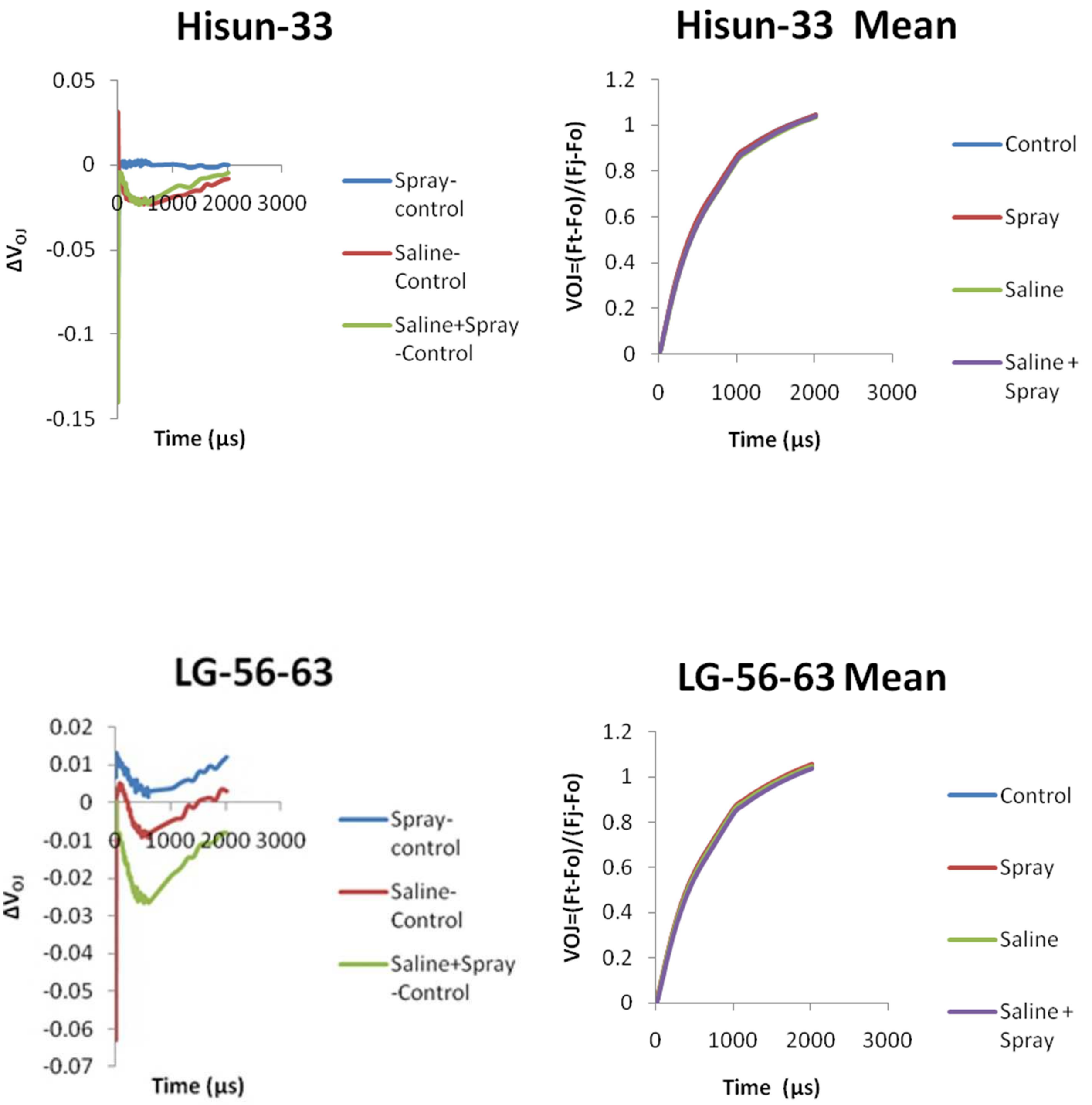

Fig. 4. Kinetic differences of Voj [ΔVoj = (Ft – Fo) / (Fj – Fo)] depicting the K-band deduced from OJIP chlorophyll a fluorescence transients

