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# Growth of chervil (*Anthriscus cerefolium*) seedlings as influenced by salinity, chitin and $GA_3$

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**Abstract**

The effect of NaCl, chitin and $GA_3$ on seedling growth of chervil (*Anthriscus cerefolium*) was studied for 35 days at a temperature of 22$^o$C under controlled growth chamber conditions. Various aqueous solutions of NaCl (80, 120, 180, 240 mM NaCl), chitin (1, 2, 3, 4%) and $GA_3$ (100, 200, 500, 1000 ppm $GA_3$) were used as growth substrates. The above solutions used solely or combined and added on Petri dishes containing fifty chervil seeds. Chitin (3%) and $GA_3$ (100, 200 and 500 ppm) increased the seedlings height. The greatest relative growth rate was observed in higher NaCl (240, 180 mM NaCl) and $GA_3$ (1000 ppm) concentrations. The addition of chitin or $GA_3$ in the substrate that contained 80 mM NaCl resulted in the increase of the seedlings height in the combinations of 80 NaCl+1% Chitin, 80 NaCl+200 $GA_3$ and 80 NaCl+500 $GA_3$. High salinity (180 and 240 mM NaCl) in combination with chitin or $GA_3$ resulted in the reduction of the seedlings height in all combinations, compared to 240 mM NaCl. In the substrate that contained NaCl the addition of chitin and $GA_3$ resulted in the reduction of the seedlings height in all combinations, save for the combination that contained 80 NaCl+3% Chitin+500 $GA_3$, where it was higher than in $H_2O$. NaCl reduced the hypocotyls and root length and $GA_3$ increased the hypocotyls length. Salinity in the substrate in combination with chitin or $GA_3$ resulted in the reduction of the seedlings root length in all combinations.

**Keywords**: NaCl; seedling growth; relative growth rate; hypocotyls; root.
**Abbreviations:** $GA_3$, Gibberellic acid; NaCl, sodium chloride.

## Introduction

The chervil (*Anthriscus cerefolium* L. Hoffm.) is a fragrant, delicate annual herb which belongs to the Apiacae family. Its principal use is as a flavouring agent for culinary purposes, but it has been used for medicinal purposes as well. Chervil is native to Europe and has finely divided pinnate leaves. It was being cultivated in England in 1597 and in America by 1806. The origin of salad chervil lies in the southeastern Europe and western Asia. The chemical composition of chervil includes flavonoids such as luteolin (Fejes et al. 2000; Milovanović et al. 2009). The *Anthriscus* herb is an interesting plant generally characterized by strong and unique flavour compounds, and in some cases providing important nutrients which can enrich the consumers' diet. The chervil seed needs light and moisture to germinate and it does not transplant. The chervil elongates cotyledons and the apical cone are thus 1,4 cm above the soil surface at the end of the second week, and 25 weeks after germination the plant begins flowering and dies without any further contraction of the root after a life-span of approx. 6 months (Pütz1 and Sukkau 2002). The chervil plants generally grow to be about 50 cm in height and have an erect, branched and hollow stem. The leaves are first light green in color and then, as they mature, they turn reddish-brown. Chervil has received relatively little research attention compared to other herbs species. Salinity is the major environmental factor limiting plant growth and productivity (Allakhverdiev et al. 2000). Salinity delimits the reproductive growth of the plants, causing physiological dysfunctions and multiple direct and indirect problems, even in low concentrations (Shannon et al. 1994). The salt stress causes a number of changes in plant metabolism (Arafa et al. 2009). The concentrations and composition of salts, the duration of exposal, the plant type, the cultivar, the underlying, the stage of growth and the environmental conditions are some of the factors that play a role in the plant's tolerance to salinity (Marschner 1995). The plant species differ in their sensitivity or tolerance to salt stress (Cony and Trione 1998). Scattered notes appear in literature on the effect of chitin amendment on plant growth. D' Addabbo (1995) mentions that when chitin concentration in the soil exceeds 1%, it has a phytotoxic effect. The addition of chitin in the soil at 1% (w/w) eliminated plant-parasitic nematodes in cotton planting, confirming long-term nematode suppressiveness induced by this organic amendment (Hallman et al. 1999). Sarathchandra et al. (1996) mention that the shoot weight of ryegrass (*Lolium perenne* L.) was greater in soil amended with chitin, most probably due to N mineralised from chitin. Chitin amendment increased the growth of the red pepper plant (Rajkumar et al. 2008). Ladner et al. (2008) report that the total plant biomass fresh weight and the shoot fresh weight at a chitin concentration of 100g in tomato plants was higher when compared to the control. Applications of plant compounds such as chitin increased tomato fruit yield compared to plants grown in untreated soil (Giotis et al. 2009). Chitin in the peat substrate did not affect the length and weight of the lemon balm plant. Chitin affected the tarragon leaves, resulting in the increase of the total chlorophyll content (Liopa-Tsakalidi et al. 2010). $GA_3$ has been reported to promote the growth of cotton, rice and some halophytes in saline conditions (Zhao et al., 1986; Lin and Kao, 1995). The exogenous application of $GA_3$ on seedling growth under salt stress conditions provides an attractive approach to encounter the effects of salinity.

