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Chlorophyll fluorescence performance of sweet almond [*Prunus dulcis* (Miller) D. Webb] in response to salinity stress induced by NaCl

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Abstract

One-year old sweet almond (*Prunus dulcis*) seedlings were submitted to four levels of salt stress induced by NaCl, namely 0.3, 0.5, 0.7, and 1.0 S m^{-1}. Effects of salt stress on a range of chlorophyll (Chl) fluorescence parameters (Chl FPs) and Chl contents were investigated in order to establish an eco-physiological characterization of *P. dulcis* to salinity. Salt stress promoted an increase in F_0, F_s, and F_0/F_m and a decrease in F_m, F'_m, F_v/F_m, q_P, $\Delta F/F'_m$, F_v/F_0, and $UQF_{(rel)}$, in almost all Chl fluorescence yields (FY) and FPs due to its adverse effect on activity of photosystem 2. No significant changes were observed for quenchings q_N, NPQ, and $q_{N(rel)}$. The contents of Chl *a* and *b* and their ratio were also significantly reduced at increased salt stress. In general, adverse salinity effects became significant when the electric conductivity of the nutrient solution (EC_n) exceeded 0.3 S m^{-1}. The most sensitive salt stress indicators were F_v/F_0 and Chl *a* content, and they are thus best used for early salt detection in *P. dulcis.* Monitoring of a simple Chl FY, such as F_0, also gave a good indication of induced salt stress due to the significant correlations observed between the different Chl FYs and FPs. Even essential Chl FYs, like F_0, F_m, F'_m, and F_s, and mutually independent Chl FPs, like F_v/F_0 and q_P, were strongly correlated with each other.

Additional key words: fluorescence quenching.

Introduction

Halophytes are able to grown on soils with high salt contents. However, most plants are non-halophytes, with either a relatively low salt tolerance or severely inhibited growth at low salinity (Sudhir and Murthy 2004). Salinity is one of the major environmental stresses that adversely affect plant growth and metabolism (Surhay *et al.* 1998). Salt stress affects plant physiology at both whole-plant and cellular levels through osmotic and ionic stress effects (Singh and Dubey 1995, Hasegawa *et al.* 2000, Ranjbarfordoei *et al.* 2002, Murphy *et al.* 2003, Sudhir and Murthy 2004). Symptoms of salinity stress are expressed at both stomatal and non-stomatal levels. At stomatal level, the plant reduces its aperture to prevent injuries (Francesco and Sebastiano 2000, Ranjbar 2005). As a result, net photosynthesis is unavoidably reduced due to a decrease in CO_2 availability, which potentially damages the photosynthetic apparatus (Lawlor and Cornic 2002). Most of the decrease in photon flux energy used for photochemistry can be explained as an increase in non-photochemical dissipation of excitation energy (Cornic 1994, Osmond 1994). Chlorophyll (Chl) fluorescence yield (Chl FY) such as minimal Chl FY (F_0) and variable Chl FY (F_v) can be used for evidencing stress and damage of the photosynthetic apparatus, and characterising the environment where plants grow in (Glynn *et al.* 2003, Papageorgiou and Govindjee 2005).

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Abbreviations: Chl, chlorophyll; DAS, dark-adapted state; EC_n, electrical conductivity of nutrient solution; F_0, minimal fluorescence; F'_0, minimal fluorescence at light-adapted state; F_m, maximal fluorescence at dark-adapted state; F'_m, maximal chlorophyll fluorescence at light-adapted state; F_s, steady state fluorescence yield; F_v, variable fluorescence; FM, fresh matter; FP, fluorescence parameter; FY, fluorescence yield; LAS, light-adapted state; NPQ, non-photochemical Chl fluorescence quenching; PS, photosystem; Q_A, quencher A; q_N, non-photochemical quenching; $q_{N(rel)}$, relative non-photochemical quenching; q_P, photochemical quenching; $q_{P(rel)}$, relative photochemical quenching; RCs, reaction centres; $UQF_{(rel)}$, relative unquenched fluorescence.

Non-stomatal effects may prevent photosynthetic activities as has been reported for eucalyptus (Rawat and Banerjee 1998), pistachio (Ranjbar *et al*. 1999, 2002), and almond (Matos *et al*. 2004, Rouhi *et al*. 2006). Salt stress can also lead to destruction of fine chloroplast structure and instability of pigment-protein complexes (Zaman *et al*. 2002).

Historical documents show that cultivated almond (*Prunus dulcis*) originated from wild species that evolved in the desert and lower mountain slopes of west-central Asia. The species became adapted to mild, wet, and dry winters and hot summers. In west-central Asia, cultivated almond can be found in parts of Iran, Turkmenistan, Uzbekistan, Tadzhikistan, and Afghanistan (Dale and Norman 1996, Ranjbarfordoei *et al*. 2002). In Iran, but also in other places worldwide, orchard crops such as almond, pistachio, and grapes are mainly confined to irrigated fields. Irrigated agricultural lands in arid regions, characterised by chronic drought, extreme temperatures, and scarce precipitation, induce accumulation of salt in soils following a combination of sustained evaporative and transpirational water losses (Goyal *et al*. 2003). More precisely, approximately 7 % of the world world's land area, 20 % of the world's cultivated land, and nearly half of the irrigated land is affected by high salt contents (Rhoades and Loveday 1990, Szalbolcs 1994).

