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Research article

Ascophyllum nodosum extract biostimulants and their role in enhancing tolerance to drought stress in tomato plants

Oscar Goñi, Patrick Quille, Shane O'Connell*

Plant Biostimulant Group, Shannon Applied Biotechnology Centre, Institute of Technology Tralee, Clash, Tralee, Co. Kerry, Ireland

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A B S T R A C T

Global changes in climate are leading to increased occurrence and duration of drought episodes with concurrent reduction in crop yields. Expansion of the irrigated land area does not appear to be a viable solution in many regions to deliver crop productivity. The development of crop drought tolerance traits by either genetic modification or plant breeding represent the principal approaches to meeting this challenge to date. Biostimulants are an emerging category of crop management products which can enhance crop productivity under abiotic stress conditions. The ability of some biostimulant products such as *Ascophyllum nodosum* extracts (ANE) to enhance the tolerance of crops to drought stress has been observed by growers. The objective of this study was to investigate if different commercial ANE biostimulants provided the same tolerance to tomato plants (cv. Moneymaker) subjected to a defined drought period. A compositional characterisation of the key macromolecules of ANEs was performed. In addition, the role of ANE biostimulants in inducing changes of chlorophyll and osmolytes levels, MDA production, dehydrin isoform pattern and dehydrin gene expression levels was assessed. The three ANE biostimulants evaluated were found to provide different levels of tolerance to drought stressed tomato plants. The level of drought tolerance provided was related to changes in the concentration of osmolytes and expression of *tas14* dehydrin gene. Taken together, our results highlight that despite the fact all ANE biostimulants were manufactured from the same raw material, their ability to maintain crop productivity during and after drought stress was not the same.

1. Introduction

Drought is a normal, recurring feature of climate which occurs in virtually all climatic regimes. Even in more humid climatic zones, drought is often a common feature. Agriculture is one of the key sectors affected by drought. The impact of drought on crop productivity has led to major consequences for food security and the economy of different world regions. World regions most impacted by drought include South-Central Asia, Southeast of South America, Central Europe and Southeast of the United States (Carrão et al., 2016). The occurrence of agricultural drought depends on the crop evapotranspiration demand and the soil moisture availability to meet this demand (Wilhite, 2011). Globally, rain fed agriculture is practised in 80% of the total agricultural area (Monneveux et al., 2013). Irrigation is the first line solution for agricultural drought but this is not without cost and problems (e.g. sustainability and salinity).

Strategies beyond irrigation for providing crop drought tolerance include speciality crop inputs, traditional plant breeding and genetic modification strategies to reduce drought stress. A key factor to successful implementation of these strategies is a better understanding of drought tolerance, which includes a series of protective mechanisms which function at the morphological and physiological levels. Typical mechanisms include development of vigorous root system, formation of epidermal wax, shedding of older leaves, regulation of stomatal closure to reduce dehydration, modulation of photosynthetic performance, repression of cell growth or induction of senescence (Wilkinson and Davies, 2010; Fang and Xiong, 2015). Reducing transpiration presents an opportunity to alleviate the adverse effects of water deficit and improve crop productivity under drought conditions (Prakash and Ramachandran, 2000). Speciality crop inputs promoted to reduce crop

Abbreviations: 2-ME, 2-mercapthoethanol; ABA, abscisic acid; ANE, *Ascophyllum nodosum* extract; DW, dry weight; EBIC, European Biostimulant Industry Consortium; FW, fresh weight; GM, genetically modified; HPAEC-PAD, High performance anion exchange chromatography with pulsed amperometric detection; LEA, late-embryogenesis-abundant; MDA, malondialdehyde; PVPP, polyvinylpolypyrrolidone; qRT-PCR, quantitative real time polymerase chain reaction; REACH, Registration, Evaluation, Authorisation and Restriction of Chemicals; RH, relative humidity; ROS, reactive oxygen species; RWC, relative water content; SDS-PAGE, sodium dodecyl sulphate polyacrylamide gel electrophoresis; TBARS, thiobarbituric acid reactive substance; TBA, thiobarbituric acid; TCA, trichloroacetic acid; TFA, trifluoroacetic acid; TW, turgid weight

* Corresponding author.
E-mail address: shane.oconnell@staff.ittralee.ie (S. O'Connell).

