

Enhancing Growth and Salinity Stress Tolerance of Pansy Using Hydrolyzed Gellan Gum – An Environmentally Friendly Plant Biostimulant

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ABSTRACT

Biostimulants are a broad group of numerous compounds that stimulate plant growth and increase plant resistance to environmental stresses. Pansies are in great demand, so new sustainability strategies for their production are still being sought. This study evaluated the effectiveness of partially hydrolyzed gellan gum (HGG) as a natural biostimulant for the production of high-quality pansy plants. In Experiment 1, the effects of drench concentrations of HGG (0, 25, 50, and 100 mg·dm⁻³) on morphological and physiological parameters of two pansy cultivars were investigated. In Experiment 2, the objective of study was to determine the effect of HGG on growth, flowering and leaf physiology of pansy grown under increasing sodium chloride (NaCl) concentrations (50 and 200 mM). Our results showed in both cultivars growth-promoting effects of HGG, and 100 mg·dm⁻³ of HGG was the most effective concentration. The increasing salinity imposed as NaCl negatively affected the growth, flowering, visual appearance, relative chlorophyll content (SPAD), stomatal conductance, and chlorophyll fluorescence (Fv/Fm) of plants. However, HGG pretreatment alleviated the adverse effects of salt stress mainly by reducing the decrease of SPAD, Fv/Fm, flower number and biomass in salt-stressed plants at 200 mM NaCl. Overall results indicated that eco-friendly HGG at the proper dose could be used as a tool to enhance growth, flowering and salt stress tolerance in pansy plants.

Keywords: microbial polysaccharide, sustainability, abiotic stress, saline irrigation, crop tolerance.

INTRODUCTION

Plant biostimulants can promote growth and protect plants against environmental stresses (Ma et al., 2022). Biopolymers from the polysaccharide group, e.g., alginate, carrageen or chitosan are an essential source of plant biostimulants due to their physicochemical properties and high bioactivity (Moenne and González, 2021). Among natural biopolymers, gellan gum is still an underexplored carbohydrate gum for plant cultivation. This microbial polysaccharide is produced by a strain of the bacterium *Sphingomonas elodea* (Sun et al., 2023) and is widely used in the food, cosmetic, pharmaceutical, and tissue engineering industries as a thickening and stabilizing agent (Dev et al., 2022). In the horticulture, gellan gum

has been used in plant tissue culture media (Ishii et al., 1998; Kikuchi et al., 2023). Few studies to date show that gellan gum can exhibit biostimulatory effects on plants with the breakdown products of this biopolymer being more effective than the native form (Salachna et al., 2019; Salachna, 2020). However, the effects of depolymerized gellan on the growth habit, flowering and aesthetic appearance of floriculture crops grown under environmental stresses has not, as yet, been reported. The excessive soil salinity is a severe problem in plant cultivation, and climate change exacerbates this phenomenon (Singh, 2022). Soil salinity is an unfriendly environment, especially for ornamental plants used in urban areas (Francini et al., 2022). Most ornamental plants react negatively to salinity stress by stunting growth

and flowering (García-Caparrós and Lao, 2018). The deleterious effects of salinity on plants can be attempted to some extent by using different plant growth regulators, plant hormones, osmoprotective compounds, and biostimulants (Awad-Allah et al., 2020; Quamruzzaman et al., 2021). Most studies evaluating the effectiveness of the abovementioned compounds under stress conditions concern crops, vegetables, fruits, and herbs (Ngearnpat et al., 2023; Mystkowska and Dmitrowicz, 2024). Much less data on this subject can be found for the floriculture industry.

