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Developmental responses of perennial ryegrass, red fescue, and Kentucky bluegrass to *In vitro* chitosan treatments

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Abstract

Effects of chitosan oligomers and polymer on *in vitro* development of perennial ryegrass (*Lolium perenne* L.), red fescue (*Festuca rubra* L.), and Kentucky bluegrass (*Poa pratensis* L.) were studied to elucidate a possible differentiation between the effects of chitosan depending on its chemical structure. The seed germination rate was enhanced after the oligomer treatments. The oligomer mixture triggered leaf elongation better than the polymer. However, the highest number of leaves was found from *L. perenne* in the polymer's presence at 10 mg·L^{-1} in the medium. The maximum leaf length was reached in *L. perenne* after oligomeric chitosan treatment at 5 mg·L^{-1}. The plant's rhizogenic response was enhanced in *P. pratensis* but decreased in *L. perenne* and *F. rubra* after 2.5 mg·L^{-1} oligomeric chitosan treatment. However, the root elongation was restricted in *F. rubra* and *P. pratensis* after chitosan treatments. Conversely, chitosan treatments augmented root elongation in *L. perenne*. This study suggested that chitosan might be preferred to ensure better turf coverage in these grass species. However, constant- or over-treatment with chitosan could reduce root growth and increase the plant's leaf elongation that might contribute to nutritional deficiency and increased mowing costs, respectively.

Introduction

Perennial ryegrass (*Lolium perenne* L.), red fescue (*Festuca rubra* L.), and Kentucky bluegrass (*Poa pratensis* L.) are cosmopolitan, cool season, and perennial grass species with dense turf production ability (Ayan et al., 2020). These species are individually cultivated for a number of uses, such as forage production, lawn production for ornamental purposes, and recreational events. *L. perenne* and *F. rubra* are also used for soil stabilization. *P. pratensis* is not preferred for soil stabilization since it has a shallow root system (Wennerberg, 2004). However, the mixture of *P. pratensis* and *L. perenne* is advantageous in establishing a more disease-resistant turfgrass with better color and year-round growth (Wilen et al., 2009). These grass species can also grow on most soil types since they have a wide range of adaptability to most soils (St. John et al., 2012; Acemi, 2021). Several cultivars of the *P. pratensis* and *L. perenne* have been shown to tolerate salt, while some cultivars of *F. rubra* have been reported to perform under drought and heat stresses (Marcum & Pessarakli, 2010; Wang et al., 2017; Bushman et al., 2020). The properties mentioned above of these species make them desirable grass species in forage and lawn production. Therefore, more scientific studies should be focused on such multipurpose species. The conventional propagation method is seed sowing for these species, although creeping species *F. rubra* and *P. pratensis* can be produced by rhizome cutting, whereas *L. perenne,* a non-creeping plant, has a fibrous root system. Therefore, cultivation practices for the improvement of desired traits in such plants also include the enhancement of seed germination rate as well as morphometric parameters of leaf and root development.

The effects of synthetic fertilizers and growth regulators are being tested to regulate growth and improve seed yield, visual quality, and traffic tolerance in many turfgrass species (McMahon & Hunter, 2012; Trethewey et al., 2016). However, natural substances with growth-promoting activities are continuously being discovered as alternatives to synthetic chemicals used on agricultural, horticultural, and ornamental plants. In this sense, the deacetylation of chitin biopolymer extracted primarily from shells of crustaceans and cell walls of fungi led researchers to produce chitosan (Tan et al., 2020), which is considered to be one of the alternatives to synthetic growth-promoters (Acemi et al., 2018). Chitosan is a linear aminopolysaccharide composed of randomly distributed β-(1→4)-linked D-glucosamine and *N*-acetyl-D-glucosamine (GlcNAc). Chitosan exhibits various effects on plants, such as enhancing seed germination, stimulating plant growth, inducing biological responses to abiotic and biotic stresses, and extending the shelf life of vegetables, ornamentals, and fruits (Romanazzi et al., 2016; Hidangmayum et al., 2019; Acemi, 2020a). Chitosan's structure may vary depending on its degree of polymerization (DP), which reflects the number of monomeric units in the polymer, and the degree of acetylation (DA), representing the molar fraction of GlcNAc in the polymer. These differences in chitosan's chemical structure have been shown to be decisive on the variation of its effects on horticultural plants, suggesting a structure-function relationship in chitosan's chemical structure and its function in plants (Acemi, 2020b).

