Scientia Horticulturae 237 (2018) 303–310

Contents lists available at ScienceDirect

Scientia Horticulturae

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

Effectiveness of bacterial inoculation in alleviation of salinity on water status, mineral content, gas exchange and photosynthetic parameters of *Viburnum tinus* L. plants

María José Gómez-Bellot[a], María Fernanda Ortuño[a], Pedro Antonio Nortes[a], Antonio Bernavé[b], Félix Fernández[b], María Jesús Sánchez-Blanco[a,*]

[a] Departamento de Riego, Centro de Edafología y Biología Aplicada del Segura (CSIC), P.O. Box 164, E-30100 Murcia, Spain
[b] SYMBORG, SL, Campus Universitario de Espinardo 7, Edificio CEEIM, E-30100, Murcia, Spain

ARTICLE INFO

Keywords:
Plant water relations
Ornamental
Microbial inoculates
Enzymes activity
Nutrients
Saline water

ABSTRACT

High concentration of salts in the irrigation water causes plant water stress due to decreased water availability and nutrients uptake from the soil. However, these effects may be minimized by the presence of soil microorganisms, which increase plant tolerance to abiotic stress. The experiment described was performed using three-year-old laurustinus (*Viburnum tinus* L.), in which the combined effect of two irrigation treatments (EC $< 0.9\ dS\ m^{-1}$ as control and EC: $6\ dS\ m^{-1}$ as the saline water) and microbial complex inoculation and non-inoculation were applied under field conditions during six months. The chemical properties of the soil as well as physiological behavior of the plants were evaluated. The higher salinity irrigation water produced plants that were lower than the control plants in the last phase of the experiment, while inoculated plants had a better visual appearance minimizing the decrease of chlorophyll levels. Salinity caused a decrease in g_s and P_n from the beginning of the experiment, the enhanced g_s due to bacterial inoculation increased the photosynthetic activity, which was accompanied by the better uptake of water by the roots (higher Ψ_{stem} values). Bacterial inoculation led to a significant decrease in Na and Cl in the aerial part of the plants. The leaf Ca, Fe, Mg and Mn contents were higher in the inoculated control plants than in non-inoculated control plants, while P decreased and K content was similar. Salinity did not affect the enzymatic activity (phosphatase and chitinase activity) or the nitrogen and total carbon content of the soil, while the total carbon was higher in inoculated plants than in non-inoculated plants. The bacterium did not significantly influence the soil phosphorus content, although it showed a slight tendency to increase at the end of experiment. Although laurustinus has natural mechanisms against saline stress, the inoculation with the selected soil microbial complex improves plant physiological behavior, which is important for plant establishment and soil protecting.

1. Introduction

Soil microorganisms are the most abundant of all the biota in soil, where they are responsible for driving nutrient and organic matter cycling, soil fertility and soil restoration (Barea et al., 2004; Giri et al., 2005). Beneficial microorganisms include those that create symbiotic associations with plant roots, increase nutrient mineralization and availability, produce plant growth hormones, and reduce the susceptibility to diseases caused by plant pathogenic fungi, bacteria, viruses and nematodes biocontrol agents (Kloepper et al., 2004). Many of these organisms are part of the soil under natural conditions, however the beneficial effects that they produce could be increased if agricultural management techniques, such as inoculation, are used to increase their abundance and activity.

Various other beneficial rhizosphere organisms, known as plant growth promoting bacteria (PGPB), have been applied to different ecosystems for their participation in fundamental processes, such as plant growth, nutrient cycling and / or pathogen control (Zahir et al., 2004). Soil microorganisms synthesize enzymes that mineralize certain nutrients and improve their supply, such as inorganic nitrogen (N) and

Abbreviations: C_{org}, Organic carbon; C_T, Total carbon; EC, Electrical conductivity; ETo, Reference evapotranspiration; g_s, Leaf stomatal conductance; N_T, Total nitrogen; OM, Organic matter content; P, Significance level; PAR, Photosynthetically active radiation; PGPB, Plant growth promoting bacteria; P_n, Net photosynthetic rate; pNP, p-nitrophenol; RCC, Relative chlorophyll content; RH, Relative humidity; Ψ_{100s}, Osmotic potential at full turgor; Ψ_{stem}, Stem water potential

