Industrial Crops & Products 120 (2018) 104–112

Contents lists available at ScienceDirect

Industrial Crops & Products

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

Stimulation of energy willow biomass with triacontanol and seaweed extract

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ARTICLE INFO

Keywords:
Phenotyping
Organ size
Woody stem
Photosynthesis
Thiamine
Nicotinamide

ABSTRACT

Biomass productivity of shrub willow plants grown in short rotation system can be improved by genetic means or by innovative cropping technologies. In the present study we analyzed the growth and physiological responses of willow plants to plant biostimulators, such as 1-triacontanol (TRIA), a saturated primary alcohol and seaweed extract (Kelpak®). Testing a novel approach, we soaked stem cuttings in TRIA or Kelpak solutions for 48 h before plantation. These treatments enlarged height and diameter of stems, furthermore increased stem and leaf weights in comparison to the water control. In agreement with these greenhouse observations, field tests showed statistically significant enhancement in height and diameter of woody stems harvested in winter. Application of 25% Kelpak solution was the most effective in stimulating all traits including stem weight per plant after pre-planting treatment of cuttings. In an alternative treatment protocol, Kelpak was applied as foliar treatment or in combination between TRIA (10 mg L^{-1}) treatment of cuttings and foliar spray with Kelpak 1 or 2% solutions in the greenhouse. The green pixel numbers revealed variable degrees of stimulation in shoot growth. These treatments resulted in an increase in stem and leaf weights. Improvement of photosynthetic functions was indicated by more efficient electron transport rates (ETRs) of photosystems. An increased nicotinamide and thiamine contents were detected in the leaves of stimulated plants. The present study can serve as a foundation for additional laboratory and field studies optimizing the application of these stimulators in energy plantations.

1. Introduction

Growing interest in the plantation of short rotation woody crops (willow, poplar and black locust) is expected to play a role in satisfying a significant portion of the increasing biomass demand for bioenergy production without interference in food production. Short Rotation Coppice (SRC) willow plantations are characterized by fast growth rate and high biomass production in a very short coppice cycle (Hanley and Karp, 2014). As concluded by Djomo et al. (2011), short-rotation woody crops (willow and poplar) yield 14.1–85.9 times more energy than coal per unit of fossil energy input, and greenhouse gas emissions are 9–161 times lower than those of coal. Besides direct burning, the biomass of short rotation energy willow can also serve as feedstock resource for releasing sugars by enzymatic saccharification that can be converted to ethanol (Serapiglia et al., 2013). These environmental benefits justify concentrated efforts to improve the biomass yield of these woody species either by genetic means (Hanley and Karp, 2014) or by optimizing cropping systems (Heller et al., 2003).

In the widely used silvicultural practice, willow hardwood cuttings are planted at high density (15000–20000 plants ha^{-1}) preferentially on liberated agricultural, marginal or contaminated lands often subjected to inland water flooding. Planting stocks of cuttings are collected from one-year-old dormant shoots during the winter period and stored at −2 to −4 °C. After cold storage, the 20–25 cm long cuttings can be soaked in water for two days prior to planting when they are pressed down into the prepared soil. During the first year, the growing shoots mature to woody stems and SRC plantations are characterized by a very short rotation cycles (1–2 years). The dry biomass yield could be as high 14.3 Mg ha^{-1} at a planting density of 24 000 ha^{-1} (Stolarski et al., 2017).

Improving a set of physiological traits by stimulator treatment can offer a novel technology for maximizing biomass yield. The saturated

Abbreviations: TRIA, 1-triacontanol; SRC, short rotation coppice; ETR, electron transport rate; PSI, photosystem I; PSII, photosystem II; PPFD, photosynthetic photon flux density; Y(II), carbon assimilation parameter; B1, thiamine; NA, nicotinamide

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https://doi.org/10.1016/j.indcrop.2018.04.047
Received 4 January 2018; Received in revised form 19 March 2018; Accepted 17 April 2018
Available online 07 May 2018
0926-6690/

primary alcohol 1-triacontanol (TRIA, $C_{30}H_{62}O$), is a natural component of plant epicuticular waxes that could function as a secondary plant growth substance. Its plant growth and yield enhancing capability was recorded after foliar spray or seed treatment of a variety of crop species in growth chamber, greenhouse, and field studies (Ries, 1985; Naeem et al., 2012). Most significant biochemical and physiological responses to application of various doses of TRIA include the enhancement of photosynthesis (Ivanov and Angelov, 1997; Chen et al., 2003; Khandaker et al., 2013). The growth promoting effects of foliar spray or seed treatment by TRIA were quantified in shoot and root lengths, fresh or dry weight of plants, leaf area, and seed yield in different plant species as reviewed by Naeem et al. (2012). Limited studies have been published on responses of woody species to TRIA application. Sitinjak and Pandiangan (2014) reported an increase in cacao seedlings growth after TRIA treatment.

Beneficial effects of seaweed products on crop plants are well-documented and the potential of their agricultural and horticultural use is outlined (Craigie, 2011; Arioli et al., 2015). Agronomic benefits include improved organ growth, and generation of tolerance to diseases and to climatic stress such as cold or drought. The seaweed product with the brand name of Kelpak® is an extract made from the kelp *Ecklonia maxima*. Its biostimulatory functions are dependent on a mixture of natural growth regulatory compounds including auxins, cytokinins, polyamines, furthermore gibberellins, brassinolide, castasterone and abscisic acid (Stirk et al., 2014). The most frequent application method of Kelpak is based on foliar spray and soil or seed treatment of crop plants including wheat, maize, bean, tomato, and grasses (Calvo et al., 2014). Studies on responses of woody fruit species to Kelpak treatment are limited. Szabó et al. (2014) reported an increased shoot mass from pretreated stockplants of *Prunus mahaleb* L.

