Industrial Crops and Products 83 (2016) 717–728

Contents lists available at ScienceDirect

Industrial Crops and Products

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

Integrated application of *Exiguobacterium oxidotolerans*, *Glomus fasciculatum*, and vermicompost improves growth, yield and quality of *Mentha arvensis* in salt-stressed soils

Nidhi Bharti[a], Deepti Barnawal[a], Samvedna Shukla[a], Shri Krishna Tewari[b], R.S. Katiyar[b], Alok Kalra[a,*]

[a] *Microbial Technology Department, CSIR-Central Institute of Medicinal and Aromatic Plants, P.O. CIMAP, 226015, India*
[b] *CSIR-National Botanical Research Institute, Rana Pratap Marg, Lucknow-226002, India*

ARTICLE INFO

Article history:
Received 6 August 2015
Received in revised form
12 November 2015
Accepted 9 December 2015
Available online 14 January 2016

Keywords:
Mentha arvensis
AM fungi
Salt
Rhizobacteria
Vermicompost

ABSTRACT

The escalating population has amplified the pressure on fertile lands for cultivation of food crops has augmented; therefore, utilization of degraded agricultural fields or wastelands including salt affected soils is a possible alternative for farming of medicinal and aromatic plants. *Mentha arvensis* L. is grown for its menthol-rich essential oil used in pharmaceutical, cosmetic, flavor, food, beverage and associated industries. The present study is aimed at identifying potential combinations of PGPR and AMF and assesses their potential in improving *M. arvensis* growth and yield under salt stressed conditions in both controlled and field conditions. Four AM fungi, viz. *Glomus aggregatum* (Ga), *Glomus mosseae* (Gm), *Glomus fasciculatum* (Gf) and *Glomus intraradices* (Gi) and two PGPR *Halomonas desiderata* (STR8, GenBank Accession no. JQ436849) and *Exiguobacterium oxidotolerans* (STR36, GenBank Accession no. JQ804988) were inoculated in *M. arvensis* in combination to assess their effects on plant growth and yield under salt stressed conditions through glasshouse trials. The plants applied with *G. fasciculatum* + *E. oxidotolerans* (Gf + STR36) recorded highest fresh weights, oil yield as compared to all other treatments as well as control plants in saline conditions. Based on the data obtained in the glasshouse experiments, *G. fasciculatum* + *E. oxidotolerans* (Gf + STR36) along with vermicompost were tested for its efficacy in promoting plant growth under naturally occurring salt stressed field conditions. The field trials indicated positive interactive effects of combined application of *G. fasciculatum* + *E. oxidotolerans* and vermicompost (Gf + STR36 + VC) as observed through improved plant growth and better AMF colonization. Our results promote the idea of multi-microbial inoculations together with vermicompost as efficient biofertilizers in promoting *M. arvensis* growth in salt affected fields.

1. Introduction

The demands for essential oil-bearing plants like *Mentha arvensis* have augmented exponentially in past few years across the globe and are anticipated to amplify in future. The supply of essential oils severely lags behind its demands, it is required to maximize the essential oil yield of such plants on cultivable lands replacing food crop causing a threat to food security. The other option could be the use of unproductive land for the cultivation of such crops. With an annual production of essential oil of 15,000–20,000 t, India is the leading *Mentha* oil producer (Chand et al., 2004) as well as an exporter of natural menthol, its processed derivative (Misra et al., 2000). *M. arvensis* L. constitutes the most important natural source of L-menthol and its essential oil has wide applications in flavoring, pharmaceutical and agrochemical industries globally (Misra et al., 2000; Tassou et al., 2004). Owing to the increasing demand of *M. arvensis* and ever-increasing pressure on agricultural fields for food production, efforts are being made to cultivate cash crops like mint on reclaimable soils affected by abiotic stress. Increase in crops productivity to meet the growing demands of industry and simultaneously maintaining a sustainable agricultural system is a challenging task.

Salinity stress has emerged as a constraint to the agricultural crops productivity worldwide and is likely to have a higher impact

* Corresponding author. Fax: +91 522 2342666.
E-mail address: alok.kalra@yahoo.com (A. Kalra).

http://dx.doi.org/10.1016/j.indcrop.2015.12.021
0926-6690/

on agriculture in coming years (Rahnama et al., 2011). Currently, 80 million hectares of agriculturally viable lands across the globe has been estimated to be salt affected (FAO, 2008; Türkan and Demiral, 2009). Rapid urbanization is pressurizing agriculture to shift into drier or more marginal lands, and global food requirements are projected to increase 70% by 2050, demanding higher gains in agricultural productivity with less productive land and quality irrigation water (Fischer et al., 2009). Developing salt-tolerant crops has been a much looked-for scientific objective however with a little success to date, as only a few major-determinant genetic traits of salt tolerance have been identified (Flowers 2004; Munns and Tester 2008; Schubert et al., 2009). An alternative approach to enhance crop salt tolerance may be the introduction of salt-tolerant microbes that improve crop growth and improve soil health. Symbiotic relationships such as arbuscular mycorrhizal fungi (AMF), ectomycorrhizal fungi (ECM) and root-associated plant growth-promoting rhizobacteria (PGPR) have been known to improve plant and soil health under salt stressed conditions (Bhardwaj et al., 2014).

More optimized use of cooperative interactions among diverse soil microbes can influence environment and crop production positively (Artursson et al., 2006). Synergistic interactions between PGPRs and AM fungi in different plants can have possible positive effectiveness, including growth promotion, disease suppression, leading to elevated production of secondary metabolites (Awasthi et al., 2011; Bharadwaj et al., 2012; Hemashenpagam and Selvaraj 2011; Liu et al., 2012). Vermicompost has been utilized as well-known organic manure in sustainable crop production. Several studies have demonstrated that vermicompost amendment may perhaps improve soil quality, by convalescing soil structure, enhancing plant available nutrients, and encouraging microbial activity, thus increasing plant production compared to conventional chemical fertilization (Ngo et al., 2011; Roy et al., 2010). A new school of thought suggests combining synergistic microbes and vermicompost for facilitating plant growth and an alternative for chemical fertilizers (Song et al., 2015).

