Journal of Plant Physiology and Breeding

2021, 11(2): 147-160 ISSN: 2008-5168

Effect of chitosan on regeneration and secondary metabolite production of *Lilium regale*

Younes Pourbeyrami Hir*, Mahsa Khalafi, Esmaeil Chamani, and Hassan Maleki Lajayer

Received: March 9, 2021 Accepted: August 31, 2021
University of Mohaghegh Ardabili, Ardabil, Iran
*Corresponding author: Email: Younes_ph62@uma.ac.ir

Abstract
Lilium spp. belong to the *Liliaceae* family. This plant contains a valuable secondary metabolite which can be increased by the stimuli treatment. The main goal of the present study was to evaluate the effects of chitosan as a stimulant on regeneration and production of secondary metabolites of *Lilium regale*. The experiment was carried out as a completely randomized design in the MS medium with 10 replications. Five different concentrations (0, 50, 100, 150, and 200 mg/L) of chitosan were used as treatments. Different morphological traits, leaf chlorophyll, and secondary metabolites were measured. Analysis of variance showed that chitosan significantly affected plant height, fresh weight, bulblet number, root number, chlorophyll a and b, total chlorophyll, phenols, flavonoids, and regeneration percentage. The highest and lowest fresh weight and root number were obtained from 200 ppm of chitosan and the control, respectively. The results also showed that the plant height and bulblet number in all concentrations of chitosan were higher than the control. The highest and lowest regeneration rate were obtained from the 200 ppm chitosan and the control, respectively. However, the highest amount of phenols, flavonoids, and chlorophyll was obtained from the 200 ppm chitosan. According to the results of the present study, chitosan at the rate of 200 ppm had a more positive effect on regeneration and production of secondary metabolites of *Lilium regale* compared to other concentrations.

Keywords: Chitosan; *In vitro* culture; Lily flowers; Regeneration; Secondary metabolites

How to cite: Pourbeyrami Hir Y, Khalafi M, Chamani E, and Maleki Lajayer H, 2021. Effect of chitosan on regeneration and secondary metabolite production of *Lilium regale*. Journal of Plant Physiology and Breeding 11(2): 147-160.

Introduction

The genus *Lilium* comprises more than 100 species worldwide (Yokota and Yahara 2012), and China is the main distribution center of this genus, with 55 species (Liang and Minoru 2000). Bulbs such as lilies have been proven to be ideal for tissue culture as their regeneration potential is usually high. Lilies are the most important bulbs produced by the tissue culture on an industrial scale (Chu and Kurtez 1990). In some cases, shoot tips can be used as raw materials (Godo *et al.* 1996) to eliminate contamination problems. Asexual propagation is mainly accomplished for its propagation using a variety of methods including aerial bulbil, bulbil cutting and division, scaling, bud stem cuttings and using the tissue culture (Azadi and Khoshkhui 2007). Tissue culture can also include tissues such as roots, leaves, anthers, flags, scales, stems, and petals of flowers. Therefore, the micropropagation is necessary to produce a large number of bulblet in Lilium. Bulbil scales, which are derived from bulblet produced by *in vitro* methods, are the best sources for bulbil production in lilium species (Han *et al.* 2004).

Lilium regale, which is usually called "Lily *Regale*", is a species of the Lilium family that quickly grows and flowers about 1-2 years after germination. Its development can be further enhanced by tissue culture (Arikat *et al.* 2004). Apart from mass multiplication of elites, plant tissue culture as an essential component of plant biotechnology, provides the means to multiply and regenerate novel plants from genetically engineered cells. This technique is widely used in obtaining disease free plants, somatic hybridization, genetics improvement of commercial

plants, and obtaining androgenic and gynogenic haploid plants for breeding programs. Tissue Culture is becoming as an alternative means to vegetative propagation of plants and can be a good platform for the preservation of native or endangering species and genotypes of nature as valuable germplasm resources (Sharma 2015; Anonymous 2009).

In addition to providing nutrients to the plant itself, the lily bulb has accumulated a variety of secondary metabolites serving as defenses for plants, including major groups such as phenolic, terpenoids, and nitrogen-containing compounds (Erb and Kliebenstein 2020). The ancient Chinese people discovered the medicinal properties of lily bulbs and recorded them in ancient medical books. The earliest known record of lily bulb efficacy is found in the book Shen Nong Ben Cao Jing, which was compiled before the second century AD (Haw 1986). Nowadays, the dry scales of three Lilium bulbs, including *L. pumilum*, *L. lancifolium*, and *L. brownii var. viridulum*, have been officially listed in the Chinese Pharmacopeia (2020 edition) and are considered meditative for bronchitis, pneumonia, and cough (Chinese Pharmacopoeia Commission 2020). On the other hands, the bulbs of *L. regale* accumulated more secondary metabolites, and, notably, more phenolic acid compounds and flavonoids (Kong *et al.* 2021). Chitosan is an organic polymer made from hard shells of aquatic animals such as crabs and shrimp. It has been recently taken into consideration for its antibacterial properties (Buschman 2004). It is a main derivative of chitin and its deacetylated form resulting from the chitin alkaline deacetylation. Chitosan is also naturally produced in some fungi, and it is much less than chitin (Baker *et al.* 2007). Therefore, the present study aimed to investigate the effect of chitosan on regeneration and production of secondary metabolites in *Lilium regale*.

