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Araştırma Makalesi/*Research Article (Original Paper)*

Effects of NaCl Stress on Chlorophyll Content and Chlorophyll Fluorescence in Sunflower (*Helianthus annuus* L.) Lines

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Abstract: Salinity is one of the important abiotic stresses that have adverse effects on photosynthesis, chlorophyll, fluorescence and their components. In this study, the influence of salinity (0, 100 and 200 mM NaCl) on chlorophyll and fluorescence was investigated in sunflower (*H. annuus*) lines (12 sunflower lines) for a period of one month in hydroponic culture system. The results showed that salt stress enhanced chlorophyll content in the first three weeks, but at last week, both levels of salinity declined it slightly. However, in non-stressed plants chlorophyll content increased during four weeks. The results indicated that salt stress affected chlorophyll content negatively only in long-term, but in short-term salinity increased chlorophyll content by secondary effects. Among the 12 sunflower lines, the highest increase in chlorophyll content was observed in both sensitive and tolerant lines. Moreover, the results showed that severe stress increased Fv/Fm ratio from week 1 to week 3, but in week 4 this ratio decreased. After week 2 and unlike control plants, results represented an increase in both F_0 and Fm values in stressed plants. It seems that Fm more increased than F_0, causing enhancement of Fv/Fm. It appeared that top (young) leaves in sunflower are adapted to remain active under salt stress to survive plant under long-term stress.

Key words: Abiotic stress, Fluorescence, Salinity, SPAD, Sunflower.

Ayçiçeği (*Helianthus annuus* L.) Hatlarının Klorofil İçeriği ve Klorofil Floresansına NaCl Stresinin Etkileri

Özet: Tuzluluk, fotosentez, klorofil, floresans ve bunların bileşenleri üzerinde olumsuz etkileri olan önemli abiyotik streslerden biridir. Bu çalışmada, 12 adet ayçiçeği (*H. annuus*) hattının klorofil ve floresansı üzerine tuzluluğun (0, 100 ve 200 mM NaCl) etkisi, hidrofonik sistemde bir aylık dönem boyunca araştırılmıştır. Sonuçlar, tuz stresinin klorofil içeriğini ilk üç hafta içinde arttırdığını, fakat son hafta hafifçe düşürdüğünü göstermektedir. Bununla birlikte, stres uygulanmayan bitkilerde klorofil içeriği, dört hafta boyunca artmıştır. Araştırma sonuçları, tuz stresinin sadece uzun vadede klorofil içeriğini olumsuz etkilediğini, ancak kısa vadede tuzluluğun ikincil etkilerle klorofil içeriğini artırdığını göstermektedir. 12 ayçiçeği hattı arasında, en yüksek klorofil içeriği artışı, hem duyarlı hem de tolerant hatlarda gözlemlenmiştir. Ayrıca, sonuçlar, aşırı stresin Fv/Fm oranını 1. haftadan 3. haftaya kadar arttırdığını, fakat 4. haftada bu oranın azaldığını göstermektedir. İkinci haftadan sonra strese maruz bırakılmış bitkilerde, kontrol bitkilerine oranla hem F_0 hem de F_m değerlerinde artış gözlenmiştir. F_0 değeri F_m değerine oranla daha fazla artmıştır, bu da Fv/Fm oranında iyileşmeye neden olmuştur. Bu üst (genç) ayçiçeği yaprakların uzun süreli stres altında daha aktif kalmaya adapte olması, bitkilerin uzun dönem stres koşullarında hayatta kalmasını sağlamaktadır.

Anahtar kelimeler: Abiotik stres, Flüoresan, Tuzluluk, SPAD, Ayçiçeği.

Introduction

Increased salinization of arable land is expected to have devastating global effects, resulting in 30% land loss within the next 25 years, and up to 50% by the year 2050 (Wang et al. 2003). Salinity is known to adversely affect production of most crops worldwide (Hasegawa et al. 2000; Bayuelo-Jime´nez et al. 2002; Ashraf 2009). Osmotic stress, ion toxicity, nutritional imbalance are the main impediments of salinity on plant growth and yield consequently bring out photosynthetic and physiological disturbance (Almodares et al. 2008).