**Table 1.** Effect of NaCl, chitin and $GA_3$ on relative growth rate (±s.e.) of chervil (*Anthriscus cerefolium*).

| $H_2O$ | 80 NaCl | 120 NaCl | 180 NaCl | 240 NaCl |
|---|---|---|---|---|
| $0.209^{c}\pm0.11$ | $0.223^{c}\pm0.04$ | $0.238^{c}\pm0.03$ | $0.388^{b}\pm0.29$ | $0.509^{a}\pm0.32$ |
| | 100 GA3 | 200 $GA_3$ | 500 $GA_3$ | 1000 $GA_3$ |
| | $0.136^{d}\pm0.05$ | $0.132^{d}\pm0.10$ | $0.152^{d}\pm0.02$ | $0.323^{b}\pm0.06$ |
| | 1% Chitin | 2% Chitin | 3% Chitin | 4% Chitin |
| | $0.225^{c}\pm0.09$ | $0.227^{c}\pm0.02$ | $0.082^{e}\pm0.05$ | $0.223^{c}\pm0.10$ |

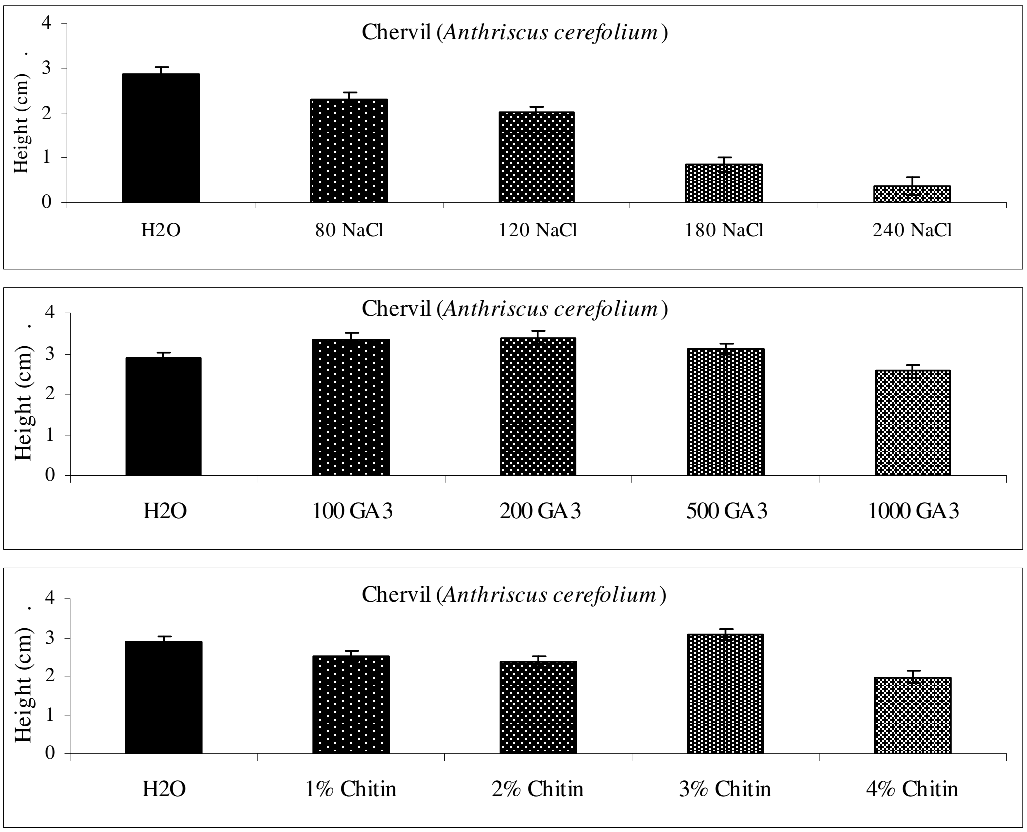


**Fig 1.** Height of chervil (*Anthriscus cerefolium*) at different NaCl, chitin and $GA_3$ concentrations (±s.e.).

Under saline conditions, soaking wheat kernels with $GA_3$ improved plant height, root length, and fresh and dry weight of stem, root and leaves (Parashar and Varma, 1988). The focus of the current study was to provide knowledge on the seedling growth of chervil on different levels of salinity, chitin and $GA_3$ under controlled conditions.