The current study aimed to answer two research questions. The first of these questions is whether there is a possible differentiation among commercially available grass species' responses to chitosan treatments. The second question is whether there is a possible differentiation between the effects of chitosan samples with different DPs on commonly cultivated grass species. Therefore, the former question places the grass species into the focus of the research while the latter focuses on the effects of chitosan's structure on its function on grass species' development. By taking advantage of the plant tissue culture technique, we aimed to answer these research questions through a controlled culture environment that eliminates the other factors contributing to the plants' development, thereby focusing only on the elucidation of chitosan's effects on *L. perenne*, *F. rubra*, and *P. pratensis*. Based on the preceding reports referred above, it is hypothesized that oligomeric and polymeric chitosan samples should also lead to different effects on the *in vitro* development of *L. perenne*, *F. rubra*, and *P. pratensis* while enhancing the seed germination and promoting the growth of the species. Also, the determination of the *in vitro* effects of well-characterized chitosans on widespread and commonly used grass species would illustrate the possible usability of chitosan as a natural alternative to synthetic growth-promoters in turfgrass cultivation and forage production.

Materials and Methods

Chitosans' source and characterization

The chitosan samples were previously produced, characterized, and provided by the Institute of Plant Biology and Biotechnology, University of Münster, Münster, Germany. The origin of the chitosan samples was shrimp shell wastes. The polymer that had the DP of 70 was previously analyzed using HP-SEC-RID MALLS following Schatz et al. (2003), while MALDI-TOF-MS was used to characterize the oligomers which had DPs ranging from 2 to 15 (Haebel et al., 2007). The DA of the samples (10%) was previously determined through 1H-NMR (Vårum et al., 1991).

Cultivars, and seeds' source, disinfection, and transplantation

The seeds of *Lolium perenne* cv. Esquire, *Festuca rubra* cv. Maxima1, and *Poa pratensis* cv. Evora were provided by the local dealer (Sekoya Tohumculuk Ziraat San. & Tic. A.Ş, Turkey) of DLF Seeds Ltd., Denmark. The seeds were kept at dark, dry, and cool place until use. A hundred seeds of each species were placed into bags (4 × 4 cm) prepared from filter paper. The seeds were then kept in 1% (w/v) sodium hypochlorite (NaOCl) solution for 8 min for disinfection. The excess NaOCl on the seeds was removed by rinsing them into sterile water several times. The bags were then opened using a sterile blade, and the seeds were transplanted onto the medium using sterile forceps. All the treatments were carried out in a laminar airflow cabinet.

Media preparation and culture conditions

The culture vessels (Magenta GA-7) were filled with 40 ml of Murashige and Skoog's medium (Murashige & Skoog, 1962) supplemented with a mixture of chitosan oligomers with DPs ranging from 2 to 15, or polymer with a DP of 70 at 2.5, 5, or 10 $mg \cdot L^{-1}$ concentrations. Sucrose at 30 g L^{-1} concentration was used as a carbon source, and the medium was solidified using 7 $g \cdot L^{-1}$ agar. One N NaOH or HCl was used to balance the pH of the medium at 5.7. The medium was sterilized through autoclaving at 121°C under a pressure of 118 kPa for 20 min. The chitosan samples were filter-sterilized and added to the medium after autoclaving. The surface-sterilized seeds were placed horizontally onto the culture medium, and the culture vessels were then incubated in a plant growth chamber. The photosynthetic photon flux density striking to the cultures was 60 µmol $m^{-2} \cdot s^{-1}$ with a 16-h photoperiod, and the temperature was 23 ± 1°C. The incubation period consisted of 30 d starting after the transplantation of the seeds onto the medium.