Notwithstanding the economic importance of almond in general and *P. dulcis* as a rootstock more specifically (Rouhi *et al*. 2006), an eco-physiological characterisation of almond species with regard to salinity has hardly been done. Shibli *et al.* (2003) studied *in vitro* effects of induced NaCl salinity on bitter almond [*Prunus amygdalus* (L.) Batsch, formerly *Amygdalus communis* L.] growth, osmotic adjustment and nutrient acquisition, and mentioned the high salt tolerance of *P. amygdalus*. Franco *et al.* (2000) mentioned limited growth and production rate of almond trees due to excessive salinity of the irrigation water, whereas Nightingale *et al.* (1991) investigated the soil salinity distribution in a trickle-irrigated almond orchard. So, to our knowledge no eco-physiological characterisation of the photosynthetic performance of *P. dulcis* with regard to salinity has been done yet.

Therefore, the aim of the present study was to evaluate the effects of salt stress, induced by NaCl, on *P. dulcis* based on Chl fluorescence characteristics, and Chl content of leaves.

Materials and methods

Plants: One-year-old almond seedlings [*Prunus dulcis* (Mill.) D.A. Webb], of a height of 0.70 m, were purchased from an almond nursery in Saman city (situated in the province of Chaharmahal-Bakhtiary, Iran). Seedlings were brought to a glasshouse located at Shahrekord University in early March 2005 and were transferred, with the least root disturbance, to 4 000 cm^3 plastic pots. In order to better control nutrient solution and salinity treatments, fine sand was selected as potting material. Plants were kept in the greenhouse with air temperature of 18–27 °C (mean daily minimum and maximum, respectively). Relative humidity of the air was around 55 %, and plants were subjected to natural irradiation. Plants were irrigated with the nutrient solution prescribed by Ranjbarfordoei *et al*. (2002) using a circulating system that consists of a water pump, gutter, and reservoir containing the nutrient solution (Ranjbarfordoei *et al*. 2000, 2002).

Salinity stress levels were chosen with respect to the pedologic conditions in the areas where salinity is a limiting factor (Dewan and Famouri 1964). The chosen salinity stresses, obtained by adding NaCl to tap water and then to the nutrient solution, were 0.3, 0.5, 0.7, and 1.0 S m^{-1} (S3, S5, S7, and S10). Control treatment consisted of no NaCl added (0.08 S m^{-1}; S0). Electrical conductivity of the nutrient solution (EC_n) was increased by 0.2 S m^{-1} per day till final salinity stress for each level was reached. From the moment final EC_n was obtained for the most severe stress level, stress in all treatments was kept constant for three weeks. Electrical conductivity of each level in the container was controlled daily and kept constant with the addition of tap water (*e.g.* Ranjbarfordoei *et al*. 2002, Rouhi *et al*. 2006). A total of 20 almond plants, *i.e*. four plants per treatment, were used in this experiment, each plant serving as an experimental unit.

Chl fluorescence was measured using a portable fluorometer *PAM-2000* (*H. Walz*, Effeltrich, Germany). Before measuring Chl fluorescence parameters (FPs), leaves were put in dark-adapted state (DAS) for 30 min (Genty *et al.* 1989) using light exclusion clips. During DAS all reaction centres (RCs) and electron carriers of the PS2 are re-oxidized; this situation is essential for rapid fluorescence induction kinetics and for recording Chl FPs. In this situation, q_N is relaxed to its minimum value (Roháček 2002, Zhang and Xu 2003).

The following Chl fluorescence yields (Chl FYs) were measured: minimum Chl FY in the dark-adapted state (F_0), minimum Chl FY in the light-adapted state (F'_0), maximum Chl FY in the dark-adapted state (F_m), maximum Chl FY in the light-adapted state (F'_m), and steady-state Chl FY in the light-adapted state (F_s). From these five essential Chl FYs, several Chl FPs were calculated. According to Roháček (2002) only some basic, mutually independent, Chl FPs such as F_0/F_m, q_P, and q_N can be calculated from these five essential Chl FYs, that give insight into the photosynthetic processes in chloroplasts and can be used effectively in photosynthesis research. Other Chl FPs, such as F_0/F_m, NPQ, and

$\Delta F/F'_m$, can be determined from the above mentioned Chl FPs (Roháček 2002). Relative photochemical and non-photochemical quenching coefficients ($q_{P(rel)}$ and $q_{N(rel)}$) (Buschmann 1995), as they would better represent the balance between energy dissipation pathways than q_P and q_N (Juneau *et al.* 2005), and the relative unquenched fluorescence ($UQF_{(rel)}$) (Juneau *et al.* 2005), as this parameter is complementary to both other relative Chl FPs, were also calculated (see Table 1).