**Results**

***Height of the seedlings***

The growth period in *Anthriscus cerefolium* seedlings ranged from 9 to 35 days. The radicle emergence from the chervil seed occurred in day 9 after the seeds were placed in Petri dishes. The height of the chervil seedlings in the control ($H_2O$) was 2.9 cm. In the 80 mM NaCl concentration there was a significant reduction of the chervil seedling height (2.3 cm) compared to the control, while in higher NaCl concentrations it was even more reduced (2.0 cm at 120 mM NaCl, 0.8 cm at 180 mM NaCl, 0.4 at 240 mM NaCl). The height of the chervil seedlings in the substrate with 1% and 2% chitin was significantly lower (2.5 and 2.4cm), while with 3% chitin it was the highest (3.1 cm) and with 4% it was still significantly lower that the corresponding in $H_2O$ (2.9 cm). The height of the chervil seedlings in the substrate with 100, 200 and 500 ppm $GA_3$ was higher, while in the 1000 ppm $GA_3$ substrate it was lower than the corresponding height in $H_2O$ (Fig 1). The greatest relative growth rate was observed in the highest NaCl (240, 180 mM NaCl) and $GA_3$ (1000 ppm) concentrations, whereas the least relative growth rate was found in the 3% chitin substrate.

Furthermore, no statistical differences were recorded for the relative seedling growth rate in the substrates with 1%, 2%, 4% chitin and with 80 and 120 mM NaCl concentrations to the respective one of the control. The relative seedling growth rate in the substrate with 100, 200 and 500 ppm $GA_3$ was lower than the corresponding one in $H_2O$ (Table 1). In the substrate that contained 80 mM NaCl the addition of chitin or $GA_3$ resulted in the increase of the seedling height in the combinations of 80 NaCl+1% Chitin, 80 NaCl+200 $GA_3$ and 80 NaCl+500 $GA_3$ compared to the seedlings height in $H_2O$, while a reduction was observed in the rest of combination. The addition of chitin or $GA_3$ in the 120 mM NaCl substrate resulted in the increase of the seedling height in the combinations of 120 NaCl+1% Chitin and 120 NaCl+100 $GA_3$, compared to the height of the seedlings in $H_2O$. High salinity (180 and 240 mM NaCl) in combination with chitin or $GA_3$ in the substrate resulted in the reduction of