Data collection and visualization, and statistical analysis

The measurements of the morphological parameters were done at the end of the incubation period. Each treatment was tested on 20 seeds in each repeat, and the experiments were done with five replications. Data were represented as mean ± standard deviation (SD). Duncan's multiple range test at a $P < 0.05$ significance level was used to compare the means after the one-way analysis of variance (ANOVA) was conducted. Statistical comparisons were made through IBM SPSS Statistics 22 software. The developmental data were standardized and shown through heatmaps for each species to visualize the degrees of the species' responses given to the treatments comparatively. The morphological differences and similarities caused by treatments in each species were analyzed through hierarchical cluster analysis (HCA) based on the Euclidean distance and complete-linkage clustering method. The clustering heatmap was created through ClustVis (Metsalu & Vilo, 2015).

Results

Effect of chitosan on seed germination rate

The germination of the seeds occurred within the first week of the incubation period. The chitosan variants did not affect the seed germination rate in *L. perenne*. The control medium gave 90 ± 3.54% mean seed germination in *L. perenne*, while the lowest mean seed germination rates (88 ± 6.71% and 88 ± 5.70%) were calculated from the medium with chitosan polymers at 5 and 10 mg·L^{-1} concentrations, respectively. However, the medium with chitosan polymer at 2.5 mg·L^{-1} gave the maximum mean seed germination rate (95 ± 3.54%) in the same species (Figure 1).

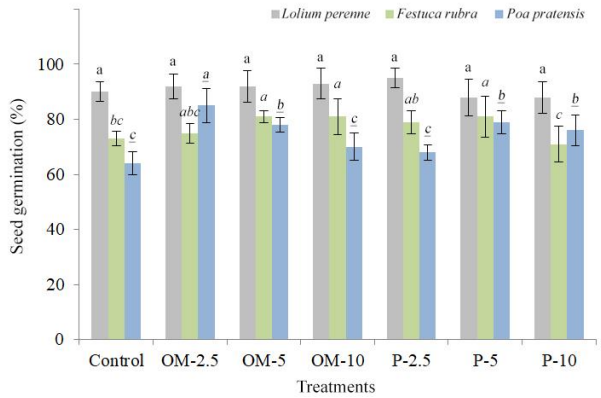

Figure 1. Comparison of the effects of chitosan treatments on *in vitro* germination of *Lolium perenne*, *Festuca rubra*, and *Poa pratensis* seeds. Data represent mean ± SD. The bars with the same-style superscript letters are not significantly different by Duncan's multiple range test ($P < 0.05$).

The mean seed germination rate from the control medium was found 73 ± 2.74% in *F. rubra*. The oligomer mixture at 5 and 10 mg·L^{-1} and polymer at 5 mg·L^{-1} increased the mean seed germination rate to 81 ± 2.24%, 81 ± 6.52%, and 81 ± 7.42%, respectively. However, the medium with chitosan polymer at 10 mg·L^{-1} gave the lowest (71 ± 6.52%) mean seed germination rate (Figure 1).

In *P. pratensis*, the control medium gave the lowest seed germination rate (64 ± 4.18%), while the medium with chitosan oligomers at 2.5 mg·L^{-1} concentration increased the mean germination rate up to 85 ± 6.12%. The mean seed germination rates from the medium with chitosan polymer at 5 and 10 mg·L^{-1} were statistically the same as that of the medium with chitosan oligomers at 5 mg·L^{-1} (Figure 1).