Materials and Methods

The present study was conducted in laboratory of tissue culture and biotechnology of department of horticulture and green space at University of Mohaghegh Ardabili (UMA) in Ardabil province in 2018 in a completely randomized design with 10 replications. The treatment applied in the study consisted of chitosan at five levels of 0 (control), 50, 100 and 150 and 200 mg/l. In this experiment, the bulblet of *Lilium regale*, which were formerly cultured in the MS medium, were used as explants. The ready-to-use MS medium as the widely used and popular medium was used in tissue culture studies. The explants were cultured in the test glasses using laminar flow. At the first stage, the bulb subculture was repeated for 2 months with an aim of increasing number of bulbs for the main treatment. The scales as explants and the hormone-free MS culture medium were used at this stage. Then, seedlings obtained from the propagation of explants were used for culture in medium containing the main treatment by obtaining sufficient number of seedlings. The experimental treatment included different concentrations of chitosan at five levels of 0 (control), 50, 100, 150, and 200 mg/l. A jar containing 20 mg of basic solid medium MS with 10 replicates and four scales per replicate was considered as the experimental unit. The cultured media were placed in a growth chamber at 23± 2 °C equipped with 2000-lx fluorescent lamps (16 h light and 8 h dark). After transferring the cultured explants to the growth chamber, daily controls were carried out to investigate changes in the growth and regeneration of explants and remove the infected cultures. After two months, the regenerated explants and seedlings were examined and the necessary data were recorded. The measured indices

and measurement methods in the present study were as follows.

After removing the seedlings from the culture medium, the surrounding culture medium was thoroughly cleaned to measure seedling fresh weight, and then the fresh weight was measured using a one-thousandth precision scale. Seedling height was measured by ruler; and number of roots and bulblet were counted. The regeneration percentage was also determined by observing the growth rate of an experimental unit.

Chlorophyll was measured by spectrophotometer at two wavelengths of 663 and 645 nm. Rates of chlorophylls a and b and total chlorophyll were calculated according to the linear equation. Total phenol was also measured based on Slinkard and Singleton (1977) with a little change using the Folin–Ciocalteu reagent (FCR). The concentration of phenol was also obtained based on values of the reaction of extract with FCR and by comparison with standard solutions of gallic acid according to the line equation obtained from the standard curve of gallic acid. Flavonoid measurement was also performed by aluminum chloride colorimetric method and a method by Kumar *et al.* (2015). The concentration of flavonoid was calculated by comparing it with standard solutions of quercetin according to the line equation obtained from the standard quercetin curve. Data were analyzed using SPSS; and the mean comparison was performed by Duncan's test; and graphs were drawn by Excel.

Results and Discussion

Seedling fresh weight

The Results of the analysis of variance of data indicated that the effects of different concentrations of chitosan was significant on seedling fresh weight at 5% probability level (Table 1). The comparison of means also showed that among different concentrations of chitosan, the highest fresh weight was observed at the rate of 200 ppm that was significantly different from control. The results showed an increase in the seedling height and fresh weight in chitosan-treated plants (Figure 1 and 2). Singla and Gary (2005) found that the fresh and dry weights of root and shoot of bean plant increased with application of different concentrations of chitosan; and the result was consistent with the results of the present study. Chitosan seems to stimulate seedling growth, thereby increasing water and nutrient uptake, and better transfer of nutrients in plant organs leading to higher fresh weight of plant.

Seedling height

According to the analysis of variance of data, the impact of different concentrations of chitosan on seedling height was significant at 1% probability level (Table 1). According to the means comparison, the maximum seedling height was obtained from 200 ppm; and the minimum rate from the control (Figure 2).

Jami (2018) examined the effect of chitosan on morphological characteristics of *Salvia leriifolia* and found that the effect of chitosan on its height was significant; and significantly increased compared to the control. Heng *et al.* (2012) investigated the stimulating effect of chitosan on medicinal plant, pennyroyal, and reported that seedling height increased using the chitosan stimulation. Dzung *et al.* (2011) also reported an increase in the height of the coffee plant at a 60 ppm of chitosan. Guan *et al.* (2009) concluded that chitosan had very positive effects on plant growth, height and yield, as well as the physiology and metabolism of different corn plants. The improved growth may be a result of

Table 1. Analysis of variance of the effect of chitosan on morphological characteristics of *Lilium regale*

Source of variation	df	Mean squares				
		Fresh weight	Plant height	Root number	Regeneration percent	Bulb number
Treatment	4	0.22*	0.28**	0.40*	0.51*	0.91**
Error	45	0.10	0..4	0.16	23.22	0.18
CV (%)		23.2	7.1	15.7	22.3	13.7

