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Effect of water stress and NaCl triggered changes on yield, physiology, biochemistry of broad bean (*Vicia faba*) plants and on quality of harvested pods

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Abstract: Salinity and drought are the most important abiotic stresses affecting crop yield. Broad bean was chosen as model plant for assessing the impact of salt stress and its interaction with drought in the field experiments. The factors examined in the experiments were the two irrigation rates (normal watering – NW with 3 L plant^{-1} and drought – D) and three salinity rates imposed by foliar application (0, 50, 100 mg L^{-1} NaCl). Highest NaCl level with normal water irrigation caused maximum reduction in plant height and production, which it was due to photosynthetic disturbances. Salt injuries were alleviated by increasing water stress. The control plants exposed to NaCl lost their ability over water control. The increased malondialdehyde (MDA) and H_2O_2 indicate the prevalence of oxidative stress due to salinity. The levels of proline and carbohydrates were higher under salinity alone than under simultaneous exposure to drought and NaCl. The protein concentration of immature and mature broad bean pods was more inhibited more by NaCl supply than by drought alone. The combination of drought and NaCl resulted in a significant increase in proteins, glucose, fructose and sucrose content. Overall, the ameliorative effect of drought under NaCl supply was quantified.

Key words: drought; lipid peroxidation; photosynthesis; proline content; respiration rate; salinity; sugar content

Introduction

Drought and high salinity, in combination, are two major environmental determinants of plant growth and productivity. The drought effects are widely observed worldwide over 1.2 billion ha in rain fed agricultural land. It is a key factor limiting plant growth, development and productivity in many regions of the world (Passioura 2007).

High soil salinity is another important environmental factor limiting distribution and productivity of major crops. Agricultural productivity in arid and semi-arid regions of the world is very low due to accumulation of salts in soils (Ashraf et al. 2002). Saline medium causes many adverse effects on plant growth, which is due to low osmotic potential of soil solution (osmotic stress), specific ion effects, nutritional imbalance or a combination of these factors. More precisely, the excess Na^+ is one of the major impact of sodium chloride (NaCl) salinity on growth inhibition. Sodium may compete with K^+ in membrane transport, interfering in enzyme activity and impairing the ability of plants to grow (Epstein 1998). Although increased uptake of salts may contribute to osmotic adjustment, plant growth is suppressed due to Na^+ and Cl^- toxicity. A range of symptoms have been described, including chlorosis on the tips of older leaves, followed by necrosis and finally death of leaves (Xu et al. 2000; Saadatmand et al. 2007).

Desertification and salinization are rapidly increasing on a global scale and currently affect more than 10% of arable land, which results in a decline of the average yields of major crops greater than 50% (Wang et al. 2009; Li et al. 2010). Therefore, understanding the mechanisms of plant response to simultaneous drought stress and salinity is a crucial environmental research topic. Generally, exposure to drought or salt stress triggers many common reactions in plants that lead to cellular dehydration with concomitant osmotic changes; removal of water from the cytoplasm into the extracellular space resulting in a decrease of the cytosolic and vacuole volumes. Another consequence of exposure to these stresses is the generation of reactive oxygen species (ROS), which in turn have a negative oxidative stress effect on cellular structures and metabolism (Wang et al. 2009).

Vicia faba L. is an annual herb known as broad bean with long tradition of cultivation in Greece and East Mediterranean countries. It is among the most ancient plants in cultivation and also among the easiest to grow. Unlike most legumes, the broad bean can be