* Corresponding author.
E-mail address: quechu@cebas.csic.es (M.J. Sánchez-Blanco).

https://doi.org/10.1016/j.scienta.2018.04.028
Received 17 October 2017; Received in revised form 27 March 2018; Accepted 14 April 2018
Available online 24 April 2018
0304-4238/

phosphorus (P). Phosphatase activity plays an important role in the transformation of organic phosphorus into the inorganic forms more appropriate for plants. Phosphorus (P) is an essential nutrient for plant and their organic form is the greater part of soil (Balemi and Negisho, 2012). Chitin is one of the dominant forms of organic N that enters the soil (Paul and Clark, 1996; Gooday, 1994), with fungi and bacteria the main producers of this polymer in soils (Gooday, 1994; Olander and Vitousek, 2000).

In a dry climate, low humidity and soil salinity are the most stressful factors for plants in terms of water absorption by the roots. In addition, in saline conditions, certain ions such as Na^+ and Cl^- may be accumulated in the plant tissues, where they cause ionic toxicity and induce nutrient imbalances, inhibiting the uptake of essential nutrients necessary for the correct functioning of the plant (Ashraf, 2004; Meloni et al., 2008; Zhu, 2001). Other consequences include metabolism alteration, such as hormonal changes, reduced enzyme activity and impaired photosynthesis, leading to a substantial losses in productivity (Munns, 2002; Acosta-Motos et al., 2014a, 2014b). Nevertheless, the response to salinity varies with the plant growth stage, climatic and soil conditions, agronomic and irrigation management and the degree of tolerance of the species (Alarcon et al., 1994; Katerji et al., 2001; Gómez-Bellot et al., 2013; Álvarez and Sánchez-Blanco, 2014). Under salt stress, PGPB rapidly have a positive effect on plants, including enhanced tolerance to abiotic stress, physical and chemical changes, and increased yield and plant growth (Mayak et al., 2004; Kokelis-Burelle et al., 2006; Medina and Azcón, 2010). *Viburnum tinus* L., also known as laurustinus or viburnum laurestine, is a popular evergreen shrub or small tree widely cultivated for its winter flowering habit in regions with mild winters (e.g., Mediterranean area). It is a perennial shrub that grows to 3 m, with dark green and oval leaves, used to attract pollinators, ornamental hedge plant (Whitehead and Peakall, 2009). According to Fox et al. (2005), this species could withstand 2.5–3 dS m^{-1} without losing its aesthetic value, because Na+ concentration in plants irrigated with water with electrical conductivity lower than 2 dS m^{-1} is higher in roots than in leaves. Nevertheless, when the electrical conductivity of the irrigation water increased to 4 dS m^{-1} the distribution changed and the concentration was higher in leaves. A similar response was also found for Cl- accumulation in this specie (Bañón et al., 2012).

The objective of this work was to determine the effectiveness of a rhizosphere microbial complex in field conditions under saline conditions in *Viburnum tinus* plants. Morphological and physiological responses of *Viburnum tinus* L. were evaluated in order to assess the potential of bacterial inoculation to mitigate the negative effect of salinity irrigation water. In addition, the activity of acid phosphatase (which mineralizes P) and a chitinolytic enzyme (N-acetyl-B-D glucosaminide) that is essential in the mineralization of N were evaluated to know how the nutrient availability and limitations influence enzyme activity.

2. Materials and methods

2.1. Plant material and experimental conditions

Nursery-grown laurustinus plants with an initial height of 10–15 cm were transplanted into the experimental plot. The soil were amended initially with 2 g L^{-1} of Osmocote Plus (14:13:13 N, P, K plus micro-elements) and a Hoagland solution (standard nutrient solution, Arnon and Hoagland, 1940) was applied every three-four months through the drip irrigation system.