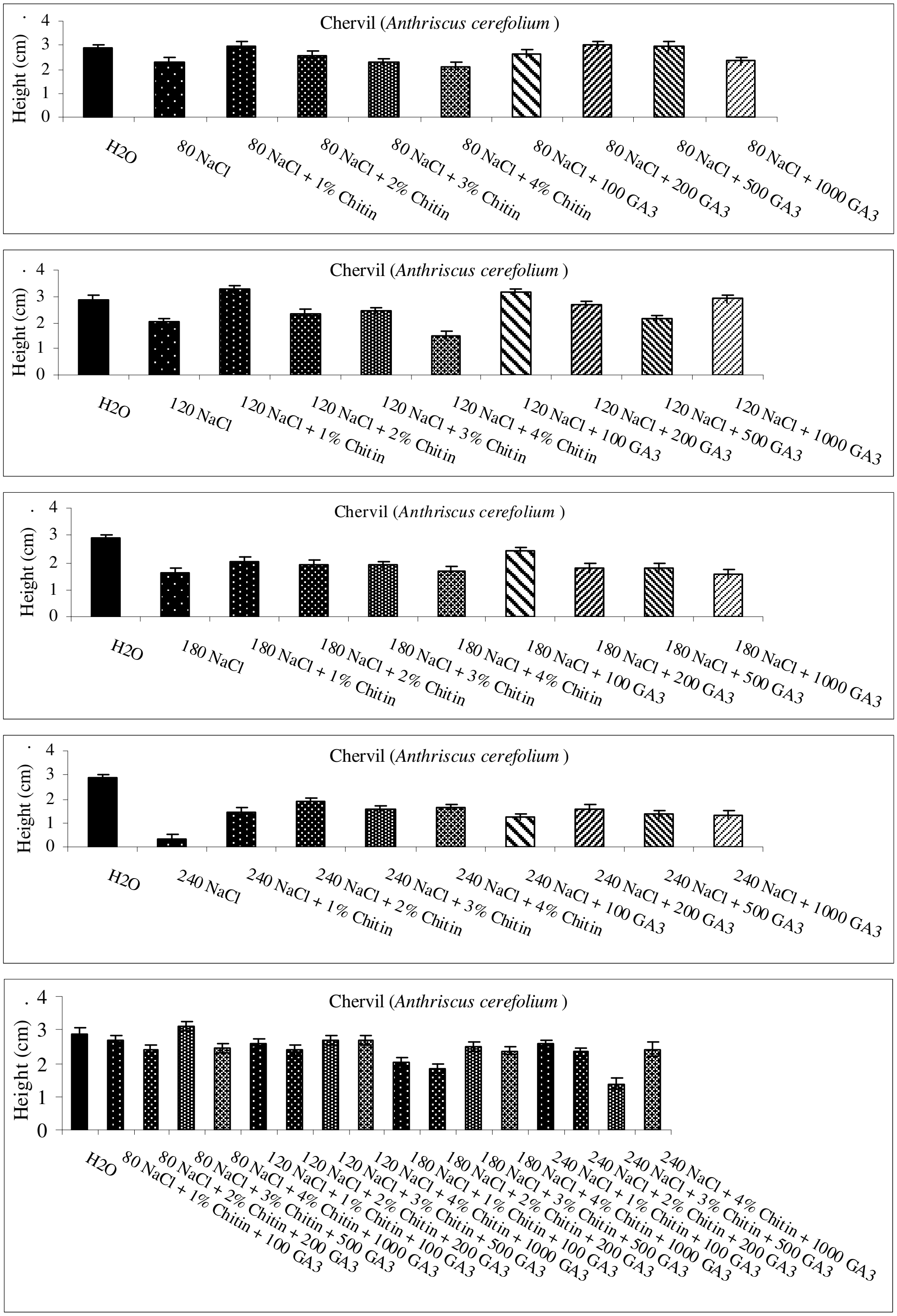


**Fig 2.** Height of chervil (*Anthriscus cerefolium*) at different NaCl, chitin and $GA_3$ concentrations (±s.e.).

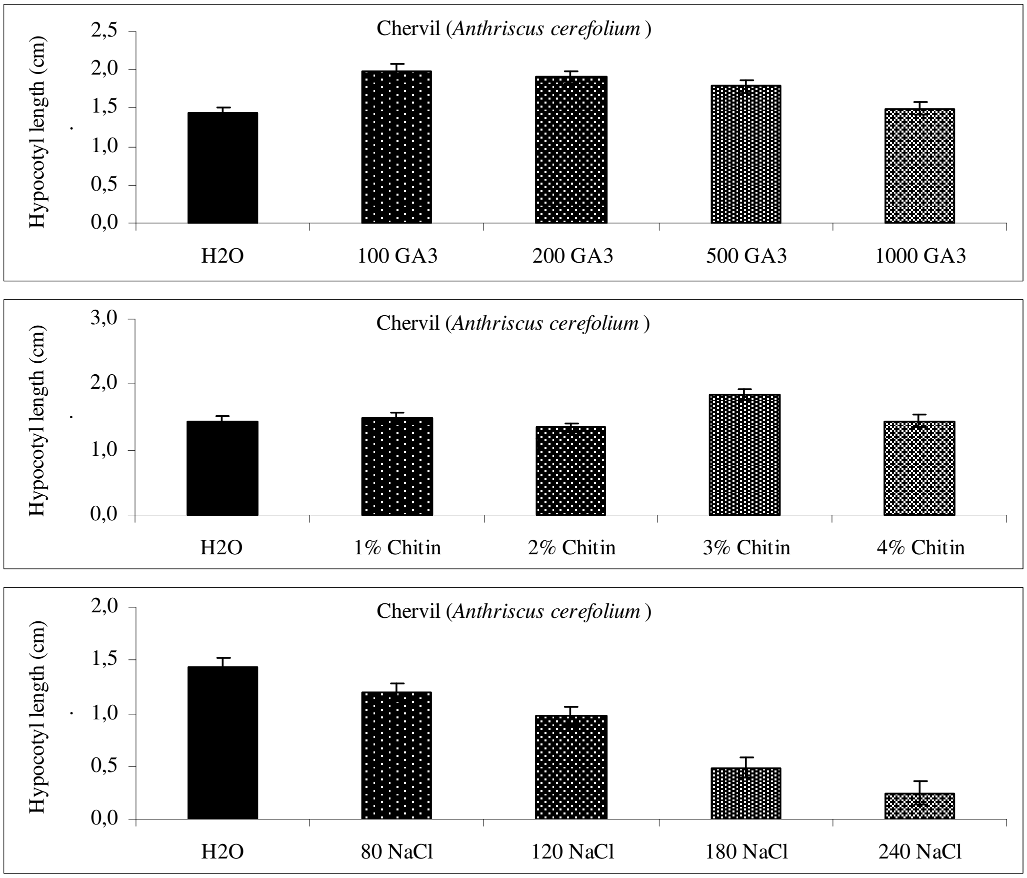


**Fig 3.** Hypocotyl length of chervil (*Anthriscus cerefolium*) at different NaCl, chitin and $GA_3$ concentrations (±s.e.).

the seedlings height in all combinations compared to 240 mM NaCl (Fig 2). In the substrate that contained NaCl the addition of chitin and $GA_3$ resulted in the reduction of the seedling height in all combinations when compared to their respective height in $H_2O$, save for the combination that contained 80 NaCl+3% Chitin+500 $GA_3$, where it was significantly higher than in $H_2O$.

