Scientia Horticulturae 233 (2018) 283–293

Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti

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

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ARTICLE INFO

Keywords:
NaCl
Covering films
Lactuca sativa l
Total and marketable yield
Osmoprotectants

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 (*Lactuca sativa* cv. *Teide* and cv. *Impulsion*) grown on a floating system. 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 (0–15 mM NaCl) would be alleviated by the greenhouse covering film (exp. #2, #3, #4 and #5) and foliar proline spray application (0–15 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.

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, research in horticulture has addressed 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 (Fernández 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 (Fernández 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

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https://doi.org/10.1016/j.scienta.2018.01.019
Received 21 August 2017; Received in revised form 11 November 2017; Accepted 10 January 2018

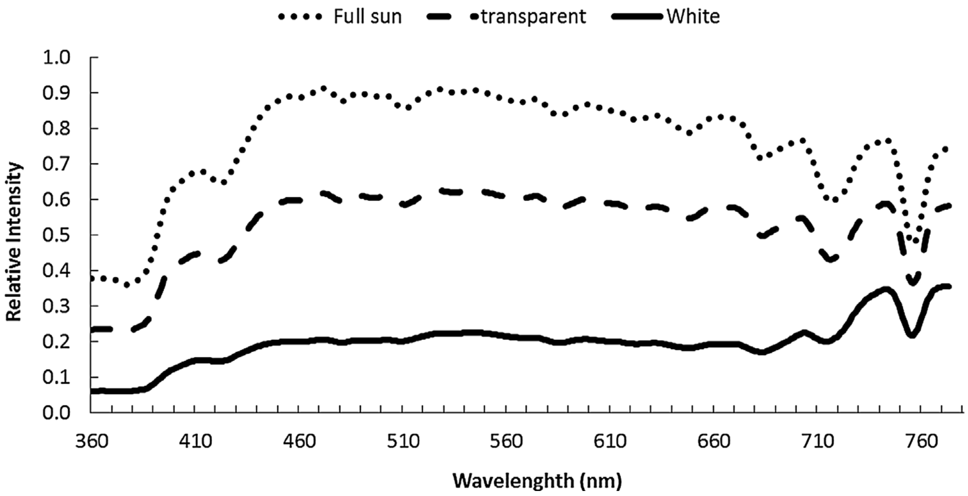

Fig. 1. Relative intensity of light spectrum outside (dotted line) and inside the greenhouse covered with transparent film (dashed line) and white film (continuous line). Data collected at noon on a sunny day on May 2010, before the first experiment took place.

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 (e.g. lettuce) even the photosynthetic rates were decreased by salinity (Han and Lee, 2005; Pérez-López 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 addressed the adoption 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 (van 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 plant growth regulators, such as Uniconazole and Daminozide (Gibson and Whipker, 2000). Similarly, UV blocking films may be used, offering an environmentally friendly solution, to control insect pests (e.g. thrips, white flies and aphids), and viral diseases (e.g. tomato yellow leaf curl virus and cucurbit yellowing stunt disorder virus) (Raviv and Antignus, 2004; Doukas and Payne, 2007). In contrast, films with high transmission in the UV fraction of the spectrum may produce potential health benefits by increasing beneficial secondary metabolites in response to the increased UV radiation (García-Macías et al., 2007). However, little evidence is 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, including the reduction in photosynthesis and stomatal conductance (Bartha et al., 2015), the biosynthesis of stress response metabolites, e.g. antioxidants (Fernández et al., 2016) or the accumulation of osmolytes (Orsini et al., 2010). For example in lettuce, the osmotic equilibrium is maintained through the biosynthesis of 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 potassium (K^+), calcium (Ca^{2+}), nitrogen (N) and phosphorus (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 chlorophyll content.

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 21 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 L 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 $\geq 86\%$ and $\leq 35\%$. 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 (Figs. 1–3, 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