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Morpho-physiological effects of Stymjod foliar application on *Dactylis glomerata* L.

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Abstract. The aim of the experiment was to study the effects of foliar application of a growth regulator containing iodine nanoparticles, with the trade name of Stymjod, on morphometrics, photosynthetic activity and chlorophyll content of *Dactylis glomerate* L. The following parameters were determined: the weight of fresh and dry matter of plants, leaves and roots, the number of shoots and leaves, potential (FV/Fm) and effective ($\Delta F/F_{m'}$) quantum efficiency of photosystem, photochemical (qP) and non-photochemical (qN) quenching, as well as chlorophyll a and chlorophyll b content in leaf blades. The pot experiment was carried out in a breeding room. Stymjod was applied at 1.5%, 3% and 4.5% concentrations in the spray solution. It is a nanotechnology-based growth regulator, with easily assimilable forms of mineral and organic ingredients, favorably affecting plant ontogenesis. Plants were treated twice with a single dose of 50 mL of spray per pot. They were sprayed till they were completely covered with the liquid. Distilled water was used to spray control plants. The results were statistically processed using analysis of variance, while the significance of the differences between means was determined with Tukey's test with $P = 0.05$. In the experiment it was found that different concentrations of Stymjod in the spray resulted in different response of plants. The best morphological effects were obtained using the concentration of the product exceeding 3%. In the test conditions Stymjod applied to *D. glomerata* increased the efficiency of the photosynthetic apparatus and the content of chlorophyll pigments.

Key words: iodine, plant morphology, photosynthetic activity, chlorophyll content.

INTRODUCTION

Products that contain iodine nanoparticles in their composition and stimulate plant growth and development such as Stymjod and Biojodis are increasingly becoming the topic of research exploring their potential to regulate physiological processes and to increase crop yields (Piotrowski et al., 2016; Romanowska-Duda et al., 2018 and 2019; Sosnowski et al., 2019a; Romanowska-Duda et al., 2020). According to many authors (Strzetelski et al., 2010a, 2010b; Piotrowski et al., 2016; Romanowska-Duda et al., 2018) the dose and form of iodine have a significant effect on plant mineral composition. Forms of water-soluble iodine are most easily taken up by the root system, but the best effects are achieved with foliar application as iodine together with water penetrates through pores in plant cuticles.

Chlorophyll fluorescence used as a physiological parameter has found wide use, such as a genetic marker of barley productivity (Planchon et al., 1989). It is also used to assess the ripeness of cabbage and coriander seeds (Jalink et al., 1998; Górnik et al., 2013), making use of the fact that the maturation process is directly related to the amount of chlorophyll monitored by the measurements of the maximum photosystem II efficiency. Górnik & Lachuta (2017) found that changes in the maximum photosystem II efficiency (Fv/Fm) in sunflower seedlings were associated with their growth. In addition, chlorophyll fluorescence was also proposed as a marker of plant reaction to herbicides (Dayan et al., 2012; Silva et al., 2014). It has been found that damage to cells by a glyphosate herbicide in *Raphanus sativus* L. resulted in changes in the value of fluorescence. Żebrowska & Michałek (2014) used the parameters of photosynthetic activity of strawberry leaves to assess their physiological condition and the level of macronutrients provided by a fertilizer. However, in the available literature there are not many reports on the effects of plant growing methods on changes in the maximum photosystem II efficiency. There is therefore a need for research on the possibility of applying the maximum photosystem II efficiency measurements as a marker of plant reaction to various growing methods and growing conditions, including the use of substances stimulating plant growth and development. The literature points to the possibility of applying the maximum photosystem II efficiency to assess Stymjod effects on the physiological condition of sorghum (Romanowska-Duda et al., 2019), or effects of *Ecklonia maxima* extract on *Medicago* × *varia* T. Martyn (Sosnowski et al., 2019b).

One of the most important perennial fodder grasses is *D. glomerata*, successfully grown in northern Africa, western and central Europe and in temperate and tropical Asia. With a large variety of ecotypes this species has an unusual ability to adapt to local soil and climatic conditions (Hultén, 1968; Tolmachev et al., 1995). Due to its good nutritive properties, high production efficiency and high tolerance to abiotic stress, *D. glomerata* has been widely used as forage for more than 100 years, especially in North America, Europe (Baležentienė & Mikulionienė, 2006; Maiksteniene & Arlauskiene, 2006; Aavola, & Karelson, 2010) and Japan (Mitui, 1981; Casler et al., 2000).

