Full Length Research Paper

Plants growth, water relations and photosynthesis of two bean genotypes *Phaseolus vulgaris* L. treated with NaCl and fluridone

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Phaseolus vulgaris **has a great variability regarding the tolerance to salinity. In this work, we used fluridone as a tool to study the herbicide's effect on two salt stressed bean genotypes since fluridone alters photosynthetic pigments and blocks normal abscisic acid biosynthesis under salinity. Plants from two bean genotypes were subjected to different NaCl concentrations during three days. Thereafter, half of these plants were treated by fluridone and then were harvested three days later. Growth, water relations, stomatal conductance and photosynthesis were reduced after NaCl application. In general, differences were highly significant for salt treatments up to 60 mM. Decreases in photosynthetic rates under salinity were attributed partly to reduce stomatal conductance ($r^2 = 0.87^{**}$), partly to membrane alterations ($r^2 = 0.69^{**}$) and partly to reduced photosynthetic pigment concentrations ($r^2 = 0.56^{**}$). Thus, when plants of both genotypes were subjected to fluridone, they showed higher sensitivity to a subsequent salinity than stressed plants that had not been exposed to the inhibitor; the negative effects were most evident with 60 mM NaCl concentration and in the presence of 90 mM NaCl, almost all physiological activities were suppressed. The superiority of the genotype** *Tema* **against** *Djadida* **genotype was attributed to quantitative rather than qualitative physiological response differences.**

Key words: Salinity, fluridone, bean, growth, photosynthesis, stomatal conductance.

INTRODUCTION

Salinity is at present one of the most serious environmental problems influencing crop growth. It has been extensively demonstrated that salinity affects several physiological processes in plant, including plant-water relations and nutritional mechanisms of most salt-sensitive crops species. Wild plants are in general more tolerant to salinity and could encounter sensitive cultivated crops in such environments. Herbicides are usually the most last effective choice for wild plant control.

Herbicides offer longer lasting control than mechanical methods, minimal expenditures of labor and equipment, and offer greater flexibility and predictability which ultimately leads to reduced costs. However, health and environmental concerns restrict the use of chemicals in eco-systems (Bevan et al., 2012). The carotenoid biosynthetic pathway constitutes an excellent target for herbicides because it is essential for plant development, but absent in animals.

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Carotenoids are polyunsaturated antioxidants that play an essential role in photosynthetic organisms because they carry out three key functions; first, accessory light-harvesting role by absorbing light (400 to 550 nm) and transferring it to the chlorophylls. Secondly, an antioxidant function by protecting the photosynthetic apparatus via quenching a triplet sensitizer (Chl^3), singlet oxygen and other harmful free radicals which are naturally formed during photosynthesis. Thirdly, a structural function for the photosystem assembly assumes the stability of thylakoid membrane and light harvesting complex proteins (Kim et al., 2004). Therefore, the pathway of carotenoid biosynthesis has been a good target for herbicide development and many different compounds, such as fluridone, norflurazon, diflufenican, and flurtamone, have been commercialized as herbicides interfering with carotenoid biosynthesis.

Plants treatment by these herbicides lead to an oxidative degradation of chlorophyll and rapid destruction of photosynthetic membranes by excessive reactive oxygen (ROS) generated from functionally active photosynthesis. Consequently, these herbicides are often referred to as bleacher or bleaching herbicides. The oxidative stress could probably explain the death of differentiated green tissue by carotenoid biosynthesis inhibitor (García et al., 2010). However, only a few inhibitors of this pathway have been commercialized because most compounds lack sufficient crop selectivity.

The herbicide fluridone belongs to the substituted tetrahydropyrimidinones class of herbicides which affect carotenoid synthesis in plants. Fluridone (1-methyl-3-phenyl-5-[3-trifluromethyl (phenyl)]-4-(1H)-pyridinone) is a selective inhibitor of carotenoid synthesis in cells whose mode of action at the molecular level has not been satisfactorily elucidated. Fluridone is a non-competitive inhibitor of the phytoene desaturase (PDS) enzyme in plants (Ren et al., 2007). Under normal conditions, phytoene does not accumulate in plant cells but is rapidly converted to the colored carotenoids phytofluene and β carotene by PDS. Carotenoids and chlorophylls are essential constituents of photosynthetic membranes. Carotenoids are present in all photosynthetic organisms where they serve as accessory pigments for harvesting light and are also components of photosynthetic reaction center complexes (Lu et al., 2001). In addition, there exist evidences that carotenoids are the main precursors for Abscisic acid (ABA) synthesis in plants which should also prevent the synthesis of ABA.

ABA is involved in the plant adaptation to abiotic stresses such as salinity (Ren et al., 2007). Many studies have shown that the physiological effects induced by salinity could be modulated by abscisic acid (ABA); it facilitates the adaptation to salinity of cells in tissue culture (Mills et al., 2001), reduces leaf abscission and increases salt tolerance in citrus plants (Gomez-Cardenas et al., 2002) and decreases total biomass and increases root to shoot ratio in poplar species (Yin et al., 2004). Previous studies of Montero et al. (1998) and Sibole

et al. (1998) showed that in salt treated beans, there is a high positive correlation between leaf Na^+ and leaf ABA content.

In this study, we used fluridone as a tool to study the herbicide's effect on plant photosynthesis and growth to discriminate between the tested genotypes since fluridone altered photosynthetic pigments on or hand and by blocking abscisic acid synthesis normally in plants under salt stress in the other hand. Among the comparisons between two genotypes of bean, we tested in the present study the effects of fluridone application, the inhibitor of ABA biosynthesis, on plants performance subjected to salt stress in order to determine further existence of intra-specific differential response against salinity by testing the involvement of carotenoids as well as by the implication or not of abscisic acid in this probable differential response. The understanding of physiological responses under these conditions could be of value in programs conducted to breed salt tolerant crop genotypes and varieties. The identification of tolerant genotype that may sustain a reasonable yield on salt affected soils constitutes a practical strategy to overcome salinity.

