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Effect of NaCl salinity on the rate of germination, seedling growth, and some metabolic changes in four plant species (umbelliferae)

Wirkung von NaCl-Versalzung auf Keimung, Sämlingswachstum und einige Stoffwechselparameter bei vier Pflanzenarten (Umbelliferae)

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The effect was studied of different salinity levels (up to 280 mM NaCl) on the germination, seedling growth, dry matter, and some related metabolic parameters of anise (*Pimpinella anisum* L.), caraway (*Carum carvi* L.), coriander (*Coriandrum sativum* L.), and cumin (*Cuminum cuminum* L.). During germination, anise tolerated salinity up to 160 mM NaCl, coriander up to 200 mM NaCl, and caraway and cumin tolerated salinity up to 240 mM NaCl. Seedling growth and dry matter production were decreased by NaCl greater than 80 mM NaCl in anise and coriander or greater than 120 mM NaCl in caraway and cumin. The soluble protein in anise and coriander, and the soluble carbohydrates in caraway and cumin remained unchanged uner low and moderate levels of NaCl. However, with higher salinity levels, different effects were recorded. Salinity induced a marked progressive increase of proline and other amino acids in anise and coriander. In caraway and cumin the increases in proline were accompanied by losses in other amino acids. The respiration rate was uneffected in anise and coriander up to 80 mM NaCl, or up to 120 mM in caraway and cumin. Above these values it increased progessively with increased salinity.

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1 Introduction

It has been generally recorded that salinity affects seed germination and seedling growth as well as some relevant metabolic processes of glycophytic plants (FLOWERS et al. 1977, AHMED et al. 1983, KHAN and NAQVI 1984, DROSSOPOULOS 1987, HAMPSON and SIMPSON 1990, SHADDAD and ZIDAN 1989, HARDEGREE and EMMERICH 1990, SCHMIDHALTER and OERTLI 1991). However, the trends and magnitudes of these changes varied according to the level and duration of salinization treatment as well as the plant species used. This variation in species response and the need to select some of our economic plants for cultivation in saline soils necessitated a series of investigations to test their ability to tolerate salinity and to adapt their physiological activities under salinization treatments. Plants apparently rely on several mechanisms by which they adapt to salinity stress. These include the accumulation of organic molecules like soluble carbohydrates, soluble proteins, proline, and possibly other compounds, which may act as non-toxic cytoplasmic osmotica in various salt-tolerant plants (FLOWER et al. 1977, MARCUM and MURDOCH 1992, MUNNS et al. 1979, SHADDED and ZIDAN 1989). Similarly, HANDA et al. (1983) using cultured tomato cells adapted to water stress, found that the concentration of reducing sugars in the cells increased the degree of adaption to salinity concentration as high as 600 mM in the cells. The aim of the present investigation is to provide information on the dees germination, seedling growth, and some metabolic changes in 4 plant species from the Umbelliferae family (anise, cumin, coriander, caraway) grown under different salinization levels.

2 Material and methods

The effect was studied of osmotic stress on the seed germination and seedling growth of anise (*Pimpinella anisum* L.), cumin (*Cuminum cuminum* L.), coriander (*Coriandrum sativum* L.), and caraway (*Carum carvi* L.). The following osmotic stresses were used: 0.0 (control), 40, 80, 120, 160, 200, 240, and 280 mM in 1/10 Hoagland solution (HOAGLAND and ARNON 1950).

Seed germination

The seed of the etest species were pretreated with 10% clorox (5.23% sodium hypochlorite) for 4 min. 25 seeds germinated in 9-cm petri dishes containing 2 layers of filter paper and 10 ml salt solutions as described above. 3 replicate petri dishes were prepared for each treatment and placed in a controlled-temperature room at 25 ± 2°C. Sedds were considered to have germinated after the radicle emerged from the testa.

Seedling development

The seeds were pretreated in the same manner as described for the germination experiment. 25 seeds were placed between folded paper towels, covered by plastic film, rolled up, and placed upright in 600 ml beakers. 80 ml solution were used to saturate the towels in each of the treatments. The beakers were placed for 10 days in a controlled-temperature room at 25 ± 2° C, under illumination from both flouorescent and incandescent lights for 10 h/d. Distilled water was added as needed to compensate for evaporation loss. At the end of the experiment, the lengths of shoots and roots of the test plants were recorded by ruler. The fresh shoots and roots were then dried at 70° C until a constant weight was reached. Free proline was determined in the seedlings (10-day old) according to the method described by BATES et al. (1973). Total free amino acids other than proline were extracted from the plant tissues and determinated according to the method of MOORE and STEIN (1948). For the determination of carbohydrates, the anthrone-sulphuric acid method (FALES 1951) was used. Soluble protein was determined according to LOWRY et al. (1951). The dark respiration rate was measured using the manometric technique developed by WARBURG (UMBRETT et al. 1959). The data of all experiments were subjected to analysis by the least significant difference test (L.S.D.).

