DE G DE GRUYTER OPEN

DOI: 10.2478/ata-2014-0020

Acta Technologica Agriculturae 4
Nitra, Slovaca Universitas Agriculturae Nitriae, 2014, pp. 86–90

EFFECT OF CHITOSAN APPLICATION ON THE PERFORMANCE OF LENTIL GENOTYPES UNDER RAINFED CONDITIONS

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In the current study, influences of chitosan solutions on morphological characteristics, growth and yield components of lentil (*Lens culinaris* Med.) under rainfed conditions have been investigated. A field experiment was conducted in the Northwest of Iran using a split-plot experiment based on a completely randomized design with three replications. The response of twelve genotypes with different origins to chitosan application at the sowing (seed soaking), vegetative and reproductive stage (spraying chitosan onto leaves) was evaluated. Results revealed that chitosan application could significantly improve the number of pods per plant, 100-seed weight, grain yield per plant and harvest index in comparison to control plants. The comparison of yield components between chitosan treatments showed that spraying chitosan during the reproductive stage was more efficient than in other stages. However, the responses of the number of pods per plants and grain yield per plants to chitosan treatments were significantly different among the genotypes. Although the highest grain yield was recorded in the 78S 26013 genotype (from Jordan), its response to chitosan treatments was different from the other genotypes and showed the best performance in plants obtained from seed soaked in chitosan solutions. We suggest that the application of chitosan as an agronomic management strategy be further investigated for an efficient technique to induce resistance in lentil plants against biotic and drought stress in semi-arid regions.

Keywords: chitosan, foliar spray, lentil production, seed soaking, yield components

Lentil (*Lens culinaris* Med.) is the second most important and widely distributed pulse crop grown under a broad range of climates. The cultivation of lentil supplies opportunities for the local production of grain legume for humans and utilization of symbiotic N2 fixation (Robson et al., 2002). Like other legumes such as dry pea and chickpea, lentil offers benefits in rotation with cereals and oilseeds, including improved water-use efficiency, increased grain yield and protein in subsequent cereals (Miller et al., 2003), and improved economic sustainability (Zentner et al., 2002). The FAO reported that the world production of lentils for calendar year 2009 was 3.917 million metric tons and about 3 % of the worldwide production of lentils is from Iran (FAO, 2009). However, Iran has a Mediterranean climate that is characterized by two distinct seasons: a wet, cool period from October to April, during which traditional rainfed cropping is practiced, and a long, dry period from May to September, when cropping is possible only with irrigation (Kassam, 1981). Available water is the most limiting factor for the production of annual crops in the Mediterranean region, especially in northern latitudes of Iran where climatic conditions vary enormously, including highly variable and unpredictable precipitation, large and diurnal ranges in temperature, warm summers, and frequent spreading of diseases and pests. Many fungal species (e.g. *Alternaria* and *Chaetomium*) and seed-borne diseases (e.g. *Botrytis* spp., and *Fusarium oxysporum*) have been reported from lentil seeds (Hussain et al., 2007). However, the impact of drought or biotic stresses can influence the relative tolerance of a genotype. Differences between genotypes in plant architecture, growth duration and internal tolerance mechanisms have been shown to be a major factor affecting the grain yield under rainfed conditions.

In order to understand the structure of the world lentil collection, landraces held at the International Center for Agriculture Research in the Dry Areas (ICARDA) were characterized into four major groups identified through the analysis of variability in quantitative and qualitative morphological traits (Erskine et al., 1993). These were the Levantine group (Egypt, Jordan, Lebanon and Syria), northern group (Greece, Iran, Turkey, USSR, Chile), Indian subcontinent group and Ethiopian group, and each group has some specific characteristics. In addition to production or selection of stress tolerant genotypes, exogenous applications of plant growth regulators seem to be another way to overcome the adverse effect of environmental stresses. Chitosan is harmless to crops, animals and humans, and is biodegradable and friendly to the environment (Dzung et al., 2011). Chitosan is a cationic polysaccharide, a derivative of chitin obtained from waste materials from seafood processing (Ye and Lou, 2009). They have been introduced as a material to improve grain yield under unfavourable conditions due to their bioactivities to plants such as inducing the plants resistance against a wide range of diseases through antifungal, antibacterial, antivirus activities (Wang et al., 2006); stimulating the growth of plants and seed germination (Chandrkrachang, 2002); improving soil fertility and enhancing the mineral nutrient uptake of plant (Dzung, 2005, 2007); increasing the content of chlorophylls, photosynthesis and chloroplast enlargement (Limpanavech et al., 2008); escalating nitrogen fixing nodes of species of leguminous plants (Dzung and Thang, 2004); and reducing the effects of abiotic stress on plants (Song et al., 2006). However, the beneficial effect of chitosan is generally depending on its concentration, application methods, environmental condition and growth status. Although there is some research about influences of chitosan application on different plants, little is known about how chitosan foliar or seed application would affect the growth and yield of lentil in semi-arid growing environments.

