Hydroponic lettuce yields are improved under salt stress by utilizing white plastic film and exogenous applications of proline

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Using white plastic covering film and exogenous proline application for the improvement of hydroponically grown lettuce performance under salt stress

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Abstract:

Greenhouse crops are often affected by salinity due to water quality decay associated with crop fertilization and irrigation managements as well as the fact that greenhouse environment may become even hotter during summer time. Thus, combined effects of salt, heat and light stresses can affect plants grown in greenhouse. This research work addressed the combined effect of white greenhouse covering film and foliar proline spray application to reduce the detrimental effects of salinity on two cultivars of lettuce (cv. *Teide* and cv. *Impulsion*) grown on a floating system and subjected to added salt stress. Accordingly, five different experiments were conducted in two twin greenhouses covered with plastic films characterized by different light permeability. The experiments aimed at identifying the most suitable nutrient solution (exp. #1), and assessing how the effects of mild salinity (0 to 15 mM NaCl) would be alleviated by the greenhouse covering film (exp. #2, #3, #4 and #5) and foliar proline spray application (0 to 15 μM) (exp. #4 and #5). Results showed that the white covering film changed the spectral light intensity and decreased the Photosynthetically Active Radiation (PAR) of the light transmitted causing a delay in the plant growth and leaf chlorophyll content. Although salinity negatively affected plant growth and leaf photosynthesis of both cultivars, using the white film partially mitigated the influence of salt stress.

The beneficial effects of the white film on salt stress mitigation were more evident during summer and in the heat sensitive genotype (cv. *Teide*) in terms of greater total and marketable yield as compared to control conditions. Exogenous application of foliar proline (up to 5 μM) increased the yield under control condition and enhanced the plant response to salinity. Overall, for summer cultivation of cv. *Teide*, in presence of saline water (15 mM NaCl), the combination of both white covering film and proline application enabled to preserve efficiently the plant growth and final yield.

Highlights:

- **In greenhouses lettuce undergoes salt and heat stress**
- **White cover and exogenous proline were tested on 2 salt stressed lettuce cultivars**
- **White film reduced temperature, PAR integrals and spectrum peaks under 700 nm**
- **Salinity reduced yield more markedly in heat sensitive cv. Teide during summer**
- **Both white cover and proline reduced salt stress in cv. Teide**

Keywords: NaCl; Covering films; *Lactuca sativa* L.; Total and Marketable Yield; osmoprotectants.

1. Introduction

Salinity constitutes one of the major threats in current agriculture and is adversely affecting crop cultivation worldwide (Mickelbart et al., 2015). The limitation of water resources of good quality is forcing growers to use water with relatively high salt concentration for crop irrigation (Singh, 2015). To mitigate the effects of salinity, ~~many researches~~ in horticulture address both the identification of cropping practices that can reduce the plant stress perception (Paranychianakis and Chartzoulakis, 2005) and the development of strategies capable to improve the plant response to the stress (Orsini et al., 2010). Lettuce (*Lactuca sativa* L.) is categorized as a moderate salt tolerant crop (Fernandez et al., 2016). Water salinity levels of more than 2.0 and 2.6 dS m^{-1} were shown to reduce lettuce yield and plant growth, respectively (De Pascale and Barbieri, 1995). It has been reported that lettuce has a salinity threshold value of 1.1 dS m^{-1} and that the relative yield decrease after this threshold is equal to 9.3% (Ünlükara et al., 2008). The detrimental effects of salinity may vary because of both air and environmental temperature ranges and light intensity (Fernandez et al., 2016). Indeed, temperature may indirectly affect plant water status by its influence on the water exchanges at leaf level (He et al., 2001), while water uptake at root level is inhibited by the difference in water potential caused by salinity (Grewal, 2010). It has been reported that light stress might intensify the damages caused by salinity on crops. In a work by Osmond et al. (1997), it was suggested that the light energy absorbed by a wilted leaf could largely exceed the photon requirement for photosynthetic electron transport, due to a reduction of the net CO_2 uptake by the leaves under an unbalanced hydration. This would in turn overload the mechanisms, which protect Photosystem II (PS II) activity from photo-inhibition. Accordingly, the combined stresses associated with excessive light and reduced water availability would overall result in increased thermal sink for the excess photons, leading to decay in the leaf photosynthetic efficiency. Nevertheless, salt stress usually did not affect significantly the photosynthetic rates per unit leaf area, such as photosynthetic efficiency, causing photo-damages, but rather decreased the stomatal conductance and transpiration (Munns and Tester, 2008), as shown in a study on salt stressed sweet basil (Mancarella et al., 2016). However, in moderate salt tolerant crops, such as lettuce, even the photosynthetic rates, as well as the stomatal conductance, ~~resulted highly~~ decreased by salinity (Han et al., 2005; Pérez-Lòpez et al., 2013). In the natural environment, salinity stress is usually associated with dry summers, where plants are exposed to intensive radiation and elevate temperature ranges. The possibility to reduce the synergistic action of thermal and light stresses may be found in the adoption of partial-shading screens that contemporarily reduce radiation and temperatures. Recent research~~es~~ addressed the adoption ~~in horticulture~~ of polyethylene films with spectral filters that block specific wavebands to improve produce quality (García-Macías et al., 2007), also resulting in changes in leaf pigmentation (Tsormpatsidis et al., 2008). Similar investigations were conducted in the past by several authors (Haeringen et al., 1998; Rajapakse et al., 1999; Runkle and Heins, 2001; Fletcher et al., 2005), mainly aimed at controlling plant growth with no use of synthetic growth regulators. Similarly, UV blocking films may be used, offering an environmentally friendly solution to control pest and diseases (Doukas and Payne, 2007). In contrast, films with high transmission in the UV fraction of the spectrum may allow an increase of beneficial secondary metabolites, ~~and therefore~~ with potential health benefits in response to the increased UV radiation (García-Macías et al., 2007). Little evidences are however available to date on the influence that the adoption of different covering films may have on the plant response to salinity.

When plants undergo salt stress, a cascade of physiological and biochemical adaptations is experienced. The osmotic equilibrium is ~~generally~~ maintained through the biosynthesis of osmolytes~~, such as, in lettuce,~~ proline (Tarakcioglu and Inal, 2002). Salt stress response may be enhanced in plants by foliar application of proline as demonstrated in barley (Cuin and Shabala, 2005), broad bean (Gadallah, 1999), tobacco (Okuma et al., 2000; Hoque et al., 2007) and tomato (Heuer, 2003). Furthermore, foliar applications of proline in drought stressed corn (Ali et al., 2008) has been shown to enhance the uptake of K^+, Ca^{2+}, N and P.

The aim of this study was to assess the mitigation effects on salt stress of different greenhouse covering films and exogenous application of proline in hydroponically grown lettuce, through the analysis of both morphological and photosynthetic parameters. For the study, conducted in northern Italy, two Batavian lettuce cultivars commonly adopted by local growers (green and red) were used. Beside morphological determinations aimed at the assessment of yield performances, plant physiological status under the two films was assessed through determination of both leaf photosynthetic performances (including net photosynthesis and transpiration) and leaf greenness.

2. Materials and methods

2.1. Plant material and growth conditions

Five experiments (exp. #1, #2, #3, #4 and #5) were carried out in two separate commercial greenhouses with same structure design located in Cadriano (Bologna, Italy, 44°32'57"N 11°24'43"E). Two cultivars of head lettuce (*Lactuca sativa* L.), namely *Teide* (red Batavian, Nunhems, De Lier, The Netherlands) and *Impulsion* (green Batavian, Rijk Zwaan seeds, De Lier, The Netherlands), were used in the first four experiments at a planting density of 42 plants m^{-2}. During the last experiment (exp. #5), only one cultivar (cv. *Teide*) was used. Twelve floating growing systems (each of 2 m^2) per each greenhouse were adopted, with seedlings transplanted onto polystyrene panels, which were then allocated on waterbeds. Each waterbed was filled at the beginning of the experiment with 500 liters of nutrient solution that was continuously aerated during plant growth by electric pumps.