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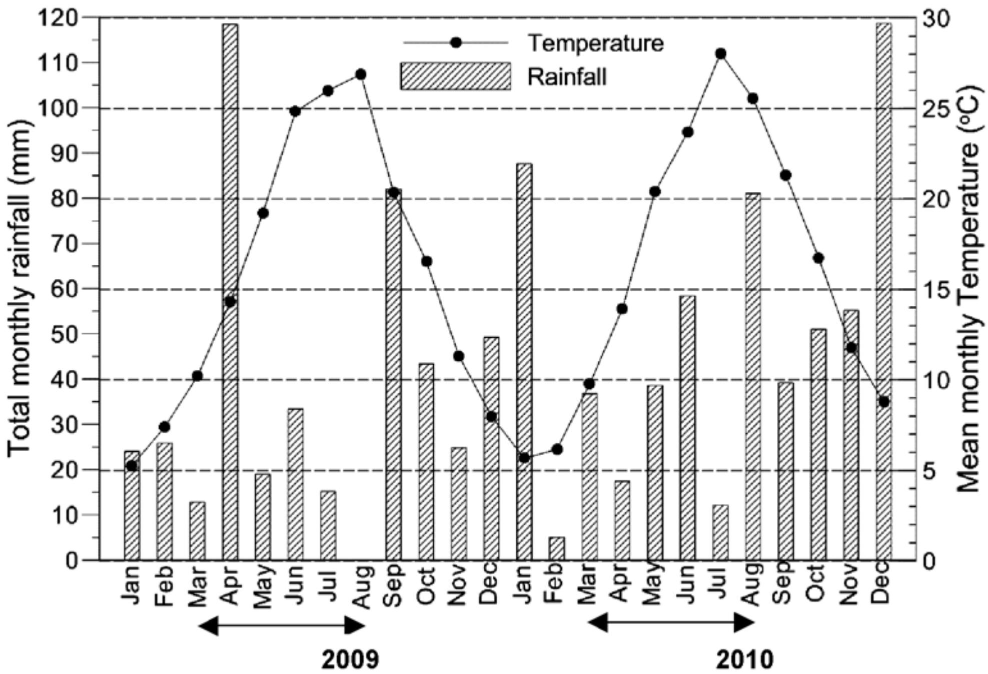

Fig. 1. Mean monthly temperature (℃) and total monthly rainfall (mm) at the experimental area during the experimental seasons 2009–2010, marked by arrows.

grown in soils with salinity, but it does prefer to grow in rich loams.

The impact of increased NaCl and water stress on crop growth is well documented; however the interaction between these two is not well understood. The objective of the present study was to evaluate the effects of salt and drought stress, imposed alone and in combination, on growth, photosynthesis and biochemistry of broad beans plants, as well as we try to assess any decay processes by the use of quality and respiration rate of immediately harvested pods.

Material and methods

Plant material and culture

Field experiment was conducted twice during the 2009 and 2010 from February through July (this period is related to the growth and ripeness of broad bean pods) at the Technological and Education Institute Farm of Thessaloniki in northern Greece. The site is located at 22°55 N, 40°38 E. Experiment was established on a sandy loam (Xerofluvent) soil consisting of 644 g sand, 280 g silt, 76 g clay, 7 g organic C kg^{-1} with a pH (1:2 H_2O) of 7.6. Electrical conductivity (EC) of the saturated soil extract in 0–60 cm depth was 2.2 dS m^{-1} and the soil moisture was 15%. Mean monthly temperature and rainfall data for 2009 and 2010 recorded near the experimental area, are given in Fig. 1. Nitrogen and phosphorus (P_2O_5) at 80 and 40 kg ha^{-1}, respectively, were incorporated as diammonium phosphate (20-10-0, F-TOP Ledra Ltd, Thessaloniki) into the soil prior to broad bean planting. Additionally, 50 kg N ha^{-1} as ammonium nitrate (33.5-0-0, F-TOP Ledra Ltd, Thessaloniki) were applied in late April. Broad bean (*Vicia faba* L. cv. Kasteloriza, local Greek variety) was sown by hand individually and randomly in eighteen experimental plots at a seed rate of 10.000 seeds ha^{-1}.

The factors examined in the experiment were the two irrigation rates (normal watering – NW with 3 L $plant^{-1}$ and drought – D) and three salinity rates (0, 50, 100 mg L^{-1} NaCl). Electrical conductivity (EC_w) of applied irrigation water was 0.6 dS m^{-1}, which is equal to TDS~384 mg L^{-1}. The plants from each plot were foliar-sprayed (to run-off) with a low pressure hand-wand sprayer and NaCl was applied 5 times at 10 days intervals. First application of NaCl was made when plants had five to six leaves. The experiment was conducted in an area where weed species were observed at very low densities and were hand-removed. Cultural practices were carried out according to the recommended production practices for the area. The broad bean plants and pods were harvested by hand early July and all measurements were made by the end of the experiments.

Pigment estimation

Chlorophylls (a + b) of the youngest fully expanded leaf were quantitatively measured in 100% acetone extract according to Ouzounidou et al. (2008).

In vivo chlorophyll fluorescence measurements

In vivo chlorophyll fluorescence was measured on the upper surface of the youngest fully expanded leaf, using a Plant Analyser (PEA, Hansatech Ltd King's Lynn, Norfolk, England). The method is described by Ouzounidou et al. (2008). Measurements were made at room temperature on intact leaves of five replicate plants from the six treatments.

Gas exchange measurements

Gas exchange was measured on the youngest fully expanded leaf with a Li-6200 portable photosynthesis meter (LiCor, Inc. Lincoln, NE) supplied with IRGA (Li-6250). Calculations of net photosynthetic rate (A), transpiration rate (E), and water use efficiency (WUE = A/E) from gas exchange measurements were according to Von Caemmer & Farquhar (1981).