Garden pansy (*Viola × wittrockiana* Gams., Violaceae) is one of the most popular bedding plant in temperate climates. Pansy flowers are edible and valuable for biologically active compounds (Zawadzińska and Salachna, 2014; de Oliveira et al., 2024). Unfortunately, pansies are sensitive to abiotic environmental stresses, including high soil salt concentrations (Villarino et al., 2011). Pansies growing in a saline medium are characterized by weaker growth and flowering and reduced chlorophyll content, leading to a significant loss of plant visual appearance (Pušić et al., 2019). Therefore, solutions are being sought in pansy production that can mitigate the adverse effects of salinity stress (Javadi et al., 2020; Zeljković et al., 2021). The goals of the study were to determine the effect of hydrolyzed gellan gum on the growth, flowering and leaf physiology of pansies grown under non-stress and salt-stress conditions. It was hypothesized that use of HGG would improve growth and flowering of plants and alleviate salinity stress in pansy cultivation.

MATERIALS AND METHODS

Two experiments were conducted in the greenhouse at the Department of Horticulture of the West Pomeranian University of Technology in Szczecin, Poland (53°25'N, 14°32'E, 25 m above sea level). Seeds of pansy cv. Fancy Carmine with Blotch and cv. Fancy Lilac Shades (Experiment 1) and cv. Fancy Yellow with Blotch (Experiment 2) were sown in the second half of September into boxes filled with a commercial peat substrate TS1 (Klasmann-Delimann, Germany) with pH 6.4 and electrical conductivity (EC) of 1.75 mS · cm⁻¹ and placed under a greenhouse condition with 21 °C day/18 °C night air temperature setting. The 5-week-old pansy seedlings were transplanted to 10-cm-diameter (total pot volume of 0.4 dm³)

black plastic pots filled with peat substrate (TS1) mixed with fertilizer (Hydrocomplex, 12N-4.8P-15K, Yara International ASA, Norway) at a dose of 2 g · dm⁻³. Plants were grown under natural photoperiod conditions and light intensity, where the air temperature was maintained at 10–15 °C.

In Expt. 1, partially hydrolyzed gellan gum (HGG) was applied in the form of aqueous solutions at concentrations of 0 (control water), 25, 50, and 100 mg · dm⁻³. The plants were drenched with HGG solutions four times, starting in mid-February, every seven days, using 50 ml of HGG solution per plant each time. HGG with molecular weight of 56 kDa was prepared by acid hydrolysis (Salachna, 2020).

In Expt. 2, HGG at a concentration of 100 mg · dm⁻³ was applied three times, starting in mid-February every seven days, using 50 ml of solution per plant each time. One week after the last HGG treatment, plants were subjected to salt stress by drenching with finally 50 or 100 mM NaCl solution. Salt treatment was carried out thrice every five days using 50 cm³ of NaCl solution per plant. The non-salt-treated plants were drenched with tap water with EC 0.65 mS · cm⁻¹.

Both experiments were conducted in a completely randomized design. Each treatment included four replicates, each replicate representing a pool of 5 plants. Biometric measurements were taken in both experiments in mid-May bloom. Plant height from substrate level to the highest point of the plant, plant width at the widest point, flower width, and days to flower were determined. At the same time, the relative chlorophyll content was measured using a SPAD 502 optical camera (Konica-Minolta Corporation, Japan), and leaf stomatal conductance was measured using a leaf porometer (SC-1; Dekagon Devices, USA). Measurements were taken on three fully expanded healthy leaves, with four readings on each leaf. Fully open flowers on each plant were counted, then the plants' aboveground part was cut and fresh weight measured. In Expt. 2, to determine the visual score of the plants, a rating of the plants on a scale of 1 to 5 points was carried out by three people, where a value of 1 meant low attractiveness, while 5 meant maximum decorative value. Leaf chlorophyll a fluorescence was measured using chlorophyll fluorometer (HandyPEA, Hansatech Instruments, England). Measurements were taken on two well-developed leaves from the central part of the plants. Based on chlorophyll a fluorescence measurements the maximum

quantum efficiency of photosystem II (Fv/Fm) was calculated following established procedures (Lichtenthaler and Rinderle, 1988).