Effect of chitosan on leaf development

Chitosan treatments greatly influenced leaf formation. The mean leaf numbers per plant from the control medium were found 1.97 ± 0.05, 1.63 ± 0.17, and 1.17 ± 0.08, respectively, for *L. perenne*, *F. rubra*, and *P. pratensis*. The mean leaf numbers calculated from the control groups were also the minimum values for the plants. All the chitosan treatments tested significantly increased leaf production in all species. The maximum mean leaf number in *L. perenne* (2.76 ± 0.13) was found from the medium with chitosan polymer at 10 mg·L^{-1}, while an increasing trend was observed in leaf numbers with the elevated chitosan concentrations. In *F. rubra*, all the chitosan treatments gave statistically the same results, while the highest mean leaf number (2.17 ± 0.09) was calculated from the medium with chitosan polymer at 5 mg·L^{-1}. A similar trend in the same parameter was also observed in *P. pratensis*. The chitosan treatments significantly increased the mean leaf numbers in *P. pratensis*, and the maximum value (2.26 ± 0.06) for the parameter was reached from the medium with chitosan oligomers at 2.5 mg·L^{-1} (Figure 2a).

Leaf elongation was also triggered after chitosan treatments. The chitosan oligomers induced longer leaves than polymer treatments. The control medium gave the mean leaf lengths 7.12 ± 0.52 cm, 6.50 ± 0.74 cm, and 2.54 ± 0.29 cm per leaf in *L. perenne*, *F. rubra*, and *P. pratensis*, respectively. The control groups also gave the lowest mean leaf lengths. The most elongated leaves in *L. perenne* (11.51 ± 0.26 cm) were found from the medium supplemented with chitosan oligomers at 5 mg·L^{-1}. In *F. rubra*, all the chitosan treatments induced close leaf lengths. The highest mean leaf length per leaf (9.76 ± 0.25 cm) in *F. rubra* was found from the medium with chitosan oligomers at 10 mg·L^{-1}. *P. pratensis* showed a similar response with *F. rubra* to the chitosan treatments. The most elongate leaves in *P. pratensis* (5.20 ± 0.30 cm) were measured from the medium with 5 mg chitosan oligomers at 5 mg·L^{-1} (Figure 2b).

Effect of chitosan on root development

Chitosan treatments affected the root formation differently in all the grass species employed in the study. However, the most significant changes were found in *P. pratensis*. The maximum number of roots per plant (3.96

