Effect of *in vitro* chitosan application on growth and minituber yield of *Solanum tuberosum* L.

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ABSTRACT

In order to investigate the effects of soluble chitosan on plantlets growth *in vitro* and increase of minituber yield in potato micropropagation, plantlets of Agria cultivar were treated *in vitro* with soluble chitosan at different concentrations including 0, 5, 15, 50, 150, 500, 750 and 1000 mg/l added to the MS tissue culture medium. Plantlets were subsequently transferred to the greenhouse and minituber yield parameters were evaluated. At the concentrations of 750 and 1000 mg/l of chitosan the culture medium failed to solidify. Application of 500 mg/l of soluble chitosan increased the shoot fresh weight, but its lower concentrations did not significantly affect this trait ($P < 0.05$). The 5 and 15 mg/l of soluble chitosan led to a significant increase in root fresh and dry weight of *in vitro* plantlets, whereas, higher concentrations, especially 500 mg/l, significantly decreased root fresh weight of *in vitro* plantlets. Application of 500 mg/l chitosan *in vitro* resulted in improved acclimatization of plantlets in the greenhouse as expressed by significant ($P < 0.05$) increase in minituber number and yield, compared to the control. The tested lower concentrations had no effect on yield parameters. The present results indicate that soluble chitosan can be successfully incorporated into potato seed production from *in vitro* plantlets.

Keywords: chitosan; *in vitro* culture; micropropagation; minituber; potatoes

Potatoes (*Solanum tuberosum* L.) are grown worldwide under a wide range of climatic conditions. No other crop can match the potato in production of food energy and food value per unit area (Sieczka and Thornton 1993). It is also high in vitamin C, niacin and vitamin B6. *In vitro* micropropagation offers a method to produce healthy potato seeds from disease-free plantlets. The main problems in commercial micropropagation are the stresses which tissue culture conditions impose on plantlets through high osmoticity, abnormal mineral nutrition, unusual hormonal treatment, high relative humidity, accumulation of gases such as ethylene, and the mechanical injury through dissection (Cassells and Walsh 1994, Gaspar et al. 2002, Joyce et al. 2003). According to Cassells and Curry (2001) the most of problems underlying plant micropropagation are in part oxidative stress damages. Therefore, the activation of plant defense mechanisms could reduce the stress caused by the *in vitro* culture microenvironment. Chitosans are polysaccharides produced from chitin, for instance from crab shells, which can be made soluble through alkalic or enzymatic deacetylation. Chitosan is reported to influence the production of substances related to stress response, such as phytoalexins (Walker-Simmons et al. 1983) and chitinases (Dörnenburg and Knoor 1994, O'Herlihy et al. 2003). It is suggested that chitosan can be used commercially for controlling tomato root rot diseases under field conditions (El-Mougy et al. 2006). Trials conducted in tomatoes (Walker et al. 2004) showed that foliar applications of chitosan resulted in yield increase of nearly 20% and a significant improvement in powdery mildew disease control.

Chitosan treatments have plant growth promoting effects, resulting in improved yields and plant health in numerous crops and fruits. The activation of protective mechanisms in plant tissues with

chitosan inhibited the growth of taxonomically different pathogens (Vasyukova et al. 2001). It has been considered as an alternative to chemical fungicides (Benhamou et al. 1994, El-Ghaouth 1994, Tiuterev 1996, Vander 1998, O'Herlihy et al. 2003). Beauséjour et al. (2003) reported that combination of *S. melanosporofaciens* EF-76 and chitosan represents a promising method of biocontrol against common scab in potato crop.

Chitosan was found to enhance secondary metabolite production in cell suspensions and calli of various species (Dörnenburg and Knoor 1994, Tumová and Backovská 1999, Yu et al. 2002, Putalun et al. 2007). Applied in micropropagation, chitosan could improve plantlet quality *in vitro*, in consequence facilitating the subsequent acclimatisation of plantlets to *ex vitro* conditions (Nge et al. 2006).

Using different concentrations of soluble chitosan on potato cv. Désirée plantlets, Kowalski et al. (2006) reported that the seed quality of minitubers derived from chitosan treatments *in vitro* was improved, giving rise to field plants with increased tuber numbers and yields. They also mentioned that the addition of chitosan to tissue culture media will further have to be adjusted to the specific requirements of different genotypes, explant types and tissue culture methods, and concentrations applied to the culture medium will have to be adjusted to improve minituber yield and quality.

The aim of this work was to investigate whether the *in vitro* application of soluble chitosan can improve plantlet growth *in vitro* and affect minituber yields in potato micropropagation.