The data from Expts. 1 and 2 were separately verified for normal distribution and variance homogeneity across samples. The results were statistically analyzed using one-way analysis of variance (ANOVA) using the Statistica® Professional 13.3.0 computer program (TIBCO Statistica, Palo Alto, USA). The significance of the variation in the means was assessed according to Tukey's HSD (Honestly Significantly Difference) test.

RESULTS AND DISCUSSION

The results from Expt. 1 showed a significant effect of HGG on the evaluated morphological parameters of the two pansy cultivars (Table 1). Drenching with HGG solution at a concentration of $100 \text{ mg}\cdot\text{dm}^{-3}$ had a particularly positive effect on the growth and flowering of pansy cultivars. In pansy cv. Fancy Carmine with Blotch, plants treated with $100 \text{ mg}\cdot\text{dm}^{-3}$ HGG were taller (+21%), wider (+15%), and had increased flower width (+15%), number of flowers per plant (+48%) and plant fresh weight (+50%) compared with the control plants. In pansy cv. Fancy Lilac Shades, after application of HGG at a concentration of $100 \text{ mg}\cdot\text{dm}^{-3}$, plants had increased plant height (+24%), plant width (+10%), flower width (+14%), number of flowers per plant (+26%) and plant fresh weight (+22%). The number of days to anthesis was not significantly affected by HGG treatments (data not shown). The stimulatory effects of depolymerized gellan gum with low

molecular weight on growth parameters were found also in seedlings of annual ornamental plants (Salachna 2020). It is so important to find the optimal dose of biostimulant for specific species and even cultivars (Mpai et al., 2022; Kocira et al., 2017). It should be noted that differences in the effectiveness of biostimulant are influenced by the application method, phenological phases of the plants, fertilization, irrigation, and environmental conditions (Matthews et al., 2022).

Drench application of HGG at all tested concentrations positively improved the relative chlorophyll content (SPAD index) and the stomatal conductance in both pansy cultivars evaluated in Expt. 1 (Table 2). Compared to the control, treatment of HGG increased leaf SPAD and the stomatal conductance by an average 16% and 30% (cv. Fancy Carmine with Blotch) and 12% and 45% (cv. Fancy Lilac Shades), respectively. The increased SPAD index due to HGG treatment may indicate increased chlorophyll biosynthesis and, thus, the optimization of leaf photosynthesis (Takai et al., 2010). Confirmation of these suppositions is demonstrated in plants treated with HGG by higher stomatal conductance, which was accompanied by elevated SPAD values. It was speculated that intensive photosynthesis due to HGG treatment could undoubtedly contribute to an increase in the number of flowers and biomass of the aboveground part of pansies (Table 1). The results obtained showing an apparent increase in relative chlorophyll content and stomatal conductance as a result of HGG application are confirmed by earlier studies (Salachna, 2020). The stimulating effect of HGG on chlorophyll biosynthesis and, consequently,

Table 1. Effects of HGG on morphological traits of pansy cultivars

HGG concentration ($\text{mg}\cdot\text{dm}^{-3}$)	Plant height (cm)	Plant width (cm)	Plant fresh weight (g)	No. of flowers per plant	Flower width (cm)
cv. Fancy Carmine with Blotch					
0 (Control)	20.2 ± 0.74^c	17.4 ± 0.10^b	22.0 ± 0.25^b	11.5 ± 1.50^b	6.70 ± 0.70^b
25	22.0 ± 0.55^b	19.5 ± 0.85^{ab}	21.1 ± 4.20^b	13.0 ± 1.33^{ab}	6.90 ± 0.15^{ab}
50	22.4 ± 0.15^b	19.6 ± 0.82^{ab}	25.4 ± 1.60^b	14.0 ± 1.00^{ab}	7.26 ± 0.75^{ab}
100	24.4 ± 0.20^a	20.0 ± 0.40^a	33.0 ± 2.10^a	17.0 ± 2.00^a	7.70 ± 0.10^a
cv. Fancy Lilac Shades					
0 (Control)	17.0 ± 0.70^b	18.8 ± 0.25^b	28.5 ± 4.15^b	11.5 ± 1.50^b	7.27 ± 0.23^c
25	20.3 ± 1.46^{ab}	19.4 ± 0.33^{ab}	31.3 ± 3.00^{ab}	12.0 ± 0.50^{ab}	7.50 ± 0.10^{bc}
50	21.1 ± 0.80^a	20.2 ± 1.15^{ab}	32.8 ± 2.65^{ab}	13.5 ± 1.33^{ab}	7.65 ± 0.05^b
100	21.1 ± 1.30^a	20.7 ± 1.35^a	34.7 ± 1.50^a	14.5 ± 0.50^a	8.25 ± 0.15^a