2.2. Covering films

Two covering films, characterized by different light permeability, transparent or white film, were used. The transparent film (LZ 17, Eiffel, Fontanellato, PR, Italy) presented initial light transmittance and diffusion respectively of ≥86% and ≤35%. The white film (Tepor, Forplast, Formignana, FE, Italy) presented initial light transmittance of 25%. Intensity of light spectrum data, PAR readings as well as maximum and minimum temperatures were recorded outside and inside the greenhouse for both transparent and white covering films (Fig.1, Fig. 2 and Fig. 3, respectively). Spectral characterizations of the light outside and inside the greenhouses were performed using a spectrophotometer CL-500A (Minolta Konica, Osaka, Japan) (Fig. 1). Radiation outside and inside the greenhouses was measured with a Photosynthetically Active Radiation (PAR) radiometer (PAR Photon Flux Sensor model QSO-S connected with data logger ProCheck, both by Decagon Devices Inc., Pullman, WA, USA) at hours 9:00, 12:00, 14:00 and 17:00 of a clear sunny day during exp. #1, #2 and #3 (Fig. 2). Finally, temperature was recorded daily with a air thermometer.

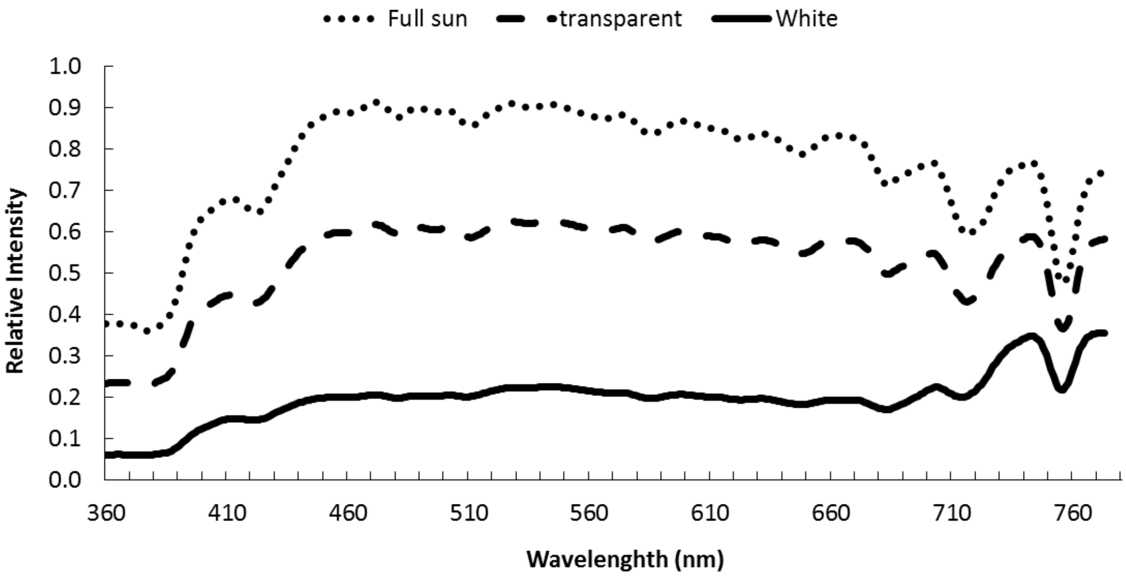

Figure 1. *Relative intensity of light spectrum outside (dotted line) and inside the greenhouse covered with transparent film (dashed line) and white film (continuous line). Data collected at noon on a sunny day on May, 2010, before the first experiment took place.*

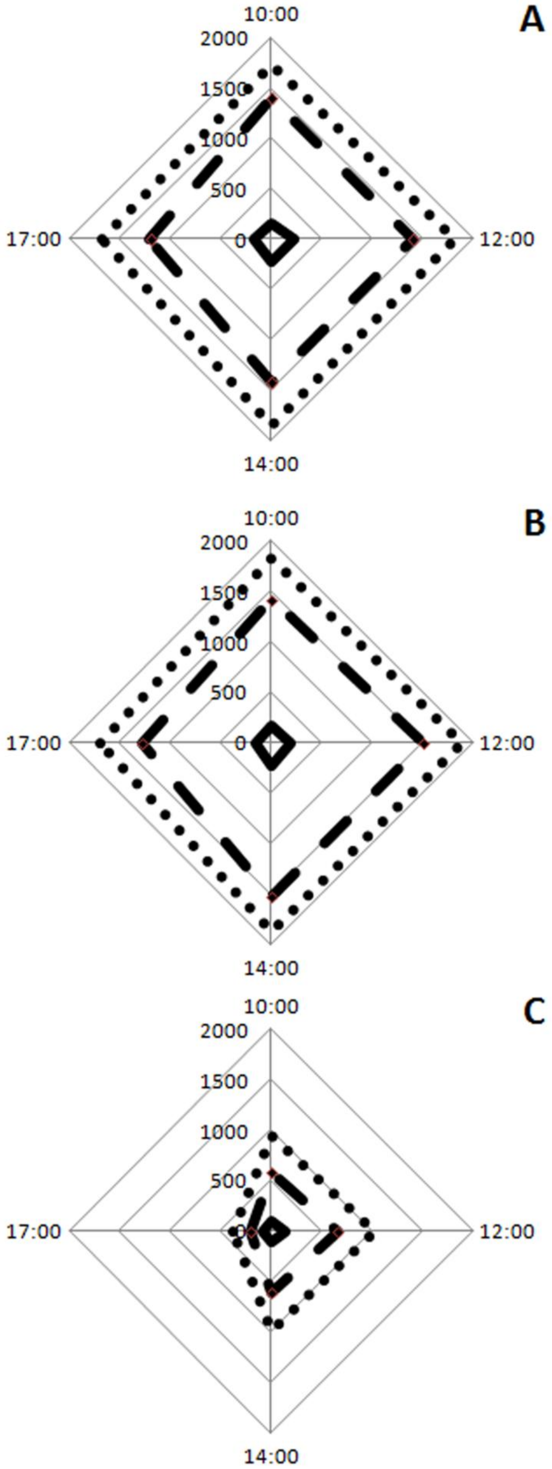

Figure 2. *PAR readings (expressed in μmol* m^{-2} s^{-1}*) outside (dotted line) and inside the greenhouse covered with transparent (dashed line) and white (continuous line). Results from exp. #1 (A, June 2010), #2 (B, July, 2010) and #3 (C, October 2010).*