*, **, indicate significant at 5% and 1% probability levels, respectively

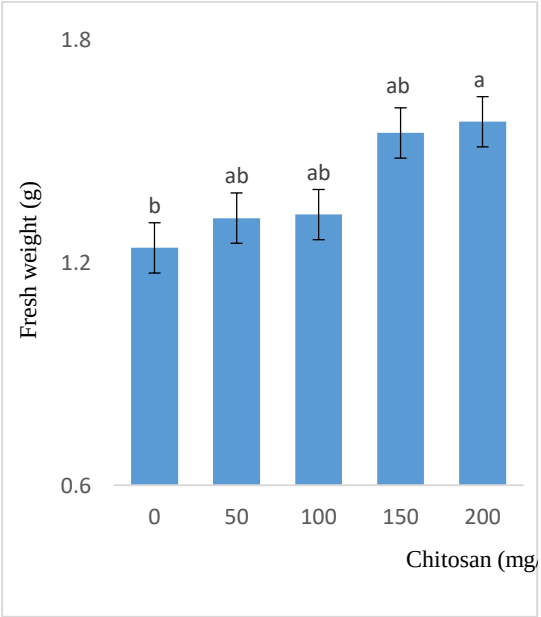

Figure 1. The effect of chitosan on the fresh weight of *Lilium regale;*

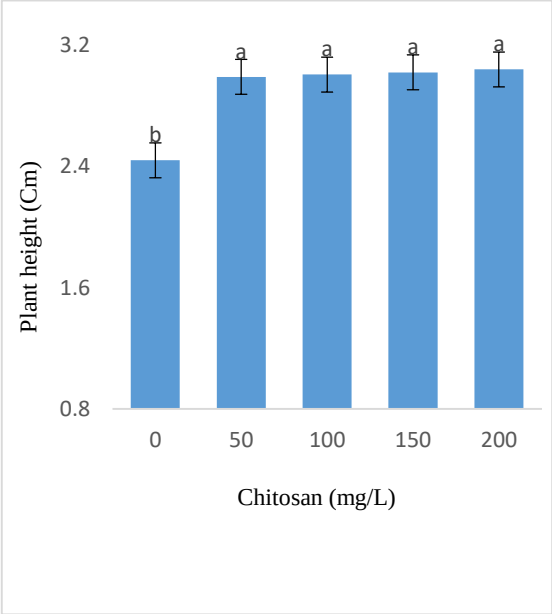

Figure 2. The effect of chitosan on the plant height of *Lilium regale*

higher cell division in the distal meristem.

Number of roots

Analysis of variance indicated the significant effects of different concentrations of chitosan on root number at 5% probability level (Table 1). The means comparison also indicated that increasing concentrations of chitosan enhanced the number of roots; and the maximum root number was obtained from 200 ppm of chitosan (Figure 3). In a study, chitosan improved rooting of grape cuttings by increasing the number and length of produced roots (Gornik *et al.* 2008). Furthermore, Nourafkan (2018) indicated that as the concentration of chitosan increased, number of roots of explants of lemon beebrush also increased. No *et al.* (2002) reported the severe effect of chitosan on root growth and development of Phalaenopsis.

Regeneration percentage

The analysis of variance of regeneration percentage indicated the significant effects of different concentrations of chitosan at a 5% probability level (Table 1). Based on results of the mean comparison of data, the maximum and minimum percentages of regeneration were observed at 200 and 0 (control) ppm of chitosan (Figure 4). The use of chitosan in tissue culture studies has increased the plant regeneration (Baker *et al.* 2007). In the study, increasing chitosan concentration enhanced the regeneration rate in explants cultivated in a medium containing chitosan treatments (Figure 5). According to Nourafkan (2018), the chitosan consumption increased the regeneration rates of lemon beebrush. Ait-Barka *et al.* (2004) reported that chitosan stimulated growth of explants in grape tissue culture; and it was consistent with results of the present study. The research results indicated that chitosan promoted growth, cell development and, consequently, increased the plant yield. Chitosan promoted growth by increasing the activities of key enzymes in nitrogen metabolism (nitrate reductase, glutamine, and protease synthase) and improving nitrogen transfer (Mondal *et al.* 2012).

Number of bulblets

The analysis of variance showed that the different concentrations of chitosan significantly ($P\leq 0.01$) affected bulblet number (Table 1). Based on the mean data comparison, the largest number of bulblet was seen at 200 ppm of chitosan, which was not significantly different from 150 ppm, but it had a significant difference with other treatments. The lowest number of bulblet was also obtained from the control treatment (Figure 6). It is worth noting that the increase of chitosan stimulation on morphological traits of plants has been proven by many researchers (Mondal *et al.* 2012). Different concentration of Chitosan were used to study the osmotic potential tolerance in safflower and the results revealed that chitosan has positive effect on bulbil count, plant growth and yield. Moreover, the results indicated that the higher concentration of chitosan significantly increased the number of bulblet (Mahdavi *et al.* 2011).

Chlorophyll a

According to results of the ANOVA, effects of different concentrations of chitosan were significant on chlorophyll a content index in *Lilium regale* at a 1% probability level (Table 2). Results of the mean comparison of effects of different concentrations of chitosan on chlorophyll a content also indicated that the highest chlorophyll rate was seen at 200 ppm, and

Table 2. Analysis of variance of the effect of chitosan on some physiological traits and production of secondary metabolites of *Lilium regale*

Source of variation	df	Mean Square				
		Total flavonoids	Total phenols	Total chlorophyll	Chlorophyll b	Chlorophyll a
Treatment	4	17.85**	14.55**	0.23*	0.31**	0.275**
Error	45	3.76	2.97	0.07	0..072	0.056
CV (%)		10.3	8.1	10.4	18.9	9.7