Note: each value represents the mean with \pm SD of $n = 4$. Different lowercase letters indicate significant differences between means at the 5% level (Tukey test).

Table 2. Effects of HGG on leaf relative chlorophyll content and stomatal conductance of pansy cultivars

HGG concentration (mg·dm ⁻³)	Relative chlorophyll content (SPAD)	Stomatal conductance (mmol H ₂ O·m ⁻² ·s ⁻¹)
cv. Fancy Carmine with Blotch		
0 (Control)	31.0 ± 0.90 ^c	17.8 ± 1.35 ^b
25	35.5 ± 0.30 ^b	22.6 ± 1.85 ^a
50	34.5 ± 0.50 ^b	24.4 ± 3.00 ^a
100	38.1 ± 0.25 ^a	22.4 ± 2.20 ^a
cv. Fancy Lilac Shades		
0 (Control)	37.0 ± 1.04 ^b	18.4 ± 3.20 ^b
25	41.0 ± 0.55 ^a	26.8 ± 3.20 ^a
50	40.8 ± 1.00 ^a	25.1 ± 2.60 ^a
100	42.3 ± 0.95 ^a	28.2 ± 0.45 ^a

Note: each value represents the mean of n = 4. Different lowercase letters indicate significant differences between means at the 5% level (Tukey test).

on plant growth may be due to its biostimulant properties. Gellan gum is produced in bioreactors by bacteria capable of producing gibberellins and indoleacetic acid (Yang et al., 2014; Douglas et al., 2020). Phytohormones can stimulate chlorophyll production, thereby contributing to increased photosynthetic efficiency in plants (Onanuga and Adl, 2012). Salt stress significantly reduced the growth parameters and visual quality of pansy plants (Table 3). The negative effect of salinity was stronger at higher concentrations of NaCl (200 mM). Regardless of HGG treatment, the application of 200 mM NaCl resulted in the reduction of plant height (-23%), plant width (-19%), flower width (-19%), number of flowers (-70%), plant fresh weight (-51%) and visual score (-73%) compared with non-treated plants. On the other hand, when treated with a lower salt concentration (50 mM NaCl), flower number per plant was not significantly different from the control. Days to flower of pansy were unaffected by salinity stress (data not shown).

The results confirm the negative effect of salinity on the morphological characteristics of pansies described in the literature (Javadi et al., 2020; Zeljković et al., 2021). Under salt stress conditions, there can be a reduction in the osmotic penetration of water into the roots, changes in the intensity of mineral nutrient uptake, and toxicity of ions, which negatively affect plant growth and flowering (García-Caparrós and Lao, 2018). The toxic effect of NaCl salts was also manifested in this study by decreasing physiological parameters (Figure 1). Treatment of 200 mM NaCl reduced the SPAD index (-19%), stomatal conductance (-62%), and chlorophyll fluorescence Fv/Fm (-11%). Reductions in photosynthetic pigments content due to a high concentration of NaCl were found in pansy cv. Queen Yellow Bee (Javadi et al., 2020). Salinity can lead to decrease in PSII efficiency and photochemical quenching parameters (Lichtenthaler and Rinderle, 1988) and increased chlorophyll degradation due to boosted chlorophyllase activity under stress