It is worth noting, however, that at high doses of mineral nitrogen *D. glomerata* tends to accumulate nitrate nitrogen, an undesirable forage component, in the aboveground parts. The use of products containing active iodine in its composition can help to reduce mineral nitrogen fertilization, without production losses. This is because such products applied to plants can improve the absorption of nutrients from the soil and increase their content in tissues. Therefore, they may be recommended to organic plant production, also because their use increases the amount of iodine in the food ration (Romanowska-Duda et al., 2018).

The aim of the experiment was to study the effects of foliar application of a growth regulator with the trade name of Stymjod containing iodine nanoparticles on morphometrics, photosynthetic activity and chlorophyll content of *D. glomerate*. To meet the objectives of the research, the effects of different concentrations of Stymjod on the following characteristics were determined: the weight of fresh and the dry weight of plants, leaves and roots, the number of shoots and leaves, the maximum (F_V/F_m) and actual ($\Delta F/F_{m'}$) photosystem II efficiency, photochemical (qP) and non-photochemical (qN) quenching coefficients, as well as chlorophyll a and chlorophyll b content in leaf blades.

MATERIAL AND METHODS

A pot experiment was conducted in 2017 in the breeding room of the Department of Grassland and Green Area Creation, Siedlce University of Natural Sciences and Humanities. The conditions of the experiment were as follows: the temperature of 24 ± 2 °C (in light) and 16 ± 2 °C (in darkness); soil moisture with 60% of field water capacity; the intensity of light of 200 µmol m^{-2} s^{-1}, obtained with high pressure sodium lamps; the photoperiod of 16 hours in light. *D. glomerata* var. Borna was used as a test plant grown in pots with a height of 300 mm and the 200 mm diameter of the base. The experiment was replicated three times and completely randomized with control units. The pots were filled with 5 kg of medium loam soil, sampled from the plough layer. Soil pH in KCl (6.3) was determined together with the concentration of $N\text{-}NO_3$ (1.4 mg kg^{-1} DM), $N\text{-}NH_4$ (60.9 mg kg^{-1} DM), and organic carbon, C_{org} (17.1 g kg^{-1}).

In mid-March, 10 *D. glomerata* seeds were planted in each of the pots at a depth of 1.5 cm. After germination and the three-leaf stage, a negative selection was carried out, leaving three plants with the largest number of leaves. In the experiment different doses of Stymjod were applied with the following variants:

- Control – plants treated with distilled water;
- 1.5% – plants treated with 1.5% concentration of Stymjod;
- 3.0% – plants treated with 3% concentration of Stymjod;
- 4.5% – plants treated with 4.5% concentration of Stymjod.

There were four replications in the experiment. The plants were treated twice: during the six-leaf stage and ten-leaf stage, using 50 mL of the solution per pot each time. According to Romanowska-Duda et al. (2019) Stymjod is a product with organic and mineral components, obtained using cold plasma in nano-technology. It consists of optimal composition of macro and micronutrients as well as iodine nanoparticles. Iodine molecules activate cell metabolism, with plants responding with an intensive growth and development and an increased resistance to biotic and climatic factors. The chemical composition of Stymjod (Romanowska-Duda et al. 2019):

- basic components: $N\text{-}NO_3^-$ (1,231 mg L^{-1}), P (6,652 mg L^{-1}), K^+ (62,720 mg L^{-1}), Ca^{2+} (943 mg L^{-1}), Mg^{2+} (11,570 mg L^{-1});
- microelements: Fe (18.9 mg L^{-1}), Mn (886 mg L^{-1}), Cu (682 mg L^{-1}), Zn (1,476 mg L^{-1}), B (576 mg L^{-1});
- other components: humic acids 3.3%, organic substances (including amino acids) 56.8%, I_n^+: aqueous iodine concentrate 0.0025%.

In the experiment the following parameters were determined: the number of stems per pot, the number of leaves per pot, fresh and dry weight of plants (g pot^{-1}), fresh and dry weight of stems (g pot^{-1}), fresh and dry weight of leaves (g pot^{-1}), fresh and dry weight of roots (g pot^{-1}).

Additionally, other parameters were determined: the leaf greenness index (SPAD), the maximum photosystem II efficiency (F_v/F_m), the actual photosystem II efficiency ($\Delta F/F_m$), the photochemical quenching coefficient (qP) and the non-photochemical quenching coefficient (qN). The content of chlorophyll a and b in *D. glomerata* leaves was also measured. All measurements were carried out when the plants were harvested, on the 15th day after the second Stymjod treatment. The SPAD measurement was conducted with the SPAD-502 portable meter, Minolta, Japan.