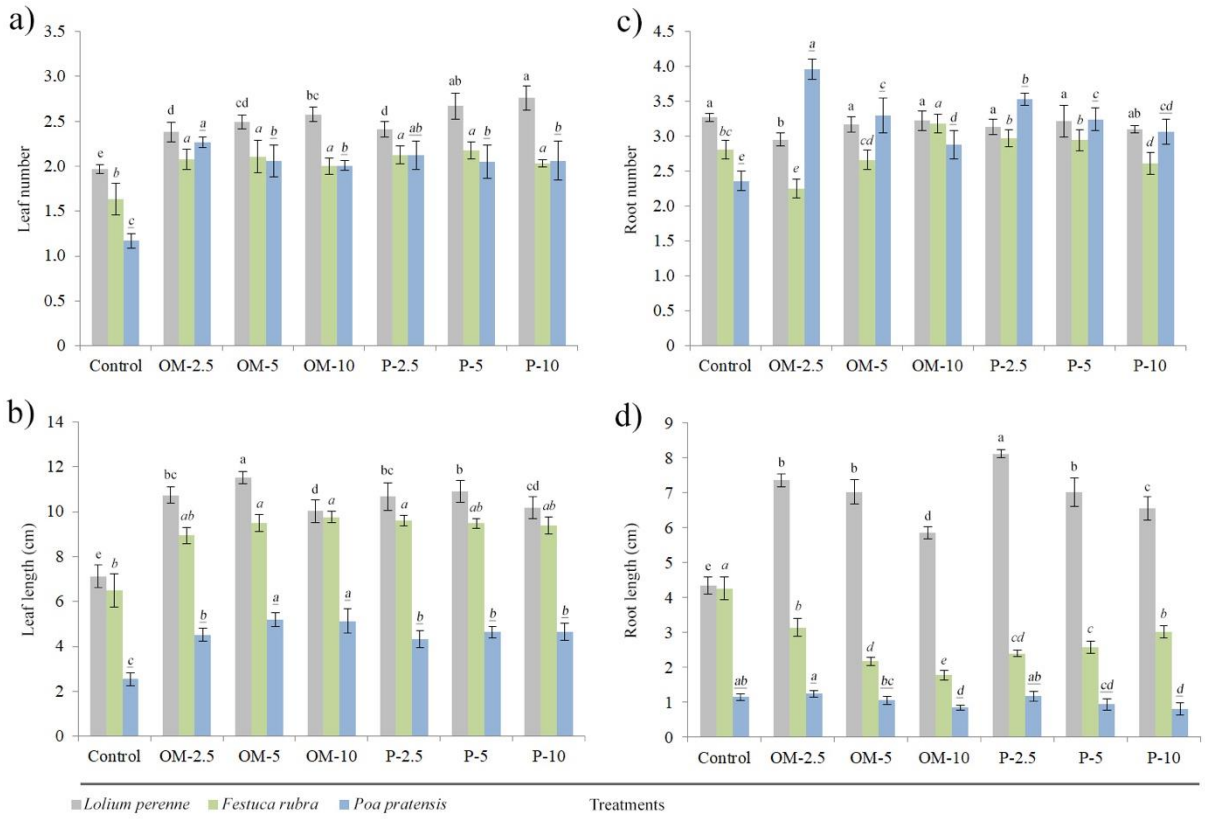

Figure 2. Comparison of the effects of chitosan treatments on *in vitro* **a**) leaf production, **b**) leaf elongation, **c**) rhizogenesis, and **d**) root elongation in *Lolium perenne*, *Festuca rubra*, and *Poa pratensis*. Data represent mean ± SD. The bars with the same-style superscript letters are not significantly different by Duncan's multiple range test ($P < 0.05$).

± 0.15) was produced in the medium with chitosan oligomers at 2.5 mg·L^{-1} concentration, while the control group gave the lowest number of roots (2.36 ± 0.14) in *P. pratensis*. The control medium in *L. perenne* and *F. rubra* gave 3.27 ± 0.06 and 2.81 ± 0.13 roots per plant, respectively. The chitosan treatments reduced the root production in *L. perenne*, and the minimum mean number of roots (2.95 ± 0.10) was found from the medium with chitosan oligomers at 2.5 mg·L^{-1}. In *F. rubra*, the detractive effects of chitosan on root production become more evident in the presence of oligomers at decreasing and the polymer at increasing concentrations. However, the polymer at 5 and 10 mg·L^{-1} in the medium gave the same results statistically. The minimum mean number of roots (2.25 ± 0.25) was calculated from the medium with chitosan oligomers at 2.5 mg·L^{-1} (Figure 2c).

In contrast to chitosan's growth-promoting effects on leaf and root formation in *P. pratensis*, root elongation reduced after chitosan treatments. The root elongation-inhibitory effect of chitosan oligomers was more evident than the polymer in *F. rubra*. However, chitosan treatments increased root lengths in *L. perenne*. The minimum mean root length (4.34 ± 0.24 cm) for *L. perenne* was recorded from the control group, whereas the most extended mean root length (8.12 ± 0.13 cm) was found from the medium supplemented with chitosan polymer at 2.5 mg·L^{-1}. In *F. rubra*, the control medium gave the highest (4.26 ± 0.33 cm) mean root length. However, increasing concentrations of chitosan oligomers and elevated concentrations of the polymer reduced the mean root length. The medium with chitosan oligomers at 10 mg·L^{-1} gave the shortest roots (1.78 ± 0.13 cm). The highest concentration of chitosan oligomers led to a reduction of root elongation in *P. pratensis*. The minimum mean root length was found 0.80 ± 0.17 cm per plant after chitosan polymer treatment at 10 mg·L$^{-1,}$ which was statistically the same as that of the oligomers at 10 mg·L^{-1} (Figure 2d).

