Effect of water salinity and osmolytes application on growth and ornamental value of *Viburnum lucidum* L.

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Abstract

The scarcity of good quality water frequently led to the use of saline water for the irrigation of ornamental shrubs. Therefore, their salt tolerance needs to be investigated, along with the possibility to counteract the effect of salinity exposure on plant growth and ornamental quality, possibly due to reduced growth, and nutritional imbalances. Under salt stress conditions, plants can activate mechanisms helping to withstand it, such as the production of several organic solutes that play a role in the osmotic adjustment. Aiming to this extent the exogenous application of osmolytes, such as glycine betaine (GB) and L-proline (L-P), has been tested on potted plants of *Viburnum lucidum* L. grown under saline irrigation. The experiment was designed as a factorial combination of two nutrient solutions (non-salt control, or 200 mM NaCl) and three osmoprotectant treatments (untreated, GB 2.5 mM, or L-P 5 mM application). Shoot and root biomass were negatively affected by salinity (-37 and -29%, respectively), but not the shoot/root ratio. A significant and positive effect of osmolytes application was found on the shoot biomass of plants treated with GB (+46%). Lateral sprouting total length per plant was also reduced by saline irrigation (-60%), but the GB application resulted in a significant increase (+102%). A positive effect of GB application was also found on the total leaf area (LA) per plant that was increased by 182% under saline conditions. Root/shoot ratio did not change with salinity. L-P application resulted in a significant increase of both shoot and root biomass per unit of LA (+40 and +85%, respectively) in comparison with the untreated control and GB.

Keywords: glycine betaine, L-proline, leaf area, ornamental shrubs

INTRODUCTION

Viburnum is an ornamental species widely diffused in Mediterranean areas due to its good adaptation to water and salt stresses (Cassaniti et al., 2009). We recently demonstrated that under high salt stress conditions the shoot biomass accumulation, Total leaf area (LA) and net CO_2 assimilation were markedly reduced in *Viburnum* (Cirillo et al., 2016). However, the former negative effects in particular those related to shoot biomass were significantly mitigated after application of glycine betaine (GB) (Cirillo et al., 2016).

Leaf succulence and specific leaf weight are generally reported as two important morphological plant adaptations to salinity (Longstreth and Nobel, 1979). In particular, leaf succulence contributes to maintain ion concentrations of plant tissues virtually unchanged under saline conditions, and generally below toxic levels thanks to a dilution of solutes. On the other hand, the specific leaf weight, which increases with salinity as a consequence of increasing mesophyll thickness (Longstreth and Nobel, 1979), may be responsible for reductions of stomatal resistance thus mitigating the negative effects of salinity on photosynthesis. Both these traits might presumably change with application of osmoprotectans. No studies are available on the use of exogenous osmoprotectans on ornamental species.

The aim of the present study was to evaluate the efficacy of exogenous application of

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osmo-protective molecules, such as L-P or GB, on counteracting the effects of NaCl salinity on *Viburnum* potted plants grown under greenhouse conditions.

MATERIALS AND METHODS

The experiment was conducted at the Experimental Station of the University of Naples Federico II, South Italy (lat. 43°31'N, long. 14°58'E; alt. 60 m a.s.l.). Rooted cuttings of 2-year-old V*iburnum lucidum* L. 'White', were transplanted on 11 January 2013 into 6-L plastic pots filled with peat moss and grown at a density of 6.2 plants m^{-2}. The experiment was designed as a six-way factorial combination of two salinity treatments: non-salinised (1.0 mM NaCl) and salinised nutrient solution (200 mM NaCl), and three osmoprotectant treatments [untreated, 2.5 mM GB, or 5.0 mM L-P]. Root applications of GB or L-P were carried out twice during the growing cycle (66 and 87 DAT), whereas tap water (electrical conductivity – ECw 0.6 dS m^{-1}; 8.63 mg L^{-1} Na and 10.3 mg L^{-1} Cl) was added to untreated plants. The treatments were arranged in a randomized complete-block design with four replicates of each treatment and each replicate contained four plants. The basic nutrient solution had an EC of 1.6 dS m^{-1}, whereas the salinised nutrient solution had an EC value of 20.5 dS m^{-1}. Saline irrigations started on January 21st 2013 and the experiment ended on April 24th 2013. Both salinised and control plants were irrigated twice a week. At the end of the experiment (103 DAT), plant dry biomass (DW), leaf number and LA were measured. The contribution of LA to shoot and root biomass was then determined as mg dry weight cm^{-2} LA. Specific leaf weight (SLW) (leaf DW/LA, mg cm^{-2}) and leaf succulence (LS, (FW-DW)/LA, mg H_2O cm^{-2}) were calculated. Leaf mineral composition was also determined.