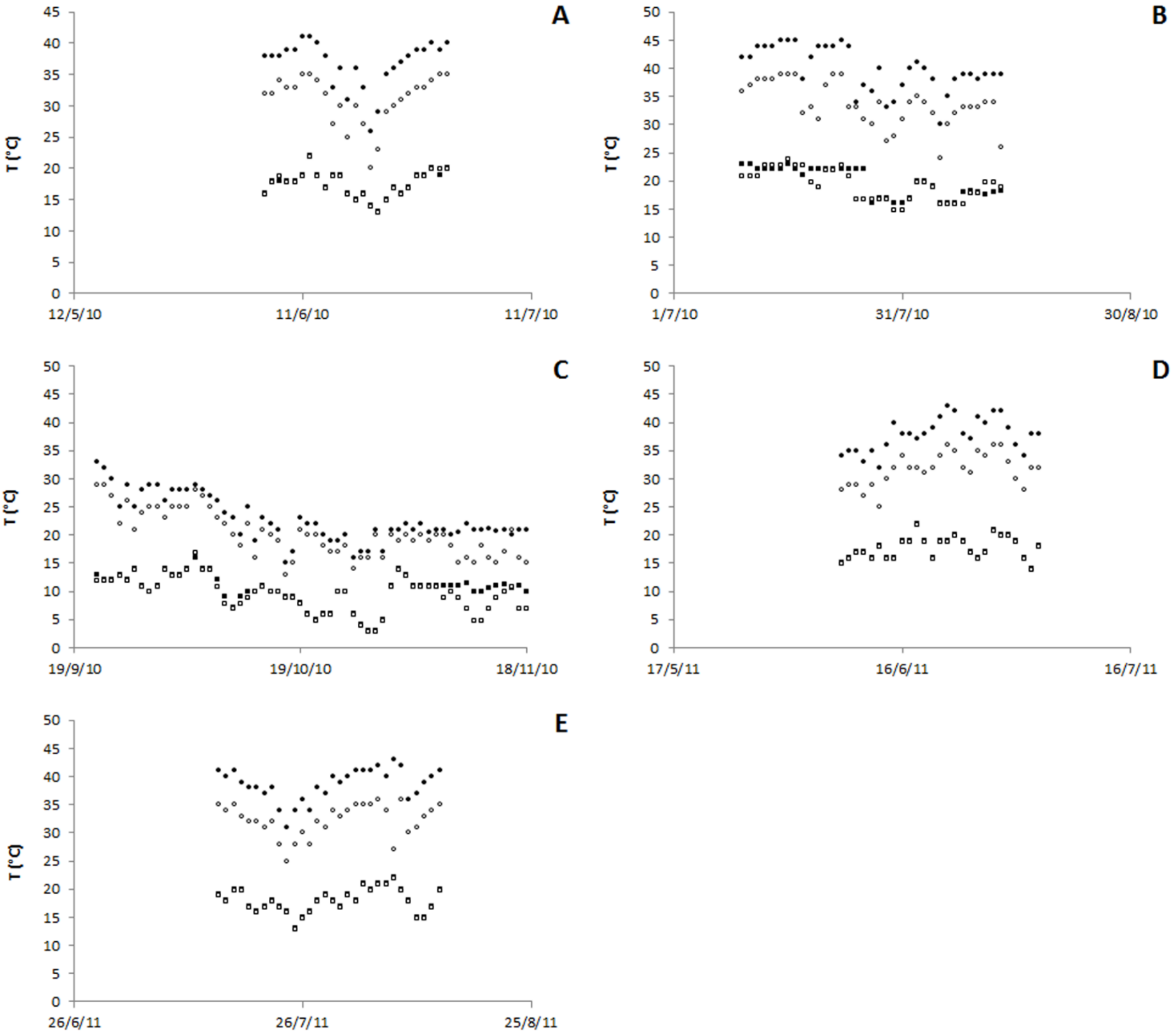

Figure 3. *Maximum (circles) and minimum (squares) temperatures in greenhouses covered with transparent (black) or white (white) covering films during exp. #1 (A), #2 (B), #3 (C), #4 (D) and #5 (E). Non visible black squares are covered by white squares due to same temperature values.*

2.3. Experimental design

Each experiment included a combination of three-factors chosen between covering film, cultivar, salt stress and foliar proline application. The plants were grown until a marketable standard size was reached. Further details on the experiments are provided below and in Table 2.

2.3.1. Exp. #1

A preliminary experiment (exp. #1) was performed to identify the best compositions in the nutrient solution to be successively used for all experiments conducted in this study. Two nutrient solutions (NS) were used, namely A (Enzo et al., 2001) and B (common nutrient solution adopted by local growers, Orsini personal communication), whose composition is detailed in Table 1. The main aim of the experiment was to identify the best nutrient solution composition for maximized plant total and commercial yield, in order to use it in the following experiments. Thus, the nutrient solution selected from exp. #1 was used in all successive experiments as control solution. The experiment took place in late spring-summer and treatments were the two nutrient solutions (A and B), two greenhouse covering films (transparent and white) and two cultivars (*Impulsion* and *Teide*).

Table 1. *Nutrient solution compositions adopted in the Exp. #1.*

Main compositions	**Concentration**	**Solution A**	**Solution B**
C-HCO_3	(mM)	0.5	0.5
N-NO_3	(mM)	15.0	18.0
N-NH_4	(mM)	5.0	3.0
P-H_2PO_4	(mM)	3.5	3.0
K	(mM)	11.0	10.5
Ca	(mM)	4.5	5.0
SO_4	(mM)	6.5	3.5
Na	(mM)	3.4	1.9
Cl	(mM)	4.0	2.5
Fe	(µM)	40.0	40.0
Mg	(µM)	3.5	3.0
Mn	(µM)	7.9	7.9
B	(µM)	35.2	35.2
Zn	(µM)	4.1	4.1
Cu	(µM)	1.0	1.0
Mo	(µM)	0.2	0.2

2.3.2. Exp. #2

The experiment was conducted with the aim of assessing the lettuce response to salinity (0 and 15 mM, resulting in a EC of the nutrient solution respectively of 1.7 and 3.2 dS m^{-1}), as affected by cultivar (*Impulsion* and *Teide*) and greenhouse covering film (transparent and white). The experiment took place in summer 2010. From exp. #2 onward, the control nutrient solution used was the one selected from exp. #1 (namely, solution A).

2.3.2. Exp. #3

The experiment had the same features of exp. #2 and was conducted during fall 2010.

2.3.3. Exp. #4

A further experiment was performed in order to address the potential role of exogenous proline spraying (0 and 5 μM in a foliar spray solution) in limiting the detrimental effect of salinity (0 and 15 mM NaCl), as affected by cultivar (*Impulsion* and *Teide*) and greenhouse covering films (transparent and white). The experiment was conducted during late spring to summer 2011.

2.3.4. Exp. #5

A final experiment was realized, addressing further concentrations of proline spraying (0, 5, 10 and 15 μM) on the salinity stress (0 and 15 mM NaCl) response in one lettuce cultivar (*Teide*) grown under two greenhouse covering films (transparent and white). The experiment took place during summer 2011.

Table 2. *Details and treatments of the five experiments carried out. Covering film (T: Transparent and W: White), nutrient solution (A and B).*

Exp.	**Sowing date**	**Harvest date**	**Covering film (Cf)**	**Nutrient solution (Ns)**	**NaCl (Sa) (mM)**	**Proline (Pr) (μM)**	**Cultivar**
Exp. #1	28/05/2010	28/06/2010	T; W	A; B	0	0	*Teide*; *Impulsion*
Exp. #2	10/07/2010	10/08/2010	T; W	A	0; 15	0	*Teide*; *Impulsion*
Exp. #3	22/09/2010	10/11/2010	T; W	A	0; 15	0	*Teide*; *Impulsion*
Exp. #4	08/06/2011	04/07/2011	T; W	A	0; 15	0; 5	*Teide*; *Impulsion*
Exp. #5	15/07/2011	13/08/2011	T; W	A	0; 15	0; 5; 10; 15	*Teide*

2.4. Physiological measurements

Physiological determinations of leaf gas exchanges were conducted in exp. #2, #3, #4 and #5 at 20 days after the salt treatment (DAT). Measurements of leaf transpiration (E) and net photosynthesis (A) were performed using a CIRAS-2 infrared gas analyzer (PPSystem, Hitchin, UK) with a Parkinson's Automatic Universal Leaf Cuvette equipped with 2.5 cm^2 area cuvette inserts. Leaf greenness was measured during experiments #2 and #3 at 20 DAT using a hand-held leaf chlorophyll meter (YARA N-Tester, Oslo, Norway). At harvest time, total plants yield and marketable yields were determined in all experiments.