Table 1. Effects of irrigation treatments (normal watering, drought) and NaCl concentration (0, 50, 100 mg L^{-1}) on broad bean yield and growth. Values followed by different letters in the same column are significantly different at $P < 0.05$. ($n = 12$, ± SE).

Treatments	NaCl concentration (mg L^{-1})	Plant height (cm)	Number of branches plant^{-1} (77 days after sowing)	Days to anthesis	Days to harvest	Number of pods plant^{-1} (whole season)
Normal watering	0	84.4 ± 0.57d	4.9 ± 0.10c	46 ± 0.2a	137 ± 1.56a	47.4 ± 0.65c
	50	69 ± 0.62 ab	4.0 ± 0.02b	48 ± 0.1a	141 ± 1.64ab	33.7 ± 0.57a
	100	65 ± 0.72a	3.6 ± 0.03a	49 ± 0.5a	145 ± 0.95bc	31.5 ± 0.44a
Drought	0	72.1 ± 0.50b	4.6 ± 0.09 bc	47 ± 0.1a	147 ± 0.88c	44.2 ± 0.24bc
	50	76 ± 0.23c	4.3 ± 0.05b	48 ± 0.6a	145 ± 0.65bc	42.1 ± 0.15b
	100	71.3 ± 0.65b	4.0 ± 0.02b	48 ± 0.3a	147 ± 0.85c	41.3 ± 0.85b

Determination of proline and carbohydrates

Shoots-leaves were cut into small pieces weighed and placed separately in glass vials containing 10 mL of 80% (v/v) ethanol, and heated at 60℃ for 30 min. The extract was then filtered and diluted with 80% (v/v) ethanol up to 20 mL (Khan et al. 2000). The shoot-leaf concentrations of free proline and carbohydrates were determined in this extract following the acid ninhydrin reagent method and the anthrone method, respectively (Khan et al. 2000; Plummer 1978).

H_2O_2 assay

H_2O_2 quantification is based on the formation of a titanium peroxide complex (Ben Hamed et al. 2014). Broad bean samples were homogenized in cold acetone (1:6 w/v), and after filtration and centrifugation the supernatant was discarded and the pellet was dissolved in 3 mL of 2N H_2SO_4. The absorbance of the solution was read at 410 nm and H_2O_2 concentration was calculated using a standard curve with concentration ranging from 0.1 to 1 mM. H_2O_2 content was expressed as nmol g^{-1} FW.

Determination of lipid peroxidation

At the end of the experiment, the level of lipid peroxidation in broad beans was measured as malondialdehyde (MDA) content determined by reaction with 2-thiobarbituric acid (TBA) reactive substances according to Ouzounidou et al. (2013). The tissue was homogenized in 0.3% TBA in 10% trichloracetic acid (TCA) at 4℃. The concentration of MDA was calculated from the difference of the absorbance at 532 nm and 600 nm using the extinction coefficient of 155 $mmol^{-1}$ cm^{-1} and expressed as nmol (MDA) g^{-1} of fresh weight.

Respiration rate measurements

A sample of 5 immature (pods harvested 10 days before the end of the experiment) and mature broad bean pods was closed in 3 L airtight glass jars with a closure containing a rubber septum on top and was used to calculate respiration rate immediately after harvest, by measuring gas exchange measurements (CO_2 and O_2 concentrations) with a Perkin-Elmer 8700 gas chromatographer. All results are expressed as mg CO_2 kg^{-1} h^{-1} (Ouzounidou et al. 2012).

Quality characteristics

Quality analyses were made using immature (pods harvested 10 days before the end of the experiment) and mature broad bean pods. Sucrose, glucose and fructose were determined with HP 1100 Series High Performance Liquid Chromatograph (refractive index detector (RID) using a reverse phase column 250 × 4 mm (Lichrosphere NH_2) bonded to microparticulate silica of 5 μm diameter maintained at 37℃ (Ouzounidou et al. 2008). The protein content was determined according to the method of AOAC (1990).

Experimental design, data collection, and analysis

Eighteen experimental plots (three replications for each treatment) were set up randomly using a split-plot design. The two drought rates were the main plot factor, while the three salinity rates were the subplot factor. Each plot contained 12 single plants in 3 rows with 4 plants per row spaced 0.40 m apart with-in each row. Distance between rows was 0.5 m and between plots 1.0 m. Data was subjected to analysis of variance (ANOVA) using the *SPSS 11.0.1 for Windows* statistical package (*SPSS*, Chicago, USA). For comparison of the means, the Duncan's multiple range tests ($P \leq 0.05$) were employed.