***Hypocotyl length***

The NaCl concentration significantly reduced the length of the hypocotyls of the chervil seedlings compared to the corresponding hypocotyl length of the control's seedlings. The reduction of the hypocotyl length increased along with the increase of the NaCl concentration. The hypocotyls length of the chervil seedlings was increasing as the $GA_3$ concentrations were increasing compared to the control. The hypocotyls length of chervil in the substrate with 3% chitin was significantly higher (1.8 cm) than the corresponding germination in $H_2O$ (1.4 cm) (Fig 3). In the substrate that contained 80 mM NaCl, the addition of chitin or $GA_3$ resulted in the increase of the chervil hypocotyls length in the combinations of 80 NaCl+1% Chitin, 80 NaCl+2% Chitin and 80 NaCl+200 $GA_3$ and 80 NaCl+500 $GA_3$, compared to the chervil hypocotyl length in $H_2O$, while in the rest of the combinations a reduction was observed. The addition of chitin or $GA_3$ in the substrate that contained 120 mM NaCl resulted in the increase of the seedlings' hypocotyls length in the combination of 120 NaCl+1% Chitin and 120 NaCl+100 $GA_3$ and 120 NaCl+1000 $GA_3$, compared to the hypocotyls length in $H_2O$. High salinity (180 and 240 mM NaCl) in the substrate in combination with chitin or $GA_3$ resulted in a reduction of the hypocotyls length of the seedlings in all combinations, when compared to $H_2O$ (Fig 4).

***Roots length***

The NaCl concentration significantly reduced the root length of the chervil seedlings when compared to the corresponding roots length of the control. The reduction of the roots length increased along with the increase of the NaCl concentration. The root length of the chervil seedlings was redusing as the $GA_3$ concentrations were increasing, compared to the control. At the high concentration of 1000 ppm $GA_3$ only the hypocotyl length of the chervil was reduced.The root length of the chervil seedlings was reducing as the percentage of chitin was increasing when compared to the control (Fig 5). All salinity treatments (80, 120, 180 and 240 mM NaCl) in the substrate in combination with chitin or $GA_3$ resulted in the reduction of the root length of the seedlings in all combinations in comparison to $H_2O$ (Fig 6).

**Discussion**

Although chervil is a fragrant culinary herb, very little attention has been paid to the leafy vegetable chervil. There is lack of knowledge about the reaction of chervil to salinity. Many plants react differently to different types of salt. The results of this study showed that the seedling growth was affected by salinity and the effect varied depending on the