Table 3. Effects of NaCl and HGG treatments on growth characteristics of pansy

Treatments	Plant height (cm)	Plant width (cm)	Plant fresh weight (g)	No. of flowers per plant	Flower width (cm)	Visual score
Control	15.4 ± 0.76 ^{ab}	24.5 ± 0.52 ^a	34.0 ± 2.47 ^a	11.7 ± 0.63 ^a	7.62 ± 0.33 ^{ab}	4.53 ± 0.22 ^b
50 mM NaCl	15.4 ± 1.18 ^{ab}	21.8 ± 0.43 ^{bc}	28.2 ± 0.66 ^b	8.27 ± 1.04 ^b	6.50 ± 0.15 ^d	2.55 ± 0.29 ^{cd}
200 mM NaCl	11.8 ± 0.66 ^c	19.8 ± 0.76 ^c	16.8 ± 1.72 ^d	3.50 ± 0.50 ^d	6.17 ± 0.29 ^d	1.23 ± 0.60 ^d
HGG	16.7 ± 0.58 ^a	24.8 ± 0.29 ^a	36.1 ± 0.64 ^a	12.8 ± 1.26 ^a	8.17 ± 0.29 ^a	4.80 ± 0.13 ^a
50 mM NaCl + HGG	15.8 ± 0.58 ^a	22.8 ± 0.25 ^{ab}	26.9 ± 0.66 ^{bc}	13.5 ± 1.50 ^a	7.33 ± 0.29 ^{bc}	4.65 ± 0.22 ^{ab}
200 mM NaCl + HGG	12.8 ± 1.76 ^{bc}	22.5 ± 1.50 ^{ab}	23.2 ± 0.79 ^c	6.80 ± 1.26 ^c	6.75 ± 0.25 ^{cd}	3.03 ± 0.07 ^c

Note: each value represents the mean of n = 4. Different lowercase letters indicate significant differences between means at the 5% level (Tukey test).