Photosynthesis is one of the most important metabolic processes in plants and its performance is greatly affected under stress conditions (Mehta et al. 2011). Measurement of chlorophyll (chl) fluorescence is a relatively new technology in recent years to study the impact of various environmental stresses such as drought, salinity and low- or high-temperature on photosynthetic efficiency of leaves in the greenhouse and field conditions has been used (Rizza et al. 2001; Rapacz et al. 2001; Ort 2002; Baker and Rosenqvist 2004; Zobayed et al. 2005). Reduction of photosynthesis with increasing salinity has been reported by several studies (Nazir et al. 2001; Raza et al. 2006). However, results of many studies indicate no significant differences in Variable fluorescence emission (Fv) / Maximum fluorescence emission (Fm) under salinity conditions (Belkhodja et al. 1999; Liu and Shi 2005; Higbie et al. 2010). In rice, Lutts et al. (1996) reported that Fv/Fm ratio decreased in response to salt treatment from day 9 in median leaves and from day 12 in youngest ones. In all treatments, the Fv/Fm ratios were slightly lower in median than in youngest leaves and NaCl did not modify the mean difference in Fv/Fm ratios between leaves of different ages. No significant differences in Fv/Fm ratio were recorded among genotypes until day 18 of stress. The main effect of NaCl upon chl fluorescence parameters consisted in increased instantaneous fluorescense emission (F_0) values. The various cultivars differed mainly in Fm values.

Chl content is considered to be one of the parameters of salt tolerance in crop plant (Srivastava et al. 1998). NaCl stress decreased total chl content of the plant by increasing the activity of the chl degrading enzyme: cholorophyllase (Rao and Rao 1981), inducing the destruction of the chloroplast structure and the instability of pigment protein complexes (Sing and Dubey 1995). It has been reported that chl content decreases under salt stress in plants such as tomato (Ciobanu and Sumalan 2009), radish (Jamil et al. 2007) and sunflower (Akram et al. 2009). However, Ezin et al. (2010) reported that no significant differences observed in tomato chl content under 0, 50, 100 and 200 mMNaCl for 4 weeks. Perbea andPetcu (2000) reported that under water deficit, all tolerant and sensitive sunflower genotypes indicate increasing in chl content and the value of sensitive genotypes was further. Increase in chl content also reported in rice (Krishnamurathy et al. 1987), soybean (Wang et al. 2001) and cotton (Higbie et al. 2010). Ciobanu andSumalan(2009) reported that chl content per unit of leaf area decreased with increasing salinity and the changes in the chl content of leaves occurred after six weeks when leaves intensified their green color.

Material and Methods

Plant materials and growth conditions

The experiment was conducted in hydroponic culture system under greenhouse conditions at Faculty of Agriculture, University of Tabriz. The experimental design consisted of 36 treatments replicated three times in a split plot in time design, with salinity as main factor and line as sub factor. Twelve sunflower lines namely R_2, R_{27}, R_{29}, R_{41}, R_{43}, R_{50}, R_{56}, B_{11}, B_{15}, B_{25}, $B_{109}$and B_{353} were subjected to three NaCl concentrations (0, 100 and 200 mM). Seeds were sterilized with sodium hypochlorite and germinated in petri dishes and seven day old seedling of uniform size were transferred into large sand tanks housed within an environmentally controlled greenhouse (15 h daily light, 600-800 µmol m^{-2} s^{-1} photosynthetic photon flux density (PPFD), thermoperiod 25±5 °C day\night, and relative humidity 45\60% day\night). The tanks were sub irrigated and flushed four times daily with a modified Hoagland nutrient solution. NaCl stress was imposed 7 days after the seedlings were transferred (Figure 1). According to before investigations and Based on growth parameters, lines R_2, R_{56} and R_{50} recognized resistant and sensitive ones were B_{11}, B_{353}, B_{25} and B_{15} (Heidari et al. 2011).

Chlorophyll content

Chl content was assessed using a chl meter (SPAD-502, Minolta). Measurements are taken on third leaves from apex. Three points from each leaf and two plants per plot were measured. Average of these six readings was considered as SPAD reading of the leaf. Recording of SPAD readings was carried out weekly from 7 days after incubation with NaCl and for 4 consecutive weeks.

Figure 1. Sunflower lines under NaCl treatment. Plants were grown in sand and irrigated with Hogland's solution.