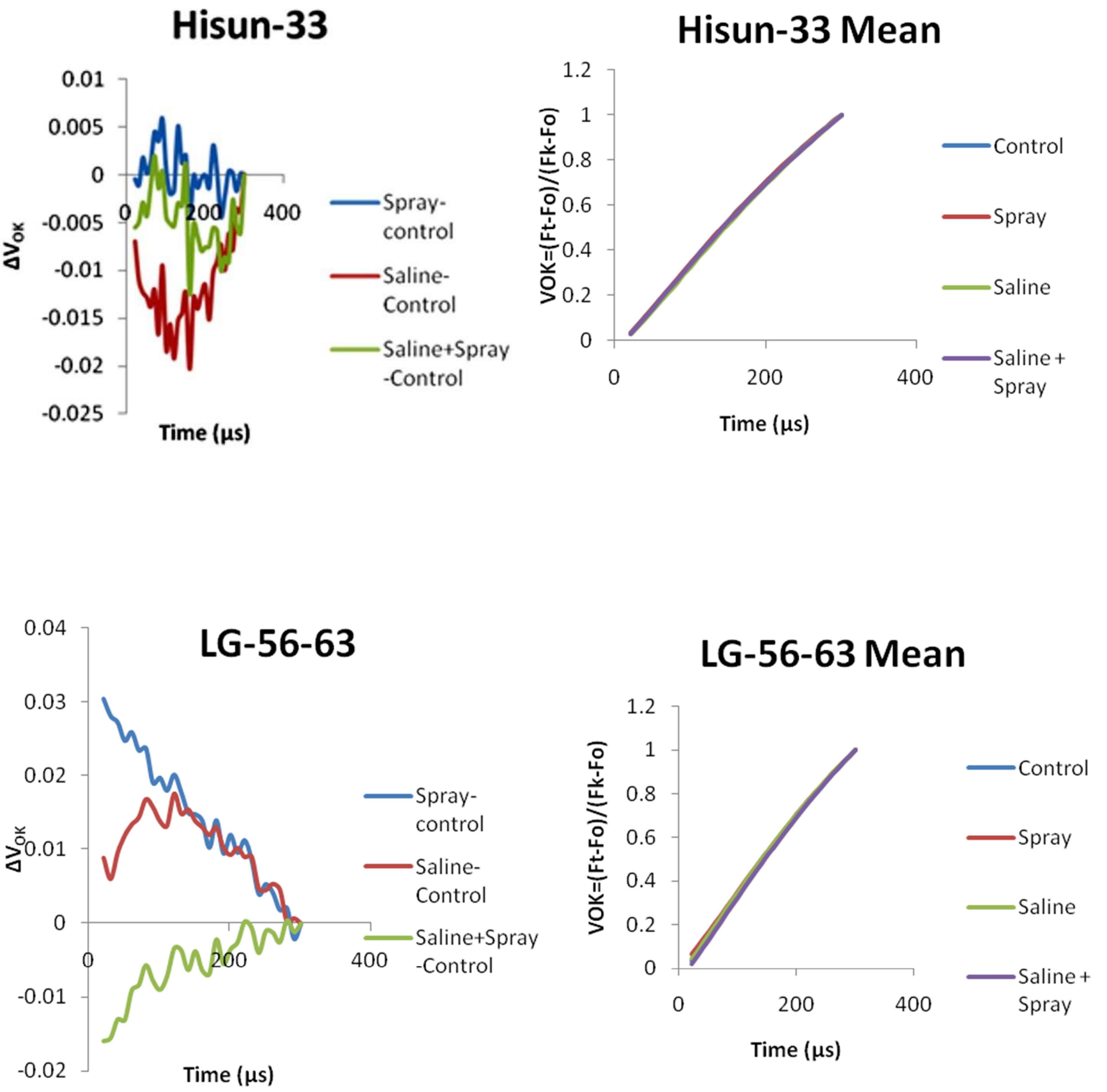

Fig. 5. Kinetic differences of Vok [$\Delta Vok = (Ft - Fo) / (Fk - Fo)$] depicting the L-band deduced from OJIP chl a fluorescence transients