Chlorophyll content determination

Chlorophyll a and b content was determined with the Arnon et al. (1956) method modified by Lichtenthaler & Wellburn (1983). The material was collected from each plant at the full flowering stage, with 40–50% flowers open. As for the photosynthetic pigments, the optical density of supernatants was determined with the Marcel Mini spectrophotometer (is produced by the Polish company Marcel) with the wavelengths of 440, 465 and 663 nm. Next, the results were calculated according to the following formulas:

– chlorophyll a content: [12.7(E663) – 2.69(E645)] w/v;
– chlorophyll b content: [22.9(E645) – 4.68(E663)] w/v;

where E – extinction at a particular wavelength; v – amount of 80% acetone (cm^3) used for extraction; w – sample weight (g).

Photosynthetic activity determination

Photosynthetic activity of plants was determined by the measurement of chlorophyll fluorescence induction using the PAM 2000, Heinz Walz GmbH, Effeltrich, Germany. The following parameters were determined:

- the maximum photosystem II efficiency (Fv/Fm) in the dark-adapted state (Bolhàr-Nordenkampf & Öquist, 1993);
- the actual photosystem II efficiency in the light-adapted state ($\Delta F/F_m$);
- qP – the photochemical quenching coefficient;
- qN – the non-photochemical quenching coefficient (Van Kooten & Snel, 1990).

All measurements were recorded during the growing season, using well developed *D. glomerata* leaves in 6 replications. By taking the measurements the leaf-clip holder 2030-B, a light emitting diode at 650 nm, and the standard intensity of 0.15 μmol m^{-2} s^{-1} PAR were used. In the dark-adapted state leaves were kept in darkness for 15 minutes.

Statistical analysis

The results were processed statistically using analysis of variance, and the differences between means were tested with Tukey's HSD test, $P < 0.05$. In tables means marked with the same letters are not significantly different. The Statistica 13-2017.3 software package was used to conduct all calculations.

RESULTS AND DISCUSSION

According to the results (Table 1) the concentration of Stymjod in the spraying liquid significantly affected the number of *D. glomerata* shoots. The largest number was in plants treated with 4.5% concentration. The average value was 25 per pot and it was greater than the number of shoots in control by about 42%. The use of 1.5% and 3% concentrations did not significantly affect the number of shoots compared to control. Stymjod treatment significantly affected the number of leaf blades developed by plants.

The most leaves were on units with the concentrations of 3 and 4.5%, with the average number of 258 per pot, which was greater by about 35% than the number of leaf blades in the control plants. The concentration of 1.5% did not affect this parameter considerably. A similar trend was also observed with fresh and dry leaf weight. The highest values of these characteristics were in plants treated with 3 and 4.5% concentrations. In addition, according to the dry weight of 100 leaf blades, the largest amounts of leaves were produced by *D. glomerata* treated with the highest concentration of Stymjod, which translated into higher fresh and dry plant weight. The largest fresh and dry plant weight (212 and 40 g per pot, respectively) was on pots with the highest concentration of the product. The use of the 3% Stymjod concentration also contributed to an increase in the value of the above parameter. The statistical analysis did not show a significant variation in the fresh and dry weight of the plant root system. The concentrations used in the experiment did not affect the development of the rhizosphere. Compared to the control units the lowest concentration of the product did not significantly affect any of *D. glomerata* characteristics.

The effect of a substance containing iodine nanoparticles on the increase in crop yields are reported by Gaurilčikienė et al. (2008) and Sosnowski et al. (2019). According to Smoleń (2009), foliar use of Biojodis increased the yield of the Chopin cabbage variety by 4.5% compared to control, and the yield of the Arivist variety by as much as 12%.