Chlorophyll fluorescence

Chl fluorescence was measured weekly from 7 days after incubation with NaCl for 4 weeks on third leaves from apex, using Opti science, OS-3OMSA portable chl fluorometer. Measurements for minimal (F_0) and maximal (Fm) fluorescence yields were made on dark-adapted (30 min) leaves and the variable fluorescence (Fv) were calculated as ($Fm-F_0$). Accordingly, maximum photochemical quantum yields of PSII (Fv/Fm) and the potential quantum efficiency of PSII (Fv/F_0) were calculated (Jian-qing et al. 2011).

Statistical analysis

Data were subjected to analysis of variance based on the statistical model of the used experimental design and mean comparison was done using LSD test.

Results

Chlorophyll content

In non-stressed plants chl content was lower than salt stressed ones (Fig. 2). Except week 2 that showed no significant difference compared with week 1, chl content at control plants was increased from week 1 to week 4 (from 25.114±0.957 to 29.834±0.698). However, the salt stressed plants show different trends during 4 weeksof salt incubation. Under mild stress, chl content at week 1 to week 2 significantly increased (from 32.817±0.819 to 34.995±0.658) and then slowly decreased at week 3 (33.200±0.615) and 4 (32.623±0.591). Interaction between line and salinity was significant only for chl content (Table 1). Under 200 mMNaCl, chl content was significantly decreased but with slowly increment at week 2 (from 31.776±0.825 to 33.868±0.892) and 3 (34.010±0.751) and then also slowly decreased at week 4 (32.703±0.824) (Figure 2). Under mild stress lowest chl (relative to control) was observed in lines B_{109}, R_{56}, B_{353}, R_2, R_{29} and R_{41}. However, lines B_{11}, R_{50}, R_{27} and B_{15} show highest chl content.Lines B_{109} and B_{353} indicate lowest chl content under severe stress conditions (Figure 3).

Table 1. ANOVA results of the chlorophyll content and chlorophyll fluorescence parameters in leaf 3 of sunflower lines under salinity stress.

Source	df	MS				
		F_0†	F_M	F_V/F_M†	F_V/F_0†	Chl content
Salinity (S)	2	0.135	94851	0.063	1.085	1676.959**
Error a	4	0.028	305094	0.022	0.517	72.575
Line (L)	11	0.025	311538**	0.008	0.136	301.858***
S × L	22	0.020	96877	0.006	0.194	26.625***
Error b	66	0.020	96347	0.008	0.213	7.921
Time (T)	3	0.178**	6723474**	0.181***	4.610***	139.444***
T × S	6	0.072***	175290	0.030**	0.754***	121.595***
T × L	33	0.012	109743	0.006	0.150	12.117
T × S × L	66	0.013	138647	0.010	0.158	6.193
Error c	210	0.017	104449	0.008	0.182	8.797

**P<0.01; ***P<0.001. F_0,minimal fluorescence, F_M, maximal fluorescence, F_V/F_M, maximum photochemical quantum yields of PSII, F_V/F_0, potential quantum efficiency of PSII, respectively. † Analysis after data transformation.

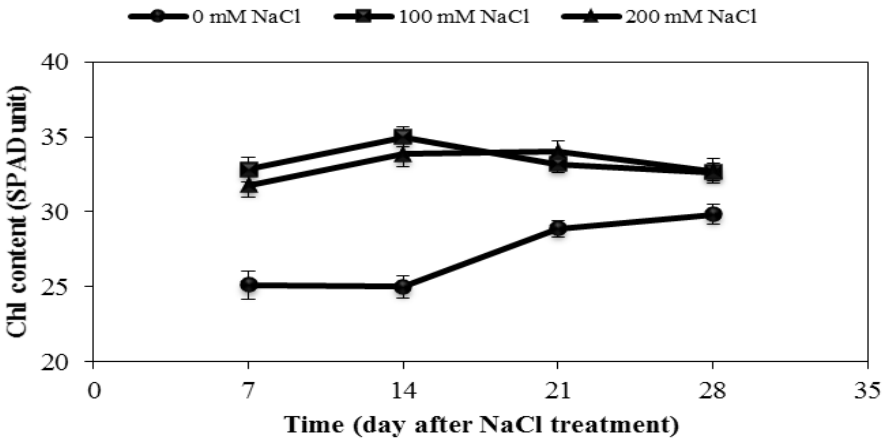

Figure 2. Chlorophyll content (SPAD unit) of 12 sunflower (H. annuus) lines grown under saline and non-saline conditions for 28 days (Mean ± SE; n = 36).