MATERIALS AND METHODS

Plant material and growth procedure

Two genotypes of *Phaseolus vulgaris* L.; *Tema* and *Djadida* commonly cultivated in Algeria, were selected on the basis of greenhouse and fields observations that showed differences in growth and yield. This experiment was conducted to examine whether there are differential responses between these genotypes from the same species against salt stress and herbicides. Seeds were surface sterilized with 5% (w/v) commercial bleach sodium hypochlorite solution (NaOCl) three times for 30 min with gentle stirring and subsequently washed in deionized water and then pre-germinated between wet paper towels at 25°C in the dark for later selection of uniform seedlings.

After 7 days, seedlings were individually transplanted into beakers filled with 300 ml of modified Hoagland nutrient solution in order to avoid over-salinization of the medium after NaCl stress application. The nutrient solution was continuously aerated using an air pump and plants were supported by inserting them through holes drilled in Styrofoam rings held in place by the beaker neck. Containers and tops were completely covered with aluminum foil to keep out light from the hydroponic culture. Solutions were renewed twice to three times a week to avoid nutrient deficiency as well as to adjust pH toward 5.5 and minimize nutrient depletion. Plants were grown in a culture chamber under controlled conditions with light intensity of about 600 $\mu mol.m^{-2}s^{-1}$ and 14 h duration, 70% relative humidity and 27/20°C day/night temperature.

Stress application

After three weeks of growth, plants of each genotype were arranged into six blocks; each block contained ten plants. Salt treatments were set up on the five blocks by adding 30, 60, 90, 120 and 150 mM NaCl to the nutrient solution while the block with the nutrient solution only served as a control. Three days after imposing salt treatments, five plants from each block were subjected to 10 µM fluridone (1-methyl-3-phenyl-5-[3-trifluromethyl]-4-(14)- an inhibitor of ABA biosynthesis, was dissolved in ethanol plus three drops of TWEEN 20 (Sigma, St. Louis, MO) (Ober and Sharp, 1994).

The same amount of ethanol and TWEEN 20 was added to the solution culture of non-fluridone-treated plants. Plants were analyzed and harvested after three days from the fluridone treatment.

Physiological measures

Before the harvest, the stomatal conductance of the most fully expanded leaves was measured with an LI-1600 steady state porometer (LiCOR Inc., Lincoln, NE). The photosynthetic rate of the same leaves was measured during the measurement period with a portable photosynthesis system (LI-6400, LiCOR, Lincoln, NE). The relative water content (RWC) of the uppermost fully expanded leaf-lets of each plant was measured according to the method of Weatherly (1950). Water potential (Ψw) was also measured using a pressure chamber (Scholander Pressure Bomb, Arimad 2, Germany).

Once plants were harvested, leaves, petioles, and stems were excised and their fresh weight (FW) was immediately recorded. Roots were rinsed three times with distilled water and carefully dampened using tissue paper before their fresh weight was recorded. Samples were dried after that during 48 h at 80°C to determine their dry weight (DW). Chlorophyll (Chl a, b) and total carotenoid (xanthophylls and β-carotene) concentrations were determined spectrophotometrically following the method of Lichtenthaler (1987). Malonaldialdehyde, routinely used as an indicator of membrane lipid peroxydation was determined according to Heath and Packer (1968) method.

Statistical analysis

Five replicates per treatment per genotype were used to arrange data into a single matrix. The variance of homogeneity of the data was assessed and conformed to the model which would permit analysis of variance (ANOVA) on the data set in order to determine significant differences between treatments of each genotype then between both genotypes. Data were analyzed using the General Linear Model (GLM) procedure implemented in the statistical software SAS (SAS Institute, Cary, NC, 1985) by ANOVA analysis. Treatment means were separated and compared using protected Student-Newmen-Keuls test. The term significant indicates differences for which P < 0.05 under the confidence level α = 95%. Collected data were used also for calculation of many regressions and correlations based on the coefficient of Pearson among examined traits.

RESULTS

The effects of the herbicide fluridone on growth, photo-syn-thesis and transpiration behavior of the analyzed genotypes subjected to salt stress were investigated. The data were expressed as percentages of mean maximum values obtained for control plants. Comparing to plants control, the observed differences were highly significant (P < 0.01**) for plants grown under salt treatments up to 60 mM. Fluridone had dramatic effects on plants. Thus, when plants of both genotypes were subjected to fluridone, they showed higher sensitivity to a subsequent salinity stress than stressed plants that had not been imposed to the inhibitor.

Reduction of the biomass in beans under saline conditions here was indicative of several growth limitations. It is striking that independent of saline level, *Tema* plants presented higher leaves biomass weight than those of

Djadida while roots biomass was much higher in the genotype *Djadida*. For genotypes, roots and leaves growth were adversely affected by the salt stress and fluridone application (P < 0.01**). With salinity increase, biomass reduction tendency was more pronounced in *Tema* than in *Djadida* genotype. Leaves and roots dry weights were significantly lower by around 40 and 44% in *Tema* after an exposition of seven days to 150 mM NaCl, however, these parameters were inferior by around 31 and 38% in *Djadida* plants compared to their respective controls. Besides, the fluridone application to the nutrient solution affected mainly leaves (33%) than roots (27%) dry weight in *Tema*. In contrary, roots were more affected in *Djadida* (23%) by the growth inhibition than leaves (19%) compared to the plants control. The combined constraint salinity-fluridone leads to severe growth inhibition in leaves and roots dry weights for both genotypes. Salinity lowered LDW about 49 and 37 respectively in *Tema* and *Djadida* genotypes in the presence of fluridone and on the other hand, it lowered RDW by 48% in both genotypes.

Water statue was highly sensitive to salinity and is, therefore, dominant in determining bean responses to salinity. NaCl affected significantly leaf water content (RWC) and water potential (Ψw) of plants (Figure 1). Differences among genotypes were significant in the control and at any salt concentration. *Djadida* had less negative values of water potential under NaCl treatments than *Tema* genotype. Reduction was higher in *Tema* than in *Djadida* genotype; in fact, it decreased by 175% when control was compared to the treatment with 150 mM NaCl in *Tema* against a decline less than 150% in *Djadida* under the same conditions (Figure 2). It should be noted that up to 90 mM NaCl decrease became more significant in both genotypes. Fluridone application reduced the ability of plants to adjust their water potential in block control. When 10 µM fluridone was applied, higher reduc-tions were recorded more obviously in *Tema* than in *Djadida* when comparing salt stressed plants to those of the control. It should be noted that up to 90 mM NaCl decrease became more significant in both genotypes following the same tendency.