Comparison of the development patterns through normalized data

In all cluster analyses, control groups were found in a separate cluster than the chitosan treatments. The polymer treatments at moderate and high concentrations were placed next to each other, while other treatments were more closely grouped in the HCA analysis for *L. perenne* (Figure 3a). At its lowest level, the oligomer treatment was found in the same cluster with the polymer treatment at the highest concentration, whereas the other treatments were found in a closer relationship in the HCA analysis for *F. rubra* (Figure 3b). In the HCA analysis for *P. pratensis*, the oligomer and the polymer treatments at moderate and high concentrations were closely grouped, whereas the lowest concentrations of both treatments were found in neighboring clusters (Figure 3c).

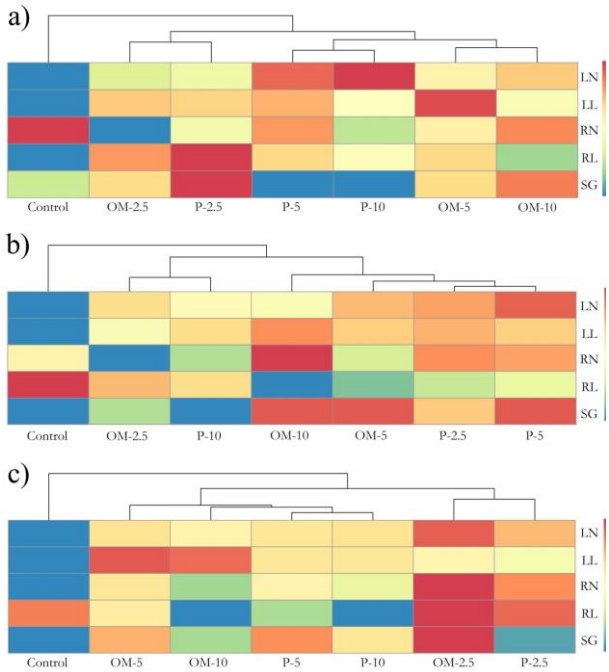

Figure 3. Hierarchical clustering heatmap-based comparison of the normalized developmental data from **a**) *Lolium perenne*, **b**) *Festuca rubra*, and **c**) *Poa pratensis*. Leaf number (LN), Leaf length (LL), Root number (RN), Root length (RL), Germination rate (SG), Chitosan polymer (P), Chitosan oligomers (OM). The treatments are represented as "chitosan variant – concentration (mg·L^{-1})".

Discussion

In turfgrass and forage management, selection and application of suitable fertilizers, growth regulators, and other types of growth-promoting chemicals according to the plants' needs cover a significant place in the cultivation practices' success. Turfgrass species are led to invade and cover the fields mainly used for sports activities at the beginning of their cultivation. The turf coverage, which is dependent on the foliar growth performance of turf species, is then enhanced through chemical fertilizers or growth promoters. However, many synthetic growth regulators such as Trinexapac Ethyl, Paclobutrazol, or Ethephon are applied primarily to suppress seedhead production (type I growth regulators) or to inhibit cell elongation (type II growth regulators) for better mowing practices and visual quality of turfgrass following the successful establishment of turf cover (Głąb et al., 2020). On the other hand, more plant biomass but lesser control of plant growth than turf grass cultivation is needed for forage production that mostly depends on vegetative parts' growth (Capstaff & Miller, 2018). Therefore, enhancement of seed germination rate and plant growth by using a biodegradable, eco-friendly, and natural growth-promoter such as chitosan would be beneficial for producers, sustainability, and nature.