*, **, indicate significant at 5% and 1% probability levels, respectively

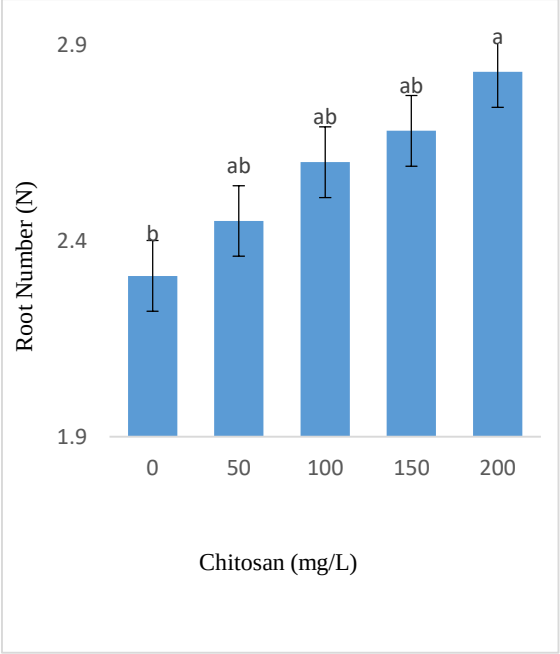

Figure 3. The effect of chitosan on the root number of *Lilium regale;*

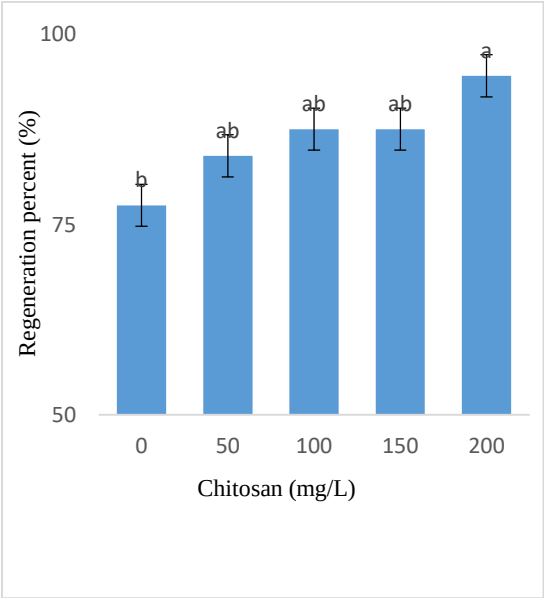

Figure 4. The effect of chitosan on regeneration percent of *Lilium regale;*

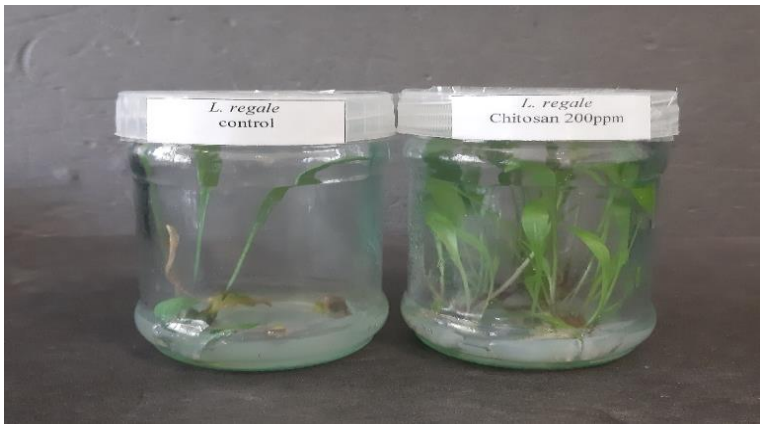

Figure 5. The effect of different concentration of chitosan on growth of *Lilium regale*

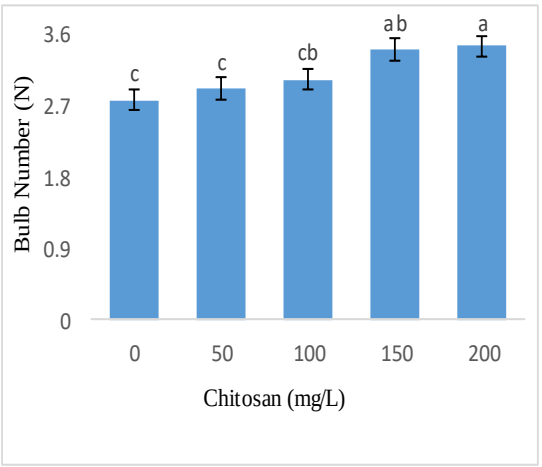

Figure 6. The effect chitosan on the bulblet number of *Lilium regale*

it was significantly different from the control (Figure 7). Mahdavi *et al.* (2011) found that chitosan consumption significantly increased chlorophyll a and b content in Safflower compared to the control. Chitosan consumption probably increased the chlorophyll production by affecting the genes responsible for chlorophyll production (Malekpoor *et al.* 2017).

Chlorophyll b

Results of the ANOVA of data indicated that effects of different concentrations of chitosan on chlorophyll b content were significant at a 1% probability level (Table 2). The mean data comparison also indicated that increasing chitosan concentration raised the chlorophyll b content; and the highest amount of chlorophyll b was obtained at 200 ppm that has a significant difference with the control (Figure 8). Sheikha *et al.* (2009) found that chitosan played a key roles in enhancing chlorophyll and photosynthesis. Furthermore, they proved that chitosan affected the leaf chloroplast gene expression. They also indicated that chitosan increased chlorophylls a, b and total chlorophyll in broad bean; and it was consistent with the results of this experiment.