Similar pattern was observed about relative water content; RWC values ranged between 85 and 75% in both genotypes with higher hydration recorded in *Tema*. The results show that relative water content of both genotypes increased slightly under 30 and 60 mM NaCl to decreased significantly thereafter with increasing salt levels in the nutrient solution (P < 0.01**). The decrease of RWC in both genotypes tissues under salt stress was around 14% and was significantly correlated with the decline in water potential (Ψw) (r^2 = -0.78**). Fluridone addition disturbed water statue on average by around 6% of decline in plants control. However, combination fluridone-salinity reduced water content by 18% in *Tema* and 16% in *Djadida* compared to their respective control.

Lipid peroxidation measure allowed the evaluation of the ability of these genotypes to maintain the integrity of
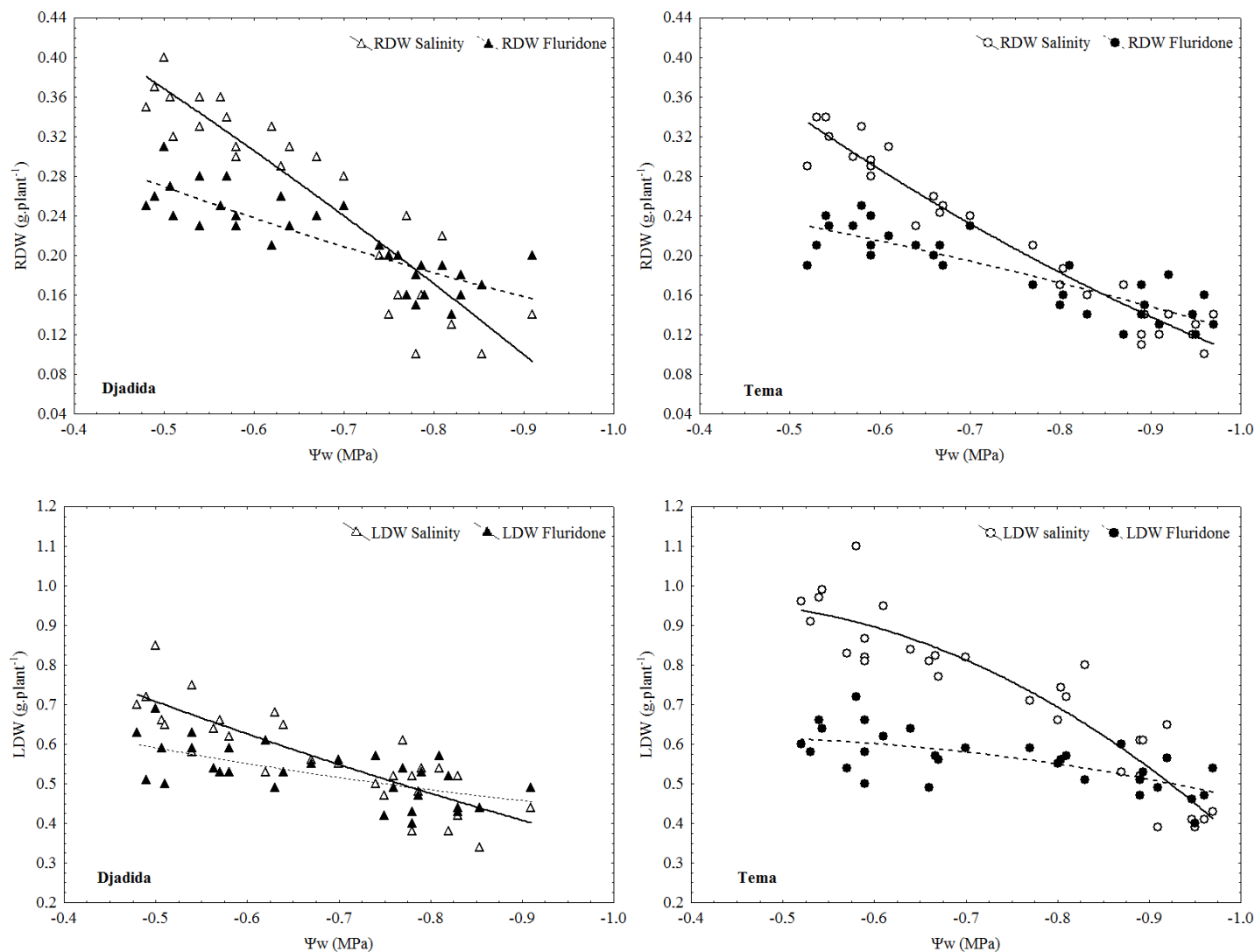
Figure 1. Leaves Dry Weight (LDW) and Roots Dry Weight (RDW) of the two *Phaseolus vulgaris* genotypes plants grown under salt stress and subjected to 10 µM Fluridone.

their cell membranes at low water potentials and ionic stress induced by salinity. Water statue of both genotypes was closely dependent to the rates of lipid peroxidation ($r^2 = 0.89^{**}$). Increase of Malonaldialdehyde content was observed for both genotypes under salinity (P < 0.05*). *Tema* genotype presented almost higher rate of peroxidation and the increase under salinity was more pronounced for treatments up of 60 mM NaCl (P < 0.01**). The membrane damages were more significant after fluridone application for both genotypes especially for *Tema* leaves where the increase reached threefold the habitual synthesis under control conditions. The increase of malonaldialdehyde content was about two fold in *Djadida* leaves under the double constraint which was caused by higher salinity and fluridone.

Stomatal conductance under unstressed conditions was higher in *Tema* than *Djadida* plants and the differen-

ces keep constant throughout salt treatments. High salinity lowered significantly the stomatal conductance by 51% in both genotypes compared to their relevant control (P < 0.01**). Applications of 10 µM fluridone decreased stomatal conductance of unstressed plants of both genotypes by 41%. However, drastic declines of stomatal conductance by around 75% were noted in plants grown under salinity combined with the herbicide fluridone (Figure 3).