All data were statistically analysed by two-way analysis of variance (ANOVA) using the SPSS 13 software package. Whenever an interaction was significant, one-way ANOVA was performed. To separate treatment means for each measured parameter, Duncan's multiple range test was performed at a significance level of $P \leq 0.05$.

RESULTS AND DISCUSSION

Leaf, root and total dry mass per plant were negatively affected by salinity (-39.5, -29.4, and -34.4%, respectively) (Table 1).

Table 1. Effects of saline irrigation (S) and osmolyte applications (O) on the total dry mass (DM) and its partitioning to leaves, stems and roots, at the end of the experimental period (103 DAT).

Treatment	Leaf DM (g DW $plant^{-1}$)	Stem DM (g DW $plant^{-1}$)	Root DM (g DW $plant^{-1}$)	Total DM (g DW $plant^{-1}$)
Saline irrigation (S)				
Control	27.6 a	11.5	22.1 a	67.7 a
200 mM	16.7 b	9.8	15.6 b	44.4 b
Osmolyte (O)				
Untreated	19.7 b	10.0	18.3	51.0 b
GB	29.6 a	10.8	17.5	65.1 a
L-P	17.1 b	11.1	20.7	52.0 b
ANOVA				
S	**	NS	*	**
O	*	NS	NS	**
S × O	NS	NS	NS	NS

*, Significant at P<0.05; **, Significant at P<0.01; NS, Not significant. Different letters indicate significant difference at P<0.05 and P<0.01. GB, Glycine betaine, L-P, L-proline; DW, Dry weight.

Under saline irrigation 7.3% of total dry mass was partitioned to leaves and 22.8% to stems compared to 23.6 and 17.4% for leaves and stems respectively in the control plants. The application of GB increased the total dry mass mainly through an increase of leaf dry mass (+ 50.2%) (Table 1).

Both leaf number and leaf area per plant were reduced at 200 mM NaCl (Figure 1). They were significantly affected by the S × O interaction, with the highest values for the non-salinised control and the GB treated plants (both control and salt-stressed) and the lowest for the salinised both untreated and L-P plants (Figure 1).

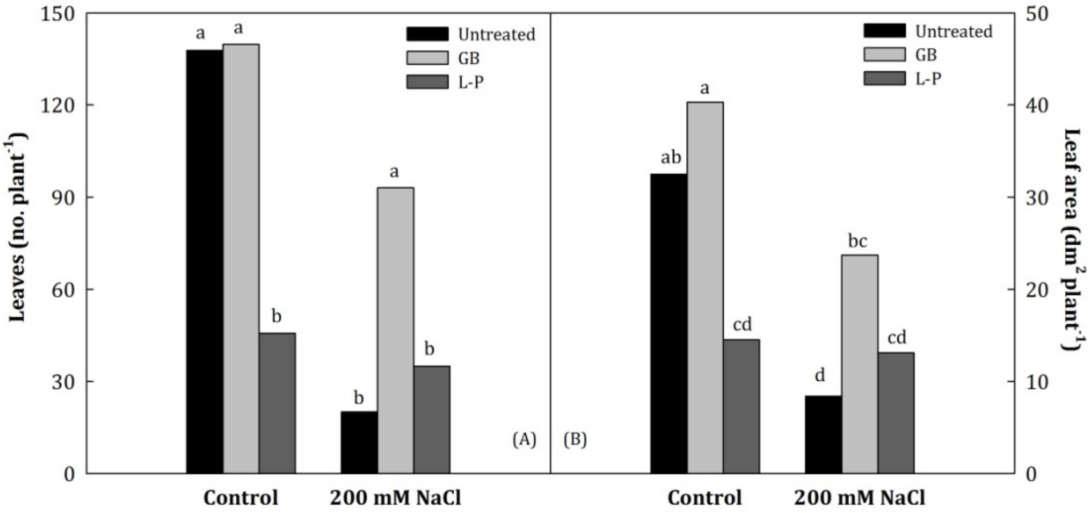

Figure 1. Effects of saline irrigation (S) × osmolyte applications (O) interaction on the leaf number (A) and total leaf area per plant (B).

Interestingly, the individual leaf mean area did not change with salinity, since saline stress equally reduced leaf number and LA per plant. Some authors reported that salinity negatively affects plant leaf area through a lower expansion of individual leaf rather than to a lower rate of production of new leaves (Terry and Waldron, 1984; Plaut et al., 2000).