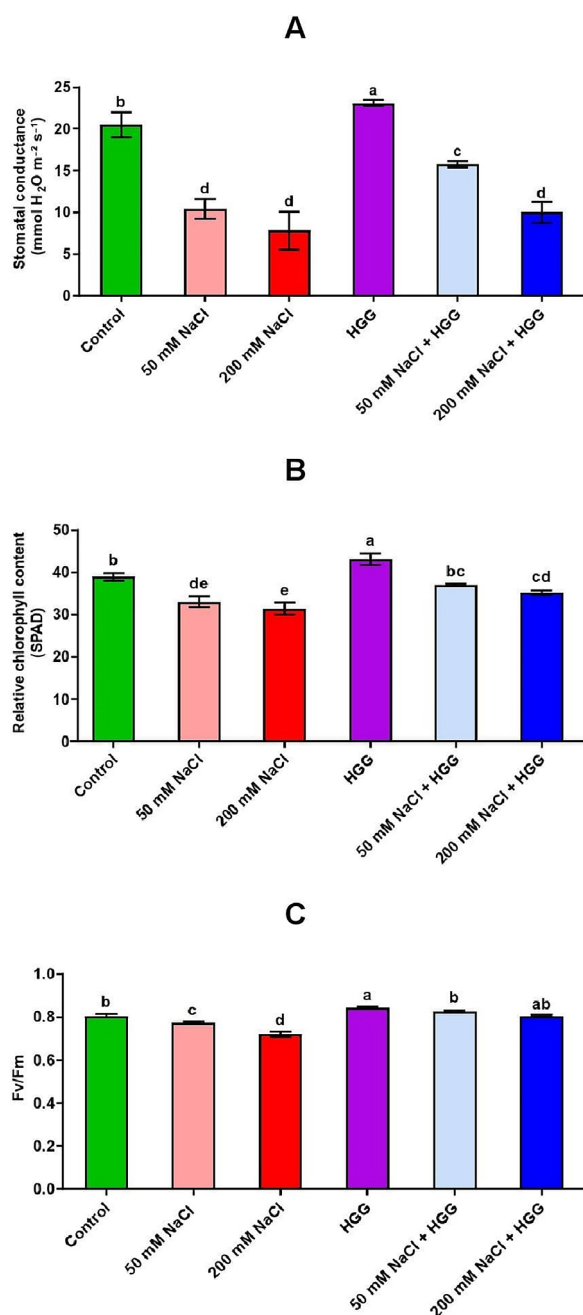


Figure 1. Effects of salt stress (50 or 100 mM NaCl) and hydrolyzed gellan gum (HGG) treatments on leaf physiology of pansy: (A) relative chlorophyll content; (B) stomatal conductance; (C) the maximum quantum efficiency of photosystem II (Fv/Fm). Different lowercase letters above the error bars indicate significant differences at the 5% level (Tukey test)

conditions (Li et al., 2015). In turn, reduced stomatal conductance in plants exposed to salinity may result from the plant's adaptation to stress through more efficient water use (Liao et al., 2022). The results from Expt. 2 confirmed the biostimulatory effect of HGG observed in Expt. 1. The pansies pretreated with HGG under nonstressed conditions

were the tallest and widest, had the widest flowers, and had the highest fresh plant weight, leaf stomatal conductance, and relative chlorophyll content (Table 3, Figure 1). Applying HGG reduced salinity's harmful effects on plant growth and flowering at higher salinity stress. Pansies grown under salt stress conditions at 200 mM NaCl pretreated with HGG were characterized by significantly increased plant width (+14%), flower width (+9%), and fresh plant weight (+38%) compared with 200 mM NaCl-stressed plants with no biostimulant pretreatment. Moreover, applying HGG counteracted the deleterious effects of excessive salinity (200 mM NaCl) on essential parameters determining the quality of ornamental plants, i.e., flower number and visual score. In addition, HGG relieved the adverse effects of salinity stress demonstrated by increased SPAD index and chlorophyll fluorescence compared to plants treated with NaCl alone. Similarly, beneficial effects of oligo-gellan on biomass accumulation were observed in *Perilla frutescens* cv. Dark Opal grown under salt-stress conditions (Salachna et al. 2019). It is believed that polysaccharides and their derivatives can stimulate plant resistance to abiotic and biotic stresses (Moenne and González, 2021). The lower susceptibility of HGG-treated plants to salinity may be related to the physicochemical properties of the biopolymer. Gellan gum is a hydrocolloid with numerous acyl groups in the molecule, which makes it capable of forming biodegradable hydrogels and retaining water in the soil (Choudhary et al., 2022). According to Wang et al. (2023), adding gellan gum to soil increases water accumulation in the soil and thus alleviates drought stress in plants. Plant resistance to salinity is highly dependent on soil moisture (Yin et al., 2023). Therefore, it can be assumed that under saline conditions, HGG reduces the formation of physiological drought in plants due to increasing the osmotic potential of the soil solution, but this requires additional studies.

CONCLUSIONS

The results from this study show that HGG can be used in pansy greenhouse production as an effective eco-friendly biostimulant to improve plant growth and flowering. The best plant quality and physiological state are characterized by pansies treated with HGG at a concentration of 100 mg·dm⁻³. Excessive salinity (200 mM NaCl) leads to drastic inhibition of flower number per plant and biomass in pansies, while pretreatment

with HGG at a concentration of $100 \text{ mg} \cdot \text{dm}^{-3}$ alleviates the mentioned effects of stress. The results described in the present work supplement the current state of knowledge on the greenhouse production of pansy and may be suitable for other floriculture crops.

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