2.5. Statistical analysis

The experimental design for all the experiments was completely randomized with 6 replicates per treatment. All data were analyzed by using SPSS program (SPSS Inc., Chicago, Illinois, USA). Multi factor ANOVA was used and the Least Significant Difference (LSD) at $P<0.05$ was used for means separations according to the highest level of significant interaction observed.

3. Results

3.1. Environmental conditions during the experiments

Inside the greenhouses, modifications of spectrum, radiation and temperature were experienced as a consequence of the covering material adopted (Fig. 1, 2 and 3). Apart from the reduction of the light relative intensity, the transparent film did not modify the spectral peaks, whereas the white film removed the peaks at wavelengths below 700 nm (Fig. 1). A constant reduction of PAR in measure of about -23% and -89% under transparent and white films, respectively, as compared to the external conditions, was observed at any time of the day in exp. #1 (Fig. 2A). Similarly, the PAR reduction from the external condition was about -21% and -90% under transparent and white films, respectively, during exp. #2 (Fig. 2B). During the winter cycle (exp. #3, Fig. 2C), measurements at 17:00 presented reduced values (-73%) as compared to morning and early noon readings; the mean daily reduction from the external conditions was of -38% and -87% in transparent and white film, respectively. Differences in thermal conditions between the two greenhouse were also evident (Fig. 3). Although no differences could be detected in the minimum temperatures during either summer or winter cycles, a general increase (+16 to 20%) in the maximum temperatures under the transparent film was registered as compared to the greenhouse covered with white film.

3.2. Effects of nutrient solution on crop performances

Exp #1.

A different response to the nutrient solution supplied was observed under transparent and white films during exp. #1 (Table 3), as evidenced by the significant interaction Cm x Ns. Under transparent film, plants of both cultivars grown with nutrient solution A presented higher total and marketable yield (+21 and +35%, respectively), as compared to those fed with solution B. Nevertheless, no statistically significant differences between the two nutrient solutions could be detected in plants grown under the white film. Furthermore, plants grown under transparent film were much bigger in size (+21 and +32% for total and marketable yield, respectively) as compared to those grown under the white one (exp. #1, Table 3).

Table 3. *Effects of the nutrient solution (A and B) and covering film (transparent and white) on yield of two cultivars of lettuce (Impulsion and Teide) grown on a floating system. Total yield considers whole plants including roots and residues. Marketable yield includes only plant marketable portion. Each value is the mean of 42 independent measures and 6 replicates. Different letters indicate significant differences at P≤0.05, while in the interaction sections significant differences at P≤0.05 are defined by *, whereas ns = not significant differences. In bold the best performances are highlighted. Data from exp. #1.*

Exp. 1#										
			Transparent				White			
Ns	Sa	Pr	Impulsion		Teide		Impulsion		Teide	
Total Yield (kg m^{-2})										
A	-	-	**5.88±0.781**	**a**	4.75±0.402	b	3.98±0.496	c	3.62±0.339	d
B	-	-	4.41±0.636	b	3.96±0.381	c	4.09±0.430	c	3.30±0.387	d
Covering material (Cm)							*			
Nutrient solution (Ns)							ns			
Cultivar (Cv)							ns			
Cm x Ns							*			
Cm x Cv							ns			
Cv x Ns							ns			
Cm x Ns x Cv							ns			
Marketable Yield (g plant^{-1})										
A	-	-	**292.3±45.71**	**a**	236.1±20.81	b	177.0±29.17	c	151.2±19.73	d
B	-	-	188.2±33.36	c	158.2±19.39	c	190.2±24.27	c	129.6±20.91	d
Covering material (Cm)							*			
Nutrient solution (Ns)							ns			
Cultivar (Cv)							ns			
Cm x Ns							*			
Cm x Cv							ns			
Cv x Ns							ns			
Cm x Ns x Cv							ns			

3.3. Effects of salt stress on crop performance

Exp. #2.

A general decrease in both total and marketable yields was associated with salinity in both cultivars and covering films during exp. #2 (Table 4).

Table 4. *Effects of salinity (0 and 15 mM NaCl) dissolved in the nutrient solution (A) and covering film (transparent and white) on yield of two cultivars of lettuce (Impulsion and Teide) grown on a floating system. Total yield considers whole plants including roots and residues. Marketable yield includes only plant marketable portion. Each value is the mean of 42 independent measures and six replicates. Different letters indicate significant differences at P≤0.05, while in the interaction sections significant differences at P≤0.05 are defined by *, whereas ns = not significant differences. In bold the best performances are highlighted. Data from exp. #2.*

Exp. 2#

			Transparent				White			
Ns	Sa	Pr	Impulsion		Teide		Impulsion		Teide	
Total Yield (kg m^{-2})										
A	0	-	**4.62±0.156**	**a**	3.22±0.120	e	4.28±0.062	b	3.92±0.050	c
A	15	-	4.26±0.143	b	2.99±0.105	f	3.65±0.047	d	3.34±0.032	e
Covering material (Cm)							*			
Salinity (Sa)							*			
Cultivar (Cv)							ns			
Cm x Sa							*			
Cm x Cv							ns			
Cv x Sa							ns			
Cm x Sa x Cv							*			
Marketable Yield (g $plant^{-1}$)										
A	0	-	**198.5±10.40**	**a**	127.4±8.00	c	**191.8±4.10**	**a**	170.3±3.30	b
A	15	-	176.5±9.50	b	115.2±7.00	d	160.1±3.10	b	139.7±2.10	c
Covering material (Cm)							*			
Salinity (Sa)							*			
Cultivar (Cv)							ns			
Cm x Sa							*			
Cm x Cv							ns			
Cv x Sa							ns			
Cm x Sa x Cv							*			

However, a significant interaction between salinity, covering material and cultivar was observed. Accordingly, while marketable yield of cv. *Impulsion* was similarly reduced by salinity in both films, namely -11% and -16% for transparent and white film, respectively, in cv. *Teide* a ~~generally~~ reduced effect of salinity was experienced when plants were grown under white film. ~~This, since~~ total and marketable yield under salt w~~ere~~ +11 and +18% higher under white film than under the transparent film. Accordingly, plants of cv. *Teide* subjected to salinity and grown under the white film showed a not significant difference in total yield as compared to the control plants grown under the transparent film. In addition to the reduced effect of salinity, the adoption of white film, as compared with the transparent film, increased the total and the marketable yield of cv. *Teide* in the control conditions. Nonetheless, the plant growth (Fig. 4A) appeared to be delayed under the white film, with plants of both cultivars reaching maximal growth rate (14.3 g plant^{-1} d^{-1}) only at the 5th week after sowing (since differences in the growth rate in the two cultivars are not statistically significant, mean values are shown). Maximum growth rate occurred conversely at the 3rd week after sowing (16.2 g plant^{-1} d^{-1}) when plants were grown under the transparent film.