Stomatal limitation to photosynthesis of bean plants under salt-stress conditions was consistent with the net photosynthetic rates ($r^2 = 0.8^{**}$). Net photosynthesis rates of salt stressed plants declined about 38% when compared to unstressed plants for both genotypes (Figure 4). Fluridone application on unstressed plants produced net photosynthetic rates decrease of 37% in *Tema* and 24% in *Djadida* (Figure 5*)*. Furthermore, on salt stressed
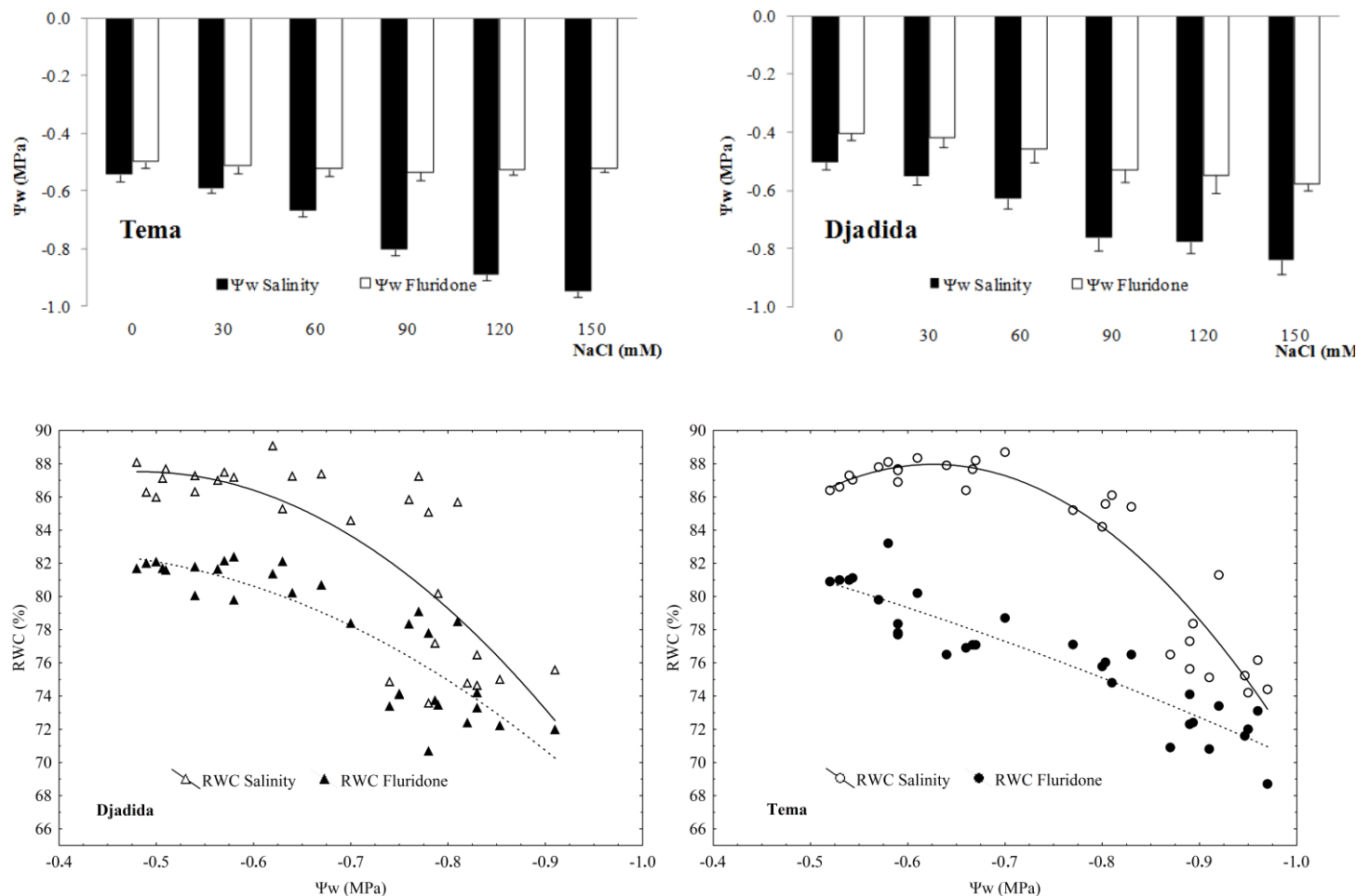
Figure 2. Salinity effects on water relations of two *Phaseolus vulgaris* genotypes plants grown under salt stress and subjected to 10 µM Fluridone.

plants, the net photosynthetic rates decreased following the same trend as stomatal conductance (73% in *Tema* and 62% in *Djadida*). Our results show an inverse relationship between salt concentration and Chl a and b contents. Whenever salt concentrations increased in the nutrient solution, Chl a content decrease by 40% in *Tema* and by 31% in *Djadida* compared to their respective control plants. Nevertheless, Chl b decrease by around 27% in both genotypes. Following carotene content during exposure of the bean plants to salt stress, it appears that salt stress was an inhibiting factor for the formation of carotenes inside the stressed bean where the carotene content decreased by 13% in *Tema* and by 11% in *Djadida*.

Analysis of the pigments composition after fluridone treatment indicated that fluridone caused a decrease of carotene (Car) and total chlorophyll content with significant changes in Chl a/Chl b ratio in all plants (Figure 6). The decrease of the Chl and Car content was at different extent; it was by 33% for Chl a and by 29% for Chl b while Car content decreased by 27% in unstressed plants. For salt stressed plants treated with fluridone, the content of Chl a decreased more obviously than Chl b (52% against 36%) which led to significant decline of the Chl a/b ratio for both genotypes. We noted also comparable reduction of 47% in Car content under the combined constraint compared to unstressed plants.