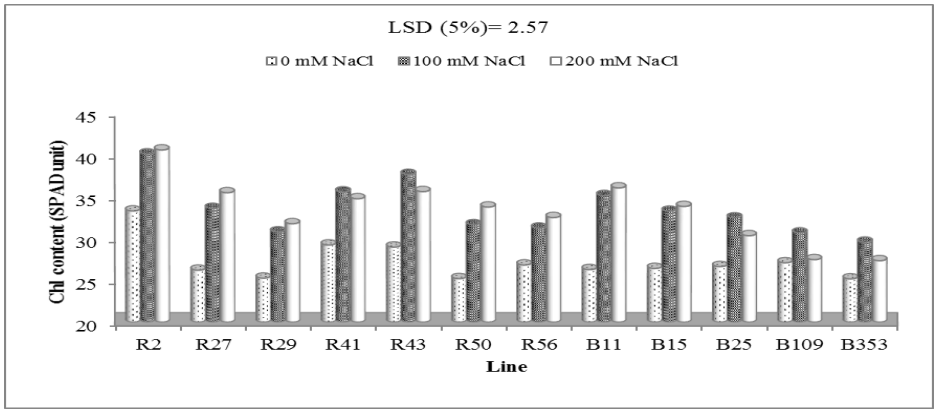

Figure 3. Chlorophyll content (SPAD unit) of 12 sunflower (H. annuus) lines grown under saline and non-saline conditions for 28 days (Mean; n = 12). LSD (5%) = 2.57; showing least significant difference.

Minimal fluorescence (F_0)

Generally, F_0 decreased in control plants from week 1 to week 4. However, this decreasing was severe and significant only from week 1 to week 2 (from 375±18 to 288±13) and then showed slowly changes in week 3 (295±11) and 4 (272±8). Whereas,F_0 decreased only from week 1 to week 2 in the salt stressed plants and unlike control plants in both salinity level this parameter increased. Plants show highest F_0value (327±21) under severe stress at week 4 (Figure 4).

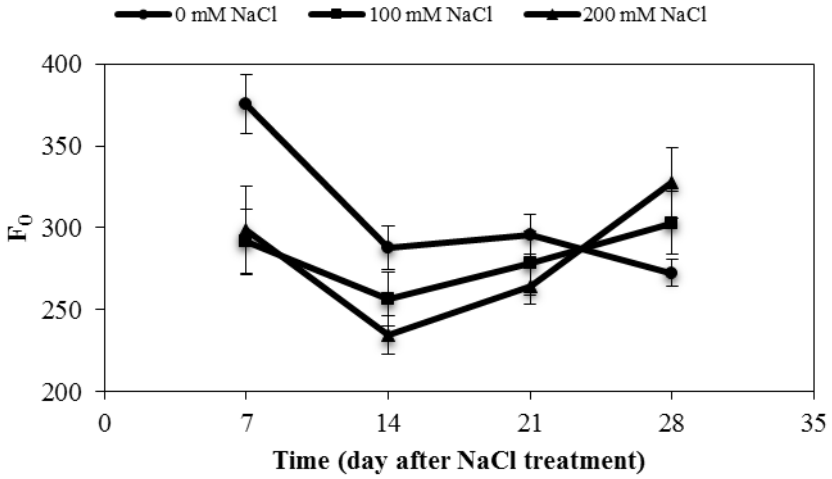

Figure 4. Minimal fluorescence (F0) of 12 sunflower (H. annuus) lines grown under saline and non-saline conditions for 28 days (Mean ± SE; n = 36).

Maximal fluorescence (Fm)

During exposureNaCl, Fm values showed significantly decreased in second week (from 1741±46 to 1646±34) and then increased in week 3 (2058±24) and 4 (2169±26). Increased Fm values in week 3 were very sharp but this increment in week 4 was slowly (Figure 5). The sunflower lines had different Fm values. Lines R_{50}, B_{353} and B_{25} indicated lowest values compared with the other lines (Figure 6).