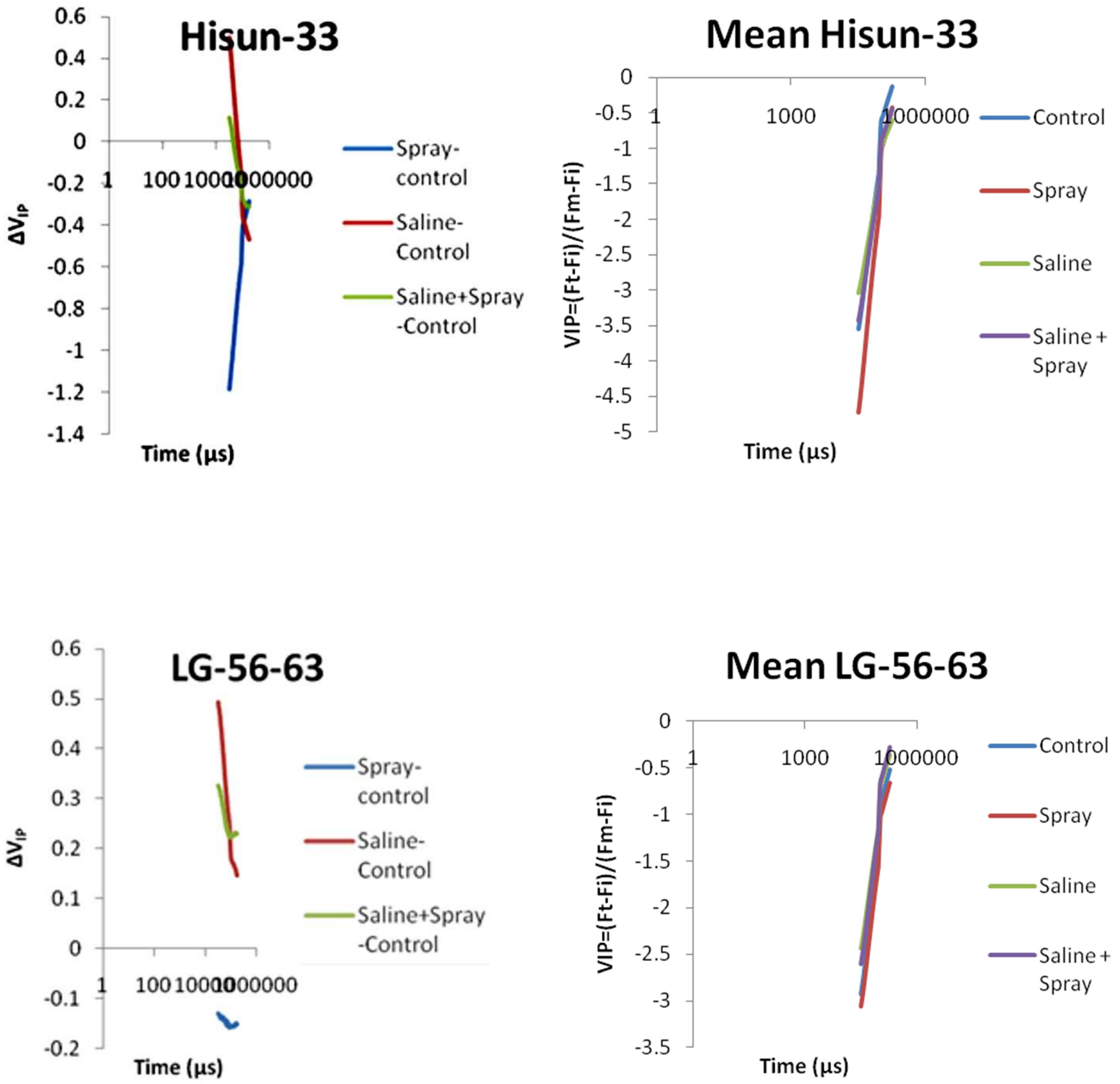

Fig. 6. Kinetic differences of Vip [ΔVip = (Ft – Fi) / (Fp – Fi)] depicting the IP-band deduced from OJIP chlorophyll a fluorescence transients

