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RESPONSE OF AZOLLA GROWTH AND NITROGENASE ACTIVITY TO SALINITY STRESS

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ABSTRACT

A greenhouse experiment was carried out for 2 weeks to test the effect of different levels of NaCl on the growth and nitrogenase activity (ARA) of *Azolla filiculoides*. Results indicate that *Azolla filiculoides* can tolerate salinity up to 20 Mm NaCl. Increasing the concentration of NaCl to 40 and 50 mM strongly inhibited the growth of *Azolla* (fresh and dry weight). *Azolla* was killed within two weeks in presence of 50 mM NaCl. Due to the nitrogen content of *Azolla* and nitrogenase activity (ARA), the effect of NaCl was harmful.

Increasing salt concentration up to 40 mM NaCl caused Nitrogen content of *Azolla* fronds to be gradually decreased ARA decreased with increasing NaCl concentration and recorded the lowest nitrogenase activity in presence of 50 mM NaCl.

INTRODUCTION

An *Azolla- Anabaena* association is the favorite biofertilizer of crops, especially in rice fields because of its ability to fix dinitrogen at high rates and low cost. In addition, Azolla is suitable candidate as animal feed, water purifier, biogas producer and suppressor of weeds. The dinitrogen- fixing ability of the association is due to the cyanobiont *Anabaena- azollae* that inhabits the dorsal leaf lobe cavity of the host *Azolla* (Peters, 1978).

In recent years, many reviews have been written on the biology, uses, factors affecting the growth and other various aspects of *Azolla* (Wagner, 1997; Rai *et al.*, 2001 and Rai and Rai 2003), but no mention is made of the response of *Azolla* to salt toxicity. Soil salinity has become a serious problem for global agricultural productivity. Growth of crop plants inhibited at salinity of 50mM and above (Downton, 1984). Water supplies always contain some dissolved salts, which on evaporation over years become more and more concentrated in the upper strata of the soil resulting in its deterioration and ultimately making it unsuitable for crops. Reduction in crop yield due to secondary salinization in many developing countries has encouraged efforts to improve salt-tolerance in crop plants. In view of the paucity of information available on the salt tolerance of *Azolla* (Moore, 1969; Zimmerman, 1985 and Ghazal, 1988), the present work describes growth behavior (fresh & dry weight), nitrogen content and nitrogenase activity (ARA) of *Azolla filiculoides* under the influence of different levels of sodium chloride salt at different incubation periods.

MATERIALS AND METHODS

An *Azolla filiculoides* strain was obtained through Prof. Dr. F.M. Ghazal, Agric. Microbiol. Dept. Soils, Water & Environ. Inst., Giza, Egypt. This strain was propagated the greenhouse experiment to study the effect of

salinity stress on *Azolla* growth, its nitrogen content and nitrogenase activity. One gram fresh *Azolla filiculoides* inoculum was grown in 750 mL of two fifth modified Hogland's solution (EL-Aggan, 1982) in round plastic pots 14 cm diameter and 7 cm depth. Sodium chloride was then, added to *Azolla* pots at different concentrations of 10, 20, 30, 40 and 50 mM. Each treatment was repeated in four replicates. *Azolla* fresh weight, dry weight, nitrogen content and nitrogenase activity (ARA) were determined at different incubation periods of 0, 3, 6, 9, 12 and 15 days of growth. At the end of each incubation period, *Azolla* fronds were collected, washed 6 times with distilled water to remove occluded salts, then put in a sieve to drain water, blotted on tissue papers and then weighed to certain the *Azolla* fresh weight. Dry weight was measured after oven drying at 65ºC for 48 hours. Nitrogen content percentage of the dried *Azolla* fronds was estimated by conventional macro-Kjeldhal technique (Bremener and Edwards, 1965) using 10.00 g K_2SO_4, 1g $CuSo_4$. $5H_2O$ and 0.1g Se as digestion agent. The distillate was collected in 10 mL boric acid indicator. Ammonia was determined by titration with 0.01 NH_2SO_4. Nitrogenase activity in the term of acetylene reducing activity (ARA) of the fresh *Azolla* fronds was measured using the method described by Hardy *et al.* (1973). Nitrogenase activity (ARA) is presented as μmole C_2H_4 g^{-1} fresh weight *Azolla* h^{-1}.