To date, various growth regulators and other chemicals have been studied on turfgrass species in order to reveal the mechanisms controlling their growth and alleviating the effects of several environmental stress factors (Ma et al., 2018; Głąb et al., 2020). However, a limited number of reports regarding the effects of chitosan application on seed germination in turfgrass species are found in the literature. In one of the reports, Kim (2014) treated *P. pratensis* with uncharacterized chitosan and reported early germination. In the present study, chitosan treatments' success in enhancing seed germination rate was demonstrated in a concentration-dependent manner. However, chitosan oligomers better enhanced the germination rates in *P. pratensis* and *F. rubra* than the polymer, whereas seeds of *L. perenne* showed a limited positive response to chitosan treatments. This finding can be explained by reducing the medium's osmotic potential through polymeric chitosan treatment, whose hydrophilic nature is proportional to the polymer's chain length (Acemi, 2020a). Therefore, lesser osmotic potential reduction in the culture medium might be expected when oligomers are used at low concentrations. Seeds need to imbibe a higher amount of water for germination, and lower osmotic potentials of the culture medium would limit the water uptake of the seeds. The reduction of germination rate in *P. pratensis*, *F. rubra*, *L. perenne*, and other turfgrass species such as *Schedonorus arundinaceus*, *Festuca brevipila*, and *F. rubra* ssp. *fallax* after decrement of osmotic potential has been demonstrated on a prediction-based model (Goatley et al., 2017). In the study, the authors reported that the seeds of *P. pratensis* are the most susceptible to the osmotic potential changes, whereas the seeds of *L. perenne* are the most tolerant of such changes among other turfgrass species. The authors also noted that *L. perenne* seeds had the highest germination rate, which is in line with our findings. The better ability of chitosan oligomers than polymers in enhancing seed germination rate in *P. pratensis* and *F. rubra* (Figure 1) might also be explained by their higher potential to stimulate the production of reactive oxygen species (ROS) in the seeds. Because ROS could play a role in regulating seed germination by oxidizing the proteins that trigger germination (El-Maarouf-Bouteau et al., 2013) and weakening the endosperm during seed swelling (Müller et al., 2009). In a recent report, the dormancy release was associated with increasing the sunflower seeds' internal H_2O_2 level (Vigliocco et al., 2019).

Leaf production and growth are considered among the parameters that determine the visual quality and cover ability of turfgrass, and the forage yield is also strictly dependent on the same parameters. The present analysis showed that chitosan successfully supported the above-listed growth parameters in the grass species tested (Figure 2a&b). Chitosan treatments have been shown to induce the synthesis of several plant growth regulators, such as benzyladenine (BA) and indole 3-acetic-acid (IAA), which involve regulating the meristematic cell division and organogenesis (Jogaiah et al., 2020). The same researchers also found that chitosan, when applied at a specific concentration,

induced callose and lignin deposition in the cucumber plant (Jogaiah et al., 2020). The promotive effects of chitosan in leaf production and elongation might be attributed to these effects possibly found also in the grass species tested in this study.

On the other hand, synthesis of callose, which is a cell wall polymer synthesized during cytokinesis and practically involved in the cell division process (Thiele et al., 2009) beside regulation of plasmodesmata and stomata closure (Nedukha, 2015), was found to be triggered in *Phaseolus vulgaris* after chitosan treatments (Franco & Iriti, 2007). Here, it should be noted that callose is degraded to form cellulose to support cell wall growth after cytokinesis (Nedukha, 2015). Also, increased cell wall lignification through the stimulation of lignin biosynthesis after chitosan treatment was reported by Acemi and Türker-Kaya (2020). However, Mondal et al. (2012) stated that chitosan's growth-promotive effects might be due to increased enzyme activities in nitrogen (N) metabolism and the increased N transportation. Therefore, the better leaf elongation performance of *L. perenne*, *F. rubra*, and *P. pratensis* treated with chitosan oligomers in this study might be explained by oligomers' better ability to trigger the synthesis of plant growth regulators and other biomolecules involved in cell division. Also, chitosan oligomers' superiority in the same parameter might be attributed to its better ability to enhance N metabolism and transportation than the polymer. Furthermore, increased leaf production after chitosan treatment was also reported in *Lactuca sativa* (Xu & Mou, 2018), *Curcuma longa* (Anusuya & Sathiyabama, 2016), and *Ipomoea purpurea* (Acemi et al., 2018), while enhanced chlorophyll content and visual quality in *P. pratensis* treated with chitosan was reported by Chang and Yoon (2011).