Material and methods

In this study, eleven lentil genotypes with different origin obtained from ICARDA (International Centre for Agricultural Research in the Dry Areas) along with a check cultivar from Iran (Kimia) were used. Genotypes consisted of PI 299127 (originated from Mexico, showed as G_1), PI 339319 (Turkey, G_2), L1278 (India, G_3), Syrian Local Large (Syria, G_4), 340 (Pakistan, G_5), Precoz (Argentina, G_6), 78S 26013 (Jordan, G_7), Flip 84-51L (ICARDA, G_8), Flip 89-71L (ICARDA, G_9), Flip 95-30L (ICARDA, G_{10}), Bari Masur4 (ICARDA, G_{11}) and Local check (Iran, G_{12}). Field experiments were carried out at the Research Farm of the University of Maragheh, Iran, during the growing season of 2013. The field was located at 46° 16ʹ east longitude and 37° 23ʹ north latitude, at an altitude of 1,485 metres above sea level. Based on Koppen's classification, this region has a semi-arid and cold temperate climate. The soil type was a clay loam, pH 6.85 and EC = 0.84 $dS.m^{-1}$. The trial was conducted in a split-split block design with three replications. The lentil genotypes were assigned to main plots, and chitosan application methods were assigned to sub-plots.

Chitosan treatments included: 1 – control (no chitosan application), 2 – seed soaking with chitosan (0.05 %w/v) for 60 min., 3 – foliar spraying with chitosan solution (0.1 %w/v) at 30 days after sowing, and 4 –foliar spraying with chitosan solution (0.1 %w/v) at a 50 % flowering stage. The row-to-row spacing was 25 cm and plant-to-plant 8 cm, and the recorded area of each sub-plot was 4 m^2. Seeds were sown on 3 March. Fertilizer at 80 kg P_2O_5 ha^{-1} was applied prior to sowing. The fertilizer P was applied in bands, 5 cm below rows. The trials were rainfed, and precipitation was 306 mm in the 2012–2013 season. The experimental lines were harvested by hand in July. The area harvested in each plot was 0.1 m^2. The experimental area was hand weeded.

Chitosan solution (0.1 %w/v) was prepared by dissolving 0.1 g of the purified chitosan powder in 100 ml of 0.15 % acetic acid solution and kept overnight, and then, the chitosan solution was adjusted to pH 6.0 by 1M NaOH solution (Reglinski et al., 2004). The plants were harvested at maturity, and the morphological traits such as plant height, the number of stems, above-ground biomass (biological yield), pods per plant, grains per pod, grains per plant and 100-grain weight were recorded on 15 randomly selected plants in each plot. Grain and biological yield was determined by harvesting the middle four rows of each plot. Data were analyzed using a SPSS software. Treatment effects were compared using LSD at a 0.05 significance level.

Results and discussion

The result of variance analysis showed that chitosan application had not a significant effect on plant height, but this trait was significantly altered among the genotypes (Table 1). Among the genotypes, the highest height was observed for 78S 26013, and the lowest value was recorded in Syrian Local Large (Table 2). Also the number of stems per plant was not statistically affected by chitosan application; however, in all of the chitosan-applied plants, stems per plant numerically were higher than untreated plants (Table 1). Among the genotypes, the highest and lowest number of stems per plant was observed in Bari Masur4 and Syrian Local Large, respectively (Table 2). However, the interaction between chitosan application treatment × genotypes was not significant for plant height and the number of stems per plant.