The experiment was performed using three-year-old laurustinus (*Viburnum tinus* L.) plants (n = 80) at the experimental farm of CEBAS-CSIC in Santomera (Murcia, Spain) (38°06′N, 1°02′W, elevation 110 m.), using a planting pattern of approximately 1 m × 1 m. The soil, classified as Lithic xeric haploxeroll, is stony (33%, w/w) and shallow, with a clay–loam texture. Analytical data showed a high lime content, low organic matter content and low cationic exchange capacity (Table 1).

Table 1
Soil characteristics data.

Soil characteristics	
pH	7.92
EC (dS m^{-1})	0.41
CEC (meq 100 g^{-1})	14.66
$CaCO_2$ (%)	56.00
OM (%)	0.34
D_b (g cm^{-3})	1.56

Starting on 24 June 2015, two irrigation treatments were applied for six months, the control, C (using water with EC < 0.9 dS m^{-1}) and the saline treatment, S (using water with EC = 6 dS m^{-1}). For each irrigation treatment, half of the plants were inoculated (10 l ha^{-1}) with a rhizosphere microbial complex (*Acetobacter fabarum, Acinetobacter jhonsonii, Candida boidinii, Nocardiopsis alba, Penicillium chrysogenum* and *Azospirillum brasilense*) with a concentration of 2.3 × 10^6 cfu stabilized at pH 3.9 twice throughout the experiment (at the beginning and at the middle). The inoculum was applied by means of the drip irrigation. The remaining plants were not inoculated, so that there were four treatments in total: Control and saline treatments with and without bacterial inoculation.

Plants were irrigated so that the stem water potential of the control plants did not decrease more than −0.9 MPa. In order to maintain this potential, the quantity of water depended on the season, climatic conditions and plant development. The plants were irrigated twice a day with one lateral pipe per plant row and one emitter (each delivering 3 l h^{-1}) per plant. The quantity of water applied was controlled every week using in-line water meters.

The EC of irrigation solution in all treatments was evaluated with a multirange Cryson-HI8734 electrical conductivity meter (Cryson Instruments, S.A., Barcelona, Spain) at the beginning of and throughout the experimental period.

Every 15 min, climatic data were recorded by an automatic weather station located next to the plot. The average maximum and minimum values of air temperature were 24 and 13 °C, respectively and of RH 84 and 35%, respectively. The annual reference evapotranspiration (ETo) determined by the FAO, Penman-Monteith equation (Allen et al., 1998) was 1091 mm, with a maximum of 194.98 mm in July. During the experiment the total rainfall was 185 mm, most of it occurring in spring and autumn.

2.2. Soil and leaf mineral content

The mineral content of three soil samples per treatment was determined by Inductively Coupled Plasma optical emission spectrometer (ICP-OES IRIS INTREPID II XDL). The soil was dried at room temperature for a week and ground and sieved through a 2 mm nylon mesh before analysis. The macronutrient concentrations were determined in a digestion extract with HNO3:HClO4 (2:1, v/v) by Inductively Coupled Plasma optical emission spectrometer (ICP-OES IRIS INTREPID II XDL). The concentration of Cl^- was analysed by a chloride analyzer (Chloride Analyser Model 926, Sherwood Scientific Ltd.) in the aqueous extracts obtained by mixing 100 mg of dry sample powder with 40 ml of water before shaking for 30 min and filtering.

The total nitrogen (N_T), total carbon (C_T) and organic carbon (C_{org}) concentrations of the soil were measured from the soil samples collected with an elemental analyser (Flash EA 1112 Series, Leco Truspec). The organic matter content (OM) of the soil was determined by multiplying TOC by 1.72. Available P was also analysed colorimetrically as molybdovanadophosphoric acid (Watanabe and Olsen, 1965).

The mineral content of leaves was determined in four plants per treatment as indicated above for the soil mineral content. The leaf samples were oven-dried at 80 °C and ground before analysis.