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drought stress include antitranspirants which can be categorised into three major groups based on their mode of action, namely films, reflecting and physiological antitranspirants (Del Amor et al., 2010).

Plants also respond and adapt to drought stress at biochemical, cellular and molecular levels. For instance, by the mobilization of stress-related hormones, production of osmolytes, elimination of reactive oxygen species (ROS) and accumulation of stress protective proteins such as LEA (Late Embryogenesis Abundance) proteins (Olvera-Carrillo et al., 2011; Fang and Xiong, 2015). Each mechanism depends on the expression and regulation of an assortment of genes with diverse functions (Nakashima et al., 2014). Conventional breeding for adaptation to drought stress is far more complicated than breeding for other traits (Fita et al., 2015). Another way to increasing yield under water stress is based on the generation of genetically modified (GM) crops with tolerance to drought (Reguera et al., 2012). The first drought tolerant GM crop was commercially launched in 2012 with market approval in USA and Canada. Monsanto, in collaboration with BASF, developed a genetically modified maize variety with improved resistance to water stress by expression of bacterial genes encoding RNA chaperones. DroughtGard™ maize remains the only drought tolerant GM crop with multi-region approval in 2017 and planting increased 15-fold from 50,000 hectares in 2013 to 810,000 hectares in 2015 reflecting high farmer acceptance (James, 2015).

Biostimulants are an emerging class of crop management products that target the modulation of crop stress to increase productivity. A number of definitions of a biostimulant have been proposed and reviewed (Yakhin et al., 2017). The European Biostimulant Industry Consortium (EBIC) are leading the international marketplace in defining and seeking regulation for biostimulant products in Europe. EBIC has defined biostimulants as “containing substance(s) and/or microorganisms whose function when applied to plants or the rhizosphere is to stimulate natural processes to enhance/benefit nutrient uptake, nutrient efficiency, tolerance to abiotic stress, and crop quality”. du Jardin (2015) assigned biostimulants into 8 categories: (i) humic substances, (ii) complex organic materials, (iii) beneficial chemical elements, (iv) inorganic salts, (v) seaweed extracts, (vi) chitin and chitosan derivatives, (vii) antitranspirants and (viii) free amino acids and other N-containing substances with microorganisms a potential ninth category.

Seaweed extracts are prominent in the biostimulant market, representing the fastest growing biostimulant product category (Watkins, 2015). The effects of seaweed extracts on plants have been reviewed (Craigie, 2011; Sangha et al., 2014) with a range of biostimulant effects reported, including drought tolerance. It is important to recognise that seaweed extract biostimulants are not a homogenous category of products. Seaweed extract biostimulants vary depending on the seaweed specie used for manufacture (e.g. brown, green or red), the spatio-temporal source of the seaweed raw material and the process used for manufacture/extraction (Khan et al., 2009; Sharma et al., 2013). Most of the commercial seaweed extracts with biostimulant effects are manufactured with brown algal species, with *Ascophyllum nodosum* Le Jol the dominant specie due to its long history of positive results in enhancing crop productivity (Craigie, 2011). *Ascophyllum nodosum* extract (ANE) biostimulants have previously been reported to increase drought stress tolerance of grasses and crops (Spann and Little, 2011; Elansary et al., 2016, 2017; Martynenko et al., 2016; Santaniello et al., 2017). Additionally, a recent transcriptome analysis of the model plant *Arabidopsis thaliana* reported the dysregulation of abiotic stress genes important for drought tolerance after the application of ANE biostimulants (Goñi et al., 2016).