Results revealed that the number of pods per plant was significantly affected by chitosan application. Pod number in plants treated with chitosan was significantly higher than control. Among the chitosan treatments, the application of chitosan at the reproductive stage resulted in the highest number of pods per plant (Table 1). However, there was not a significant difference between chitosan application during seed sowing (seed priming through chitosan solution) and foliar spraying with chitosan solution at 30 days after sowing (vegetative stage). The number of pods per plant was also significantly affected by genotypes. The highest and lowest values were recorded for L1278 and Flip 89-71L, respectively. Furthermore, the interaction between chitosan application treatment × genotypes on pods per plant was significant. In some of the genotypes like L1278 and 78S 26013, seed soaking in chitosan solution at sowing time had better effect on the number of pods per plant in comparison with other chitosan treatments. Zeng et al. (2012) reported that chitosan protection by exclusion occurs with soybean seed treatments. In this case, the benefit was protection from insects such as agarotis, ypsilon, soybean pod borer, and soybean aphids. Also the chitosan treatment developed an antifeedant rate greater than 80 % against all these insects.

Table 1 Effect of different chitosan treatments on growth attributes of lentil

Chitosan	Plant height in cm	Stems $plant^{-1}$	Pods $plant^{-1}$	Grains pod^{-1}	Grains $plant^{-1}$	100-seed weight in g	Biological yield in gm^{-2}	Economic yield in gm^{-2}	Harvest index in %
Control	22.18^a	5.69^a	25.1^c	1.13^a	29.0^b	4.46^b	76.6^a	34.1^b	44.28^b
Sowing	23.01^a	5.78^a	28.3^b	1.33^a	31.3^a	4.35^{ab}	77.9^a	34.3^b	42.98^b
Vegetative	22.79^a	5.79^a	28.7^b	1.17^a	31.4^a	4.03^{bc}	76.1^a	35.5^b	46.39^b
Reproductive	22.65^a	5.81^a	30.1^a	1.15^a	31.2^a	4.74^a	75.6^a	39.9^a	52.77^a

Different letters in each column indicate significant difference at $P \leq 0.05$

Table 2 Some morphological traits, yield and yield components of 12 lentil genotypes

Genotypes	Plant height in cm	Stems plant^{-1}	100-seed weight in g	Biological yield in gm^{-2}	Economic yield in gm^{-2}	Harvest index in %
PI 299127	20.9^{cd}	6.01^{b}	2.83^{g}	72.2^{d}	47.1^{c}	65.3^{b}
PI 339319	25.0^{b}	6.73^{ab}	3.45^{ef}	84.2^{b}	17.1^{g}	20.4^{f}
L1278	23.3^{b}	6.55^{ab}	3.86^{cde}	79.5^{c}	56.3^{b}	70.9^{a}
SYRIAN LOCAL LARGE	16.3^{e}	3.58^{f}	2.97^{g}	60.3^{f}	28.2^{e}	47.1^{c}
340	23.8^{b}	6.46^{b}	4.29^{c}	78.8^{c}	30.7^{e}	39.1^{d}
PRECOZ	22.8^{bc}	5.33^{c}	5.22^{b}	69.1^{de}	45.3^{c}	66.0^{b}
78S 26013	27.7^{a}	6.73^{ab}	6.92^{a}	89.0^{a}	64.9^{a}	73.5^{a}
FLIP 84-51L	19.1^{d}	4.15^{e}	4.11^{cd}	67.7^{e}	15.9^{g}	23.5^{f}
FLIP 89-71L	23.2^{b}	4.63^{d}	7.13^{a}	85.4^{ab}	54.6^{b}	64.1^{b}
FLIP 95-30L	24.1^{b}	6.02^{b}	5.02^{b}	83.2^{bc}	35.6^{d}	43.1^{cd}
Bari Masur4	20.7^{cd}	6.97^{a}	3.18^{fg}	66.8^{e}	22.3^{f}	33.6^{e}
Local check	24.5^{b}	6.49^{b}	3.71^{de}	83.1^{bc}	12.7^{h}	15.3^{e}

