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Effect of foliar application of a nitrophenolate–based biostimulant on the yield and quality of two bean cultivars

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ABSTRACT

The use of Atonik, a synthetic biostimulant, to improve the yield and quality of red and white bean was studied. The 0.1% and 0.3% solutions of Atonik biostimulant were applied by a single and double spraying of plants. The yield of the investigated common beans increased after Atonik treatment with the highest impact recorded upon the double spraying with the 0.3% solution. The studied treatments had no significant effect on phenolics content; however, some differences were found in the antioxidant potential. The applied biostimulant had no significant effect on the reducing power either. The angiotensin-converting enzyme inhibitory activity was noted only for Aura bean; however it remained unchanged upon the biostimulant treatment. All the analyzed types of application had no significant effect on starch and protein contents; however, a slight, insignificant decrease was observed in starch content in Aura cultivar. An insignificant decrease of globulins in beans obtained from plants double-sprayed with 0.1% Atonik should also be noted. Most importantly, the studied treatment did not enhance the activity of α-amylase and trypsin inhibitors. In summary, results of the study demonstrate that Atonik effectively improves the yield and nutraceutical potential of beans without any negative effects on their nutritional quality.

1. Introduction

An increasing demand for high-quality products of plant-origin is a challenge in modern agriculture. Currently, crop production is focused on boosting the yield and quality of plants keeping to the principles of production safety. Therefore, the cultivation of plants with a limited use of pesticides is more common and better perceived by consumers (Szymanowska et al., 2015).

Stress factors (adverse environmental conditions, diseases, pests) negatively affect crop yield. In such cases, it is fully justified to use the preparations containing biologically-active substances (biostimulants), which may improve the yield of plants by supporting the natural mechanisms of their resistance (Djanaguiraman et al., 2005a; Przybysz et al., 2014). Biostimulants may be of natural or synthetic origin. Natural biostimulants are preparations based on free amino acids, seaweed and fruit extracts, effective microorganisms, humic substances and chitosan (Calvo et al., 2014). Synthetic biostimulants include growth regulators, phenolic compounds, inorganic salts, essential elements, and other substances (Przybysz et al., 2014). Atonik, also known as Chapperone or Asahi SL, is a synthetic biostimulant. It is a water solution of phenolic compounds: sodium para-nitrophenolate PNP (0.3%), sodium ortho-nitrophenolate ONP (0.2%) and sodium 5-nitroguaiacolate 5NG (0.1%). Atonik has a positive effect on the yield of some important crops including cotton (Djanaguiraman et al., 2005a), bean (Kocira et al., 2013), oilseed rape (Przybysz et al., 2014), and carrot (Kwiatkowski et al., 2013). So far, the mechanism of nitrophenolate–based biostimulant action has not been fully explained; however, biomass accumulation and elongated growth,

Abbreviations: A_A, absorbance of sample; ABTS, antiradical activity; A_C, absorbance of control; ACE, angiotensin converting enzyme; ACEI, ACE inhibitory activity; BAPNA, α-*N*-benzoyl-DL-arginine-*p*-nitroanilidehydrochloride; C, control; Cy3-GE, cyanidin 3-glucoside equivalent; DM, dry matter; DNSA, standard dinitrosalicylic acid; DS 0.2%, double spraying by 0.2% solution of Kelpak SL; DS 0.4%, double spraying by 0.4% solution of Kelpak SL; EDTA, ethylenediaminetetraacetic acid; GAE, gallic acid equivalent; MW, molecular weights; ND, not detected; QE, quercetin equivalent; RP, reducing power; SS 0.2%, single spraying by 0.2% solution of Kelpak SL; SS 0.4%, single spraying by 0.4% solution of Kelpak SL; TAC, total anthocyanins content; TCA, trichloroacetic acid; TFC, total flavonoids content; TIA, trypsin inhibitor activity; TPC, total phenolic compounds; ε, molar absorptivity.

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Table 1
Temperature (°C) and rainfall (mm) during the vegetative seasons of common bean.

Months	Years			
	2012		2013	
	Temperature (min/max) (°C)	Rainfall(mm)	Temperature (min/max) (°C)	Rainfall(mm)
V	16.1 (0.5/28.7)	90.2	15.9 (6.3/26.0)	116.6
VI	16.2 (7.8/31.2)	101.3	17.9 (8.8/29.6)	77.3
VII	21.6 (7.3/33.4)	79.6	20.4 (12.1/31.2)	88.3
VIII	18.2 (6.9/31.6)	85.7	18.9 (6.8/33.4)	112.6
Average/Total	18.0	356.8	18.3	394.8

observed after its application, are usually linked with an increase of auxin concentration (Djanaguiraman et al., 2005b). The foliar application of Atonik additionally increases the inhibition of IAA oxidase, which ensures a greater activity of naturally-synthesized auxins and a greater number of high-affinity binding sites of IAA (Davies, 1987; Przybysz et al., 2014; Stutte and Clark, 1990).