Results and discussion

Effect of NaCl and drought on growth and yield performance

NaCl toxicity symptoms first appeared as marginal chlorosis of broad bean leaves and gradually changed to necrosis. The symptoms were more pronounced in normal watered than in drought plants. The number of branches and pods per plant decreased with the increase in NaCl, while there was no significant changes on days to anthesis under both stressors (Table 1). Drought conditions imposed alone had no significant negative effects on broad bean growth (Table 1). However, plant height significantly decreased by increasing salinity at NW and D (Table 1). A decline of 22% in plant height at NW under 100 mg L^{-1} NaCl was recorded, whereas at drought and salinity combination the suppression was 15% of the plants grown at NW. Similar results for height and weight of pistachio trees, grown under salinity and water stress were found by Saadatmand et al. (2007). Increasing water stress reduced salt injuries of broad beans. Highest NaCl level with normal water irrigation resulted in a maximum reduction in plant height and production. The deleterious effects of salt stress are commonly thought to result from a combination of osmotic and ionic stresses (Li et al. 2010). According to Saadatmand et al. (2007) a greater content of Cl^- and Na^+ ions is accumulated by plants under normal watering and salinity which is responsible for growth inhibition; whereas less salinity-induced suppression occurs under drought. Syvertsen et al. (1988) reported that drought stress had no additive effect at high salinity on osmotic potential of mature leaves. Moreover, the complex nature of the plant response to salt and water stress may result in