In a column, within treatment, figures having the same letter(s) do not differ significantly at $P \leq 0.05$

The treatment was accompanied by increases in seed germination, plant growth and soybean yield. This chitosan application fulfilled the major objective of replacing high toxicity pesticides (Zeng et al., 2012). However, the highest number of pods per plant in some genotypes like PI 339319, 340, Bari Masur4 and Local check (Kimia) was obtained through foliar spraying during the vegetative stage. Though, the highest number of pods per plant was observed in Precoz with foliar spraying during the reproductive stage (Figure 1). Turner et al. (2001) reported that abiotic stress during the reproductive phase of pulse crops leads to flower abortion, poor pod set and formation of infertile pods. It seems that chitosan by alleviating the adverse effect of unfavourable condition, such as terminal drought that takes place at the reproductive stage, can enhance pods formation.

The result showed that the number of grains per pod was not affected by chitosan treatment and genotypes. The number of grains per pod is genetically controlled and environmental factors had little effect on this trait; however, in this study, significant difference was observed among the genotypes. The evaluation of grains per plants between chitosan treatments revealed that chitosan application, regardless of the utilization methods, significantly increased the number of grains per plants in comparison with control (Table 1). It can be attributed to reduction of poor pod set and pod abortion under rainfed conditions. Fang et al.

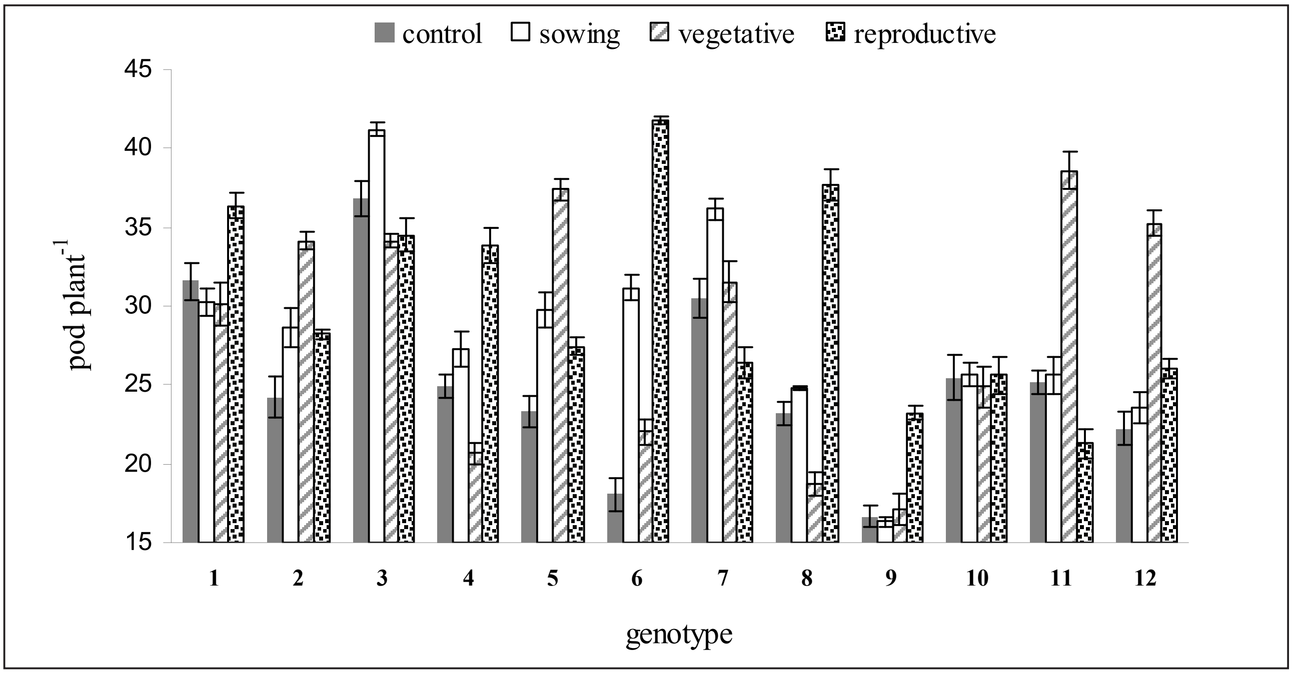

Figure 1 Response of pods per plant of lentil genotypes to different chitosan applications. The values and standards errors (vertical bars) of three replications are shown