Tomato (*Solanum lycopersicum* L.) is one of the most important vegetable crop plants around the world and is particularly sensitive to a number of environmental stresses, including drought. Responses of tomato to drought stress depend on several factors including duration and severity of the drought period as well as its inherent tolerance mechanisms (Iovieno et al., 2016; Patanè et al., 2016). Due to the agronomic and economic relevance of tomato, different approaches to reduce the impact of drought on fruit yield and quality have been proposed, including the application of a biostimulant enriched in betaines (Petrozza et al., 2014). GM tomato plants have been shown to have increased drought stress tolerance without affecting plant growth under non-drought conditions. Successful gene modifications include the introduction of genes encoding dehydrins or enzymes involved in the synthesis of osmoprotectants (Gerszberg and Hnatuszko-Konka, 2017).

This study focused on generating data to support answers to the following questions: Do ANE biostimulants have a role in maintaining crop productivity during periods of drought; Are all ANE biostimulants the same in terms of their ability to induce drought tolerance in tomato; What are the effects of biostimulants on some of the molecular players involved in mediating drought tolerance in tomato.

2. Material and methods

2.1. Plant material and growth conditions and drought stress treatment

Tomato seeds (*Lycopersicon esculentum*, cv. Moneymaker) were purchased from Liscahane Nurseries, Tralee. Seeds were surface sterilised with sodium hypochlorite for 1 min before being thoroughly rinsed with distilled water. Seeds were set in plug trays using growth medium of compost: vermiculite: perlite (5: 1: 1). On day 21, seedlings were then transferred to 2 litre pots (same growth medium as previous) and 2g of slow releaser fertilizer containing $N/P_2O_5/K_2O$ (7/7/7, w/w/w) was applied to each pot. The resultant plants were raised in a growth room at a temperature of 27/22 ± 2 °C (day/night; 16/8 h) and 70 ± 5% relative humidity (RH) under a light intensity of 120 μmol $m^{-2}·s^{-1}$ in a complete randomised block design. Plants were irrigated with 125 mL water every other day in order to create equal soil moisture conditions in all the pots. Temperature and relative moisture content were recorded regularly with a portable USB data logger (Log32TH, Dostmann electronic GmbH).

2.2. Treatment application and drought stress conditions

Three commercially available liquid seaweed extracts of *A. nodosum* (ANE A, ANE B and ANE C) manufactured using different methods were applied to plants as biostimulant treatments. ANE A was manufactured using a proprietary process at high temperatures and neutral pH. ANE B and ANE C were manufactured using a proprietary process at high temperatures and alkaline pH. Prior to imposition of severe drought, ANE biostimulants and control treatments were applied by foliar spray at a dilution of 0.33% (v/v) on 35-day-old tomato plants. Distilled water was applied as a control. After 24 h, drought stress was induced by withholding water for 7 days. To minimize the influence of any positional effect on drought stress responses, the relative position of the pots in the growth room was changed every other day. After the drought treatment, plants were re-watered, and 24 h later ANE treatments were applied again as foliar spray at 0.33% (v/v). Control plants were sprayed with equal volume of distilled water. Recovery stage after water withdrawal was maintained for 2 weeks under conditions described at section 2.1 to obtain 56-day-old plants. This experimental protocol is evaluating the drought tolerance stage (until T1) and growth promotion effects after stress (from T1 to T3). The 2 applications programme before and after stress period is based on current farmer practice for the use of ANE biostimulants. Leaf tissue was sampled before first ANE biostimulant application (T0), at 7 days after subjecting plants to drought stress (T1), at 48 h after the second ANE treatment in the 3rd day of the recovery stage (T2) and at the end of the recovery stage (T3). The samples were snap-frozen in liquid nitrogen, ground and kept in −80 °C until further analysis. Similar tomato plants were selected and grown under unstressed conditions for 56 days. ANE biostimulants and control treatments were applied by foliar spray as described above to evaluate growth promoting effects on non-drought