The present study is aimed at studying the combined effects of PGPR-AMF on *M. arvensis* plants in ameliorating salt-induced stress and the possibility of employing such combinations in naturally occurring salt stressed fields. The study is an effort to understand the specificity of interactions between halotolerant PGPR (*Halomonas desiderata* (STR8); *Exiguobacterium oxidotolerans* (STR36)) and AM fungi (*Glomus* spp.).

2. Materials and methods

2.1. Micro-organisms used

Two halotolerant plant growth promoting rhizobacteria viz., *H. desiderata* (STR8, GenBank Accession no. JQ436849) and *E. oxidotolerans* (STR36, GenBank Accession no. JQ804988) and four species of *Glomus* viz., *Glomus mosseae* (Gm), *Glomus aggregatum* (Ga), *Glomus fasciculatum* (Gf), *Glomus intraradices* have (Gi) were obtained from the microbial culture collection of Microbial Technology Department, CSIR–Central Institute of Medicinal and Aromatic Plants (CSIR–CIMAP), Lucknow, India. Rhizobacteria *H. desiderata* (STR8) and *E. oxidotolerans* (STR36) were primarily isolated from the rhizosphere of naturally growing plants of the grass family (Poaceae) on the nearly unproductive saline soils of Rae Bareilly, Uttar Pradesh, India. Ece, pH and Exchangeable Sodium Percentage (ESP) of the soil were 1.462 dS m^{-1}, 10.71 and 50.154%, respectively (Bharti et al., 2014).

The following four species of *Glomus* viz., *G. mosseae* (Gm), *G. aggregatum* (Ga), *G. fasciculatum* (Gf), *G. intraradices* (Gi), obtained from microbial culture collection of CSIR–CIMAP, initially disseminated on maize roots (Zea mays L.) were proliferated in sterilized vermicompost on maize for 10 weeks and were shade dried for 2 weeks. The potential mycorrhizal inoculum was prepared using AMF mycelium containing maize roots cut down into 1-cm segments, and uniformly mixed into the potting medium (vermicompost) (Soni et al., 2014).

2.2. Vermicompost used

To generate vermicompost (VC), a mix of distillation waste (plant-spent de-oiled herb) of aromatic grasses (*Cymbopogon winterianus* and *Cymbopogan flexuosus*) was composted in a vermicompost unit for 90 days, using adult epigeic species of clitellate earthworms, *Eudrilius eugineae*. This vermicompost mixture was employed as the organic manure component in the glasshouse and field trials. The vermicompost consisted of 1.05% N, 0.65% P and 0.71% K.

2.3. Pot experiment

The experiment consisted of a randomized complete block design with two bacterial treatments in combination with four AM fungi at two salinity levels of 0 mM (control) and 300 mM NaCl. The study was carried out under glasshouse conditions at CSIR–CIMAP, Lucknow, India. The experiments were performed two times and the data presented is the mean of the two experiments. Suckers of one-inch length of *M. arvensis* cv. Kosi obtained from Genetic Resource Management Division, CSIR–CIMAP, were surface sterilized in 0.1% $HgCl_2$ and then planted in the earthen pots. The soil utilized in this experiment was obtained from the agricultural fields of CSIR–CIMAP, Lucknow. The soil, a sandy–loam (Ustifluvent), had the following physicochemical properties: pH 7.29, EC 0.45 dS m^{-1}, 4.40 g kg^{-1} organic carbon, 124 kg ha^{-1} available N (alkaline permanganate extractable), 10.5 kg ha^{-1} available P (0.50 M $NaHCO_3$ extractable), and 98 kg ha^{-1} available K (1 N NH_4OAc extractable). The soil was sterilized through autoclaving for 1 h at 121 °C and 15 psi to remove existing microbial propagules. Four kilograms of the autoclaved soil was distributed into each earthen pot (20 cm high and 20 cm internal diameter).

The NaCl treatment was initiated two weeks after sprouting of suckers. To evade salt effects on AM fungus establishment and osmotic shock to the fine *M. arvensis* roots, NaCl was commenced gradually by successively increasing the NaCl concentration at a gradient of 50 mM every 7 days until it reached 300 mM NaCl. Leaching was reduced by keeping the soil water below field capacity at all times. The plants maintained at 0 mM NaCl were irrigated with sterile water not supplemented with NaCl (Bharti et al., 2013).

2.4. Microbial inoculation

An amount of 50 g of vermicompost based inoculum (containing 8–10 AM fungal propagules g^{-1} soil) of four species of *Glomus* viz., *G. mosseae* (Gm), *G. aggregatum* (Ga), *G. fasciculatum* (Gf) and *G. intraradices* (Gi) was added to each pot, placed 3 cm deep along with suckers. Control pots contained an equal amount of autoclaved sterilized vermicompost based inoculums.

The bacterial inoculums were prepared using the method illustrated in Bharti et al. (2014) with slight modifications. Rhizobacterial strains were grown in nutrient broth (NB) with 5% (w/v) NaCl upto late exponential growth phase to prepare bacterial inoculums. The broth in each flask was inoculated with isolated rhizobacterial strain and incubated at 28 °C for 24 h in an orbital shaking incubator at 100 rpm. Optical density was measured to achieve a uniform population of bacteria [$\sim 10^8$ colony forming units (CFU) ml^{-1}] in the broth prior to inoculation. The inoculums