Total Chlorophyll

The results showed that the effects of different

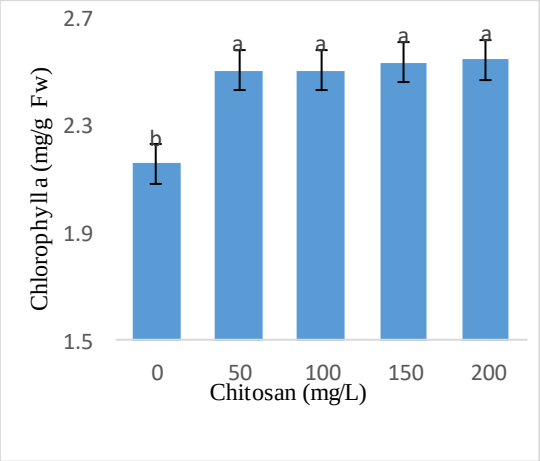

Figure 7. The effect chitosan on chlorophyll a content of *Lilium regale*

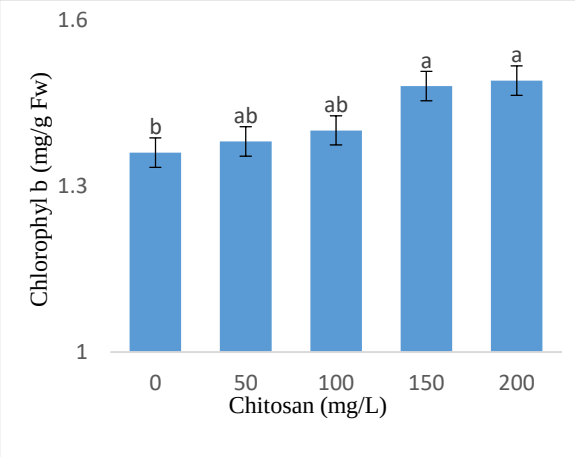

Figure 8. The effect of chitosan on chlorophyll b content of *Lilium regale*

concentrations of chitosan on total chlorophyll content in *Lilium regale* was significant at 5% probability level (Table 2). Comparison of means also revealed that the highest and lowest chlorophyll content was observed in chitosan at the rate of 200 ppm and 0 ppm (control) respectively (Figure 9). In a study, the use of chitosan increased the coffee leaf chlorophyll content (Salachna and Zawadzinska 2014). Given the presence of nitrogen element in the chitosan stimulus and the structural role of this element in the Tetrapyrrole loops of chlorophyll, such an increase is expected (Malekpoor *et al.* 2017).

Effects of different concentrations of chitosan on secondary metabolites

Total phenol content: Different concentrations of chitosan significantly ($P \leq 0.05$) affected the total phenol content (Table 2). Comparing the effects of different concentrations of chitosan on total phenol also indicated that increasing the concentrations of chitosan enhanced the total phenol concentration; and the highest amount was observed in 200 ppm chitosan (Figure 10). As a biological elicitor, chitosan increased the production of secondary metabolites in

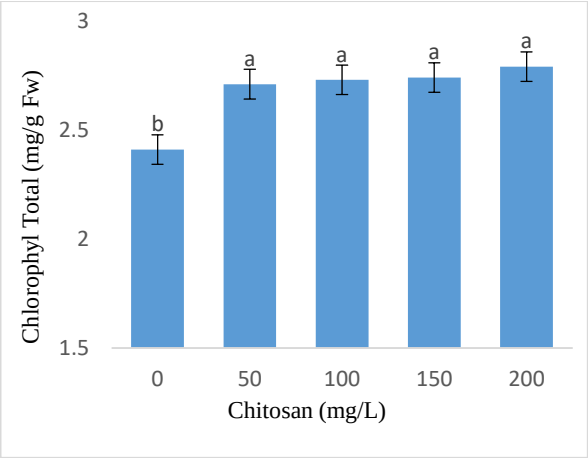

Figure 9. The effect of chitosan on total chlorophyll content of *Lilium regale*

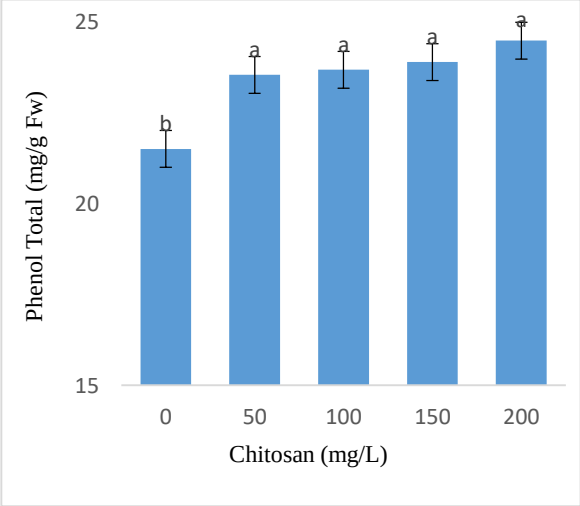

Figure 10. The effect of chitosan on total phenols of *Lilium regale*

many *in vitro* plants (Esmaeilzadeh and Sharifi 2013). Phenolic compounds are potent inhibitors of oxidative stress and participate in the collection or removal of hydrogen peroxide in collaboration with peroxidases (Kovacik *et al.* 2009). Plants release phenolic compounds in response to some signaling compounds that play important defense roles against the elicitors (Mandal 2010). Recent studies also indicated that chitosan as a biological elicitor might have a potential to eliminate free radicals, thereby increasing the amounts of phenolic compounds. Michalac (2006) conducted a study on Ajwain plant and found that chitosan increased amounts of phenolic compounds compared to the control. In another study, Pallida *et al.* (2014) found that chitosan application in tea plant caused a significant increase in phenol content of the plant compared to the control treatment.

Total flavonoids content*:* According to the results of the variance analysis, effects of different concentrations of chitosan on total flavonoid index were significant at a 1% probability level (Table 2). The mean comparison of different concentrations of chitosan also indicated that the highest and lowest total flavonoid levels were observed at 200 and 0

(control) ppm respectively (Figure 11). Biological elicitors such as chitosan have been widely used in the production of secondary metabolites. In general, the elicitors identify them by stimulating cellular signals and molecular interactions between plant receptors at the cell membrane surface with cytoplasm. As a result, the signal received by plant cells stimulates the expression of pathway-related genes and results in the synthesis of secondary metabolites in plants (Zhao and Sakai 2003). Different issues, including elicitor source, its specificity, elicitor concentration, plant growth stage, elicitor addition time, and duration when a plant is exposed to the elicitor affect the increased production of secondary metabolites (Vasyukova *et al.* 2001). Coqueiro *et al.* (2011) reported that chitosan application increased the flavonoid content in tomato. In another study, Esmaeilzadeh and Sharifi (2013) found that chitosan in white flax cell culture caused a significant increase in flavonoid content.