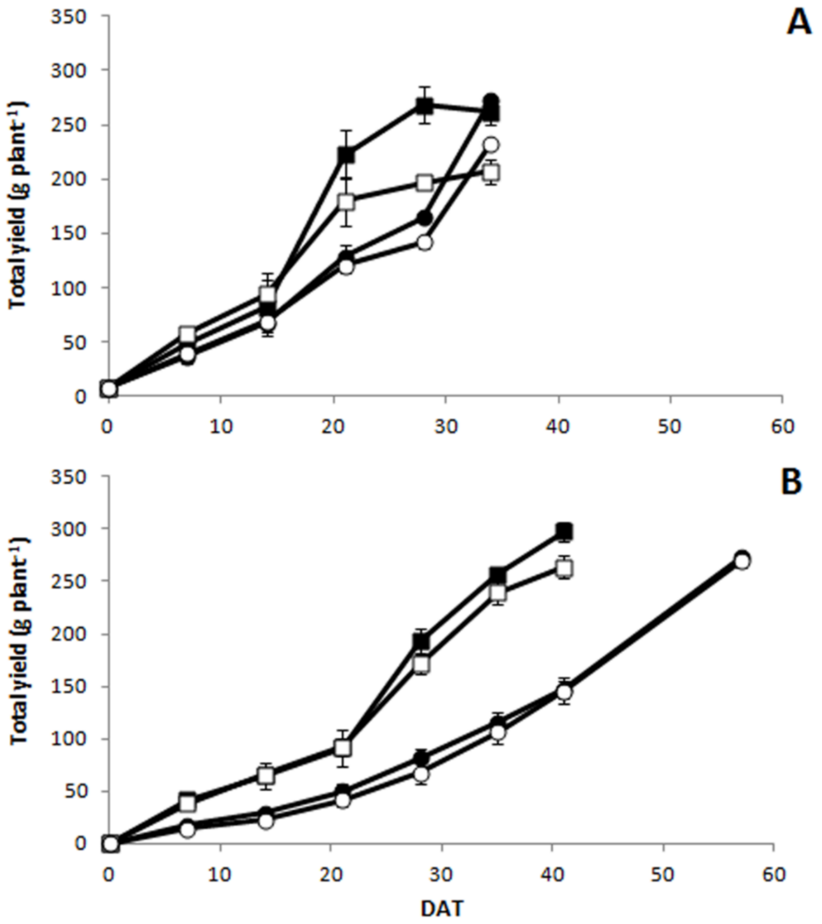

Figure 4. Effect of covering film (transparent, squares, or white, circles) and salt stress (0, black symbols, and 15, white symbols, mM NaCl dissolved in the nutrient solution) on growth of lettuce (cv. Impulsion and Teide, (cvs. Impulsion and Teide, mean values since no significant differences could be associated with cultivars) during summer, exp. #2(A) and winter, exp. #3(B) cycle. Bars indicate standard errors of 6 replicates.

In both cultivars, a similar behavior in both leaf gas exchanges and greenness was observed (Fig. 5A, C and E), and accordingly mean values between the two cultivars were represented in the dedicated charts. The net photosynthesis was higher in plants grown in the control solution and covered with a transparent film (20.1±0.89 μmol CO_2 m^{-2} s^{-1}), while 15 mM NaCl caused a net photosynthesis reduction of 27% (Fig. 5A). On the other hand, under white film, the net photosynthesis averaged 14.5±1.01 μmol CO_2 m^{-2} s^{-1}, but it was not affected by salinity (Fig. 5A). Leaf transpiration was similar among treatments (mean value 14.1±1.39 mmol H_2O m^{-2} s^{-1}), with the exception of salinized plants grown under the transparent film, where the transpiration was reduced by -42% (Fig. 5C).

The cultivar effect on leaf greenness was negligible, and once again mean values between the two cultivars were presented in the charts. Moreover, salinity did not impair leaf greenness under any of the films, whereas the films were actually responsible of great reduction in N-tester values (-45%) in plants grown under white film (Fig 5E).

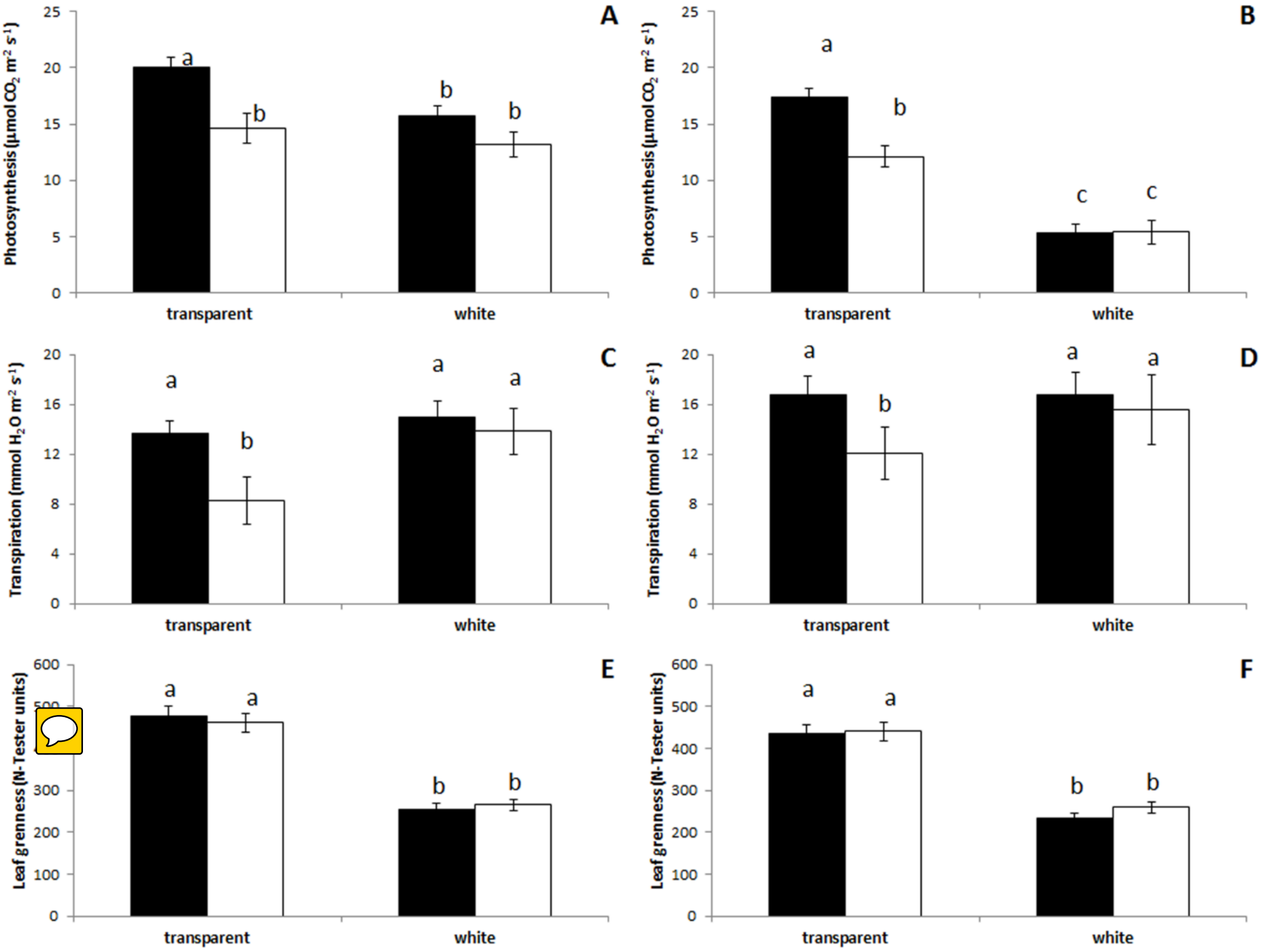

Figure 5. *Effect of covering films (transparent or white) and salt stress (0, black bars, and 15, white bars, mM NaCl dissolved in the nutrient solution) on net photosynthesis (A, B), transpiration (C, D) and greenness (E, F) of lettuce leaves (cvs. Impulsion and Teide, mean values since no*

significant differences could be associated with cultivars). Values from exp. #2 (A, C, E) and #3 (B, D, F). Bars indicate standard errors (n=6) and different letters indicate significant differences at $P \leq 0.05$.

Exp. #3.