Table 1. The effect of Stymjod on morphometric parameters of *D. glomerata*

Characteristic	Stymjod concentration			
	control	1.5%	3%	4.5%
	(pieces pot^{-1})			
Shoot number	17.6 (± 1.1) b	17.2 (± 1.4) b	19.8 (± 1.7) b	25.0 (± 2.5) a
Leaf number	191 (± 18) b	213 (± 22) b	254 (± 25) a	262 (± 24) a
	(g pot^{-1})			
Leaf fresh weight	67.8 (± 4.1) b	77.4 (± 6.9) b	97.4 (± 6.0) a	118 (± 8.7) a
Leaf dry weight	12.5 (± 1.1) b	13.3 (± 1.3) b	19.5 (± 1.4) a	22.4 (± 2.2) a
Root fresh weight	80.5 (± 18) a	71.8 (± 11) a	67.7 (± 17) a	93.5 (± 21) a
Root dry weight	16.1 (± 1.4) a	14.2 (± 1.6) a	12.2 (± 1.2) a	17.6 (± 2.1) a
Plant fresh weight	148 (± 21) c	149 (± 22) c	165 (± 29) b	212 (± 31) a
Plant dry weight	28.6 (± 2.2) c	27.5 (± 2.0) c	31.7 (± 2.7) b	40.0 (± 3.2) a
	(g)			
Dry weight of 100 leaves	6.54 (± 0.52) c	6.24 (± 0.70) c	7.68 (± 0.31) b	8.55 (± 0.83) a

Standard deviation (± SD).

In the cultivation of pickled cucumber the same product increased the yield of fruits by 10%. Substances containing iodine have had a beneficial effect on the yield of cereals. Gaurilčikienė et al. (2008), who conducted studies involving spring barley treated with a Biojodis foliar spray, a product containing iodine nanoparticles, found that the average yield of barley increased by 3.2% compared to control. The effect of increased concentrations of Stymjod on the growth of plant aboveground biomass was noted in the studies of Romanowska-Duda et al. (2018). The authors found that sorghum plants responded very well to the growing concentrations of Stymjod. Concentrations of 1.5 and 3% significantly increased plant growth, the leaf greenness index, According to the authors Stymjod increased the yield by improving the parameters of plant physiological activity. In addition, they also pointed out that the morphological effects of Stymjod on sorghum plants were in line with the general recommendation of its manufacturer. It indicates that Stymjod increases the growth of biomass, improves plant resistance to adverse weather conditions and relieves the negative effects of stress.

The impact of Stymjod on chlorophyll a and b content in *D. glomerata* leaves (Table 2) was statistically significant. In the leaves of control plants the chlorophyll content was 172 mg 100 g^{-1} per fresh weight, but upon the treatments of 3% and 4.5% solutions the chlorophyll an increased accordingly to 227 and 225 mg 100 g^{-1} per fresh weight. The average increase in chlorophyll a content compared to control was 31.4%. A similar difference of 30.8% was recorded for chlorophyll b. According to the literature (Yokoya et al., 2007; Zhao et al., 2016) these photosynthetic pigments are responsible for collecting light and moving the absorbed light to photosynthetic reaction centres; their concentration is associated with the effectiveness of photosynthesis. Increasing the content of these pigments may be one of the causes of an increase in photosynthetic activity (Zhao et al., 2016). Similarly, in the present studies *D. glomerata* leaves responded to Stymjod application with a higher content of photosynthetic pigments and an increased photosynthetic activity.

Table 2. The effect of Stymjod on chlorophyll pigment content (mg 100 $g^{-1}$of fresh weight) and SPAD leaf greenness index in *D. glomerata* leaves

Characteristic	Stymjod concentration			
	Control	1.5%	3%	4.5%
Chlorofil a	172 (± 11.2) c	198 (± 18.1) b	227 (± 20.1) a	225 (± 21.1) a
Chlorofil b	91 (± 10.4) b	119 (± 12.3) a	120 (± 11.7) a	118 (± 10.1) a
SPAD	39.8 (± 4.1) c	44.1 (± 5.0) b	47.7 (± 3.8) a	49.7 (± 3.9) a

Standard deviation (± SD).

According to Netto et al. (2005) and Stankowski et al. (2019) the SPAD index is a good parameter for diagnosing the integrity of the photosynthetic system in leaves, and thus can help with advanced interpretations of the photochemical process. In addition, the content of chlorophyll in leaves depends on many factors, one of which is the availability of nitrogen to the plant. According to the above authors SPAD readings lower than 40 indicate some impairment of the photosynthesis process. In the present experiment (Table 2) SPAD values for *D. glomerata* were between 39.8 for control and 49.7 for plants treated with the highest concentration of Stymjod. Each concentration resulted in a significant increase in the SPAD value, compared to control. According to the research of other authors (Netto et al., 2005; Uddling et al., 2007) the relationship between chlorophyll concentration and SPAD values determined for coffee, birch, wheat and potato was statistically significant. For all species the relationship was nonlinear with a rising slope as the SPAD grew. The relationship between chlorophyll concentration and SPAD values for coffee, birch and wheat were strong ($r^2 = 0.9$), while for potatoes it was weaker ($r^2 = 0.5$). For birch and wheat the relationship was very similar when chlorophyll levels were expressed with a unit of measurement for leave surface area, but it differed when expressed with a unit of measurement for fresh weight. In addition, the relationship values between chlorophyll concentration and SPAD values of wheat for two different varieties and during two different growing seasons were similar. The curved shape of those relationships matched the simulated effect of uneven chlorophyll distribution on the leaf surface and its rotatory dispersion, causing deviations from linearity in the upper and low SPAD range, respectively.