Conclusion

The present study aimed to investigate the effect of chitosan on regeneration and secondary metabolites production of *Lilium regale*. The results indicated that its effects were significant on most traits such as fresh weight, number of roots, percentage of regeneration, and total chlorophyll at a 5% probability level, and also significant on plant height, number of bulblet, Chlorophyll a and b, and secondary metabolites such as phenol and flavonoid at a 1% probability level. The production of secondary metabolites under *in vitro* conditions was influenced by some important factors such as type of medium, medium compounds, and plant growth regulators. The use of chitosan in the medium as a stimulator to improve regeneration could be an important aid in the propagation of Lilium. Maximum fresh weight and number of bulblet, seedling height, root number, percentage of regeneration, chlorophyll a, b, total phenol, and total flavonoid were obtained at a concentration of 200 ppm. The results of the another experiment of the authors showed that chitosan significantly ($p \leq 0.01$) affected most of the morphological traits such as fresh weight, plant height, root numbers, bulblet numbers,

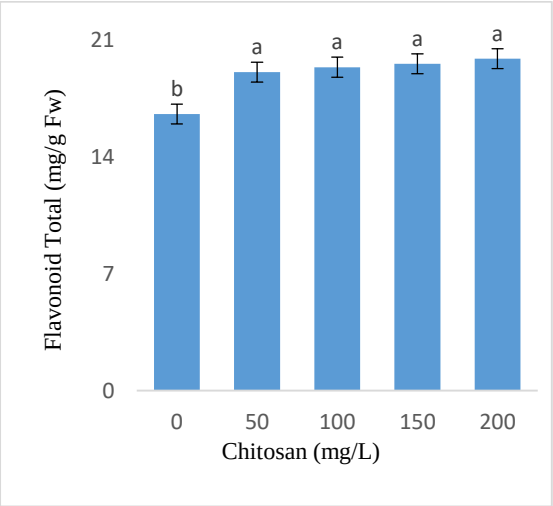

Figure 11. The effect of chitosan on total flavonoids of *Lilium regale*

percentage of regeneration, chlorophyll b, a and total and secondary phenol metabolites (Khalafi *et al*. 2021), which were similar to this results. The results indicated that a concentration of 200 ppm had more positive effect on the regeneration and secondary metabolites of Lilium regale in comparison with other treatments; and a high concentration of chitosan was a general process for the proper plant growth (Figure 12). The growth process change in line with changes at different concentrations of chitosan was most likely due to the ability of *Lilium regale* species to absorb chitosan.

Acknowledgments

We are gratefully thankful the Mohaghegh Ardabili University, Iran, for its financial support.

Conflict of Interest

The authors declare that they have no conflict of interest with any organization concerning the subject of the manuscript.

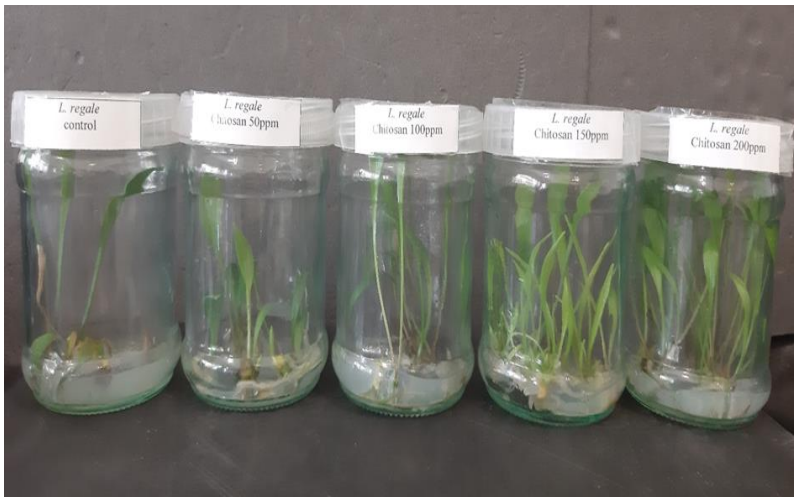

Figure 12. The effect of different concentrations of chitosan on growth of *Lilium regale*

References

Ait-Barka E, Eullaffroy P, Clement C and Vernet G, 2004. Chitosan improves development, and protects *Vitis vinifera L.* against *Botrytis cinerea*. Plant Cell Report 22: 608-614.

Anonymous S, 2009. Lilies the Flower Expert-Flower Encyclopedia. http://www.theflowerexpert.com/content/mostpopularflowers/lilies

Arikat NA, Jawad FM, Karam NS and Shibli RA, 2004. Micropropagation and accumulation of essential oils in wild sage (*Salvia fruticosa Mill*). Scientia Horticulturea 100: 193-202.

Azadi P and Khosh-Khui M, 2007. Micropropagation of *Lilium ledebourii bioss* as affected by plant growth regulator, sucrose concentration, harvesting season and cold treatments. Electronic Journal of Biotechnolgy10(4): 583-591.

Baker LG, Specht CA, Donlin MJ and Lodge JK, 2007. Chitosan the Deacetylati Form of chitin is Necessary for cell wall Integrity in Cryptococcus neoformans. Eukaryotic Cell 6 (5): 855-867.

Buschman JCM, 2004. Production of bulbs and bulb cut flowers in the world present and future. *Ix*th International symposium on flower bulbs. Niigata. Japan, 1-2.