DISCUSSION

When plants of both genotypes were subjected to fluridone supply, they showed higher sensitivity to a subsequent salinity than salt stressed plants that had not been subjected to fluridone. Reduction of the biomass in beans under saline condition was indicative of several growth limitations (Kaymakanova and Stoeva, 2008). The assayed common bean plants are salt sensitive, able to grow at salinity levels below 60 mM NaCl but not surviving well above 90 mM NaCl. Reduction of plant growth and dry-matter accumulation under saline condi-tions has been reported in several important grain legumes (Tejera et al., 2006). However, at these levels, treatment of plants with 10 µM fluridone caused a considerable decrease in the rate of growth and bleaching of the plants. Salinity
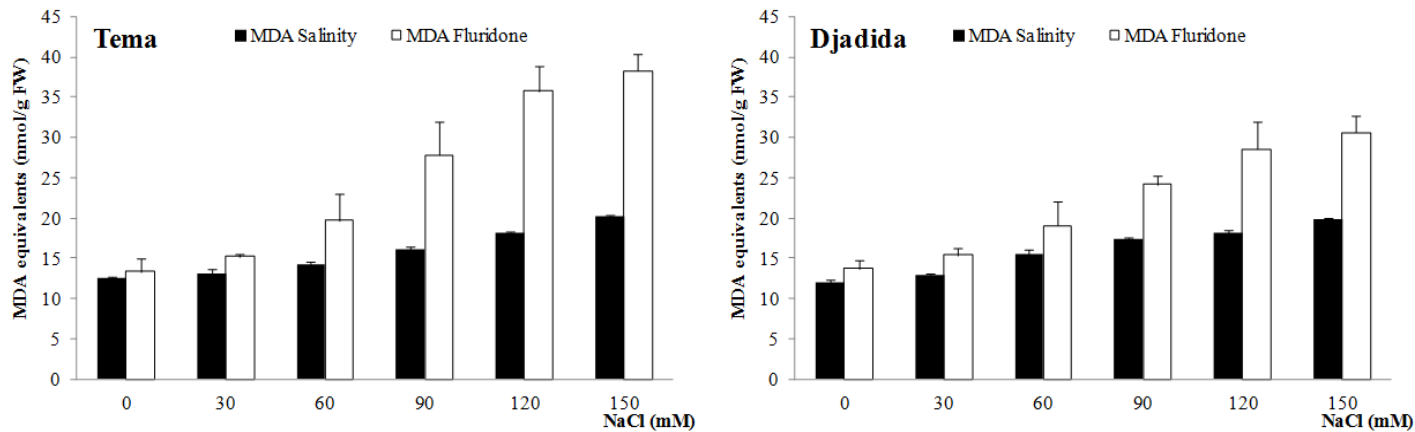
Figure 3. Salinity effects on Malonaldialdehyde content of two *Phaseolus vulgaris* genotypes plants grown under salt stress and subjected to 10 μM Fluridone.
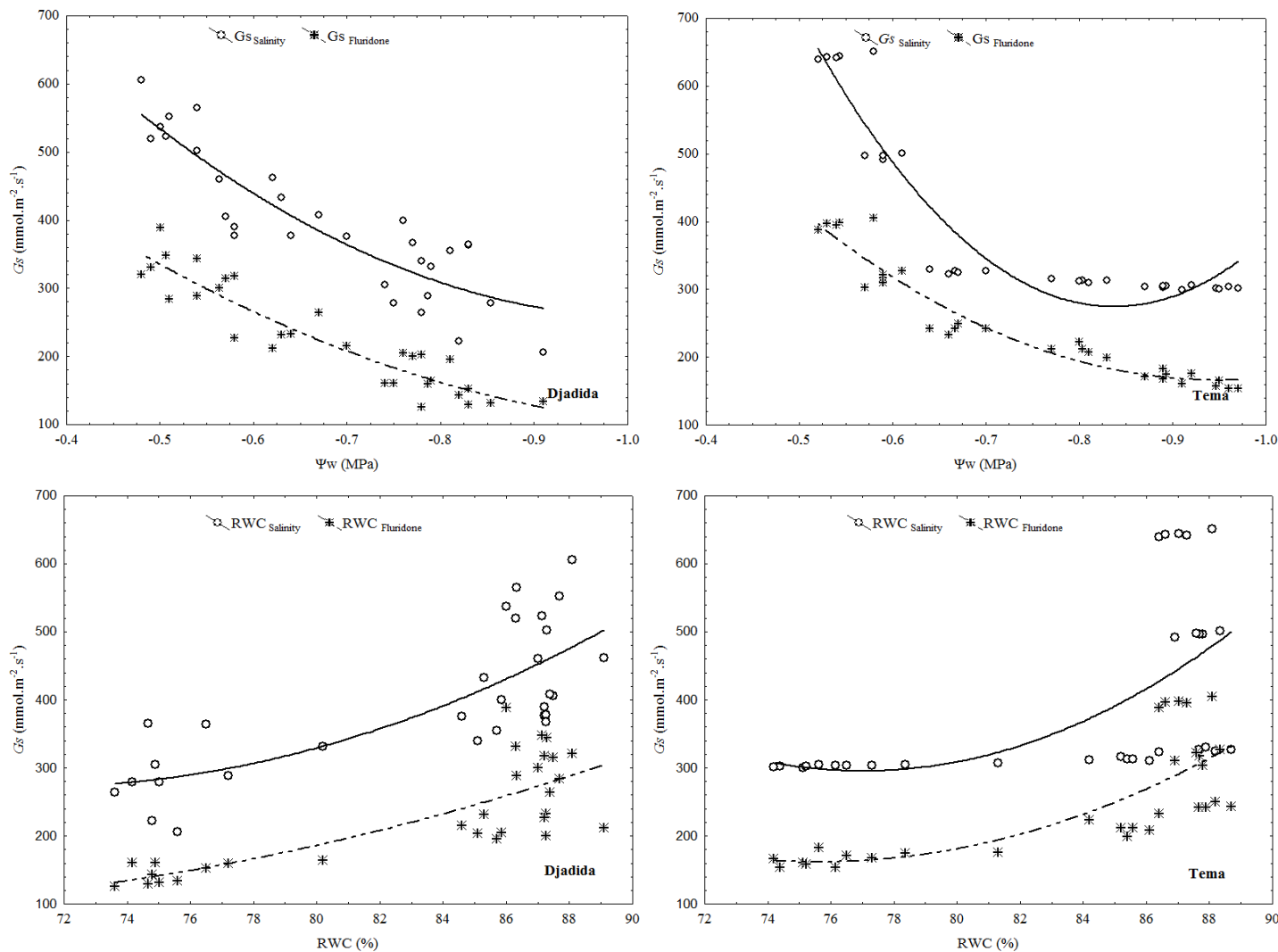