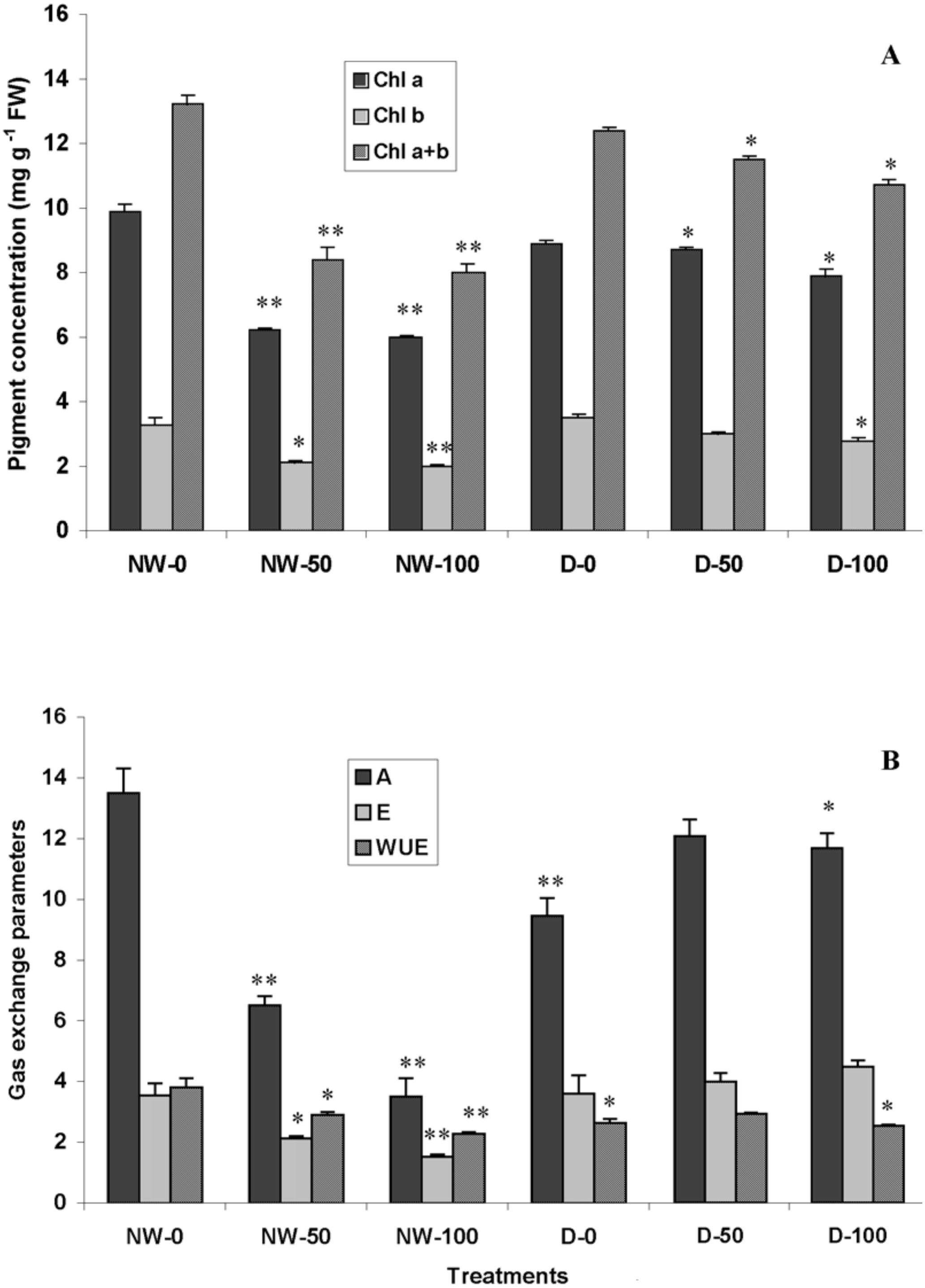

Fig. 2. Effects of irrigation treatments (normal watering – NW, drought – D) and NaCl concentration (0, 50, 100 mg L^{-1}) on (A) chlorophyll *a*, chlorophyll *b* and total chlorophyll ($a + b$) content and (B) A, leaf net photosynthetic rate, (μmol CO_2 m^{-2} s^{-1}); E, transpiration rate, (mmol H_2O m^{-2} s^{-1}); WUE, water use efficiency, (μmol CO_2 $mmol^{-1}$ H_2O) of the youngest fully expanded leaf of broad bean. Values are presented as mean ± standard error ($n = 5$). *significant at a P value < 0.05, ** significant at a P value < 0.01 as compared with NW-0 plants.

a response that is not necessarily equal or additive when the two stress factors are imposed simultaneously (Shani & Dudley 2001). Vanaja et al. (2011) observed an ameliorative effect on maize and sunflower growth under simultaneous exposure to elevated CO_2 and drought.

Effect of NaCl and drought on the chlorophyll concentration

A concentration dependent response of salinity stress was observed on photosynthetic pigments. Total chlorophyll concentration was significantly affected by NaCl and drought treatment (Fig. 2A) and decreased from

Table 2. Effects of irrigation treatments (normal watering, drought) and NaCl concentration (0, 50, 100 mg L^{-1}) on initial chlorophyll fluorescence (Fo), maximum chlorophyll fluorescence (Fm) and maximal photochemical efficiency of PSII, (Fv/Fm) of broad bean plants. Values followed by different letters in the same column are significantly different at $P < 0.05$. ($n = 5$, ± SE).

Treatments	NaCl cconcentration (mg L^{-1})	Fo	Fm	Fv/Fm	P. index
Normal watering	0	485 ± 12ab	2934 ± 54c	0.835 ± 0.08b	5.58 ± 0.21c
	50	501 ± 20b	1722 ± 65a	0.738 ± 0.03a	1.42 ± 0.05a
	100	473 ± 9ab	1630 ± 48a	0.709 ± 0.05a	1.15 ± 0.02a
Drought	0	690 ± 17c	3817 ± 62d	0.819 ± 0.04b	3.12 ± 0.11b
	50	418 ± 11a	2209 ± 46b	0.811 ± 0.05ab	3.51 ± 0.02b
	100	440 ± 19a	2048 ± 32b	0.785 ± 0.03a	3.72 ± 0.09b

13.2 mg g^{-1} FW in the normal watering to 8 mg g^{-1} FW (reduction equal to 40%) and 10.7 mg g^{-1} FW (reduction equal to 13% of D-0 and 19% of NW) for plants grown at NW-100 NaCl and D-100 NaCl, respectively. The lower Chl $a + b$ at high salinity level occurred during normal watering, indicating higher stress and damage to the photosynthetic apparatus whereas, a marked maintenance in pigment content under simultaneous salinity and drought stress was observed. Our results resemble those of Li et al. (2010) who reported a decrease in chlorophyll concentration under NaCl exposure in alfalfa. The decline in the level of photosynthetic pigments may be attributed to salinity-induced inhibition of chlorophyll biosynthesis (Khan 2006) that may be caused by nutrient deficiency induction or imbalance (Li et al. 2010).