Similarly to exp. #2, during exp. #3 a significant interaction between salinity, covering material and cultivar was observed (Table 5). Accordingly, yield of cv. *Impulsion* was reduced by salinity independently from the covering film: a mean reduction of -10 and -13%, respectively, for total and marketable yield, was reached. On the contrary, yield of cv. *Teide* was enhanced in plants grown under white film even upon salinity: a mean increase of +5 and +8%, respectively, for total and marketable yield, was observed, as compared to the control conditions (Table 5).

Table 5. *Effects of salinity (0 and 15 mM NaCl) dissolved in the nutrient solution (A) and covering film (transparent and white) on yield of two cultivars of lettuce (Impulsion and Teide) grown on a floating system. Total yield considers whole plants including roots and residues. Marketable yield includes only plant marketable portion. Each value is the mean of 42 independent measures and 6 replicates. Different letters indicate significant differences at P≤0.05, while in the interaction sections significant differences at P≤0.05 are defined by *, whereas ns = not significant differences. In bold the best performances are highlighted. Data from exp. #3.*

Exp. 3#

			Transparent				White			
Ns	Sa	Pr	Impulsion		Teide		Impulsion		Teide	
Total Yield (kg m^{-2})										
A	0	-	**4.86±0.103**	**a**	4.06±0.09	c	4.27±0.055	b	3.92±0.049	d
A	15	-	4.27±0.107	b	3.65±0.112	e	3.99±0.050	d	4.10±0.043	c
Covering material (Cm)							*			
Salinity (Sa)							*			
Cultivar (Cv)							ns			
Cm x Sa							*			
Cm x Cv							ns			
Cv x Sa							ns			
Cm x Sa x Cv							*			
Marketable Yield (g plant^{-1})										
A	0	-	**203.8±5.74**	a	166.6±5.07	d	189.1±2.31	b	174.5±2.32	c
A	15	-	166.2±5.21	d	140.4±5.46	e	176.5±2.39	c	189.0±2.10	b
Covering material (Cm)							*			
Salinity (Sa)							*			
Cultivar (Cv)							ns			
Cm x Sa							*			
Cm x Cv							ns			
Cv x Sa							ns			
Cm x Sa x Cv							*			

For this cultivar, no significant differences in total yield were found between control plants under the transparent film and salt stressed plants under the white film. The growth delay caused by the white film was more dramatic during this experiment than for exp. #2, resulting in about two additional weeks needed for completing the growth cycle (Fig. 4B). Once again, since no statistically significant differences between *Teide* and *Impulsion* were observed, mean values of the two cultivars are shown in the figure.

As occurred in exp. #2, also in exp. #3 a similar physiological response to salinity and covering material was observed in the two studied cultivars, therefore mean values between the two cultivars were used for the analysis (Fig. 5B, 5D and 5F). Accordingly, highest net photosynthesis values were observed in plants grown with control solution and covered with a transparent film (17.4±0.85 μmol CO_2 m^{-2} s^{-1}), while 15 mM NaCl under the same film caused 30% net photosynthesis reduction (Fig. 5B). On the other hand, under white film, the net photosynthesis averaged 5.39±0.81 μmol CO_2 m^{-2} s^{-1}, but it was not affected by salinity (Fig. 5B). Leaf transpiration was again similar among treatments (mean value 16.3±2.41 mmol H_2O m^{-2} s^{-1}), with the exception of salinized plants grown under the transparent film, where the transpiration was reduced by -36% (Fig. 5C).

As observed in exp. #2, the cultivar effect on leaf greenness was negligible, thus allowing to use mean values between the two cultivars for the analysis. Again, negligible effects of salinity were observed, whereas a substantial difference was recorded between the two films, resulting in lower N-tester values (-44%) in plants grown under white film (Fig 5F).

3.4. Effects of salt stress and foliar proline spraying on crop performances

Exp. #4.

The application of 5 mM of exogenous proline improved crop performances under control conditions, independently from cultivar or covering film (+5 and +8% for total and marketable yield, respectively) (exp. #4, Table 6).

Table 6. *Effects of salinity (0 and 15 mM NaCl) dissolved in the nutrient solution (A), covering film (transparent and white) and foliar proline spraying (0 and 5 μM proline) on yield of two cultivars of lettuce (Impulsion and Teide) grown on a floating system. Total yield considers whole plants including roots and residues. Marketable yield includes only plant marketable portion. Each value is the mean of 21 independent measures and six replicates. Different letters indicate significant differences at P≤0.05, while in the interaction sections significant differences at P≤0.05 are defined by *, whereas ns = not significant differences. In bold the best performances are highlighted. Data from exp. #4.*

Exp. 4#

		Transparent				White			
Sa	Pr	Impulsion		Teide		Impulsion		Teide	
Total Yield (kg m^{-2})									
0	0	5.32±0.110	b	4.09±0.135	h	4.73±0.045	e	3.75±0.063	i
15	0	4.64±0.040	f	3.62±0.059	i	4.62±0.030	f	3.69±0.033	i
0	5	**5.69±0.056**	**a**	4.43±0.099	g	4.84±0.029	d	3.96±0.034	h
15	5	4.99±0.095	c	4.05±0.096	h	4.68±0.027	e	3.82±0.032	i
Covering material (Cm)						*			
Salinity (Sa)						*			
Proline (Pr)						*			
Cm x Sa						*			
Cm x Pr						*			
Pr x Sa						*			
Cm x Sa x Pr						*			
Marketable Yield (g plant^{-1})									
0	0	233.0±6.41	b	175.7±7.86	e	215.2±2.29	d	170.6±2.83	e
15	0	185.0±2.00	e	140.0±1.46	f	208.0±1.35	d	165.2±1.58	e
0	5	**264.7±2.84**	**a**	200.1±5.53	d	222.5±1.43	c	176.9±1.30	e
15	5	212.3±4.69	d	168.4±5.05	e	209.9±1.37	d	168.3±1.24	e
Covering material (Cm)						*			
Salinity (Sa)						*			
Proline (Pr)						*			
Cm x Sa						*			
Cm x Pr						*			
Pr x Sa						*			
Cm x Sa x Pr						*			

When salt stress was present, foliar proline caused a mean increase of total and marketable yield of +6 and +8%, respectively, compared to plants without proline application. Once again, in the treatments without proline application, the white film did not improve *Impulsion* response to salinity, while it did in *Teide* (+12% for marketable yield), compared to using the transparent film. A significant interaction between salinity, covering material and proline application was however observed (Table 6). Accordingly, the effect of the white film on reducing salt stress symptoms was partially hidden by the simultaneous proline application, which increased yield under the transparent film more than under the white one.

Exp. #5.

The exp. #5, which included only cv. *Teide*, highlighted how best performances could be achieved by combining 5 μM proline spraying and the covering with a white film (Table 7).

Table 7. *Effects of salinity (0 and 15 mM NaCl) dissolved in the nutrient solution (A), covering film (transparent and white) and foliar proline spraying (0, 5, 10 and 15 μM proline) on yield lettuce (cv. Teide) grown on a floating system. Total yield considers whole plants including roots and residues. Marketable yield includes only plant marketable portion. Each value is the mean of 21 independent measures and 6 replicates. Different letters indicate significant differences at P≤0.05, while in the interaction sections significant differences at P≤0.05 are defined by *, whereas ns = not significant differences. In bold the best performances are highlighted. Data from exp. #5.*