Table 1. Chlorophyll (Chl) fluorescence parameters related to the slow Chl *a* fluorescence induction kinetics. DAS/LAS: dark/light-adapted state, PS: photosystem. Chl yields are explained in the text.

Symbol State	Meaning of the fluorescence parameter Definition	Reference
Φ_{P0} DAS	Maximum quantum yield of PS2 photochemistry, F_v/F_m ratio $\Phi_{P0} = \frac{F_v}{F_m} = 1 - \frac{F_0}{F_m}; F_v = F_m - F_0$	Kitajima and Butler 1975
Φ_{N0} DAS	Basal quantum yield of non-photochemical processes in PS2 $\Phi_{N0} = \frac{F_0}{F_m} = 1 - \Phi_{P0}$	Roháček 2002
q_P LAS	Photochemical quenching of variable ChlF $q_P = \frac{\Delta F}{F'_v} = \frac{F'_m - F_s}{F'_v} = 1 - \frac{F_s - F'_0}{F'_m - F'_0}; \Delta F = F'_m - F_s$	Bilger and Schreiber 1986
q_N LAS	Non-photochemical quenching of variable ChlF $q_n = \frac{F_v - F'_v}{F_v} = 1 - \frac{F'_v}{F_v} = 1 - \frac{F'_m - F'_0}{F_m - F_0}; F'_v = F'_m - F'_0$	Bilger and Schreiber 1986
NPQ LAS	Non-photochemical ChlF quenching $NPQ = \frac{F_m - F'_m}{F'_m} = \frac{F_m}{F'_m} - 1 = \frac{q_N\Phi_{P0} + q_0\Phi_{N0}}{1 - q_N\Phi_{P0} - q_0\Phi_{N0}}$	Bilger and Björkman 1990
Φ_2 LAS	Effective quantum yield of photochemical energy conversion in PS2 $\Phi_2 = \frac{\Delta F}{F'_m} = 1 - \frac{F_s}{F'_m} = \frac{q_P(1 - q_N)\Phi_{P0}}{1 - q_N\Phi_{P0} - q_0\Phi_{N0}}$	Genty *et al.* 1989
$q_{P(rel)}$ LAS	Relative photochemical quenching $q_{P(rel)} = \frac{F'_m - F_s}{F_m - F'_0}$	Buschmann 1995
$q_{N(rel)}$ LAS	Relative non-photochemical quenching $q_{N(rel)} = \frac{F_m - F'_m}{F_m - F'_0}$	Buschmann 1995
$UQF_{(rel)}$ LAS	Relative unquenched fluorescence $UQF_{(rel)} = \frac{F_s - F'_0}{F_m - F'_0}$	Juneau *et al.* 2005

All Chl FPs were measured once a week during the three weeks of the experiment, after treatments had reached the final salinity stress level. Analysis of variance to test for significant (p=0.05) differences between salinity stresses and control plants was carried out using the average of the recordings over the three experimental weeks, with four replicates per salt stress level per week.

Chl content: After three weeks, at the end of the greenhouse experiment, a fully expanded, non-senescent, and undamaged leaf was collected from each plant. Leaves were immediately wrapped in aluminium foil to avoid degradation of pigments by light. Soon afterwards, 0.5-g samples were taken from the collected leaves. These samples were then pulverized with liquid nitrogen. Subsequently, 0.25 g of each sample was extracted by 80 % acetone and put in the freezer at –5 °C for 24 h. Pigments were determined according to Lichtenthaler (1987) using a spectrophotometer (*Uvikon 930*). Amounts of Chl *a* and *b* [g kg^{-1}(FM)] were calculated according to Wellburn (1994).

Results

Salinity effects on Chl FY: Exposure of almond plants (*P. dulcis*) to the selected salt stresses induced remarkable alterations in Chl FYs. Within three weeks of salt imposition, F_0 which indicates the impairment of the light-harvesting complex of PS2, was significantly affected by the applied salinity stress at S7 and S10 (Fig. 1*A*), whereas F_0 was not significantly different between S0, S3, and S5. A continuous reduction in F_M was observed with increasing salinity stress (Fig. 1*B*), but only the S0 and S3 levels were statistically different from S10. A comparable response as for F_0 and F_m with increasing salt stress was observed for F_s and F'_m (see Fig. 1*D*,*C*),