Figure 4. Relationship between stomatal conductance (Gs) and water relations of plants of *Phaseolus vulgaris* grown under salt stress and subjected to 10 μM Fluridone.
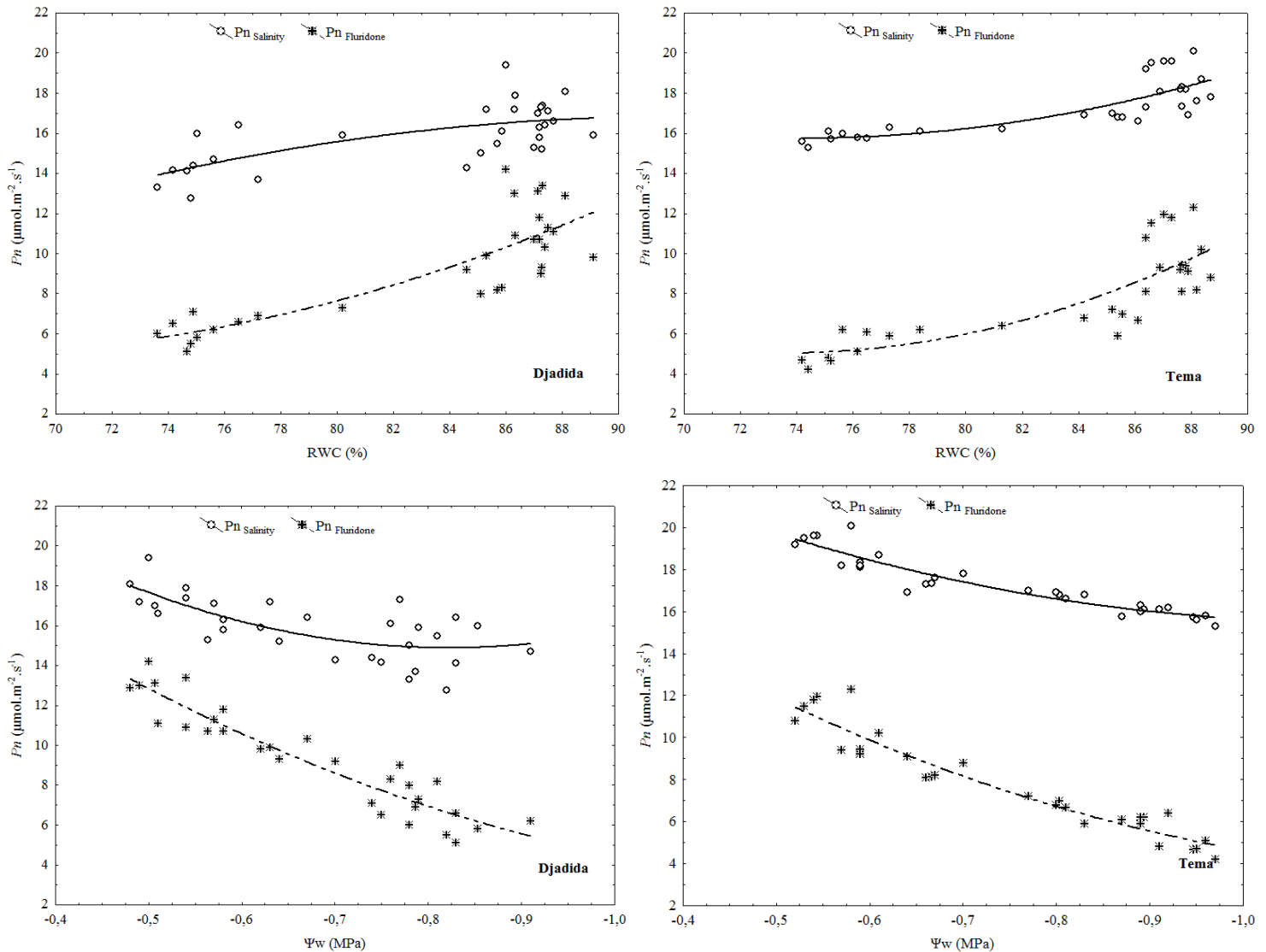
Figure 5. Relationship between photosynthesis rate (Pn) and water relations of plants of *Phaseolus vulgaris* grown under salt stress and subjected to 10 µM Fluridone.

and fluridone had adverse effects for both genotypes not only on the biomass, but also on other morphological parameters such as plant height, root length and shoot/ root ratio. Shoot growth was more extensively affected than root growth in *Tema*, dissimilar to *Djadida* characterized by determined shoot growth, and consequently, increases of root to shoot ratio with NaCl were higher in *Tema*. Our results are in accordance with experiments of Creelman et al. (1990) in soybean, where authors considered this behavior profitable since could improve plant water status.