Common bean (*Phaseolus vulgaris* L.) is one of the most commonly cultivated legumes in the world. It is considered as a nutraceutical legume due to the high content of bioactive components such as protein, peptides, polyphenols, unsaturated fatty acids, dietary fiber, vitamins and minerals (Kutos et al., 2003). Studies concerning the effect of nitrophenolates on legumes are sparse; however the previous research suggests that the application of other biostimulants such as Nano-Gro and Kelpak had a positive effect on common bean by increasing its yield and nutritional quality (Kocira et al., 2015a, 2016). Such treatments as well as other biostimulants including chitosan, yeast extract, jasmonic, arachidonic or β-aminobutyric acid, may also induce the accumulation of phenolics in the treated plants, which usually resulted in an increase of the nutraceutical quality of foods based on such crops (Złotek et al., 2016). This paper, for the first time, provides the information about the effects of a nitrophenolate-based Atonik biostimulant on the nutraceutical and nutritional quality of common bean (Aura and Toska cultivars).

The aim of the present studies was firstly to determine the optimum Atonik treatment regime for two bean cultivars to ensure the maximum yield and secondly, to determine if the biostimulant influenced the nutritional and nutraceutical quality of the crop.

2. Materials and methods

2.1. Chemicals

ABTS (2,2-diphenyl-1-picrylhydrazyl), potassium ferricyanide, EDTA (ethylenediaminetetraacetic acid), PMSF (phenylmethanesulfonyl fluoride), BAPNA (α-N-benzoyl-DL-arginine-*p*-nitroanilidehydrochloride), HHL (Hippuryl-L-histidyl-L-leucine), and pepstatin A were purchased from Sigma–Aldrich (Poznan, Poland). All other chemicals were of analytical grade. Atonik was purchased from Arysta LifeScience (Warsaw, Poland).

2.2. Plant materials and growth conditions

The study was carried out in 2012 and 2013 in Perespa (50°66′N; 23°63′E), Poland. The soil type was characterized as Brown rendzina belonging to the Rendzinas soil group. It is alkaline (pH in 1 M KCl around 7.4–7.5) and rich in phosphorus, potassium, and magnesium. The experiment was established in a randomized block design in four replications with an elementary experimental plot area of 6 m^2 (1.80 × 3.33 m). The study was carried out with two bean cultivars commonly cultivated in Poland and processed by the food industry; Aura – white seed coatings and Toska – red seed coatings. Seeds were sown in the first 10-day period of May at a depth of 3–4 cm, with the spacing of drills set at 45 cm to achieve a density of 30 plants m^2. The bean crop was harvested in the first (Aura cultivar) and second 10-day periods of August (Toska cultivar). During the growing season, a 0.1% and 0.3% solution of Atonik was applied by single spraying (at the 2–3 leaf stage) and double spraying of plants (first at the 2–3 leaf stage and second at the beginning of bean blooming). The biostimulant was applied with a GARLAND FUM 12 B battery field sprayer (Lechler LU 120–03) at a pressure of 0.30 MPa, using 300 L liquid per hectare. Four different types of Atonik treatment were compared with each other and with the control, where plants were treated with the same volume of water (no biostimulant applied). Tillage for bean was done using good agricultural practices. Mineral fertilization in kg of nutrient per hectare was as follows: 30 kg N ha^{-1}, 60 kg P ha^{-1}, 120 kg K ha^{-1}. No pesticides were used (pests did not exceed the thresholds of harmfulness). Average temperature and rainfalls during the growing season of common beans are presented in Table 1.

2.3. Plant yield

Upon harvesting (13th week after planting for Aura cultivar and 14th week after planting for Toska cultivar), the number and weight of seeds, the number of pods and the weight of thousand seeds were recorded for each plot (four replications of each combination). The seeds were dried, ground in a laboratory mill and sieved (sieve size 0.310 mm). Flours were stored at −20 °C and used for further analysis.

2.4. Nutrient analysis

2.4.1. Protein sequential fractionation based on solubility criteria

Albumins and globulins from common beans were isolated on the basis of solubility criteria according to the method by Ribeiro et al. (2004) with some modification (Durak et al., 2013). Proteins were subsequently sequentially extracted and purified using appropriate extraction solutions. Albumins were extracted in distilled water containing 10 mM $CaCl_2$ and 10 mM $MgCl_2$ (1 g·30 mL^{-1}). The insoluble proteins were removed by centrifugation at 9000 × *g* and 4 °C for 30 min. The resultant supernatant accounted for the albumin fraction. For globulin extraction, the pellet was resuspended in 30 mL of 100 mM Tris-HCl buffer (pH 7.5), containing 10% (w/v) NaCl and 10 mM EDTA. The solubilized globulins were obtained by centrifugation at 9000 × *g* and 4 °C for 30 min. The resultant supernatant accounted for the globulin fraction.

2.4.2. Protein content

The protein content was determined with the Bradford method (Bradford, 1976), using bovine albumin as the standard protein.

2.4.3. Starch analysis

The total starch content was determined after dispersion of the starch granules in 2 M KOH (50 mg sample, 6 mL KOH) at room