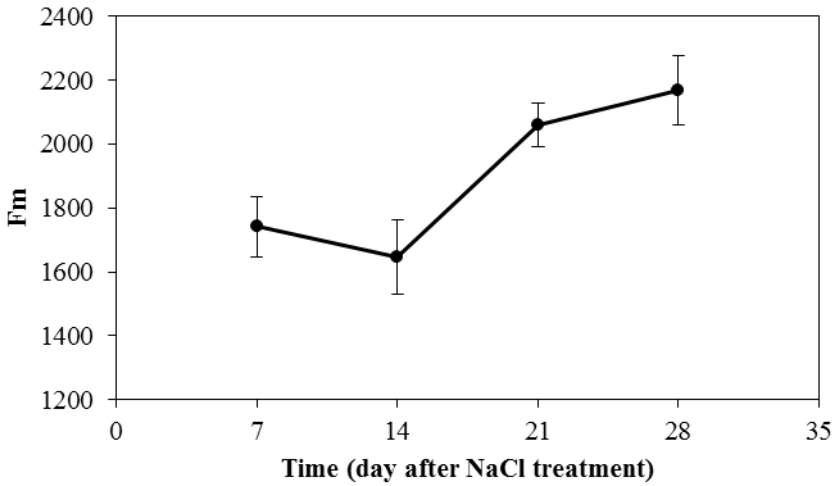

Figure 5. Maximal fluorescence (Fm) of 12 sunflower (H. annuus) lines grown under saline and non-saline conditions for 28 days (Mean ± SE; n = 108).

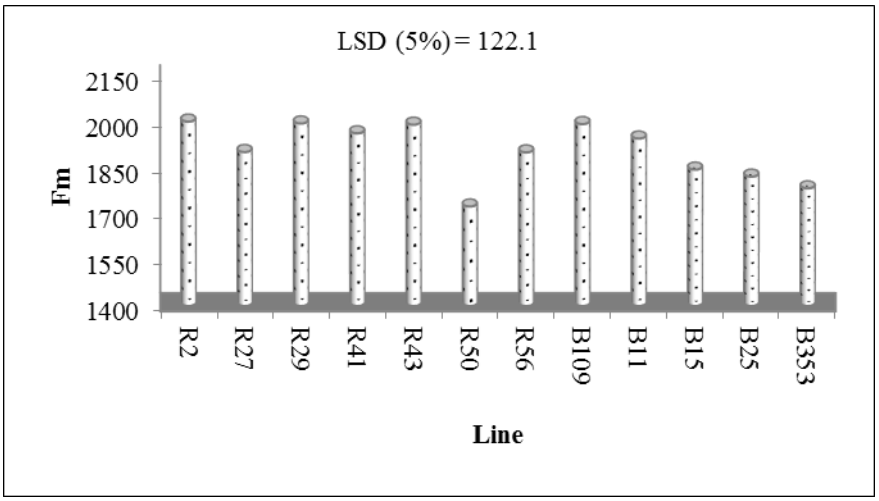

Figure 6. Maximal fluorescence (Fm) of 12 sunflower (H. annuus) lines grown for 28 days.LSD (5%)=122.1; showing least significant difference between mean values of the sunflower lines.

Maximum photochemical efficiency of PSII (Fv/Fm)

In non-stressed plants Fv/Fm ratio increased during the four weeks. Minimum value in control plants was in week 1 (0.775±0.010) and maximum value was observed in week 4 (0.863±0.006). Fv/Fm ratio decreased in response to 100 mMNaCl treatment in week 2 (from 0.843±0.006 to 0.832±0.018) and 4 (0.858±0.007) but this ratio was the highest in week 3 (0.866±0.007). Under severe stress, Fv/Fm ratio showed increasing trend from week 1 to week 3 (from 0.820±0.010 to 0.868±0.005) but in week 4 this ratio was decreased (0.855±0.007) (Figure 7). However, these variations in stressed plantswere slight compared with control plants changes under both salinity levels.