Effect of NaCl and drought on fluorescence induction
The maximum quantum efficiency of PSII photochemistry (Fv/Fm) showed a progressive reduction under the two NaCl levels (50 and 100 mg L^{-1}) equal to 12 and 15% of the control, respectively (Table 2). On the other hand, the Fv/Fm was not significantly influenced by water stress when supplied alone. This is in accordance with the findings of Santos et al. (2009) findings in five common bean genotypes. The simultaneous exposure of broad beans to drought and salinity induced alleviation of this ratio. It seems that drought mitigated the negative impact of NaCl on photosynthetic efficiency. The toxic action of NaCl focuses (among others) on a significant decrease in the chlorophyll levels, which in turn are associated with the reaction centers of PSII. This effect could be mainly attributed to the reduction of variable fluorescence (Fv) under salt stress, indicating a disturbed status of the acceptor side of PSII (Ouzounidou et al. 2006). As the stresses progressed, biochemical constraints might limit photosynthetic CO_2 fixation more directly. The observed diminution of Calvin cycle function generates an excess of reducing power and finally leads to the formation of active oxygen species (AOS) which then leads to photoinhibitory and photooxidative damage.

Effect of NaCl and drought on leaf gas exchange
In order to estimate the effect of NaCl and drought on Calvin cycle, we measured photosynthesis and chlorophyll fluorescence, simultaneously. When compared to the control treatment (normal watered plants), assimilation rate was markedly reduced by 75% ($P < 0.01$) under 100 mg L^{-1} NaCl, whereas under simultaneous exposure to salt and drought a slight depression in the photosynthesis of broad beans was observed (11%, Fig. 2B). Drought stress alone caused less photosynthetic changes than salinity did; in addition drought stress alleviated salinity phytotoxicity in plants (Fig. 2B). The enhanced supply of photo-assimilates and improved energy supply (net photosynthetic rate) to the growing parts of beans under drought stress might be one of the important factors contributing to growth improvement under salinity. Similar pattern was displayed by the transpiration rate, which was more suppressed under salt and normal watering. The WUE also decreased more under NaCl exposure in the control plants (by 40% of the control) than under water deficit regime (by 30% of the control), due to rather large reduction of net photosynthesis than transpiration decline (Fig. 2B). According to Santos et al. (2009) stomatal and non-stomatal limitation of photosynthesis has been reported under drought stress. In our study, the low CO_2 availability measured by gs (stomatal conductance, data not shown) affects negatively net photosynthesis under both NaCl and drought treatments. However, factors other than CO_2 availability seem to be involved like pigments concentration, chloroplast structural and functional damage (Fig. 2A, Table 2). Changes of gas exchange indices under drought and salt have been referred to different plant species (Tezara et al. 2002; Jabeen et al. 2008; Chaves et al. 2009).

Effect of NaCl and drought on lipid peroxidation and H_2O_2
MDA is the decomposition product of polysaturated fatty acids of biomembranes and has frequently been used as a biomarker for lipid peroxidation and damage on the cell membrane and ultrastructure. The increase of MDA content is displayed in plants under high-level antioxidative stress (Pan et al. 2006). In our experiment, significant increases in MDA concentration in broad beans were observed, especially under salinity and normal watering (Table 3). Its concentration displayed a linear relationship with NaCl levels in the solution at both water regimes. MDA values were 3-times higher under NW-100 compared to the NW-0, while under D-100 the increase was only 1.5-times of the D-0 and 2-times of the NW-0 (Table 3). Similar results are reported for *Salicornia persica* and *S. europaea* (Aghaleh

Table 3. Effects of irrigation treatments (normal watering, drought) and NaCl concentration (0, 50, 100 mg L^{-1}) on carbohydrate (mol g^{-1} FW), proline (mol g^{-1} FW) lipid peroxidation (nmol g^{-1} FW) and H_2O_2 (nmol g^{-1} FW) concentrations of broad bean plants. Values followed by different letters in the same column are significantly different at $P < 0.05$. ($n = 5$, ± SE).

Treatments	NaCl concentration (mg L^{-1})	Proline	Carbohydrates	MDA	H_2O_2
Normal watering	0	1.48 ± 0.2a	20.92 ± 2.6a	65 ± 1.8a	6.0 ± 0.1a
	50	2.10 ± 0.03c	31.81 ± 2.4c	139 ± 2.6c	8.6 ± 0.1c
	100	2.19 ± 0.07c	32.00 ± 0.9c	148 ± 2.8d	8.8 ± 0.1c
Drought	0	1.87 ± 0.2b	21.62 ± 0.05a	95 ± 1.8b	7.2 ± 0.1b
	50	1.94 ± 0.01b	27.68 ± 1.0b	98 ± 4.2b	7.0 ± 0.1b
	100	1.77 ± 0.01b	28.77 ± 1.4b	125 ± 4.3c	8.4 ± 0.1c

Table 4. Effects of irrigation treatments (normal watering, drought) and NaCl concentration (0, 50, 100 mg L^{-1}) on proteins (g 100 g^{-1} FW) and sugars (g 100 g^{-1} FW) of broad beans pods under two growth stages (1, immature and 2, mature beans). Values followed by different letters in the same column are significantly different at $P < 0.05$, ($n = 3$), *significant differences ($P < 0.05$) between the immature and mature beans for the same factor.