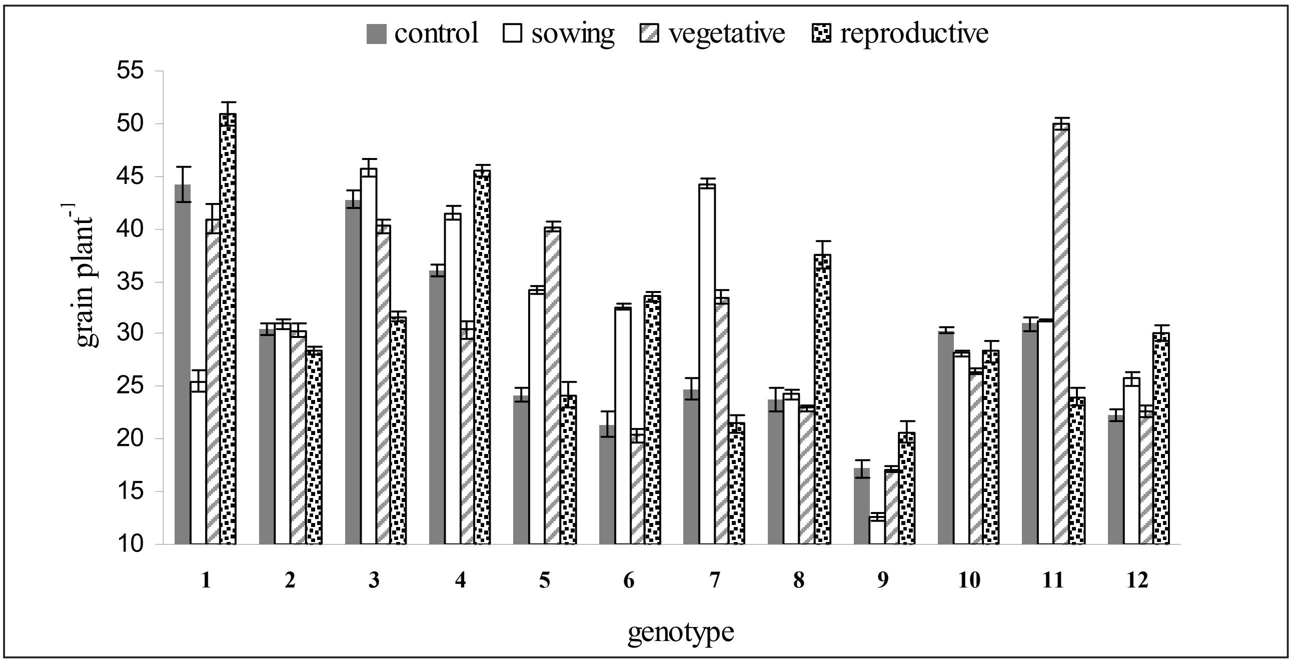

Figure 2 Effect of different methods of chitosan application on the grain yield of lentil

(2009) reported that stressful condition during the flowering stage impairs both pollen and stigma/style function, and the impairment of pistil function is an important factor related to flower abortion. Abiotic and biotic stress can be decreased photosynthetic rate and disrupted carbohydrate metabolism in leaves (Kim et al., 2000); both could lead to a decreased availability of assimilate for export to the sink tissue, thereby escalating the rate of reproductive abortion (Fulai et al., 2004). This trait was also affected by genotype and interaction between genotypes × chitosan treatment. Among the genotypes, PI 299127 and L1278 showed the highest grain yield per plant, and conversely the lowest value was recorded for Flip 89-71L. The positive influence of chitosan treatments on genotype grains mostly depends on application methods; thus, a particular method cannot be recommended for all conditions and genotypes.