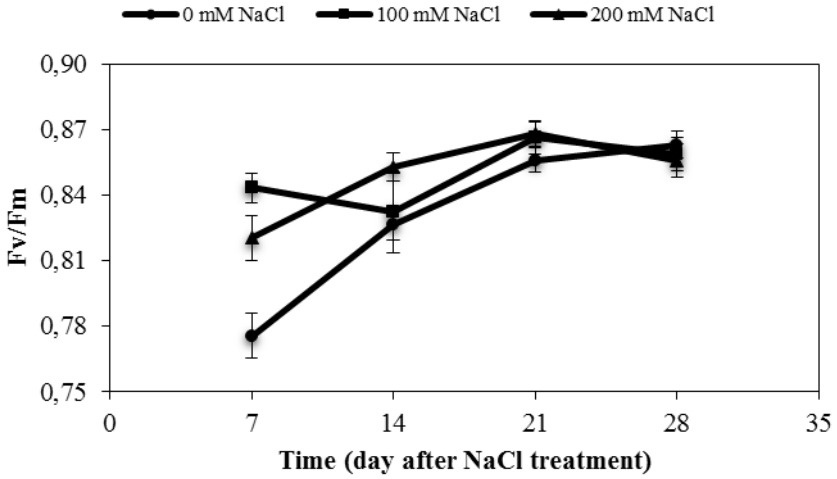

Figure 7. Maximum photochemical efficiency of PSII (Fv/Fm) of 12 sunflower (H. annuus) lines grown under saline and non-saline conditions for 28 days (Mean ± SE; n = 36).

Potential quantum efficiency of PSII (Fv/F_0)

Fv/F_0 ratio similar to Fv/Fm increased in control plantsduring 4 weeks. Fv/F_0 ratio also increased in response to 100 mMNaCl treatment in first 21 days (from 5.651±0.300 to 7.262±0.431) after stress exposure. However, in day 28 this ratio decreased (6.587±0.380) slowly. Fv/F_0 changes under severe stress were similar to 100 mMNaCl treatment. In first 3 weeks under severe stress Fv/F_0 ratio increased (from 5.033±0.255 to 7.135±0.375) and then decreased in week 4 (6.421±0.385) slowly (Figure 8).

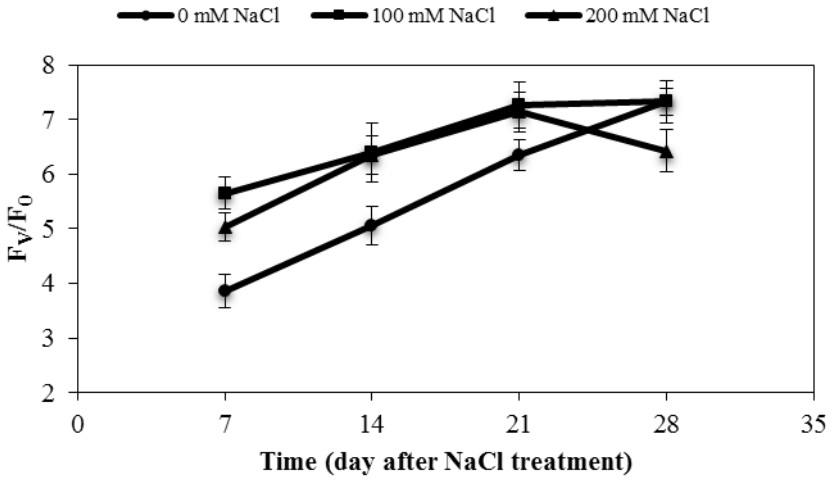

Figure 8. Potential quantum efficiency of PSII (Fv/F0)of 12 sunflower (H. annuus) lines grown under saline and non-saline conditions for 28 days (Mean ± SE; n = 36).

Discussion

In this study, NaCl increased chl content in all sunflower lines. Although, this increment was not remarkable in last weeks. These findings are in agreement with the results reported in rice (Krishnamurathy et al. 1987), soybean (Wang et al. 2001) and cotton (Higbie et al. 2010). Munns (2002) has reported that salinity causing plant cell lose water and shrink and then cell elongation rates show reduction. Over days, changes in cell elongation and cell division lead to slower leaf appearance and smaller final size. Flowers et al. (1977) and Sohan et al. (1999) stated that salinity reduce leaf area index and lead to increase in specific leaf weight. In other words,reduction in leaf area under salt stress associated with increasing in leaf thickness. Papp et al. (1983) have reported that increase in thickness of leaves under salinity stress causes enhancement in chl content. Misra et al. (1997) concluded that increase in the chl content under salt stress could be due to an increase in the number of chloroplasts in stressed leaves. However, in soybean, Ommen et al. (1999) observed a rise in SPAD reading only up to 10 $dsm^{-1}$of NaCl stress. Husain et al. (2003) reported that genotypes which accumulate low concentration of Na^+ in their leaves indicate high SPAD values, 40 days after salinity exposure, and retain their greenness for more time. In contrast, genotypes which showed suddenly reduction in greenness after 50 days had more Na^+ in their leaves. Nevertheless, Zhao et al. (2007) showed that in naked oat under increasing salinity condition, chl content reduced and this reduction was more obvious after 25 days. Reduction in chl content under salinity stress also has been reported in radish (Jamil et al. 2007) and wheat (El-Hendawy et al. 2005).