Treatments	NaCl concentration (mg L^{-1})	Proteins 1	Proteins 2	Glucose 1	Glucose 2	Frucose 1	Frucose 2	Sucrose 1	Sucrose 2
Normal watering	0	3.6c	12.0d*	1.3b	0.8b*	0.6c	0.5c	0.8c	2.0e*
	50	2.3a	9.3b*	1.4b	0.8b*	0.6c	0.4b*	1.0d	0.8b*
	100	2.1a	7.2a*	1.0a	0.7b*	0.5b	0.3a*	0.9c	0.6a*
Drought	0	3.0b	10.6c*	0.9a	0.4a*	0.2a	0.2a	0.4a	1.1c*
	50	3.1b	11.4cd*	1.2b	0.7b*	0.5b	0.3a*	0.6b	1.5d*
	100	3.2bc	11.2cd*	1.2b	0.5a*	0.3a	0.4b*	0.5a	1.3cd*

et al. 2009). A similar trend for H_2O_2 was observed with its highest concentration measured under NW-100 (Table 3). Thus, the enhanced MDA and H_2O_2 indicate the prevalence of oxidative stress and this may be one of the possible mechanisms by which toxicity due to salinity could be manifested in the plant tissues. The combined application of drought and salinity reduced MDA content of broad beans indicating that drought attenuated the lipid peroxidation caused by salt. Our findings agree with other studies concerning interactive effects of multiple stresses on plants (Yao et al. 2009; Wang et al. 2009).

Effect of NaCl and drought on proline and carbohydrates

Proline and carbohydrate fluctuations of broad beans under drought and different concentrations of salinity are given in Table 3. Both proline and carbohydrates increased gradually under drought and salinity levels. The maximum proline accumulation was recorded at NW-100 (increased by 47% of the control), in addition the maximum level of carbohydrates was revealed also at NW-100 (increased by 53% of the control). Induction of such substances in the shoot-leaves of broad bean during drought and salinity indicates their involvement in stress response. The level of their induction is greater under salinity alone than under simultaneous exposure to drought and NaCl. Proline and carbohydrates are important osmolytes to adjust the plant under both drought and salinity conditions and their accumulation in response to excess NaCl was described in several plants (Ozturk et al. 1981; Ozturk et al. 1986; Ashraf et al. 1998; Liu & Staden 2001; Khan 2006; Pan et al. 2006; Ozturk et al. 2011).

Effect of NaCl and drought on post-harvest physiology of pods

The quality characteristics of broad bean pods were measured immediately after harvest and are given in Table 4. Comparing the two growth stages of the broad bean pods it can be concluded proteins and sucrose contents enhance with maturity, but there is a depression in hexose (glucose and fructose) content at both watered and drought plants. The protein concentration of immature and mature broad bean pods was more inhibited by NaCl supply (by 41 and 40%, respectively) than drought supply alone (by 16 and 12%, respectively, Table 4). Under NW the two NaCl concentrations induced loss of sugars both to immature and mature pods, whereas, during drought stress a significant loss of sucrose content by 45%, compared to the control, was observed. The hydrolysis in this case provides the elevated fructose and glucose content of mature pods. A cleavage of sucrose has also been reported by Hockema & Etxeberria (2001) in oranges under mild drought. On the other hand, the combination of drought and NaCl induced a significant increase in proteins, glucose, fructose and sucrose content of both immature and mature pods compared to the effects caused by salinity alone. The interpretation for the quality indices inhibition may be the retardation of the whole metabolism and the decrease of pod growth under salt exposure (Tazuke et al. 2009). According to Saito et al. (2008), salinity led to an increase in sugar concentration of tomato fruit, but this is still not elucidated.