MATERIAL AND METHODS

Rooting medium contained MS basal medium (Murashige and Skoog 1962), 1.0 mg/l thiamine HCl, 30×10^3 mg/l sucrose and 6×10^3 mg/l agar. Soluble chitosan (ChitoPlant, ChiPro GmbH Bremen, chitosan content 99.9%) was added to the rooting medium prior to autoclaving and the pH was adjusted to 5.7–5.8. Tested concentrations of soluble chitosan were 0, 5, 15, 50, 150, 500, 750 and 1000 mg/l. Nodal cuttings of *Solanum tuberosum* cv. Agria were placed on rooting medium (25 explants in each of three replicates per treatment). *In vitro* plants were maintained at 20 ± 2°C, 120 μmol/m^2 s, 16 h photoperiod (fluorescent light tubes) in 25 × 150 mm test tubes containing 10 ml of culture medium for 21 days. *In vitro* parameters including shoot and root fresh and dry weights, plantlet length and leaf number per plantlet were recorded.

Plantlets from chitosan treatments *in vitro* and controls were transferred to the greenhouse and cultivated at plastic pots (10 cm) containing Biolan peat. Sample size for each treatment was 30 plantlets. Seventy days after planting in greenhouse, minitubers were harvested, counted and weighed. The trial site was the Vilcage potato seed production cooperation, province of Ardabil (Northwest Iran), situated 1100 m above sea level with the

Table1. Effect of different concentrations of soluble chitosan applied in the culture medium on growth and minituber yield of potato *Solanum tuberosum* L. cv. Agria plantlets expressed as the means of *in vitro* plantlets and greenhouse plants characters

In vitro plantlets and greenhouse plants characters	Chitosan concentration (mg/l)						LSD (5%)
	0	5	15	50	150	500	
Shoot fresh weight (mg/plantlet)	302.2	326	337.8	299	316.8	429.2*	69.32
Shoot dry weight (mg/plantlet)	29.8	36.8	39.2*	34	36	49.2*	7.64
Root fresh weight (mg/plantlet)	96	108.76*	136.78*	79.04*	78.44*	65.66*	12.45
Root dry weight (mg/plantlet)	8.24	9.4	12.2*	8.222	8.776	7.58	1.74
Plantlet length (cm)	13.7	14	13.1	12.1	13.5	10.1*	2.43
Leaf number per plantlet	8.8	9	8.2	8.6	9	9.8	1.8
Minituber number per plant	2.444	2.833	2.2	2.833	2.778	3.333*	0.542
Minituber yield (g/plant)	13.818	14.889	14.646	13.39	14.486	15.56*	1.156

LSD – the values of least significant difference test at $P < 0.05$; * significant difference (positive or negative) from control at $P < 0.05$