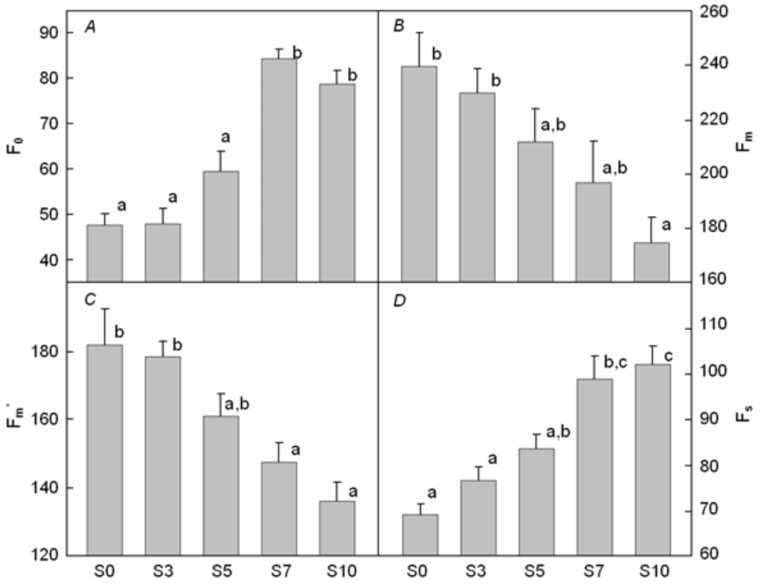

Fig. 1. Chlorophyll fluorescence yields in relation to salinity of the nutrient solution (S0, S3, S5, S7, S10). (*A*) Minimal fluorescence F_0, (*B*) maximal fluorescence F_m, (*C*) maximal fluorescence at light-adapted state F'_m, and (*D*) steady state fluorescence yield F_s. *Different letters* express significantly different results among salinities at the 5 % level. Mean values (± 1 SE) of the weekly measurements from the moment final stress level was reached.

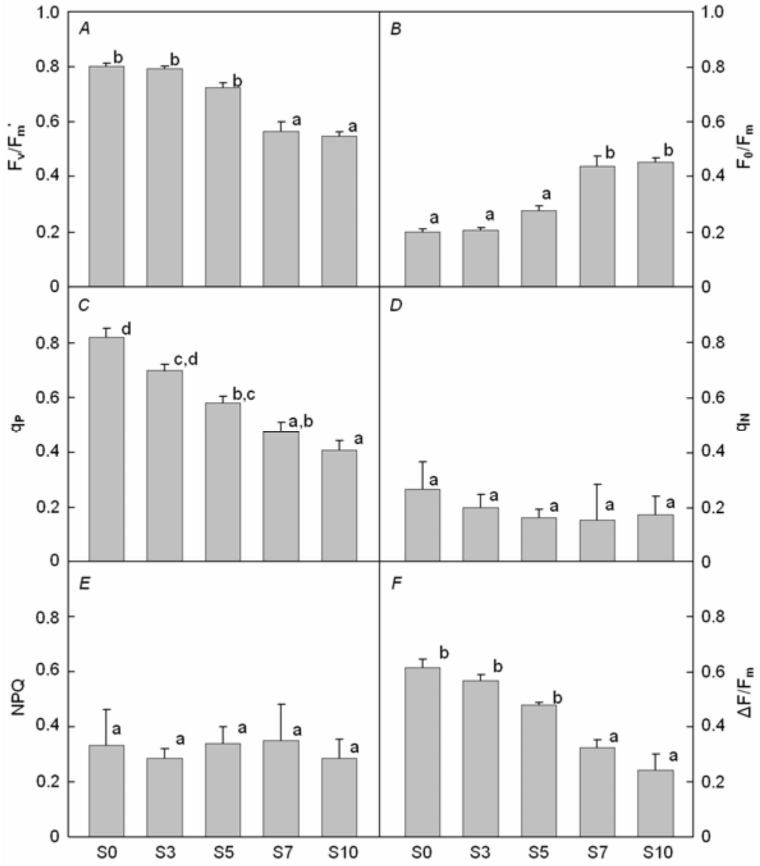

Fig. 2. Chlorophyll fluorescence parameters in relation to salinity of the nutrient solution (S0, S3, S5, S7, S10). (*A*) Variable fluorescence F_v, (*B*) maximum quantum yield of photosystem 2 (PS2) photochemistry F_v/F_m, (*C*) photochemical quenching of variable fluorescence q_P, (*D*) basal quantum yield of non-photochemical processes in photosystem 2 F_0/F_m, (*E*) non-photochemical ChlF quenching NPQ, and (*F*) effective quantum yield of photochemical energy conversion in PS2 $\Delta F/F'_m$. *Different letters* express significantly different results among salinities at the 5 % level. Mean values (± 1 SE) of the weekly measurements from the moment final stress level was reached.

respectively. In general, there was a significant difference between salinity effects of the control and low salt stress on the one hand, and the severe stress on the other.

Salinity effects on Chl FPs: F_v/F_m, q_P, and $\Delta F/F'_m$ showed the highest and significant decrease following an increase in EC_n, F_0/F_m significantly increased, while q_N and NPQ did not change significantly in function of an increasing salinity (Fig. 2). In former four cases the Chl FPs at S0 and S3 were statistically different from those observed at S7 and S10.