Under saline irrigation both the untreated and the osmolyte (GB and L-P) treated plants showed an increase of LS (+46%) compared to the non-salinised control (Figure 2). The SLW was generally increased by salinity, with the highest values in untreated and L-P treated plants (Figure 2). The effect on these two parameters of L-P application was significantly increased under non-saline conditions too (Figure 2).

The L-P application resulted in a significant increase of both shoot and root dry mass per unit LA (+40 and +85%, respectively) in comparison to the untreated control and GB, thus indicating that under saline conditions and L-P application LA appeared to give a significant contribution to shoot and root growth too (20.3 vs. 17.4 mg cm^{-2} LA; Table 2).

In the present study, the developed LA under saline conditions appeared to give a significant greater contribution to shoot growth than that of not salinised plants (20.3 vs. 17.4 mg cm^{-2} LA; Table 2) which might explain the lower effect of salinity on shoot biomass with respect to the LA (-37 and -48%, respectively). No significant contribution to root growth of the survived LA was instead recorded due to salinity (Table 2).

LS increased at 200 mM NaCl by 21% with respect to the not salinized control (Table 2).

As expected, SLW increased also significantly with salinity (Table 2) due to more dry matter invested per leaf because of nutrient poor condition or lack in water supply under saline conditions (Shipley et al., 2005).

Shoot and root biomass produced per unit of LA, LS and SLW were generally lowered by GB application (Table 2; Figure 2).

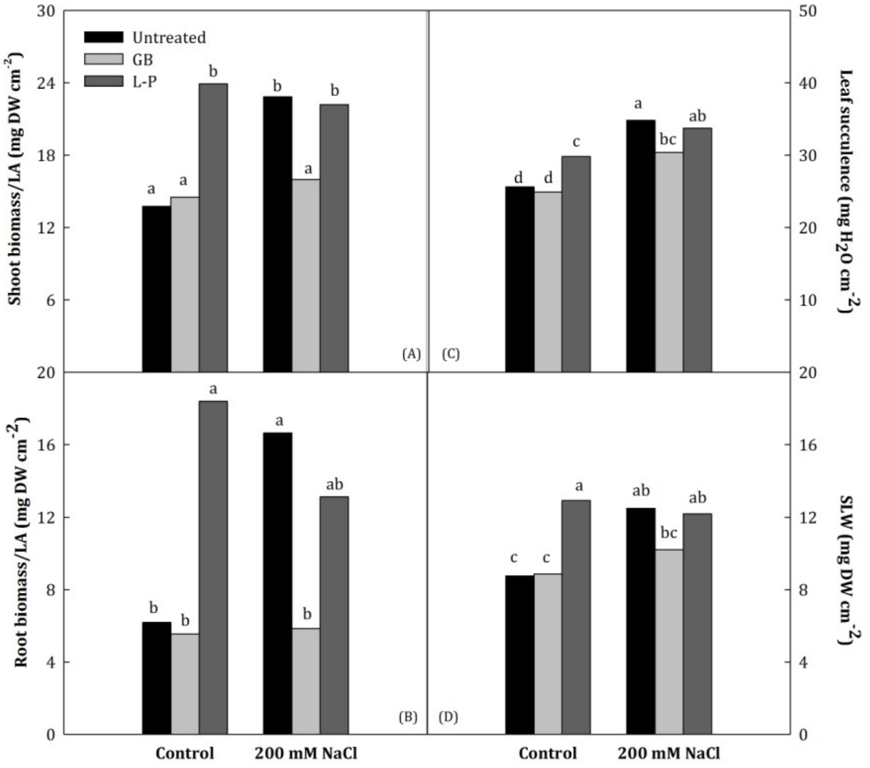

Figure 2. Effects of interaction S × O on shoot (A) and root (B) biomass per unit of LA, leaf succulence (SL; C) and specific leaf weight (SLW; D).

Table 2. Effects of saline irrigation (S) and osmolyte applications (O) on shoot and root biomass per unit of LA, leaf succulence and specific leaf weight, at the end of the experimental period (103 DAT).