The analysis of 100-seed weight showed that this yield component was significantly affected by chitosan treatments and genotypes, but their interaction effect was not statistically significant. Among the treatments, the application of chitosan during the reproductive stage resulted in the highest 100-seed weight. Interestingly, foliar spraying with chitosan solution at 30 days after sowing (vegetative stage) resulted in small seeds compared with control (Table 1). Moreover, the foliar application of chitosan during the vegetative stage slightly increased the number of grains per plants. Therefore, determinate assimilate partitioning should be conducted between a large number of seeds that will result in little accessible assimilate for individual seed. The foliar application of chitosan at the reproductive stage may supply more N, a fundamental element for crops at the seed filling period, for grains, therefore increase their weight. Among the genotypes, 785 26013 and FLIP 89-71L had the highest 100-seed weight and the lowest value was recorded for Syrian Local Large and PI 299127 (Table 2). The result of variance analysis showed that biological yield was only influenced by genotypes. The highest and lowest biological yield was observed in 78S 26013 and Syrian Local Large, respectively (Table 1). Economic yield and harvest index were affected by both chitosan treatments and genotypes. Among the chitosan treatments, foliar application during the reproductive stage significantly enhanced the harvest index and also economic yield; although, the other treatments could not statistically affect the mentioned traits (Table 1). The improvement of economic yield can be attributed to 100-seed weight enhancement achieved by foliar application. It seems that chitosan application during the reproductive stage can induce the partitioning of assimilates to reproductive organs. The comparison of genotypes indicated that the highest and the lowest economic yield was produced by 785 26013 and Local check (Kimia), respectively (Table 2). Totally, it seems that after chitosan application subsequent changes occur in: cell membranes, chromatin, DNA, free calcium concentration, the activity of MAP kinase pathway, oxidative burst, the concentration of reactive oxygen species (ROS), callose, pathogenesis-related (PR) genes/proteins, and phytoalexins (Hadwiger, 2013).

This study proved that chitosan could act as a biostimulant of lentil plants under rainfed conditions. In addition, previous investigations confirmed that chitosan induces the production of phenolic acids and the phytoalexins substances that protect plant tissues and seeds from microorganism attacks and undesirable environmental conditions (Dzung, 2007; Wang et al., 2006). Our result revealed that the foliar application of chitosan had better influence in comparison with seed soaking. In fact, foliar spraying is the need of the hour in Mediterranean arid to semi-arid soils having high pH values where plants fail to uptake these substances from the soil. Plants are able to absorb some essential elements and substances through their stomata as well as through their waxy cuticle layer. Two technologies that appear especially appropriate to foliar spraying of chitosan deserve to be mentioned.

The first is the use of electrostatic sprayers, which impart a charge to spray particles and cause them to adhere more readily to plants. The second technology uses sound to increase the leaves' absorption of substances. Furthermore, nanotechnology, by good quality of nanomaterial-related properties, has potential agro-biotechnological applications for alleviation of some adverse environmental effects. Chitosan nanoparticles hold great promise regarding their application in plant protection due to their size-dependent qualities, high surface-to-volume ratio and unique visual properties, and it can be an appropriate subject for future investigations.

Conclusion

Overall, low soil moisture content during the late reproductive stage is the main constricting factor for lentil yield under rainfed conditions, especially in susceptible genotypes in comparison with tolerant genotypes. In summary, our results revealed chitosan, as a yield enhancing agent (YEA), significantly affected the growth and yield components of lentil genotypes in the semi-arid region. The results suggest that the foliar application of chitosan during the reproductive stage could be effective; however, its effect depends on genotypes. Among the genotypes, 78S 26013 had the highest economic yield. It may be revealed that environmental condition for the genotype originated from Jordan was similar to rainfed conditions in NW Iran. Local check showed the lowest economic yield; it may be attributed to low breeding practices that were conducted on this genotype. Therefore, the foliar application of chitosan may be recommended for genotypes derived from the semi-arid Mediterranean climate after few more field trials. These positive benefits from the chitosan application must be seen in the light of chitosan modes of actions that result in the induction of stress resistance responses in plant.

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