RESULT AND DISCUSSION

NaCl was added with different concentrations to *Azolla* growing pots to study the effect of salinity stress on *Azolla filiculoides*. Sodium chloride at concentrations of 0, 10, 20 and 30 mM had no effect on *Azolla* fresh or dry weight compared to the control treatment (Table 1).

All salt concentrations caused all the measured parameters to be less than that of the control (Table 1) at all incubation periods. The highest fresh and dry weights of 4.81 and 222.00 g pot^{-1} and mg pot^{-1} were obtained due to the pots received 10 mM NaCl (15 days incubation period). Afterwards the fresh and dry weights of *Azolla* started to decline with increasing the salt concentrations over 30 mM at all incubation periods. Increasing the concentrations to 40 and 50 mM strongly inhibited the growth of *Azolla* (fresh and dry weights). *Azolla* was killed within two weeks in 50 mM salt. In this concern, Rai andRai (2003) reported that inclusion of 20mM NaCl into *Azolla pinnata* growth medium led to reduce its dry weight and chlorophyll content. They explained that salt stress causes an earlier decrease in chlorophyll content, Chl, a/b ratio and whole chain electron transport activity. This has consequently resulted in growth reduction of *Azolla*.

Due to *Azolla* nitrogen content and nitrogenase activity (ARA), the effect of NaCl was harmful. Increasing NaCl up to 40 mM caused *Azolla* nitrogen content to be gradually decreased gradually (Table 1). A sharp decrease occurred with 50 mM at 15 days resulting in *Azolla* death.

ARA was affected by NaCl at any concentration. The lowest ARA was obtained due to the effect 50 mM NaCl. It continuously decreased with the time and stopped at 15 days which corresponded to the death of *Azolla*. The

other NaCl concentrations (10, 20 and 30 mM) only reduced ARA as compared to the control. The percentage of inhibition at 15 days roughly correlated with NaCl concentration. This harmful effect of salinity stress against *Azolla* nitrogenase activity was confirmed by Rai and Rai (2003), while it was previously explained by Rai *et al.* (2001) who noted that NaCl induced inhibition nitrogenase activity is reported to be due to disturbed electron transport, loss in ability of the heterocyst symbionts to protect nitrogenase from oxygen due to disturbed plasmamembrane permeability, inadequate supply of reductants and energy (ATP).

Table (1): Effect of different levels of NaCl on *Azolla filiculoides*

NaCl mM	Days of growth				
	3	6	9	12	15
Fresh weight (g pot^{-1})					
(0) control	1.76	3.34	3.77	5.97	6.24
10	1.38	1.2	2.52	4.38	4.81
20	1.33	1.68	2.14	4.12	4.19
30	1.26	1.52	1.99	3.10	3.93
40	1.22	1.42	1.71	2.12	0.94
50	1.12	1.23	1.42	1.21	0.00
Dry weight (mg pot^{-1})					
(0) control	83.3	108.3	182.00	271.00	293.00
10	66.00	95.00	118.30	201.80	222.00
20	61.50	81.50	100.50	186.50	192.50
30	58.50	70.00	95.80	145.80	182.50
40	55.00	66.00	78.00	99.80	94.50
50	52.00	57.50	62.50	49.00	0.00
Total nitrogen (%)					
(0) control	3.05	3.4	3.8	4.23	4.48
10	2.30	2.93	2.87	3.06	3.29
20	2.88	2.84	2.77	2.81	3.13
30	2.93	2.79	2.61	2.60	2.53
40	2.64	2.58	2.51	2.14	2.03
50	2.52	2.41	2.24	1.85	0.00
ARA (μmole C_2H_2 g^{-1} FW h^{-1})					
(0) control	2.65	7.42	7.54	8.83	9.26
10	2.80	1.70	3.43	4.51	5.71
20	3.22	3.02	4.13	2.24	2.13
30	1.74	3.82	2.15	1.15	4.72
40	1.65	3.26	1.82	1.41	1.91
50	0.59	1.31	0.72	0.53	0.00