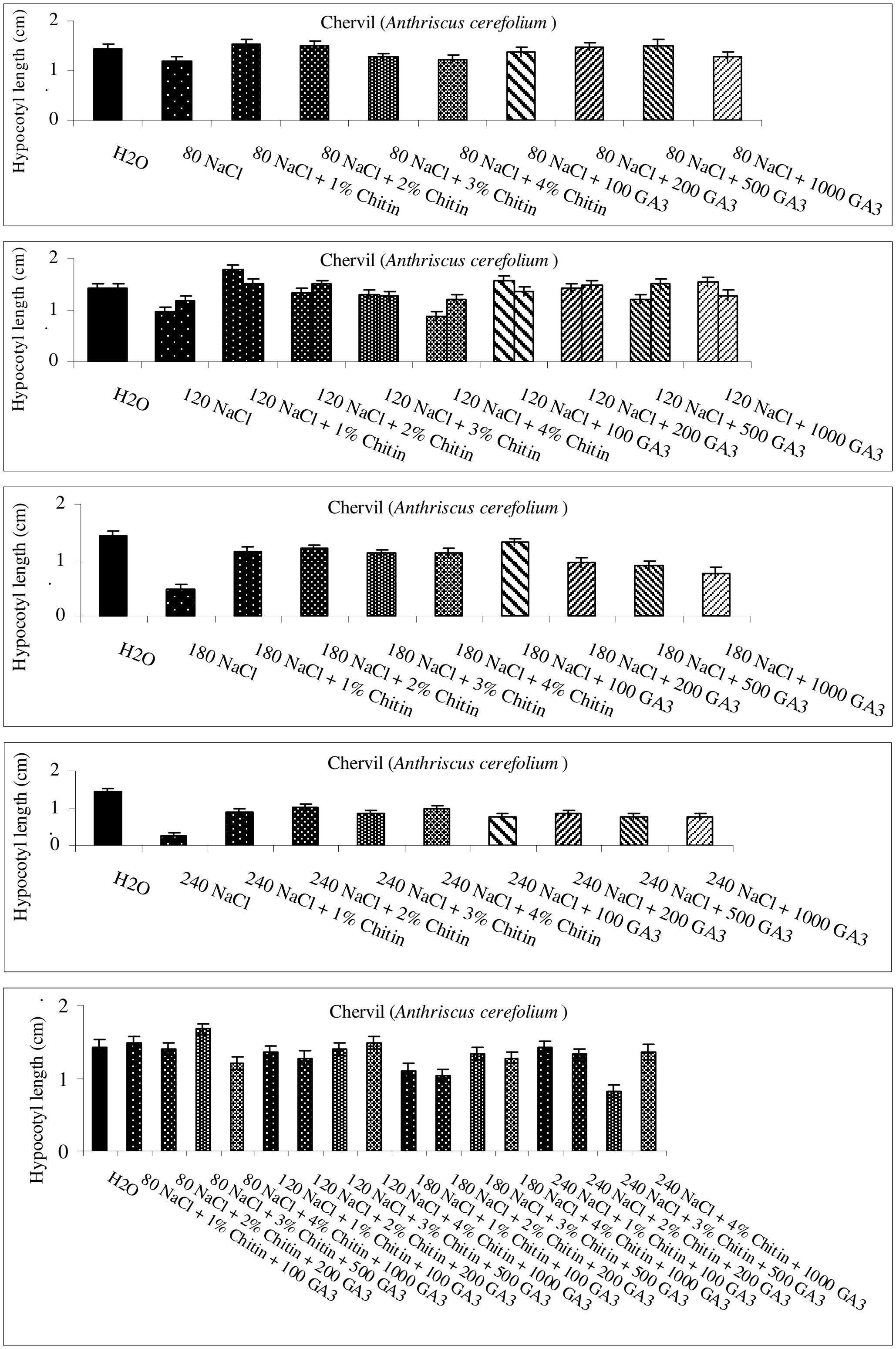


**Fig 4.** Hypocotyl length of chervil (*Anthriscus cerefolium*) at different NaCl, chitin and $GA_3$ concentrations (±s.e.).