Water status is highly sensitive to salinity and therefore is dominant in determining the plant responses to stress (Stepien and Klobus, 2006). The results show that water potential (Ψw) decreased considerably in salt treated plants because salinity increased cellular water loss. Subsequently, leaves became succulent in order to survive in salt-stressed environments and maintain adequate leaf water content. The reduced water potential could be explained by the fact that during stress carbon allocation, osmotic adjustment and accumulation of soluble sugars compete with other sinks and can affect growth (Kaymakanova and Stoeva, 2008). Generally, there is substantial evidence that glycophytic as well as halophytic species adjust to high salt concentrations by lowering tissue osmotic potentials with an increase of inorganic ions accumulation in tissues (Cachorro et al., 1995). Lipid peroxidation measured as the amount of tiobarbituric acid reactive substance or malondialdehyde is produced when polyunsaturated fatty acids in the membrane undergo
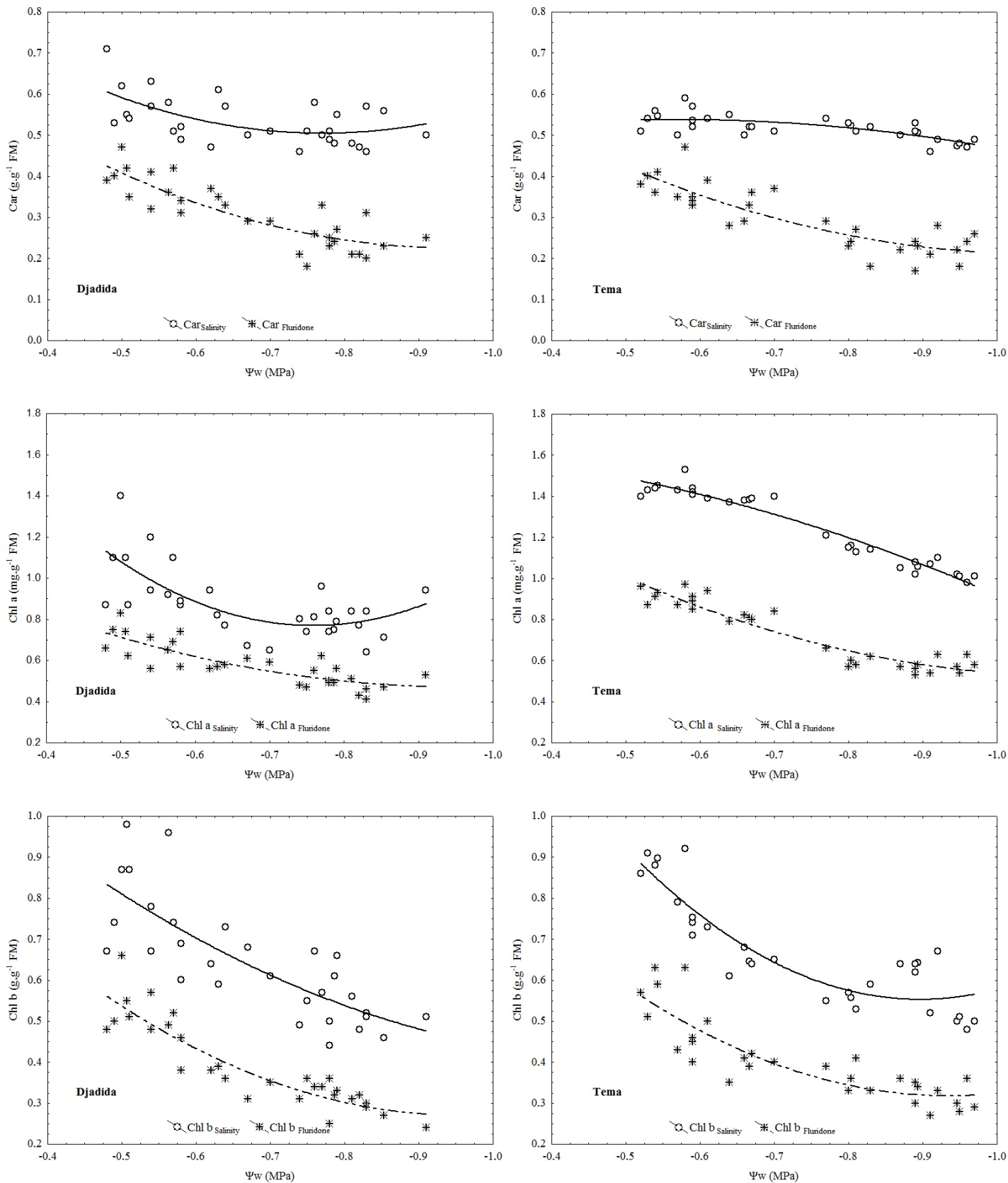
Figure 6. Salinity effects on photosynthetic pigments; Chlorophyll a (Chl a), Chlorophyll b (Chl b) and Carotene (Car) of two *Phaseolus vulgaris* genotypes plants subjected to 10 µM Fluridone.
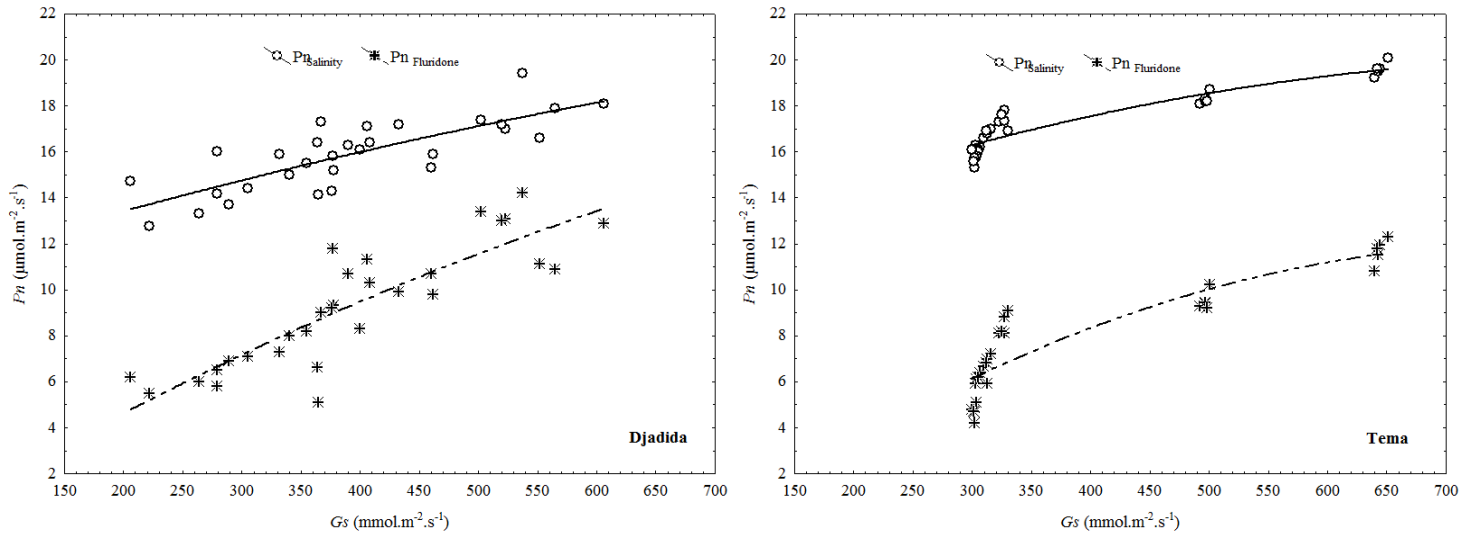
Figure 7. Relationship between photosynthesis rate (Pn) and stomatal conductance (Gs) of *Phaseolus vulgaris* plants grown under salt stress and subjected to 10 µM Fluridone.