DISCUSSION

Salicylic acid is an endogenous hormone, which is involved in number of processes, such as growth and development, seed and fruit yield, flowering initiation, glycolysis and defense system to quench the production of ROS [Arfan et al. 2007, Noreen and Ashraf 2008, Hasanuzzaman et al. 2015]. Moreover the modulating effect exerted by external application of salicylic acid enhanced the photosynthetic capacity and stomatal conductance [Noreen and Ashraf 2008]. Moreover the quantity and proportionate amount of ions was also altered as induced by spray of salicylic acid under stressful condition. [Tufail et al. 2013]. Various researchers have reported that oxidative stress caused by ROS under salinity stress could be quenched by increased the quantum of salicylic acid in the plant system [Noreen et al. 2012]. In a recent study, Hasanuzzaman et al. [2015] also reported that salicylic acid induced antioxidative system resulted in ameliorating the adverse effect of environmental stresses.

The result of the present study demonstrated that biological yield and stem length were decreased in response to salinity in both sunflower lines. The production of biomass and attainment of higher growth rate is dependent upon progression of cell division, higher photosynthetic activity, and higher turgor pressure for sustaining the life cycle. Under saline condition, the growth and development of sunflower crop was affected to a greater extent thereby, lowered quantity of biomass. The similar results have been reported by other researchers [Djanaguiraman and Parad 2013] that progression in growth and development and steering a series of physiological functions including photosynthetic activity were impacted to a greater extent in response to salinity stress. The reduction in photosynthetic capacity resulted in decreased production of biological yield and stem length ultimately, which led to loss of yield [Noreen et al. 2009, Hessini et al. 2015]. The reduction in stem length was an outcome of lowering photosynthetic efficiency in relation to high salt contents in growth medium. Various researchers [Zhang et al. 2014] also found a close correlation between photosynthetic capacity and progressive growth and development in various plant species. The exogenous application of salicylic acid alleviated the adverse affects of salinity stress, with concurrent enhanced the growth in both lines of sunflower. The similar result have also been reported by various researchers [Loatfy et al. 2012, Kong et al. 2014, Mirsa et al. 2014, Hasanuzzaman et al. 2015] that raising the level of salicylic acid through foliar spray would enhance the tolerance capacity of plants under salinity for improving growth and development of crop species. The existence of negative association between photosynthetic capacity and biomass production under salt stress has also been reported in other crop plants i.e. wheat seedlings [Loatfy et al. 2012], roses [Mirsa et al. 2014], peanut seedlings [Kong et al. 2014], rape seed [Parashar et al. 2014], soybean and mung bean [Yang et al. 2013], cotton [Noreen et al. 2013] and sunflower [Noreen et al. 2012].

The salts in the rooting medium caused toxicity through absorption of Na^+ and Cl^- ions, disturbing the ionic balance and thereby impacting physiological processes of the plants [Hasanuzzaman et al. 2015]. The chlorophyll content was greatly limited in response to salinity stress. The process of photosynthesis is regulated by chlorophyll 'a' and 'b' [Ashraf and Harris 2013]. These findings of our study are analogous to evidence of other researchers [Rivas et al. 2011, Hasanuzzaman et al. 2015] that the total chlorophyll content decreased due to salt stress which caused reduction in photosynthetic capacity in most of crop plants. In analogous to this, chlorophyll values in both sunflower lines (Hysun-33 and LG-56-63) was decreased due to salt stress and resulted in the reduction of photosynthetic capacity. Salt stress considerably reduced the chlorophyll content in sorghum [Bavei et al. 2011], wheat and bean [Radi et al. 2013], maize [Rashad and Hussain 2014], however the exogenous application of salicylic acid improved the chlorophyll content under both saline and non-saline condition. The similar results have also been found in sunflower [Noreen et al. 2012], fenugreek [Babar et al. 2014] and canola [Baghizadeh et al. 2014]. The results of our study indicated that quantity of soluble protein increased in both sunflower lines, when exposed to salinity stress compared to non-saline condition. Various researchers [Kong et al. 2012, Yi et al. 2014] also reported that de novo synthesis of protein occurred, when plants were subjected to stressful conditions. The findings of our study are similar to the earlier findings that salt tolerant cultivars exhibited increase in soluble protein in response to salt stress in crops namely barley, sun flower, pearl millet and rice [Radi et al. 2013]. Different researchers [Ahmad et al. 2011] also reported that quantity of soluble protein increased with increasing level of salinity in barley and chlorella plants. However, contrary to these findings, that Kausar et al. [2014] reported that salt stress caused reduction in soluble protein contents in sorghum and tomato crops, respectively.