In general, F_v/F_m of almond plants decreased as salinity of nutrient solution increased (Fig. 2*A*). A clear reduction in F_v/F_m was observed starting from S5. The ratio remained almost constant at about 0.56 at S7 and S10. F_0/F_m did not significantly change with increasing salinity from S0 to S3. A further increase in salinity, however, led to a significant increase of F_0/F_m, from about 0.208 at S5 to 0.452 at S10 (Fig. 2*B*).

A steadily descending trend was observed for q_P at increasing salt stress (Fig. 2*C*). Photochemical quenching at S10 decreased to 52 % of control q_P. Increasing salt stress induced also a descending trend in $\Delta F/F'_m$ (Fig. 2*F*).

Table 2. Chlorophyll *a* and *b* contents [g kg^{-1}(FM)] in *P. dulcis* at control (S0) and four increasing salt stresses (S3, S5, S7, and S10) induced by NaCl. Means ± SE (*n* = 4). *Different letters* express significantly different results among salt stresses at the 5 % level.

Stress	Chl *a*	Chl *b*	Chl *a/b*
Control, S0	1.21 ± 0.03c	0.55 ± 0.02c	2.20c
Low, S3	1.19 ± 0.03c	0.53 ± 0.03c	2.24c
Moderate, S5	1.03 ± 0.02b	0.48 ± 0.03b	2.14b
High, S7	0.89 ± 0.02a	0.43 ± 0.02a	2.07a
Severe, S10	0.85 ± 0.03a	0.41 ± 0.03a	2.07a

Salinity effects on contents of Chl *a* and *b* (Table 2): Salt stress caused a significant decrease in both pigments when EC_n exceeded 0.3 S m^{-1}, and again when EC_n exceeded 0.5 S m^{-1}. At S10, Chl *a* and *b* contents were reduced by 30 and 25 % compared to S0, respectively. Reduction of Chl *a* was somewhat greater than reduction of Chl *b*. Consequently, the ratio Chl *a/b* decreased significantly, compared to the control, when EC_n exceeded 0.5 S m^{-1}.

Discussion

Responses in leaf Chl fluorescence to increasing salt stress in *P. dulcis* changed significantly through an increase in F_0, F_s, and F_0/F_m and a decrease in F_m, F'_m, F_v/F_m, q_P, $\Delta F/F'_m$, and $UQF_{(rel)}$. Such alterations in fluorescence parameters have been reported for annual and perennial plants subjected to salinity (Netondo *et al.* 2004), ozone (Meinander *et al.* 1996), freezing (Percival and Fraser 2001), and drought stress (Lu and Zhang 1998). Plants exposed to S7 and S10 experienced severe salt stress, *e.g.* F_0 normally does not vary significantly unless plants are exposed to extreme environmental stress that causes structural alteration of the PS2 pigment content (Krause and Weis 1984).

The observed increase in F_0 from S5 onwards (Fig. 1), which indicates the impairment of the light-harvesting complex of PS2, and the concomitant decrease in F_m induced an overall reduction in F_v (see Table 1, data not shown). A lower F_v indicates a slower reduction rate of quencher Q_A and a decrease in PS2 quantum yield (Fernandez *et al.* 1997). Efficiency and stability of PS2, the major component of photosynthetic apparatus, was monitored during the experiment by means of F_v/F_m. Variation in F_v/F_m implies changes in photochemical conversion efficiency of PS2 and, therefore, possible photoinhibition of photosynthesis. The maximum quantum yield of PS2 photochemistry, expressed through F_v/F_m, is almost constant for different plant species measured under non-stressed conditions, with $0.80 \le F_v/F_m \le 0.86$ (Björkman and Demmig 1987). Our control value of F_v/F_m was 0.800±0.011, indicating that plants growing in control conditions were non-stressed. For the most severe salt stress, F_v/F_m decreased to 0.588±0.019, which provides clear evidence of the significance of thermal dissipation processes during stress build-up (Scarascia-Mugnozza *et al.* 1996). However, to support this statement concurrent measurements of heat production and/or photochemistry are required. According to Björkman and Demmig (1987), F_v/F_m is indeed markedly reduced in stressed plants. Because of the sensitiveness of this parameter it is frequently used as indicator of photoinhibition or another kind of injury caused to PS2 complexes (*e.g.* Rees *et al.* 1990, Krause and Weis 1991, Lazár and Nauš 1998, Roháček 2002).