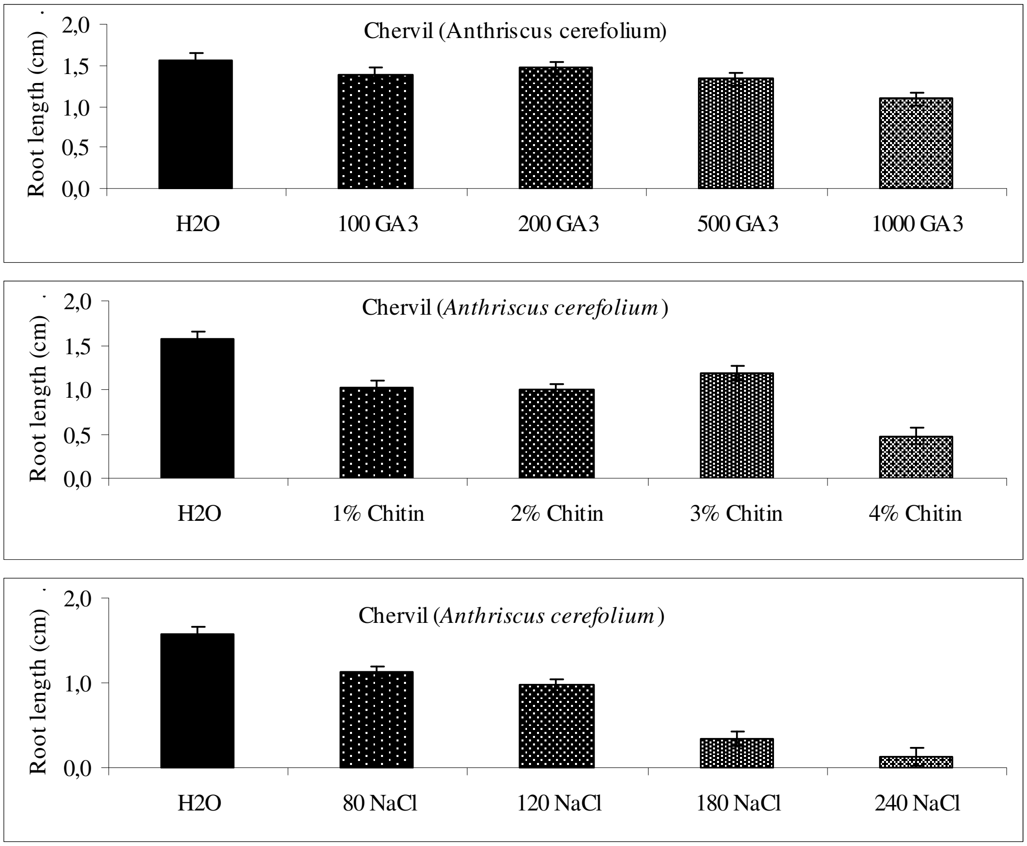


**Fig 5.** Roots length of chervil (*Anthriscus cerefolium*) at different NaCl, chitin and $GA_3$ concentrations (±s.e.).

salinity level. In this study the different measured parts of chervil decreased with the increasing salinity. It can therefore be considered as a salt-sensitive plant. The results of this study showed that the effect of 3% chitin in the substrate had a positive effect on the aboveground parts hypocotyl length of chervil but did not affect the root, and the effect of 1, 2,

4% chitin decreased the seedling growth. The present study indicated that 1% chitin addition in lower concentrations of NaCl (80 and 120 mM) in the substrate increased the seedling growth significantly in chervil. This result showed that the increasing chitinolytic activities in the substrate, which improved with 3% chitin presence, might be a major factor in the increase of plant length. But the results of this study are not in agreement with the results of D' Addabbo (1995) who mentions that when chitin concentration in the soil exceeds 1%, it has a phytotoxic effect. The relations between the chitin addition and the growth features of the plants are obviously complex and hard to assess. Several reports have indicated that $GA_3$ application on crops produced some benefit in alleviating the adverse effects of salt stress (Xiong and Zhu, 2002). Shah (2007) studied the effects of $GA_3$ on growth of salt-stressed mustard. The application of $10^{-5}$ $GA_3$ appeared to mitigate the adverse effects of salinity stress on the overall performance and productivity of mustard. Chakrabarti and Mukherji (2004) investigated the efficiency of pretreatment as foliar spray of gibberellic acid, in restoring the metabolic alterations imposed by NaCl salinity in *Vigna radiata.* The used $GA_3$ was able to overcome the adverse effects of stress imposed by NaCl to these parameters to variable extents. This study showed that the addition of 200, 500 ppm $GA_3$ in the lower 80 mM NaCl substrate alleviated the inhibitory effects of salinity on chervil growth and increased the seedling height. In conclusion, our study show that the 100 to 500 ppm GA3 treatments increased seedling height as well as 3% chitin, even though D' Addabbo (1995) mentions that if chitin concentration in the soil exceeds 1%, it has a phytotoxic effect. However, more research is needed to confirm these results.

## Materials and methods

### *Plant material and growth conditions*

Seeds of chervil (*Anthriscus cerefolium*) were used in this study. Fifty seeds were placed on filter paper in 10cm Petri dishes and moistened with 5ml of distilled water (control) or with an equal quantity of the respective test solution. The following treatments were designed: A) $H_2O$ (control), B) 80, 120, 180, 240mM NaCl solution, C) 1, 2, 3, 4% (w/v) chitin (Sigma C7170) D) 100, 200, 500, 1000ppm $GA_3$ solution ($GA_3$, Sigma-Aldrich) respectively, E) 80 mM NaCl +1%, 2%, 3%, 4% chitin and 80 mM NaCl +100, 200, 500, 1000 ppm $GA_3$ F) 120 mM NaCl +1%, 2%, 3%, 4% chitin and 120 mM NaCl +100, 200, 500, 1000 ppm $GA_3$ G) 180 mM NaCl +1%, 2%, 3%, 4% chitin and 180 mM NaCl +100, 200, 500, 1000 ppm $GA_3$ H) 240 mM NaCl +1%, 2%, 3%, 4% chitin and 240 mM NaCl +100, 200, 500, 1000 ppm $GA_3$. Three dishes for each treatment were placed in completely randomized design in a controlled plant growth chamber at a 24h photoperiod, 12 klx light intensity, 22 ± 1°C temperature regime, and 70 ± 5% relative humidity. Distilled water or test