It is evidenced from our present study that total free amino acid were increased in both sunflower lines when they were grown under saline condition. These results agree with those of various researchers [Akhtar et al. 2013, Kausar et al. 2014], who reported that quantity of total free amino acids in most of the crops were elevated. Furthermore, various studies showed that application of salicylic acid produced simulating effect in increasing the quantity of total soluble protein and total free amino acid in response to salt stress in maize crop [Sahar et al. 2011, Akhtar et al. 2013]. The results from our study reveal that salt stress depressed the absorption of K^+ ion and enhanced the uptake of Na^+ and Cl^- ions in both sunflower lines. However contrary to it, foliar application of salicylic acid favoured the uptake of K^+ ion at the cost of depressing of Na^+ Cl^- ions in the plant system. Recently, similar results were reported by Tufail et al. [2013] that increased salinity due to NaCl caused increase in uptake of Na^+ and Cl^- ions with concurrent reduction in K^+ ion in maize and barley plants, respectively. However, Benzarti et al. [2014] found that pre-sowing treating the broad bean seed with salicylic acid enhanced the level of tolerance to salt stress. The plants accumulated greater quantities of K^+ ions in their tissues, while the absorption of Na^+ and Cl^- ions were reduced markedly, thereby improved the growth and development.

The photosystem II is highly sensitive to salt stress resulting in reduction in photosynthetic capacity of the crop plants [Ashraf and Harris 2013]. Data of our study also indicate that photosynthetic capacity was reduced due to damage to photosystem II in both sunflower lines. It was observed that salt stress also mediated the fall in light energy moving towards reaction centers and reducing quantum yield of photosystem II [Mehta et al. 2010]. Moreover, there was a drop in the fluorescence yield at J, I and P contents in plants subjected to salinity. Mehta et al. [2010] reported that shrinkage in QA, QB and PQ pool size of photosystem II or hindrance of electron transport at the donor sites were the main cause of reduction in different phases of fluorescence yield.

Chlorophyll 'a' fluorescence transients double normalized between Fo and Fm as Vop exhibited the single and multiple turnover phases for localization of other action sites. The relative variable fluorescence at J step (Vj) is the degree of the fraction of the primary quinine of PS II [Ni et al. 2012]. Data from our present study also indicate that Vj escalated in Hysun-33 due to salinity stress. These results correlate with those of Mehta et al. [2010] and Cuchiara et al. [2013] who noticed escalation in Vj under salinity. However, treatment of salicylic acid caused acclimatization of salt stress and alleviated the level of these parameters. Furthermore, the quantities of ETo/RC and TRo/RC were decreased in reaction to salinity level. The efficiency of these parameters were reduced due to inactivation of RCs tap and excitation of energy in the system. Jajoo [2013] reported that RCs are instantly converted into inactive RCs under stressful condition. However the rate of inactivation is dependent upon the degree of severity of the prevalent stress condition.

CONCLUSION

The negative aspects of salinity on sunflower crop could be alleviated by spray of salicylic acid at the rate of 200 mg L^{-1} solution. The levels of various physiological and biochemical parameters could be augmented by spray of salicylic acid under saline conditions. The improvement in growth and development would result in enhancing the yield of sunflower crop on saline soils.

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