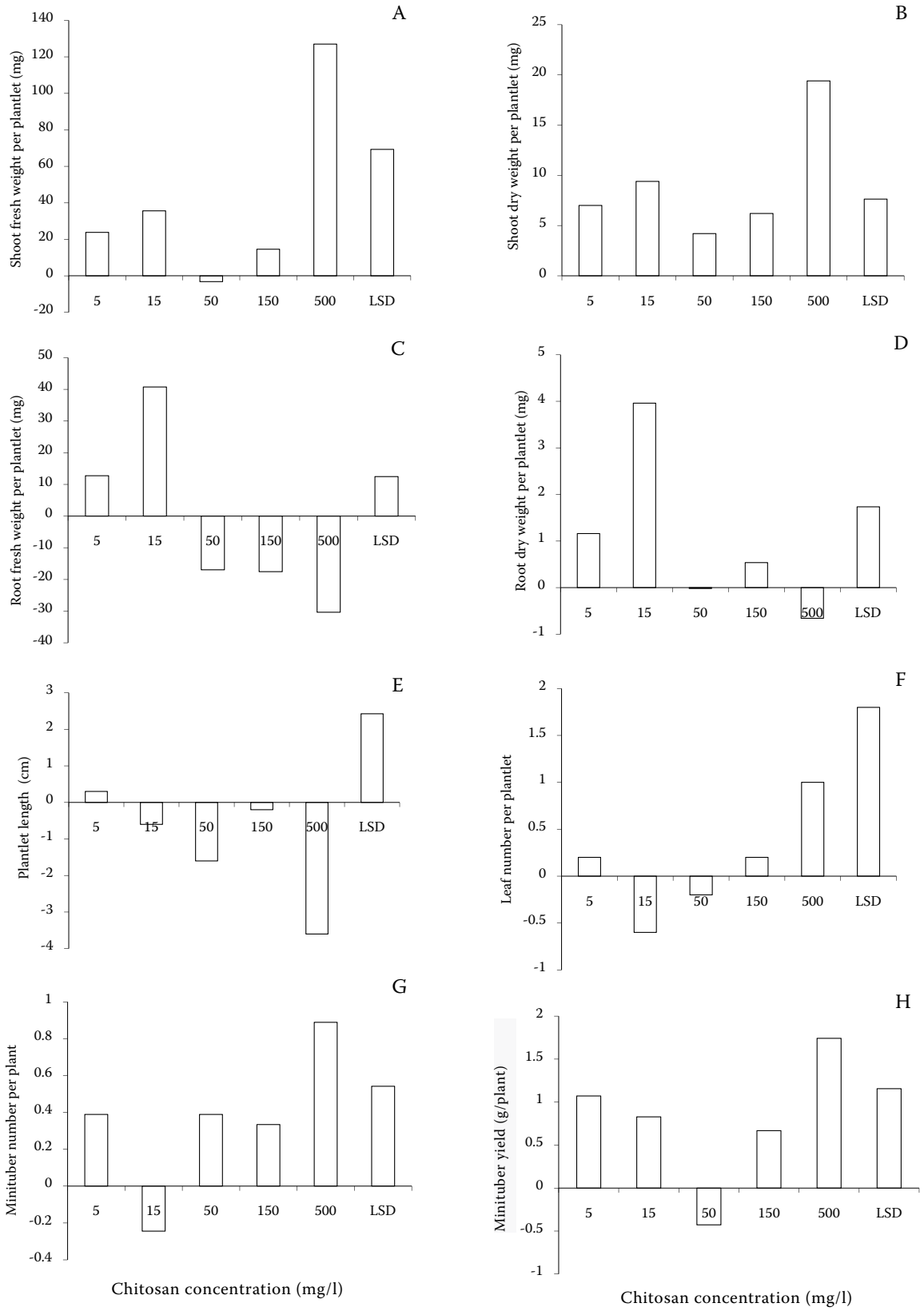

Figure 1. Effects of different concentrations of soluble chitosan on growth and minituber yield of potato plantlets demonstrated as their differences with control using least significant difference test for mean comparing. (A) shoot fresh weight (mg/plant); (B) shoot dry weight (mg/plantlet); (C) root fresh weight (mg/plantlet); (D) root dry weight (mg/plantlet); (E) plantlet length (cm); (F) leaf number; (G) minituber number; (H) minituber yield (g/plant)

average maximum temperatures of 27°C, minimum temperatures 10°C and humidity 70%.

Analysis of variance (one-way ANOVA) was carried out, using the least significant difference (LSD) test at $P < 0.05$ to compare means.

RESULTS AND DISCUSSION

At the concentrations of 750 and 1000 mg/l of chitosan, the culture medium failed to solidify and these concentrations were removed from the experiment. The effects of different concentrations of soluble chitosan applied in the culture medium on growth and minituber yield of potato *Solanum tuberosum* L. cv. Agria plantlets was shown in Table 1 and Figure 1.

Application of 500 mg/l of soluble chitosan increased the shoot fresh weight, but its lower concentrations did not significantly affect this trait. According to Figure 1, all of the chitosan concentrations showed a positive effect on shoot dry weight, the effects of 15 and 500 mg/l were significant (500 mg/l was more significant).

The 5 and 15 mg/l of soluble chitosan led to a significant increase in root fresh and dry weight of *in vitro* plantlets, whereas higher concentrations, especially 500 mg/l, significantly decreased root fresh weight of *in vitro* plantlets. Root dry weight was not significantly affected by high concentrations of chitosan (Table 1, Figure 1). It seems that soluble chitosan prevents the proper growth of roots at higher concentrations, seeing that the number of roots ultimately decreased (data not shown). The leaf number of *in vitro* plantlets was not affected by chitosan, but the plantlet length was significantly decreased at 500 mg/l of soluble chitosan, showing a decrease of inter-nodal distances.

Application of 500 mg/l chitosan *in vitro* resulted in improved acclimatization of plantlets in the greenhouse as expressed by a significant increase in minituber number and yield, compared to the control. The lower concentrations tested had no effect on yield parameters (Table 1, Figure 1). Some authors showed that the quality of *in vitro* plants has influenced acclimatization and subsequent development of plantlets in the field (Cassells et al. 1999, Kowalski et al. 1999, 2006). A good physiological condition of *in vitro* plants is a guarantee of the later successful seed production process in greenhouse. The present results showed that chitosan as a growth promoter and elicitor of plant defense mechanisms could alleviate stress caused by *in vitro* conditions and acclimatization (Table 1). Application of chitosan *in vitro* at 500 mg/l concentration mainly influenced growth in the greenhouse and minituber production.

Soluble chitosan can be added without extra manipulation to the culture medium before autoclaving. The chitosan is used in small amounts and so the additional costs do not rise. Increase of yield and minituber number after application of 500 mg/l chitosan was 12.6 and 36.3%, respectively.

The present results indicate that soluble chitosan can be successfully incorporated into potato seed production from *in vitro* plantlet.

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