The basal quantum yield of non-photochemical processes in PS2, F_0/F_m, covers the influences of both Chl fluorescence and non-radiative energy dissipation in DAS when all PS2 RCs are open (Roháček 2002). Based on the definition of F_v/F_m and F_0/F_m (see Table 1) and the values of F_0/F_m quoted above, F_0/F_m for un-stressed, undamaged plants is therefore $0.14 \le F_0/F_m \le 0.20$. Bilger *et al.* (1987) and other researchers report that F_0/F_m is markedly increased for stressed or damaged plants, as is also observed here (Fig. 2*B*). The principal cause of this salt-induced increase in F_0/F_m can be attributed to a loss of excitation energy during its transfer from the pigment bed to the RCs, and to an increase of energy loss through non-photochemical quenching processes (Yordanov *et al.* 1997, Roháček 2002). Based on the data for both F_v/F_m and F_0/F_m (Fig. 2*A,B*), it can be concluded that S7 and S10 imposed a serious salt stress on *P. dulcis*, negatively influencing the photosynthesis system.

The ratio F_v/F_m over F_0/F_m expresses the maximal ratio of quantum yields of photochemical and concurrent non-photochemical processes in PS2 related to DAS (Roháček 2002). This ratio is thus a very sensitive indicator of maximum efficiency of photochemical processes in PS2 and/or the potential photosynthetic activity of healthy as well as stressed plants. Fig. 4 supports this statement as, besides the S7 and S10 treatments, also the S5 treatment generated significantly different values from those of S0 and S3. The observed decrease in F_v/F_0 reflects that the efficiency of the photochemical process and the electron transport chain in PS2 were affected by salt stress. Our results of F_v/F_0 are similar to those of Yordanov *et al*. (1997) on maize (*Zea mays*) and sunflower (*Helianthus annuus*).

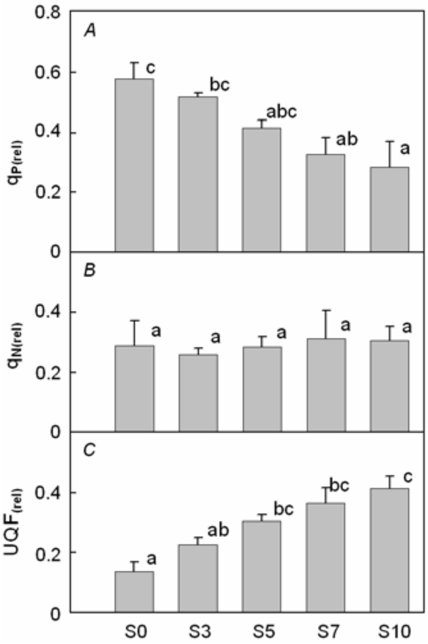

Fig. 3. Relative chlorophyll fluorescence parameters in relation to salinity of the nutrient solution (S0, S3, S5, S7, S10). (*A*) Relative photochemical quenching $q_{P(rel)}$, (*B*) relative non-photochemical quenching $q_{N(rel)}$, and (*C*) relative unquenched fluorescence $UQF_{(rel)}$. *Different letters* express significantly different results among salinities at the 5 % level. Mean values (± 1 SE) of the weekly measurements from the moment final stress level was reached.

q_P indicates the photochemical capacity of PS2 in LAS and quantifies the actual fraction of PS2 RCs that are in open state, *i.e*. with re-oxidised Q_A (Krause *et al.* 1982). Or, as defined by Juneau *et al.* (2005), q_P is proportional to the photon energy captured by open PS2 RCs and dissipated *via* photosynthetic electron transport, while q_N indicates all the non-radiative processes of de-excitation. These two parameters can not be summed to a constant value, partly because they are normalised to different physiological states (*e.g*. Buschmann 1999) and partly because their derivations are based on different models of PS2 connectivity (*e.g*. Roháček 2002). Although q_P is insensitive to some environmental factors, because it does not take into account the efficiency of open PS2 RCs (Genty *et al.* 1989), it may be a sensitive indicator of salt stress (Fig. 2*C*). This was to be expected as in higher plants salt stress inhibits PS2 activity (*e.g.* Belkhodja *et al.* 1994, Singh and Dubey 1995). In several cases, q_N was a much more sensitive indicator of stress response than q_P, *e.g.* in plants exposed to copper, mercury, and some herbicides (Juneau *et al.* 2002, Frankart *et al.* 2003). However, in our study this was not the case. So, it seems that as an indicator of salt stress q_P is much more sensitive than q_N.

Juneau *et al.* (2005) mentioned that when plants are exposed to some stress conditions there is no relationship between the indicators of q_N and q_P energy dissipation processes in the light-adapted steady state (Juneau *et al.* 2001). This may be due to the fact that these parameters do not refer to the same state of energy storage and dissipation *via* the photosynthetic apparatus (Buschmann 1995, 1999). As a solution to this problem, Buschmann (1995) proposed two new parameters, $q_{P(rel)}$ and $q_{N(rel)}$ (see Table 1). However, Fig. 3*A,B* shows that these two parameters yielded similar results compared to q_P and q_N, whereas significant responses to salt stress were only found for $q_{P(rel)}$. The relative photochemical quenching coefficient $q_{P(rel)}$ has been criticised because it combines both photochemical and non-photochemical effects (Roháček 2002) and has not been widely used.