Chinese Pharmacopoeia Commission 2020. Pharmacopoeia of the People's Republic of China (Part I) 2020th ed. China Pharmaceutical Science and Technology Press; Beijing, China p. 137.

Cho MH, No HK and Prinyawiwatkul W, 2008. Chitosan treatments affect growth and selected quality of sunflower sprouts. Journal of Food Science 73: 570-577

Chu IYE and Kurtz SL, 1990. Commercialization of plant micropropagation. In: Ammirato PV, Evans DA, Sharp WR and Bajaj YPS (eds.). Handbook of Plant Cell Culture. Volume 5. Ornamental Species, pp. 126-164. McGraw-Hill Publishing Company, New York, USA.

Coqueiro DSO, Maraschin M and Piero RMD, 2011. Chitosan Reduces Bacterial Spot Severity and Acts in Phenylpropanoid metabolism in Tomato Plants. Journal of Phytopatholgy 159(7):488-494.

Dzung NA, Phuong VT and Dzung TT, 2011. Research on impact of chitosan oligomers on biophysical characteristics, growth, development and drought resistance of coffee. Carbohydrate Polymers 84:751755.

Erb M and Kliebenstein DJ, 2020. Plant secondary metabolites as defenses, regulators, and primary metabolites: The blurred functional trichotomy. Plant Physiology 184(1):39–52.

Esmailzadeh behabadi SV and Sharifi M, 2013. Increasing the production of plant secondary metabolites using bio-electrics. Journal of Cell and Tissue 119-128. (In Persian).

Godo T, Matsui K, Kida T, and Mii M, 1996. Effect of sugar type on the efficiency of plant regeneration from protoplasts isolated from shoot tip-derived meristematic nodular cell clumps of *Lilium* x formolongi hort. Plant Cell Reports 15: 401-404.

Gornik K, Grzesik M and Romanowska Duda B, 2008. The effect of chitosan on rooting of grapevine cutting and on subsequent plant growth under drought and temperature stress. Journal of Fruit Ornamental Plant Research 16: 333-343.

Guan YJ, Hu J, Wang XJ and Shao CX, 2009. Seed priming with chitosan improves maize stress germination and seedling growth in relation to physiological changes under low temperature. Journal of Zhejiang University-Science- A 10: 427-433.

Han BH, Yu HJ, Yae BW and Peak KY, 2004. *In vitro* micropropagation of *Lilium longiflorum* 'Georgia'by shoot formation as influenced by addition of liquid medium. Scientia Horticurea 103(1), 3949.

Hasandokht M and Ebrahimi R, 2006. Principle of plant tissue culture. Marze danesh pub. Press.

Haw SG, 1986. The history of lilies in China. In: Haw S.G., editor. The Lilies of China. Timber Press; London, UK: pp. 43–56.

Heng Y, Xavier CF, Lars PC and Kai G, 2012. Chitosan Oligosaccharides Promote the Content of Polyphenols in Greek Oregano (*Origanum vulgare* ssp. hirtum). Journal of Agriculture and Food Chemistry 60:136-143.

Jami S, 2018. The effect of chitosan on micropropagation, secondary metabolites and antioxidant activity of *selvia lerifolia Benth.* M.Sc. Thesis. University of Zabol. (In Persian).

Khalafi M, Pourbeyrami Hir Y, Chamani E and Maleki Lajayer H, 2021. The Effect of Chitosan on Regeneration and Secondary Metabolite Production of Two Species of Lily Flower. Journal of Plant Research (Iranian Journal of Biology). Accepted Manuscript, Available Online from 07 March.

Kong Y, Bai J, Lang L, Bao F, Dou X, Wang H, and Shang H, 2017. Floral scents produced by *Lilium* and *Cardiocrinum* species native to China. Biochemical Systematics and Ecology 70: 222-229.

Kong Y, Wang H, Lang L, Dou X and Bai J, 2021. Metabolome-Based Discrimination Analysis of Five Lilium Bulbs Associated with Differences in Secondary Metabolites 26(5): 1340.

Kovacik J, Backor M, Strnad M and Repcak M, 2009. Salicylic acid-induced changes to growth and phenolic metabolism in *Matricaria chamomilla* plants. Plant Cell Report 28 (1): 135-143.

Kumar S, Kanwar JK and Sharma DR, 2015. *In vitro* propagation of Lilium: Review paper. Advances in Horticultural Science Vol. 20 (2): 181-188.

Liang S and Minoru NT, 2000. Lilium. In: Wu Z, Raven PH, and Hong D (eds.) Flora of China. Volume 24: Flagellariaceae through Marantaceae. Pp. 135-149. Scidence Press & Missouri Botanical Garden Press, Beijing, China; St. Louis, MO, USA.

Mahdavi B, ModarresSanavy SAM, Aghaalikhani M and Sharifi M, 2011. The Effect of water stress and chitosan on Germination and proline of seedling in safflower (*Carthamus tinctorius* L.). Journal of Plant Research 26(3): 352 - 365. (In Persian).

Mahdavi B, Modarres Sanavy SAM, Aghaalikhani M, Sharifi M and Dolatabadian A, 2011. Chitosan improves osmotic potential tolerance in safflower (*Carthamus tinctorius* L.) seedlings. Journal of Crop Improvement 25 (6): 728–741.

Malekpoor F, Salimi A and Ghasemi Pirbalouti A, 2017. The Effect of bio-elicitor of chitosan on physiological and morphological properties in purpule basil (*Ocimum basilicum* L.) under water deficit. Journal of Plant Ecophysiology 8(27): 56-71. (In Persian).