oxidation by the accumulation of free oxygen radicals. As lipid peroxidation is the symptom mostly ascribed to oxidative damage, it is often used as an indicator of increased damage (Khan and Panda, 2008). The results reported in this study show that the degree of accumulation of MDA was higher in *Tema* compared to *Djadida*, indicating higher rates of lipid peroxidation. Our explanation is based on the facts that lack of carotenoid (especially after fluridone treatment) results in a block of membrane formation, of membrane constituents and assembly (Popova, 1995). Sibole et al. (1998) found decreased levels of soluble protein content in common bean plants under salt stress which could explain membrane alteration due to the osmotic rather than the salt-specific effect.

Regarding the stomatal conductance of bean plants under non saline conditions, the existence of some differences between genotypes was observed; the best results being registered at *Tema*. Salt stress in beans caused significant differences in gas exchange parameters in the plants of both genotypes; stomatal conductance decreased by around 51% (P < 0.01**). This reduction in conductance caused by salinity was similar to that found for kidney bean (Seeman and Critchley, 1985). Stomatal limitation to photosynthesis in common bean plants under salt-stress conditions, as shown by Brugnoli and Lauteri (1991) was consistent with the net photosynthetic rates obtained in our experiment. It has been established that salinity strongly decreased stomatal conductance (gs), which reduced photosynthesis rates (Figure .7) since they are integral elements of the photosynthetic apparatus of plants (Gama et al., 2007).

Decline in stomatal conductance with increasing salinity may be due to the fact that lowered water potentials in roots can trigger a signal from roots to shoots, such as abscisic acid, which has been suggested to be the opera-

ting mechanism (Wieland, 2004). However, an alternative hypothesis could be that the inhibition of photosynthesis caused by salt accumulation in the mesophyll produces an increase in intercellular CO_2 concentration, which reduces the stomatal aperture (Josefa et al., 2003). The observed decline in photosynthesis could be attributed to stomata factors; during salt stress, the concentration of CO_2 in chloroplasts decreased because of the reduction in stomata conductance, in spite of the apparent stability of CO_2 concentration in intercellular spaces (Tourneux and Peltier, 1995). Brugnoli and Lauteri (1991) also indicated that reduced photosynthetic carbon assimilation was attributed to reduced stomata conductance. Limited CO_2 fixation due to stress conditions led to a decrease in carbon reduction by the Calvin cycle and decrease in oxidized $NADP^+$ to serve as an electron acceptor in photosynthesis (Khan and Panda, 2008). It has been also reported that fluridone lead to decrease in photosynthesis (Lem and Williams, 1981), ribosome number per plastid and plastid rRNA synthesis (Bartles and Watson, 1978), alter lipid composition (Lem and Williams, 1981) and also chlorophyll amount per leaf (Vaisberg and Schiff, 1976) which is in agreement with our results where an inverse relationship was established between fluridone and Chl a, Chl b and total chlorophyll contents. Moreover, our results regarding the decrease in chlorophyll a, b, and total Chl under salinity, agree with those of Tort and Turkyilmaz (2004) on barley (*Hordeum vulgare* L.), Turan et al. (2007) on bean plant (*Phaseolus vulgaris* L.), Cheruth et al. (2008) on *Catharanthus roseus* (L.), Taffouo et al. (2009) on cowpea (*Vigna unguiculata* L.) and on *Vigna subterranean* (L.) when salt concentrations increase in the medium led to severe decrease in chlorophyll a, b and total chlorophyll content. Carotene content decreased under salt conditions by 11 and 13% respectively in *Djadida* and *Tema*

genotypes; these results concur with those found by Tort and Turkyilmaz (2004) on Barley and Mustard and Renault (2006) on dogwood (*Cornus sericea* L.). Carotenoid biosynthesis is one of the major targets for fluridone; analysis of the pigment composition after fluridone treatment of bean plants indicates that carotenoid biosynthesis inhibitor caused a significant decrease in both the Car and total chlorophyll contents with changes in Chl a/b ratio for both genotypes. Treatment with fluridone leads to the inhibition of phytoene desaturase and a decrease of the amount of carotenoids in the thylakoid membranes (Bartels and Watson, 1978). Kim et al. (2004) showed similar changes of the Car and total chlorophyll after herbicide treatment. It was suggested that the decrease of the chlorophylls could be a result of a carotenoid deficiency-induced photo-oxidation of the chlorophylls which could be explained by the increase of MDA of plants under this constraint ($r^2 = 0.68^*$) (García et al., 2010). In addition, our results indicate that the degradation of Chl b was smaller than that of Chl a. Such a decrease in the chlorophyll content and Chl a/b ratio could be attributed to damage of the chlorophyll a-binding proteins (Dankov et al., 2009). This fact is in agreement with the observation that core antenna complexes of PSII are more sensitive to illumination than the peripheral complexes, the degradation of these pigment–protein complexes could be due to the degradation of their newly synthesized molecules, as a consequence of chlorophyll photo-oxidation (Dalla Vicchia et al., 2001), which influences pigment–protein interaction and apoprotein stabilization (Mullet et al., 1990). It should be noted that MDA contents increase under stress treatments to higher values after fluridone application ($P < 0.05^*$).

In summary, the present study shows that stomatal con-ductance, photosynthetic rate, leaf area, and leaf water relations were reduced after NaCl application. Decreases in photosynthetic rates under salinity were attributed partly to reduced stomatal conductance, partly to membrane alterations and partly to reduced photosynthetic pigment concentrations especially Chlorophyll a and Carotenoids. Fluridone had a dramatic effect on plants response to stress in both genotypes. Thus, when plants of both genotypes were subjected to Fluridone, they showed higher sensitivity to a subsequent salinity stress than stressed plants that had not been imposed to the inhibitor. This finding illustrates the efficiency of the selective herbicide fluridone used on bean plants growth and survival especially under salinity constraint.

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