Fluorescent measurements of *D. glomerata* leaves showed that the effect of Stymjod on the maximum photosystem II efficiency (Fv/Fm) in the dark-adapted state was statistically significant (Table 3). Stymjod application resulted in an increase from Fv/Fm of 0.549 on the control units, to Fv/Fm exceeding 0.6 for plants treated with different concentrations (1.5% – 0.618, 3% – 0.647, 4.5% – 0.644); the average percentage increase was 26.6%. On the other hand, the actual photosystem II efficiency ($\Delta F/F_{m'}$), determined under the same conditions, was on average 28.9% higher for leaves of plants treated with Stymjod. According to et al. (1997) such an increase indicates growing demand of plants for products constituting assimilatory power and a lack of disruption in the growth and development process.

Table 3. The effect of Stymjod on photosynthetic activity of *D. glomerata* leaves

Characteristics	Stymjod concentration			
	Control	1.5%	3%	4.5%
The maximum photosystem II efficiency (F_v/F_m)	0.549 (± 0.02) b	0.618 (± 0.03) a	0.647 (± 0.03) a	0.644 (± 0.03) a
The actual photosystem II efficiency ($\Delta F/F_{m'}$)	0.398 (± 0.01) a	0.512 (± 0.02) a	0.509 (± 0.02) a	0.518 (± 0.03) a
The photochemical quenching coefficient (qP)	0.537 (± 0.02) a	0.543 (± 0.02) a	0.549 (± 0.03) a	0.556 (± 0.03) a
The non-photochemical quenching coefficient (qN)	0.108 (± 0.01) c	0.120 (± 0.03) b	0.141 (± 0.03) a	0.138 (± 0.04) a

Sstandard deviation (± SD).

In addition, an increase in Fv/Fm means activation of PSII due to the absence of photoinhibition in plant cells with nitrogen deficiency. That is, the energy consumed for the transport of electrons is not reduced. At the same time, an increase in the activity of PSII reaction centres of nitrogen-rich cells indicates a high activity of the photosynthetic apparatus and increased light-energy conversion efficiency (Nishiyama et al., 2006). It is therefore worth pointing out that Stymjod treatment of *Dactylis glomerata* L. may have resulted in better nitrogen nutrition of plant cells, as evidenced by increases in photosynthetic parameters such as the maximum (Fv/Fm) and the actual ($\Delta F/F_{m'}$) photosystem efficiency of leaves. The use of Stymjod also contributed to an increase in the non-photochemical quenching coefficient (qN). As the data in Table 3 indicates, it increased by 23.2% compared to control pots. However, no statistically significant effect of the product on the photochemical quenching coefficient (qP) of leaves was recorded. The literature (Laisk et al., 2014; Körner et al., 2015) points to a very large impact of the genetic conditions of plants on the value of fluorescence parameters, which may explain the lack of differentiation of qP values under the effect of Stymjod.

CONCLUSIONS

Stymjod applied at the concentration of 4.5% significantly increased the number of shoots, leaf blades and dry weight of 100 leaf blades, which translated into greater fresh and dry *D. glomerata* weight. There was no effect of Stymjod on the increase in fresh and dry root weight relative to the control pots. Under the research conditions, *D. glomerata* treated with Stymjod, regardless of the concentration, was characterized by better efficiency of the photosynthetic apparatus. This is evidenced by, among others, the values of the maximum photosystem efficiency (Fv/Fm), the actual photosystem efficiency ($\Delta F/F_{m'}$) and of the non-photochemical quenching coefficient (qN). All of the Stymjod concentrations increased the photosynthetic activity of plants, resulting from an increase in the content of photosynthetic pigments and the value of the SPAD leaf greenness index. Beneficial effects of Stymjod application to D. glomerata and the possibility of decreasing mineral nitrogen doses without negative physiological consequences for plants were observed in the research.

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