Treatment	Shoot/LA (mg DW cm^{-2})	Root/LA (mg DW cm^{-2})	LS (mg H_2O cm^{-2} LA)	SLW (mg DW cm^{-2} LA)
Saline irrigation (S)				
Control	17.4 b	10.1	27.1 b	10.2 b
200 mM	20.3 a	11.9	32.9 a	11.6 a
Osmolyte (O)				
Untreated	18.3 b	11.4 a	30.7 a	10.6 ab
GB	15.3 b	5.7 b	27.6 b	9.5 b
L-P	23.0 a	15.8 c	31.7 a	12.6 a
ANOVA				
S	*	NS	**	*
O	**	**	**	**
S × O	*	*	*	*

*, Significant at P<0.05; **, Significant at P<0.01; NS, Not significant. Different letters indicate significant difference at P<0.05 and P<0.01. GB, Glycine betaine, L-P, L-proline; DW, Dry weight; LA, Leaf area; LS, Leaf succulence; SLW, Specific leaf weight.

Exposure to high concentration of NaCl generally increases LS, which is a morphological strategy adopted by plants to lower salt concentration in the cells. While in the halophytes it depends on the enlargement of cells which counteracts the lower cell division due to salinity, in the glycophytes the mechanism is not yet completely understood but the main consequence is ions dilution in the cell.

No significant differences among treatments were observed in the concentrations of N, P, and K (data not shown). The Na concentration in leaves of salinised with respect to non-salinised plants was about 5-fold, and 2-fold for Cl (Figure 3).

The lowest values of leaf Na concentration were recorded under salt stress conditions in GB treated plants (Figure 3). Exogenous glycine betaine has been reported to suppress the Na uptake via apoplastic flow, leading to mitigation of the detrimental effects of salt stress on crop performance in *Viburnum*.

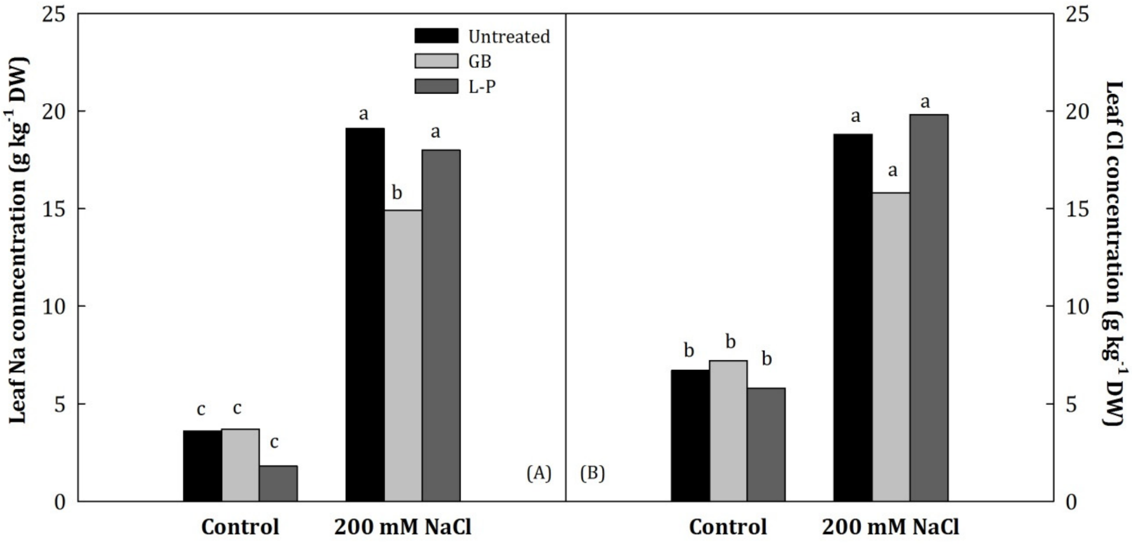

Figure 3. Effects of saline irrigation (S) × osmolyte applications (O) interaction on Na (A) and Cl (B) leaf concentration.

Similar reduction of Na, together with a maintenance of the K uptake in the shoots, was previously reported as a consequence of exogenous application of GB. The response observed in control plants treated with L-P might indicate that the exogenous application of this osmolyte induced the plants to act as salt-stressed even when the stress was not applied. The observed differences in the growth matched well with this finding. On the contrary, the GB applications appeared to be able to better counteract the negative effects of saline irrigation as confirmed by the moderate reduction of total leaf area and by the increased in total dry mass produced.

CONCLUSIONS

The reduced plant growth parameters and DW production of *Viburnum* plants under saline conditions was due to detrimental effects of Na and Cl accumulation in the shoot. Our results demonstrated that exogenous application of osmolytes generally mitigated the negative effects of salinity. Indeed, glycine betaine application alleviated the deleterious effect of salt stress on most morphological parameters measured. The better crop performance of plants treated with glycine betaine under salt stress conditions may be attributed to the partial suppression of Na uptake, and better nutritional status.

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