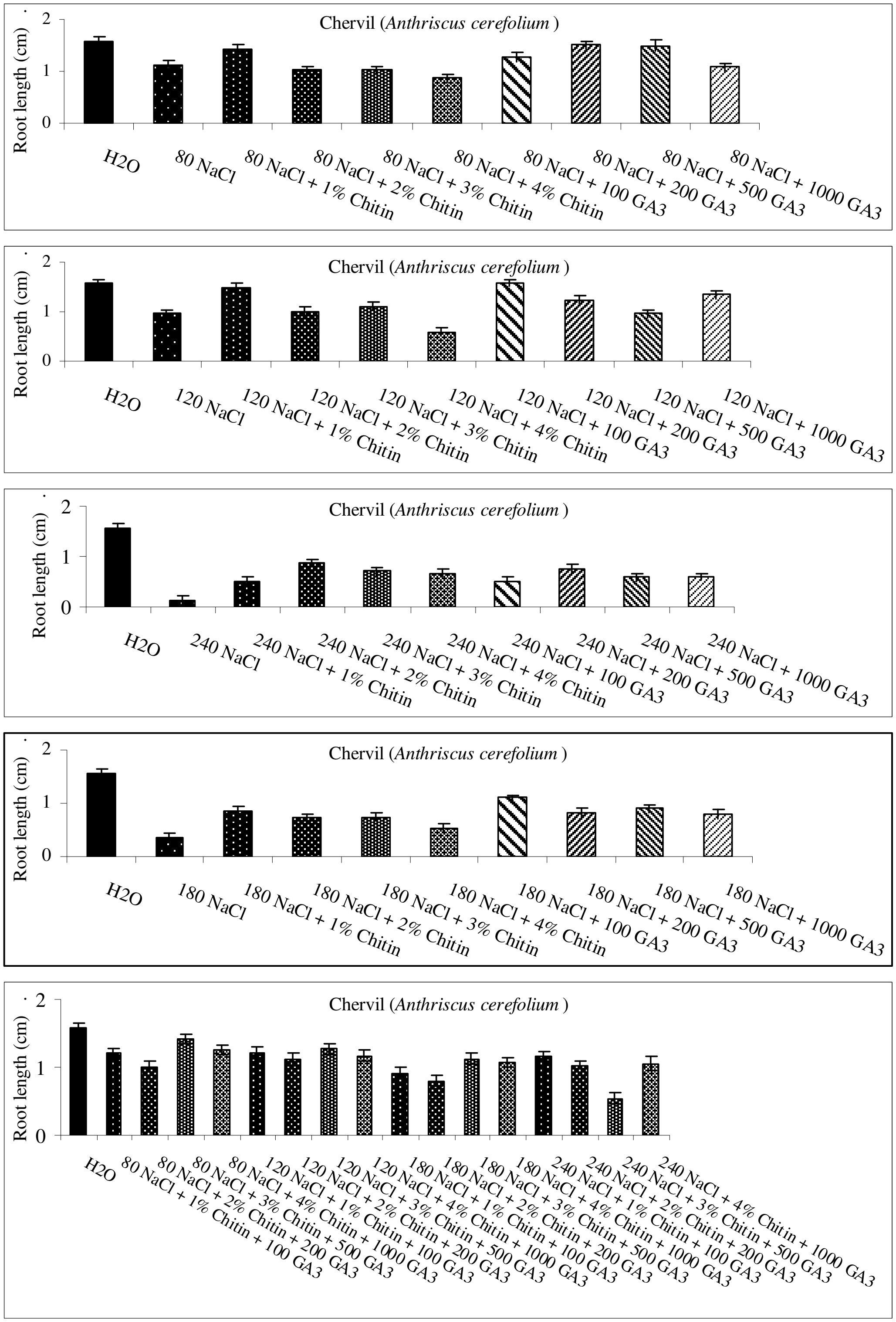


**Fig 6.** Roots length of chervil (*Anthriscus cerefolium*) at at different NaCl, chitin and $GA_3$ concentrations (±s.e.).

solutions were added to each Petri dish during the experiment, according to their water requirements. The seedling length was recorded every five days, starting from day 9 after the seeds were initially placed on the Petri dishes. The seedling length was measured with the help of a measuring tape in cm with an 1 mm accuracy. The experiment was conducted three times.

***Data analysis***

In growth analysis, relative growth rate (RGR) is calculated as

$$RGR = \frac{(\ln L2 - \ln L1)}{(t2 - t1)},$$

where $L_1$ and $L_2$ is the seedling length at times $t_1$ and $t_2$ at 7-day intervals (Hunt, 1982). The data analysis for the chervil (*Anthriscus cerefolium*) seedling growth in different levels of salinity, chitin and $GA_3$ was performed according to the randomized complete block design. The means of the examined traits were ranked according to Duncan's multiple range test and the Post Hoc comparison was used alternatively with the Student-Newman-Keuls (SNK), Dunnett and Tukey methods.

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