Grass root growth that extends deep into the soil is one of the most significant factors helping prolong grass life and reduce fertilizer use. The current study's findings indicated that chitosan use induced a reliably more robust rhizogenic response in *P. pratensis* than *F. rubra* and *L. perenne* (Figure 2c). However, root elongation was reduced in *P. pratensis* when both of the chitosan variants were used above 5 $mg \cdot L^{-1}$ (Figure 2d). In *F. rubra*, chitosan treatments reduced the root elongation regardless of its DP. However, *L. perenne* was the only grass species with longer roots after chitosan treatments (Figure 2d). In a recent report that the authors showed the alteration in auxin homeostasis and the accumulation of IAA after chitosan polymer (DP 70, DA 15%) treatments between 0.1 and 1 $mg \cdot mL^{-1}$, arrested root elongation in the apical root meristem of *Arabidopsis* after chitosan treatments was reported (Lopez-Moya et al., 2017). The authors attributed their findings to the reduced expression of the WUSCHEL-RELATED HOMEOBOX 5 (*WOX5*) gene, which controls the stem cells' activity in the quiescent center of the root tissue where the cell division is regulated. Therefore, increased root production, however, reduced root elongation in *F. rubra* and *P. pratensis,* might be explained by the possible accumulation of IAA and downregulation of *WOX5*, respectively. However, this discussion should be proven with further studies, and the physiological mechanism behind chitosan's success in triggering root elongation in *L. perenne* should be investigated. In the root tissues of another monocotyledonous plant, *Serapias vomeracea*, chitosan polymer treatment was reported to decrease water-associated cellulose content, while oligomer treatment led to an increase in the same parameter (Acemi & Türker-Kaya, 2020). In other reports conducted with uncharacterized chitosan, increased leaf number, chlorophyll content, and fresh and dry weight were reported in *P. pratensis* (Yoon & Kim, 2007) and *Agrostis palustris* (Yoon et al., 2006) treated with 500× diluted chitosan solution. In those studies, the species were cultured in soil, where their roots were not continuously in contact with chitosan due to soil drainage. However, in tissue culture, the culture medium is more stable and has no drainage like soil, making the roots exposed to the test treatments continuously. This condition might be the reason behind that the researchers in both reports found longer roots in the plants treated with chitosan than control, which is partly in contrast with the current study.

In light of the outcomes derived from the analyses, the possible use of chitosan should be taken into consideration to enhance foliar growth, which would be a favorable trait in turf and forage production. However, it should be noted that root growth might decline in such cases, which would be a disadvantage for turfs encountering dense traffic and soil stabilization. Therefore, foliar application of chitosan should be tested in further field studies since foliar fertilization has a minimum impact on root growth (Liu et al., 2008). Also, molecular evaluation of the effects of chitosan on root development should be conducted to reveal the exact physiological mechanism behind its effects.

Conclusion

This study showed that the effects of chitosan treatment on turfgrass species could be altered in response to chitosan's chemical structure. Therefore, to ensure a high germination rate and improved leaf growth in *L. perenne*, *F. rubra*, and *P. pratensis* when establishing turf coverage on recreational fields, treatment with oligomers could be a better option than the polymer. However, excessive chitosan applications might reduce root development, which would lead to nutrient deficiency in the plants. After establishing turfgrass, continuous chitosan application would also increase mowing frequency due to leaf elongation and increase maintenance costs. For forage production, chitosan oligomers are suggested to be used more frequently in these species' cultivation since biomass production is more critical in such usage. It is necessary to reduce chemical fertilizers and growth regulators to

mitigate the harmful effects of cultivation on the environment. Therefore, characterized chitosan could be safely employed in the stages mentioned earlier of turfgrass and forage cultivation instead of synthetic growth regulators to minimize the harmful effects of excessive use of chemicals on nature. However, this suggestion should be tested in field conditions before large-scale application of chitosan.

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Author Contributions

Conceptualization: AA, Data curation: AA, DT, SY; Formal analysis: AA; Investigation: AA, DT, SY; Methodology: AA; Resources: FÖ; Visualization: AA; Writing - original draft: AA; Writing - review and editing: AA.

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