Exp. 5#

	Transparent				White			
Pr (μM proline)	0 mM NaCl		15 mM NaCl		0 mM NaCl		15 mM NaCl	
Total Yield (kg m^{-2})								
0	1.45±0.058	e	1.07±0.033	f	2.78±0.017	b	2.04±0.018	d
5	1.45±0.043	e	1.14±0.037	f	**3.20±0.027**	**a**	2.71±0.016	b
10	1.19±0.027	f	1.11±0.036	f	2.88±0.017	b	2.58±0.014	c
15	1.22±0.030	f	1.01±0.026	f	2.46±0.019	c	2.19±0.022	d
Covering material (Cm)					ns			
Salinity (Sa)					ns			
Proline (Pr)					ns			
Cm x Sa					*			
Cm x Pr					*			
Pr x Sa					*			
Cm x Sa x Pr					*			
Marketable Yield (g plant^{-1})								
0	144.9±3.87	b	101.5±2.18	d	135.8±1.11	c	102.2±1.17	d
5	145.2±2.88	b	113.6±2.50	d	**156.2±1.78**	**a**	129.2±1.08	c
10	131.7±1.81	c	105.3±2.37	d	140.4±1.15	b	126.0±0.95	c
15	128.8±1.97	c	101.2±1.75	d	114.4±1.26	d	104.3±1.46	d
Covering material (Cm)					ns			
Salinity (Sa)					ns			
Proline (Pr)					ns			
Cm x Sa					*			
Cm x Pr					*			
Pr x Sa					*			
Cm x Sa x Pr					*			

Confirming results from exp. #4, a significant interaction between salinity, covering material and proline application was observed (Table 7). Under the transparent film, exogenous proline did not mitigate the adverse effect of salt stress. On the other hand, under the white film, the proline application improved yield on control and salt treated plants. Accordingly, adding 5 μM of proline resulted in a greater growth in non-stressed plants (+13% as compared to control plants without proline application) and on plants treated with 15 mM of salt (+25% as compared to salt stressed plants without proline application). The 10 μM concentration of proline mitigated the effects of salt stress (+21% as compared to stressed plants without proline), while 15 μM of proline did not show mitigation effects on salt stress (exp. #5, Table 7). No significant differences in leaf gas exchanges were detected under the white film due to proline application: mean values of 15.2±1.47 μmol CO_2 m^{-2} s^{-1} and 19.4 mmol H_2O m^{-2} s^{-1} for leaf photosynthesis and transpiration, respectively, were recorded (data not shown). Conversely, a significant interaction ($P \leq 0.05$) between salt and proline was observed in plants grown under transparent films, in both leaf photosynthesis and transpiration (Fig. 6). The net photosynthesis decreased along with the increase of proline concentration in control plants, although the detrimental effects of salinity were mitigated by the foliar application of the proline, which increased photosynthesis by +20% as the mean of 5 μM, 10 μM and 15 μM, compared to salt stressed plants without proline application (Fig. 6A). Transpiration was also decreased by the application of proline in both control and salt stressed plants. However, at 15 μM proline application, salt stressed plants presented higher transpiration (+32%) as compared to non-salinized plants (Fig. 6B).

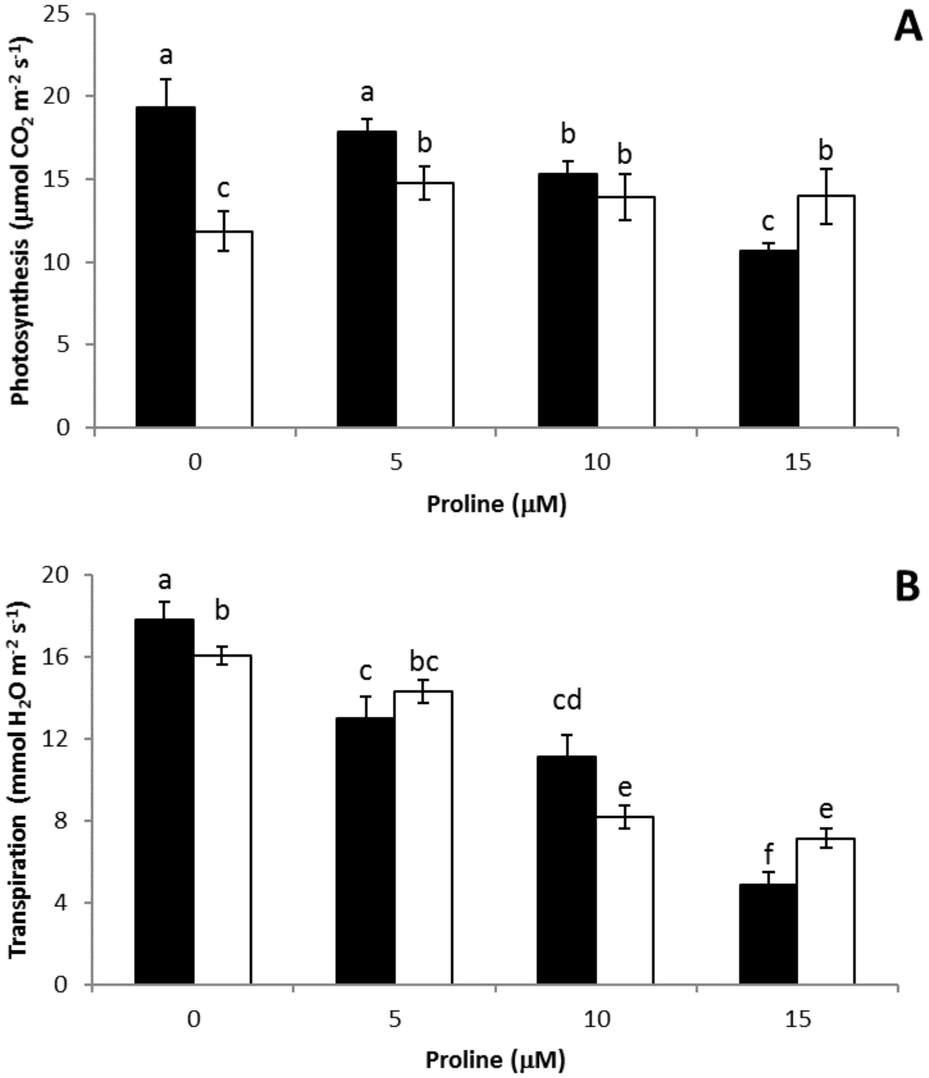

Figure 6. *Effect of foliar spray proline application (0, 5, 10 and 15 µM) and salt stress (0, black bars, and 15, white bars, mM NaCl dissolved in the nutrient solution) on net photosynthesis (A) and transpiration (B) of lettuce leaves (cv. Teide) grown under transparent film. Results from exp. #5. Bars indicate standard errors (n=6) and different letters indicate significant differences at P≤0.05.*

4. Discussion

4.1. Effect of covering films and cultivar on yield of lettuce.

UV blocking films have been extensively studied in relation to nutritional quality of greenhouse-grown vegetables (Espi et al., 2006; García-Macías et al., 2007; Tsormpatsidis et al., 2008), with most studies being focused on the impact of the films for reducing Near Infrared wavelengths (NIR) (Kittas et al., 1999; Hoffmann and Waaijenberg, 2001; Kempkes, 2008). On the other hand, few reports until now have confirmed the effect of a white PAR reducing film (Hoffmann and Waaijenberg, 2001; Kittas et al., 2006), and to our knowledge there are no studies on the effects of this covering film to improve plant response to salt stress. The hereby studied transparent film was highly permeable to PAR (77-79% PAR transmission during summer cycle), while the white film had limited PAR permeability, that is, PAR inside the greenhouse was limited to 10% as compared to external conditions (Fig. 2). However, the transmission of PAR decreased in winter cycle and the daily mean reduction from external condition was of 38% and 87% in transparent and white film, respectively. Furthermore, the white film reduced the permeability of the light at the wavelengths useful for chlorophyll absorbance, reducing by 77.5% the intensity in the range of wavelengths between 470 and 480 nm, which is one of the peaks of absorbance of chlorophyll (Wellburn, 1994; Lin et al., 2013).