Effective quantum yield of photochemical energy conversion in PS2, $\Delta F/F'_m$, reflects electron transport rate. It indicates the plant's capacity to convert photon energy into chemical energy once steady-state electron transport has been achieved (Genty *et al.* 1989). According to Roháček (2002), $\Delta F/F'_m$ is related to the actual fraction of photochemistry active PS2 RCs in LAS. In contrast to the dark-adapted state FYs, the fluorescence obtained in the light-adapted state (F_s and F'_m) is sensitive to any alteration of the overall PS2-PS1 electron transport and to biochemical reactions linked to photosynthesis (Lazár 1999). Considering these definitions in regard to the data in Fig. 2*F*, we conclude that the overall PS2-PS1 electron transport is damaged at salinities above 0.3 S m^{-1}. The statement of Juneau *et al.* (2005), that $\Delta F/F'_m$ and q_N (see above) are more sensitive stress indicators in many cases compared to q_P, was probably not valid in our research on salinity stress.

NPQ is often used as indicator of the excess radiant energy dissipation to heat in the PS2 antenna complexes (Bilger and Björkman 1990, Demmig-Adams *et al*. 1996, Gilmore 1997). As NPQ does not vary significantly in response to an increasing salt stress (Fig. 2*E*), it is clear that this radiant energy dissipation process is not affected by salt stress.

Table 3. Bivariate Pearson correlations between the different Chl FYs (see text) and Chl FPs (see Table 1); **bold** indicates significant interactions at the 0.05 level, ***bold italics*** indicates significant interactions at the 0.01 level.

	F_m	F'_m	F_s	F_v	F_v/F_m	q_P	F_0/F_m	NPQ	$\Delta F/F'_m$	q_N	$UQF_{(rel)}$	$q_{P(rel)}$	$q_{N(rel)}$	F'_v	F_v/F_0	Chl *a*	Chl *b*	Chl *a*/*b*
F_0	**–0.903**	**–0.946**	***0.962***	**–0.945**	**–0.958**	**–0.916**	***0.986***	0.167	**–0.951**	–0.714	**0.898**	**–0.942**	0.858	**–0.926**	***–0 978***	***–0.969***	***–0.958***	***–0.961***
F_m		***0.986***	***–0.974***	***0.994***	***0.981***	***0.969***	**–0.957**	0.209	***0.992***	0.698	***–0.964***	***0.974***	–0.701	**0.955**	***0.960***	***0.966***	***0.978***	**0.888**
F'_m			***–0.983***	***0.994***	***0.985***	***0.978***	***–0.976***	0.047	***0.993***	0.743	***–0.970***	***0.991***	–0.772	**0.952**	***0.991***	***0.995***	***0.996***	**0.942**
F_s				***–0.987***	***–0.978***	***–0.984***	***0.982***	–0.071	***–0.991***	–0.786	***0.977***	***–0.990***	0.726	**–0.931**	***–0.986***	***–0.983***	***–0.992***	**–0.909**
F_v					***0.995***	***0.970***	***–0.983***	0.113	***0.999***	0.704	***–0.961***	***0.982***	–0.763	***0.970***	***0.982***	***0.984***	***0.990***	**0.927**
F_v/F_m						**0.944**	***–0.992***	0.072	***0.995***	0.650	**–0.931**	***0.964***	–0.813	***0.987***	***0.979***	***0.978***	***0.978***	**0.945**
q_P							**–0.941**	0.104	***0.972***	0.852	***–0.999***	***0.996***	–0.644	**0.881**	***0.970***	***0.973***	***0.987***	0.871
F_0/F_m								0.021	***–0.986***	–0.684	**0.926**	***–0.963***	0.835	***–0.970***	***–0.987***	***–0.982***	***–0.978***	**–0.956**
NPQ									0.107	–0.067	–0.130	0.045	0.416	0.082	–0.061	–0.047	0.210	–0.241
$\Delta F/F'_m$										0.715	***–0.963***	***0.983***	–0.760	***0.966***	***0.984***	***0.985***	***0.991***	**0.925**
q_N											–0.863	0.822	–0.332	0.519	0.766	0.765	0.787	0.614
$UQF_{(rel)}$												***–0.991***	0.612	–0.864	***–0.959***	***–0.963***	***–0.980***	–0.850
$q_{P(rel)}$													–0.712	**0..911**	***0.988***	***0.990***	***0.997***	**0.912**
$q_{N(rel)}$														–0.851	–0.809	–0.803	**–0**.756	**–0.935**
F'_v															**0.941**	**0.941**	**0.935**	**0.938**
F_v/F_0																***0.999***	***0.995***	***0.961***
Chl *a*																	***0.997***	***0.961***
Chl *b*																		**0.936**

Juneau *et al*. (2005) defined the relative unquenched fluorescence $UQF_{(rel)}$, which is a complement to the other relative quenching components ($q_{P(rel)}$ and $q_{N(rel)}$), and takes into account the fraction of non-quenched fluorescence yields related to the proportion of closed PS2 RCs present under continuous radiation. They stated that the relative parameters represent more adequately the proportion of the different quenching processes occurring in the light-adapted state. Our results (Fig. 3) show that $q_{P(rel)}$ and $UQF_{(rel)}$ are sensitive to increasing salt stress. However, just as observed for q_N (Fig. 2*D*), $q_{N(rel)}$ does not seem to be a sensitive Chl FP for indicating salt stress.