Mandal S, 2010. Induction of phenolics, lignin and key defense enzymes in eggplant (*Solanum melongena* L.) roots in response to elicitors. African Journal of Biotechnology 9 (47):8038-8047.

Michalak A, 2006. Phenolic compounds and their antioxidant activity in plants growing under heavy metal stress. Polish Journal of Environmental Studies 15(4):523–530.

Mondal MA, Malek MA, Puteh AB, Ismail MR, Ashrafuzzaman M and Naher L, 2012. Effect of foliar application of chitosan on growth and yield in okra. Australian Journal of Crop Science 6 (5): 918-921.

No HK, Park NY, Lee SH and Meyers SP, 2002. Antibacterial activity of chitosan and chitosan oligomers with different molecular weights. International Journal of Food Microbiology 74(1-2): 65–72.

Nourafkan H, 2018. The effect of chitosan on physiological and morphological traits (*lippie citriodora L.)* under in-field and in vitro conditions. Journal of Crop Ecophysiology Vol.1 (49): 73-86. (In Persian).

Palida S, Rath P and Supachitra C, 2014. Chitosan increased phenolic compound contents in tea (*Camellia sinensis*) leaves by pre- and post-treatments. Journal of Chitin and Chitosan Science 2 (2): 93- 98.

Salachna P and Zawadzińska A, 2014. Effect of chitosan on plant growth, flowering and corms yield of potted freesia. Journal of Ecological Engineering, 15(3): 97– 102.

Sharma GK, 2015. General Techniques of Plant Tissue Culture. Lulu Press Inc. Raleigh, North Carolina, United States. ISBN; 978-1-329-73251-3.

Sheikha SAAK and AL-Malki FM, 2009. Growth and chlorophyll Responses of Bean Plants to the Chitosan Applications. European Journal of Scientific Research 50 (1): 124-134.

Singla R and Gary N, 2005. Influence of salinity on growth and yield attributes in chickpea cultivars. Turkish Journal of Agriculture and Forestry 29 (4): 231-235.

Slinkard K and Singleton VL, 1977. Total Phenol Analysis: Automation and Comparison with Manual Methods. American Journal of Enology and Viticulture 28: 49-55.

Vasyukova NI, Zinovèva SV, ilìnskaya LI, Perekhod EA, Chalenko GI, Gerasimova G, Il'ina V, Varlamov P and Ozeretskovskaya L, 2001. Modula-tion of plant resistance to diseases by water-soluble chitosan. Applied Biochemistry and Microbiology 37(1): 103-109.

Yokota S and Yahara T, 2012. Pollination biology of *Lilium japonicum* var. *abeanum* and var. *japonicum*: evidence of adaptation to the different availability of diurnal and nocturnal pollinators. Plant Species Biology 27(1): 96-105.

Zhao J and Sakai K, 2003. Multiple signaling pathways mediate fungal elicitor induced beta-thujaplicin biosynthesis in *Cupressus lusitanica* cell cultures. Journal of Experimental Botany 54(383): 647-656.

بررسی تأثیر کیتوسان بر باززایی و تولید متابولیت‌های ثانویه در گل سوسن گونه رگال

یونس پوربیرامی هیر*، مهسا خلفی، اسماعیل چمنی و حسن ملکی لجایر

دانشگاه محقق اردبیلی، اردبیل
*مسئول مکاتبه؛ Email: Younes_ph62@uma.ac.ir

چکیده

گونه‌های *Lilium* متعلق به خانواده Liliaceae هستند. این گیاه حاوی یک متابولیت ثانویه ارزشمند است که با تیمار محرک می‌توان آن را افزایش داد. هدف اصلی مطالعه حاضر بررسی اثرات کیتوزان به عنوان یک محرک بر باززایی و تولید متابولیت های ثانویه در *Lilium regale* بود. آزمایش در قالب طرح کاملا تصادفی در محیط کشت MS با ۱۰ تکرار انجام شد. پنج غلظت مختلف (۰، ۵۰، ۱۰۰، ۱۵۰ و ۲۰۰ میلی گرم در لیتر) کیتوزان به عنوان تیمار مورد استفاده قرار گرفت. صفات مورفولوژیکی مختلف، کلروفیل برگ و متابولیت‌های ثانویه اندازه گیری شد. تجزیه واریانس نشان داد که کیتوزان بر ارتفاع بوته، وزن تر، تعداد پیازچه، تعداد ریشه، کلروفیل a و b، کلروفیل کل، فنل‌ها، فلاونوئیدها و درصد باززایی تأثیر معنی‌داری داشت. بیشترین و کمترین وزن تر و تعداد ریشه به ترتیب از ۲۰۰ پی پی ام کیتوزان و شاهد به دست آمد. همچنین نتایج نشان داد که ارتفاع بوته و تعداد پیازچه در تمامی غلظت‌های کیتوزان بیشتر از شاهد بود. بیشترین و کمترین میزان باززایی به ترتیب از کیتوزان ۲۰۰ پی پی ام و شاهد به دست آمد. با این حال، بیشترین میزان فنل، فلاونوئیدها و کلروفیل از کیتوزان ۲۰۰ پی پی ام حاصل شد. با توجه به نتایج مطالعه حاضر، کیتوزان به میزان ۲۰۰ پی پی ام نسبت به سایر غلظت‌ها تأثیر مثبت بیشتری بر باززایی و تولید متابولیت‌های ثانویه *Lilium regale* داشت.

واژه‌های کلیدی: باززایی؛ کشت درون شیشه‌ای؛ کیتوزان؛ گل سوسن؛ متابولیت‌های ثانویه