Having recognized the increased demand for biomass produced in short rotation energy plantations, we tested two biostimulators (TRIA and Kelpak®) alone or in combination for application in willow cultivation. The primary aim was to quantify responses of two energy willow cultivars to the treatments of planting stocks of stem cuttings or to spraying shoots with these commercial products. In greenhouse studies we use organ size and biomass data, furthermore a set of physiological parameters such as photosynthetic electron transport rates and the amounts of nicotinamide and thiamine in leaves for monitoring plant responses to these stimulators. The stimulatory effects of TRIA and Kelpak® were also confirmed under field conditions. We discuss the potential use of these technologies described in woody biomass production.

2. Materials and methods

2.1. Experimental setup in greenhouse studies

2.1.1. Plant material and stimulator treatments

Commercial willow production in Hungary has primarily been based on Swedish clones. For the present studies we used two high-yielding clones, Tordis (*Salix schwerinii x Salix viminalis*) and Inger (*Salix triandra x Salix viminalis*). 20 cm stem cuttings were harvested in winter and stored at between 0 °C and −4 °C. Before planting 10 cuttings were presoaked in 3 L water or alternatively in stimulator solutions: 10 mg L^{-1} TRIA or Kelpak 25% solution. Nutri-Stim Triacontanol™ (2.5%) solution was purchased from Nutri-Tech Solutions (Yandina, Queenstand, Australia). KELPAK® stock solution (100%) was delivered by Kelp Products International Ltd. South Africa Simon's Town. The stem pieces were incubated for 48 h in various stimulator solutions as indicated in the text and directly placed into the soil, each stem piece with a 1.5-2-cm segment above ground. In this study we determined the actual amounts of active compounds taken up by 20 cm stem rods. During the two-day incubation time the solution uptake ranged between 1.7-2.9 g/cutting. We calculated absorption of the following active compounds into cuttings: TRIA 10 mg L^{-1}: 21.6 µg; Kelpak 25%: 446 µg. These values can be influenced by the actual water content of cuttings, the cold storage of wood pieces or the incubation temperature.

In the combined methodology of stimulator treatment, cuttings were treated with 10 mg L^{-1} TRIA solution and with foliar spray of 1% or 2% Kelpak solution in the greenhouse. Green shoots developed during two weeks after the plantation of stem cuttings into phenotyping pots were sprayed with equal volumes of these solutions that covered the leaves completely.

2.1.2. Growth conditions

Technical details of pot experiments were described previously for characterization of tetraploid willow plants (Dudits et al., 2016). Briefly single, water- or stimulator-treated stem cuttings were planted into radio-tagged plexiglass columns with a mixture of 80% Terra peat soil and 20% sandy soil. Five plexiglass columns surrounded with polyvinyl chloride tubing were placed on a metal rack. Two racks were used for each treatment combination with random arrangement. The racks were rearranged every week after each measurement during the experiments. The level of illumination in the greenhouse was approximately 400 µmol photons $m^{-2}s^{-1}$. Watering was performed once per week.

2.1.3. Biomass and growth rate data collection

Shoot height and basal stem diameter were measured at weekly intervals. The height of the longest stem was determined from the top of the planted cutting upto the shoot tip. Stem diameter was measured on the same stem at 5 cm above the top of the planted cutting. Imaging of shoot growth was carried out by a semi-automatic phenotyping platform. Shoots developed from dormant buds were photographed with an Olympus C-7070WZ digital camera from seven different side positions, produced by 51.4° step rotation of the pot. The shoot and leaf surface that corresponds to the plant-related pixel number was provided as the average of green pixel counts derived from photographs of seven projections to minimize the variations in superposition of leaves and shoots. After a four- or seven-week growing period, fresh weights of the outgrown green stems and leaves from all shoots cut from each plant were immediatlely measured in each treatment combination.

2.1.4. Measurements of the electron transport rates (ETRs) of photosystems I and II

For the characterization of photosynthetic events, the fifth or sixth fully opened young leaf from the top was screened from five plants per treatment combination. We applied Pulse Amplitude Modulation (PAM) based chlorophyll fluorescence and P700 absorbance measurement techniques using Dual-PAM-100 (Walz GmbH) to determine ETR through PSII and PSI, respectively in dark-adapted leaves of Inger and Tordis as described in Dudits et al. (2016). The ETRs through PSII [ETR (II) = 0.5* Y(II)* PPFD* 0.84] as well as through PSI [ETR(I) = 0.5* Y (I)* PPFD* 0.84] were simultaneously measured according to Genty et al. (1989); Klughammer and Schreiber (1994). The light response curves of PSI and PSII were generated as described by Dudits et al. (2016).

2.1.5. Nicotinamide and thiamine content determination in leaves

Pulled sample of 5 g of leaf tissues from ten plants per combination was used after cutting out the main veins. Samples were rubbed in 50 mL of distilled water mixed with silica sand. After rubbing, the samples were filtrated. The filtrates were kept at −20 °C, and lyophilized. Dried samples were resolved in 1 mL of water and centrifuged for 5 min at 1000 rpm. The supernatant was used in further analysis. For easier separation of the compounds studied, a simple fractionation of the supernatant was performed using Strata X-C tubes. The tubes were conditioned with 2 mL of methanol and 2 mL of 1% formic acid. The samples were eluted with methanol containing 2% NH_4OH. The purified samples were lyophilized and resolved 1 mL of 3% formic acid each, prior to HPLC analysis.