The respiration rates, measured immediately after harvest, were higher for immature and drought effected broad beans pods than for mature and normal watering (Fig. 3). Precisely, immature broad beans presented

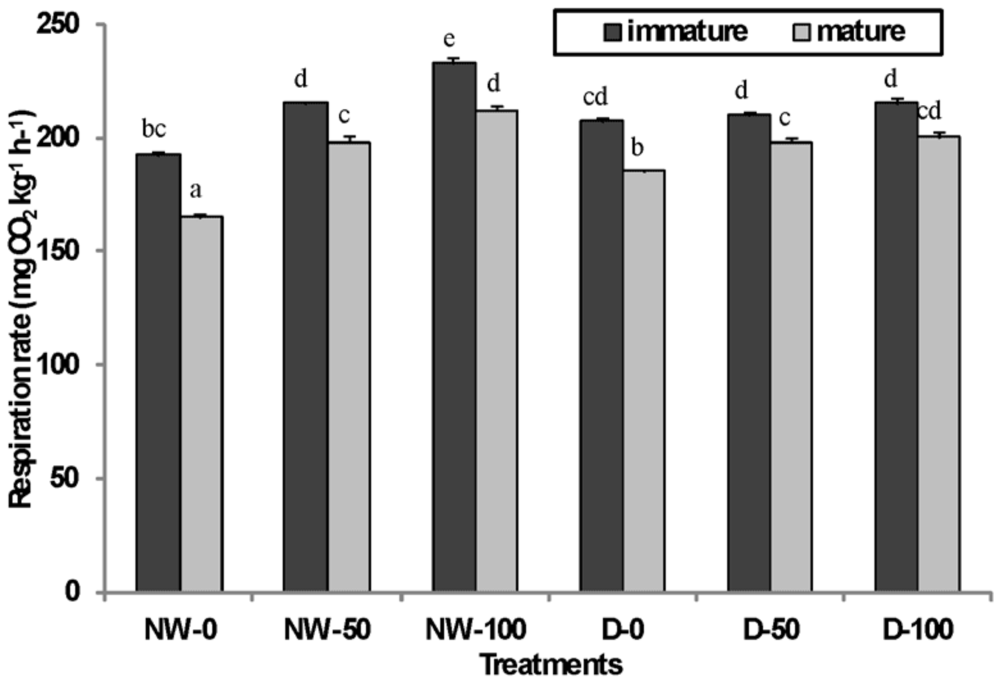

Fig. 3. Effects of irrigation treatments (normal watering – NW, drought – D) and NaCl concentration (0, 50, 100 mg L^{-1}) on respiration rate (mg CO_2 kg^{-1} h^{-1}) of immature and mature broad beans pods measured immediately after harvest. Values are presented as mean ± standard error ($n = 5$). Means that differ at $P < 0.05$ are shown by different letters.

the highest respiration rate at both normal (NW-0) and drought (D-0) conditions, showing a significant increase by 17 and 12% of the mature pods. Addition of 50 and 100 mg L^{-1} NaCl enhanced CO_2 production that was more significant under normal watering conditions. The application of both stresses alone, accelerated markedly the CO_2 production by 28% at NW-100 and 12% at D-0 compared to NW-0 mature pods (Fig. 3). The combination of stresses (drought and salinity, D-50, D-100) enhanced markedly the respiration rate in immature and mature broad beans (Fig. 3).

The increased respiration in immature pods coupled with the lack of storage reserves, as shown in Table 4, lead these beans to deteriorate rapidly. In general, the faster is the respiration, the lower is the shelf life of the fruit. Measurements of respiration rates and maximum fluorescence (Fm) provide direct information on the functioning of mitochondria and chloroplasts, respectively, because these organelles are very sensitive to early stages of deterioration in plant tissue (Ouzounidou et al. 2008). The enhanced respiration rate and lipid peroxidation (MDA) of broad beans correlate well with the decreased chlorophyll fluorescence (P. index) under drought and salinity application, representing the reduction in freshness and the beginning of senescence. The decreased photosynthetic activity and/or growth of tissues are followed by reduction of plant productivity and pod's quality.

Overall, the effects of NaCl on plant yield and physiology were suppressive since growth markedly decreased with increasing NaCl level in growth medium, but under drought conditions the salt injury was attenuated. We have confirmed the alleviation of drought in broad bean performance under salinity stress. NaCl stress had more negative influence than drought stress imposed alone, on both broad bean plants and pods. The highest growth reduction was revealed in the higher salinity treatments (100 mg L^{-1}) with normal irrigation, because in this situation greater amounts of salt were added to soil and taken by the root system causing higher injuries to the plant tissues. Under these conditions, the functioning of the aerial parts such as photosynthesis, respiration and water relations are severely disturbed. Moreover, in broad beans grown at normal watering, H_2O_2 and MDA were produced in larger amounts than under simultaneous drought conditions. Thus, it seems likely that under lack of water a possible protective strategy against salinity can be developed. However, additional experiments are in progress to ascertain other defense strategies to drought and salinity.

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