***Azolla* inoculum at initial time**
Fresh weight = 1 g
Dry weight = 47 mg
Total N (%) = 2.57
Nitrogenase activity = 2.4 μmole C_2H_2 g^{-1} FW-*Azolla* h^{-1}

Generally, in the cumulative review, Moore (1969) indicated that *Azolla* species were killed within three weeks in Knop's solution (1500 ppm salt). Haller *et al.* (1974) found that *A. caroliniana* grew in a sea and pond water mixture of 3000 ppm solute and was not killed by a salt content of 16.000 ppm. Holst and Yoop (1979) reported that 2000 ppm did not affect significantly the growth of *Azolla* and at 4000 ppm, growth was slightly reduced. They also (1979a) noted that no reduction in nitrogenase activity of *Azolla* grown with up to 8000 pmm NaCl. Most plants can tolerate low salinity about 10 –20 % sea water (about 50- 100 mM NaCl) (Downton, 1984), but *Azolla pinnata* is unable to survive even 40 mM external NaCl. Same results were observed by Johanson (1985) who reported that 10 meq L^{-1} NaCl stimulated *Azolla* growth and 50 meq L^{-1} Nacl depressed the growth (86% of the control dry weight value) while at 100 meq L^{-1}NaCl the growth decreased to 42% of the control value.

The present results are comparable to the observations collected from the literatures; Zimmerman (1985) found that 25 and 85 mM NaCl was toxic to *Azolla* sp. and *Azolla mexicana*, respectively. *Azolla pinnata* R. Br., reported to tolerate 40 mM NaCl in the medium with 73 % reduction in the yield, was unable to survive 50 mM NaCl (Rajrathinam and Padhya, 1989). *Azolla pinnata* is an extremely NaCl-sensitive plant and can not tolerate an external NaCl concentration beyond 30 mM (Vanda and Ashwani, 1999). However, it could be concluded that *Azolla filiculoides* is very sensitive to salt presence in their natural growing habitat.

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استجابة نمو الازولا ونشا ط انزيم النيتروجينيزلاجهاد الملوحة

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أجريت تجربة فى الصوبة لمدة ١٥ يوم لدراسة تأثير تركيزات مختلفة من كلوريد الصوديوم (صفر، ١٠، ٢٠ ، ٣٠ ، ٤٠ ،٥٠ ميلليمولر على نمو الـ *Azolla fliculoides* وكذلك على نشا ط انزيم النيتروجينيز. ولقد اوضحت النتائج مايلى:

١- أن الأزولا تستطيع تحمل الملوحة حتى تركيز ٢٠ ملليمولر من كلوريد الصوديوم.

٢- زيادة تركيز كلوريد الصوديوم ٤٠ و ٥٠ ملليمولر أدى الى انخفاض حاد فى نمو الأزولا تسبب فى موتها تماما بعد اسبوعين.

٣- كان تأثير الملوحة على محتوى الأزولا من النيتروجين وكذلك نشا ط انزيم النيتروجينيز ضارا.

٤- لقد انخفض محتوى الأزولا من النيتروجين تدريجيا بزيادة كل من تركيز كلوريد الصوديوم حتى ٤٠ ميلليمولر وكذلك فترة التحضين.

٥- لقد انخفض نشا ط انزيم النيتروجينيز بزيادة تركيز كلوريد الصوديوم حتى٥٠ ميلليمولر وكذلك بزيادة فترة التحضين.