PAR reducing films have been reported to decrease the temperature inside the greenhouse (Siwek et al., 2009), and in the present study the white film decreased the maximum temperature in both winter and summer cycles as compared to using a transparent film (Fig. 3). Therefore, during summer cv. *Teide* had better performance under a white film (exp. #2 Table 4), which was sensibly able to reduce the maximum temperature (Fig. 3A). It should be pointed out that many reports state~~d~~ that the adoption of a thin transparent covering film in spring might lead to plant burning as a consequence of the increased air temperature (Libik and Siwek 1994, Tsormpatsidis et al., 2008). Moreover, cv. *Teide* produced a higher marketable yield under white films even during winter cycle (exp. #3, Table 5). Hence, we can conclude that cv. *Impulsion* had a higher tolerance to the elevated temperatures occurred, and produced always greater biomass under the transparent film. Conversely, for the potentially heat sensitive cv. *Teide*, a white film would preserve yield during warmer periods.

In the hereby presented study, the white cover affected negatively the leaf greenness, an indirect measure of leaf chlorophyll content (Gianquinto et al., 2009), which is directly correlated with plant access to light. Being the chlorophyll concentration a critical determinant in defining plant photosynthetic activity (Ashraf and Harris, 2013), it may be advanced that the higher light available under transparent film enhanced leaf chlorophyll (Fig. 5E and 5F) and therein photosynthetic rates (Fig. 5A and 5B), which caused an acceleration of plant growth, leading to higher yield (Table 4 and 5) and a shorter growing cycle (Fig. 4B). Consequently, the obtained results on photosynthetic rate and chlorophyll content were a further confirmation that the transparent film ~~– overall transmitting more light –~~ was the most favourable cover during summer time for cv. *Impulsion*. Although photosynthate accumulation in plant tissue has been linked with optimal light access also in lettuce (Miyagi et al., 2017), the differences in light integrals could have been responsible of the different cycle lengths under the two films (Fig. 4, Fernandez et al., 2016).

4.2. Effect of white film adoption on detrimental effects of salinity

Salinity affects plant productivity and causes a significant reduction in crop yield (Munns and Tester, 2008). Significant decreases in total and marketable yields for both cultivars upon salt stress were experienced in the present study, regardless of the covering film types and seasonal plant growth (exp. #2 and #3, Table 4 and 5). ~~Nevertheless,~~ in winter and upon salinity, the white film, as compared with the transparent one, promoted growth in cv. *Teide* and limited salt-induced growth limitations in cv. *Impulsion* (exp. #3, Table 5). ~~Consistently,~~ similar photosynthetic rates between salt-stressed and control plants were observed under the white film (Fig. 5A and 5B). Furthermore, salt stress did not affect transpiration in plants grown under the white film (Fig. 5C and 5D), probably due to the lower environmental temperatures (Lai and He, 2016). ~~Contrarily,~~ a significant salt-induced reduction of photosynthesis and transpiration was observed under the transparent film, overall resulting in a decrease of yield, confirming that salt stress symptoms were enhanced when combined with high temperatures (Xiong et al., 1999). However, the reduced growth rate under the white film (Fig. 4) probably limited salt-induced ion toxicity, which enabled cv. *Teide* to eventually increase yield under salt stress (exp #3, Table 5) (Fernandez et al., 2016; Bartha et al. 2015).

It appears that reducing incident radiation and ~~therefore~~ plant light access through application of a white cover resulted in reduced growth. Although this may be seen as a drawback of the white covering film, when salt stress was present its symptoms on the plants were reduced thanks to the slower growth rate (and eventually ion loading in shoots) and the limitation of concurrent heat stress. In recent years, the importance of studying the plant response physiology to multiple environmental stresses has been matter of debate among scientists, particularly with regard to the transferability of results obtained in controlled environment onto real agricultural contexts (Deikman et al., 2012). Not only a crop generally undergoes a range of different stresses, but also it appears that the same response mechanisms are often activated when different stresses occur, as evidenced in Arabidopsis for heat and salt stress (Liu et al., 2011). How do biochemical response mechanisms to combined heat and salinity stresses translate in variations of sensitivity or tolerance is still under investigation (Savvides et al., 2016). Nonetheless, first evidences of common pathways are yet available in literature, including, for instance, biosynthesis of antioxidant enzymes (Gill and Tuteja, 2010), Salycilic Acid (Khalifa et al., 2016) and A (Suzuki et al., 2016) as well as ROS accumulation (Savvides et al., 2016).

Lettuce adaptation to sodium accumulation in plant tissues has also been associated with proline biosynthesis (Bartha et al., 2015). Accordingly, further improvements in salt response where addressed by exogenous proline application in exp. #4 and #5.

4.3. Effect of exogenous foliar proline spraying on salt stress symptoms

In order to maintain turgor and water uptake for growth, plants undergoing salinity reduce their water potential through osmotic adjustments. This is generally balanced with metabolically compatible solutes that can be alternatively absorbed from the root zone or newly synthesized within the plant (Tester and Davenport, 2003). Compatible solutes, such as proline, are known to be accumulated under conditions of environmental stresses and to play a role in the process of osmotic adjustment in many crops, including lettuce (Bartha et al., 2015). They are supposed to protect plant cells against the ravages of salt by preserving the osmotic balance, stabilizing sub-cellular structures, such as membranes and proteins, and scavenging reactive oxygen species (Heuer, 2003; Ashraf and Foolad, 2007). In the present study, a low concentration of proline foliar spraying (5 μM) enhanced the plant performance (exp. #4, Table 6) and resulted in a significant increase in the photosynthetic rate in salt stressed plants (Fig.6A). However, both photosynthesis and transpiration decreased in control plants when higher doses of proline (10 to 15 μM) were supplied (Fig. 6A and 6B). Proline application in high concentrations has shown to present harmful effects, such as an inhibition of growth and cellular metabolism (Ashraf and Foolad, 2007). The hereby presented results confirm previous indications, where the foliar application of proline (5 to 10 μM) improved water status in salt stressed melon (Kaya et al., 2007) and rice (Hasanuzzaman et al., 2014) plants. This phenomenon has been associated with a combined inhibition of water efflux via effects of solutes on membrane stability and reduced transpiration via effects on stomatal regulation (Raghavendra and Reddy, 1987). In exp. #4, the application of 5 μM of proline led to the greatest production in absence of salt stress (Table 6). This probably happened because its application limited some detrimental effects caused by other suboptimal conditions, such as heat stress caused by the extreme temperature under the transparent film or the low light access under the white film (Ashraf and Foolad, 2007). In addition, 5 μM of proline affected positively the yield of lettuce grown upon salt stress, which resulted similar to the yield of the control plants without proline application (exp. #5, Table 7). However, while these results were statistically confirmed under the white film, no significant differences in yield were observed under the transparent film, revealing that the elevate temperatures reached in the greenhouse were too stressful for the cv. *Teide*, which was therefore confirmed as heat sensitive.

5. Conclusions

The obtained results indicate that the adoption of a white covering film enabled to mitigate the detrimental effects of salinity on hydroponically grown lettuce. These results were more evident in the summer season and in the potentially heat sensitive genotype (cv. *Teide*) resulting in improved yield. Exogenous application of foliar proline spray (up to 5 μM) resulted in yield increasing under control conditions and mitigating the adverse effect of salinity stress. Overall, for summer cultivation of cv. *Teide*, in presence of mildly saline water (15 mM NaCl), the combination of white film covering and of 5 μM foliar spray proline application enabled to efficiently preserve plant growth and final yield. Previous experiences on lettuce plants exposed to salt stress indicate that the biochemical composition and more specifically the biosynthesis of functional compounds may play an active role in the plant response to salinity. Accordingly, the evaluation of the plant biochemical response to salt stress should be further addressed in order to quantify and compare the accumulation of secondary metabolism products under both transparent and white films and in presence of proline application as plants undergo salt stress.

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