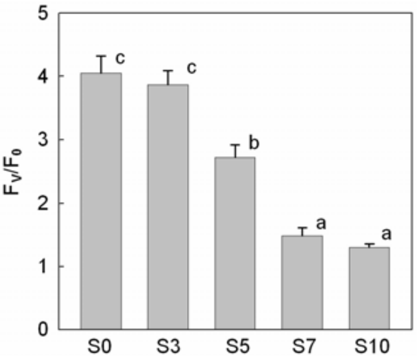

Fig. 4. F_v/F_0 in relation to to salinity of the nutrient solution (S0, S3, S5, S7, S10). *Different letters* express significantly different results among salinities at the 5 % level. Mean values (± 1 SE) of the weekly measurements from the moment final stress level was reached.

Just as observed for other species, *e.g*. *Pistacia* (Ranjbarfordoei *et al*. 2002), rice (Singh and Dubey 1995), and *Sorghum bicolor* (Jagtap *et al*. 1998), Chl content decreased in response to an increasing salt stress. A decrease in contents of both Chl *a* and *b* of plants subjected to salt stress can be attributed to destruction of Chl *a*, which is more sensitive to salinity than Chl *b* (Singh and Dubey 1995), and an increased activity of the Chl degrading enzyme chlorophyllase which is more active under salt stress (Neera and Ranju 2004). The larger destruction of Chl *a* compared to Chl *b* is reflected in a significantly decreasing ratio of Chl *a*/*b* with increasing EC_n (Table 2). Ranjbarfordoei *et al*. (2002) found a strong relationship between the decreased Chl *a* content and the decreased net photosynthetic rate in *Pistacia* caused by salt stress. Although in many cases more parameters than just Chl *a* are affects by salt stress, *e.g*. antenna systems, electron transport, enzymes of the Calvin-Benson cycle, it can be expected that because of the observed decrease in Chl *a* and *b* contents (Table 2), net photosynthetic CO_2-uptake and thus productivity will be significantly reduced at salt stress above 0.3 S m^{-1}.

Most Chl FYs and Chl FPs are significantly correlated (at the 0.05 and even 0.01 level) to each other (Table 3). Even the essential Chl FYs such as F_0, F_m, F'_m, and F_s and the mutually independent Chl FPs (Roháček 2002), such as F_v/F_m and q_P, are strongly correlated. Only NPQ, q_N, and $q_{N(rel)}$ are not significantly correlated to the described Chl FYs and Chl FPs. These Chl FPs also showed no significant response to increasing salt concentrations (Figs. 2 and 3).

Although F_0/F_m did not reveal any new discriminating information compared to F_v/F_m (Fig. 2*A*,*B*), as F_0/F_m is defined as the complement of F_v/F_m (Table 1), the most sensitive Chl FP seems to be F_v/F_0 (Fig. 4). This latter parameter is also significantly correlated with most Chl FYs and Chl FPs, and is almost perfectly correlated with Chl *a* (Table 3).

We conclude that Chl fluorescence and Chl *a* and *b* contents demonstrate a serious decline in eco-physiological performance of *P. dulcis* in response to salt stress induced by NaCl through alteration of PS2 structure. Especially salt stresses above 0.3 S m^{-1} negatively influenced the eco-physiological behaviour of *P. dulcis* for which a significantly lower yield can be expected. The most sensitive salt stress indicators seem to be the Chl FP F_v/F_0 and the Chl *a* content, and are thus best suited for early salt stress detection. Due to significant correlations observed between the different Chl FYs and FPs, the monitoring of a simple Chl FY, like F_0, is already a good indicator of the induced salt stress.

For a better understanding of mechanisms of salt stress, comparative studies should be made using different almond species, or cultivars within species (differing in salt stress resistance). Moreover, attention should be given to the exact action of the stressor, *e.g*. whether it operates through an osmotic shock, ionic stress, enzyme inactivation (Sudhir and Murthy 2004), stomatal closure or oxidative stress (Sharma *et al*. 2005). Comparing species and knowledge of the exact operation modus of the stressor would increase the relevance of the findings for other species. In addition to such detailed salinity studies, mainly executed on seedlings, validation based on field experiments remains necessary to correctly estimate salinity effects on growth and productivity.

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