Chl fluorescence is a very sensitive tool in the study of stress-induced damage to PSII (Li et al. 2007). DeEll et al. (1999) reported that the Fv/Fm ratio for an active leaf varies between 0.75 and 0.85 and a decline in this ratio indicates photoinhibitory damage. In our study, average of Fv/Fm ratio was not below 0.775±0.010 under salinity treatments and during 4 weeks. It has been reported that salt accumulates prevalently in old leaves that eventually drop (Bongi and Loreto1989). This would allow to make photosynthesis more efficient and young leaves to be relatively unaffected by salt (Munns 1993).In this study, Fv/Fm ratio rose in first 3 weeks after salt exposure, at least under severe stress, but this ratio declined very slightly at last week. Seems, at least in sunflower, salt stress improves Fv/Fm ratio at top leaves.

Al-aghabary et al. (2004) reported that 100 mMNaCl stress (10 and 17 days after salt exposure) in fully expanded leaves of tomato, increased Fv/Fm ratio, but it showed slight decrease after 27 days. In rice, Lutts et al. (1996) monitored that Fv/Fm ratio decreased in response to salt treatment from day 9 in median leaves and from day 12 in youngest ones and NaCl did not modify the mean difference in Fv/Fm ratios between leaves of different ages.Lutts et al. (1996) also reported that main effect of NaClstress upon chl fluorescence parameters consisted of increased F_0values but Fm values showed mainly differed in various cultivars. In our investigation, Fm values increased and lines showed significant difference in

this characteristic, but F_0 indicated increment only in stressed plant under salinity levels during weeks. Thedirections of F_0 changes depend on the dominant factor between the energy dissipation and the inactivation or damage of PSII. An increase in NPQ (non-photochemical quenching) leads to a decreasing F_0 (Ogren and Oquist 1984) and the inactivationor the damage of PSII causes the increase of F_0 (Xu and Wu 1996). Perez-Perez et al. (2007) reported that salinity did not affect F_0 but the other fluorescence parameters, Fm and Fv/Fm,had been decreased.Jamil et al. (2007) indicated that in radish Fv/Fm reduced under salinity conditions and it was lowest under severe stress. In celery Fm value indicated no significant change but F_0 and Fv/Fmincreased and decreased, respectively under 300 mMNaCl stress (Everard et al. 1994). Santos (2004) showed that reduction of the Fv/Fm ratio is mainly due to reduction of the Fm. In contrast, our results represent increase in both of F_0 and Fm in stressed plants. It seems Fm more increased than F_0, causing Fv/Fm enhancement. To emphasize this result, we calculated Fv/F_0 ratio and got accurately same results for Fv/Fm. Some reports show Fv/Fm reduction under NaCl stress (Hasegawa et al. 2000; Munns 2002; Ashraf andShahbaz 2003).

Conclusion

Salt stress causes chl destruction that lead to decreasing of SPAD reading; but on the other hand it causes leaf area decrease that following pseudo-enhancement in SPAD reading. These two factors are in conflict with each other and the strength and weakness of each one depends on the crop, stress intensity and stress duration. Nevertheless, it seems leaf area reduction may has more influence in mild salinity that causes SPAD increase in SPAD reading, but when salinity is more severe chldestruction become further and SPAD reading shows reduction. In addition, salinity exposure for long-term also causes more chl destruction. Among the lines, the highest increasing of SPAD reading observed in both sensitive and tolerant lines. However, it seems this increase in sensitive and tolerant lines caused by different processes. In sensitive lines increasing of SPAD is due to leaf area reduction but in tolerant lines increasing caused by chl biosynthesis ability of lines. Under salinity stress tolerant lines of sunflower produce further proline compare with sensitive ones (Heidari et al. 2011) and considering that proline and chl synthesize from a common compound, (Kaya et al. 2001; Houshmand et al. 2005) chl decreasing in some tolerant lines is explicable. Hence, to compare the chl content in sensitive lines, they should be compared with each other individually and also tolerant lines should be compared with each other individually. Results of fluorescence indicate the existence of an especial evolution in sunflower to maintain Fv/Fm ratio under salinity stress. It seems that top leaves in sunflower adapted to remain safe and active under long-term salt stress to survive plant.

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