3 Results

The final germination percentage of anise was up to 160 mM NaCl in anise, upto 200 mM NaCl in coriander, and 240 mM NaCl in caraway and cumin. Above these

Tab. 1: Effect of various concentrations of NaCl salinity on the germination of anise, coriander, caraway, and cumin seeds (Data expressed as % of control)

	Anise			Coriander			Caraway			Cumin		
NaCl Mol m^{-3}	24h	48h	72h	24h	48h	72h	24h	48h	72h	24h	48h	72h
0.0	83	100	100	100	100	100	93	100	100	90	100	100
40	62*	100	100	100	100	100	80*	100	100	90	100	100
80	62*	100	100	81*	100	100	80*	90	100	87	100	100
120	48*	75*	100	68*	88	100	72*	92	100	87	93	100
160	25*	75*	100	42*	88	100	70*	84*	100	75*	85	100
200	15*	65*	85*	25*	80*	100	55*	73*	100	50*	80*	100
240	0	0	20*	0	22*	23*	20	73*	100	35*	73*	100
280	0	0	0	0	0	0	0	32	60*	0	28*	35*
L.S.D. at 1%	10	13	14	12	14	16	13	15	18	14	16	17

* Significant difference as compared with the control

values it decreased (Table 1). The high level (280 mM NaCl) completely inhibited the germination of anise and coriander seeds but not on cumin (35% germination) or caraway (60% germination) (Table 1).

Low salinity levels (40-80 mM NaCl) stimulated the shoot and root length and the production of dry matter in caraway and cumin. Thereafter, the values smoothly decreased with the increase of the NaCl concentrations, especially under higher concentrations. In anise and coriander the shoot and root length and dry matter remained unaffected up to 80 mM NaCl. Above that, they decreased sharply (Table 2). The water content of anise and coriander seedlings remained unchanged up to 80 mM NaCl; thereafter an increase was observed (Table 2). In the case of caraway and cumin, the water content of the seedlings remained unchanged up to 120 mM NaCl; above that, it smoothly increased. The soluble carbohydrate content of anise and coriander seedlings remained more or less unaffected up to 80 mM NaCl, after which they decresead with increasing salinity. In the case of caraway and cumin seedlings,

Tab. 2: Effect of various concentrations of NaCl salinity on the shoot and root length (cm/seedling), water content (g/100g dry wt) and dry matter (mg/100 seedling)

Salinity Species	0.0	40	80	mM 120	160	200	L.S.D.(1%)
Anis							
Shoot length	6.20	6.00	6.10	4.50*	4.00*	3.20*	0.55
Root length	8.40	8.10	8.20	6.50*	5.10*	4.20*	0.86
Water content	91.68	91.8	91.68	92.20	92.99	93.50	2.00
Dry matter	8.32	8.2	8.31	7.80	7.01*	6.50*	1.22
Coriander							
Shoot length	5.30	5.20	5.30	3.30*	2.80*	2.20*	1.05
Root length	6.40	6.20	6.30	4.10*	3.20*	2.80*	1.01
Water content	90.58	90.49	90.60	91.47	92.60*	93.20*	1.72
Dry matter	9.42	9.51	9.40	8.53	7.40*	6.80*	1.82
Caraway							
Shoot length	7.30	8.10	8.80	7.00	6.50*	6.10*	0.78
Root length	10.10	10.70	11.30	10.00	9.31	7.50*	1.13
Water content	93.67	93.48	93.10	93.75	93.85	94.1	2.20
Dry matter	6.33	6.52	6.90	6.25	6.15	5.81	0.95
Cumin							
Shoot length	8.20	9.40	10.80	9.50	7.20	6.50*	1.48
Root length	10.20	11.2	12.50	10.00	9.50	8.10*	1.85
Water content	92.75	92.59	92.38	92.85	93.50	94.00	2.72
Dry matter	7.25	7.41	7.62	7.15	6.50	6.00*	1.20

* Significant difference as compared with control seedlings.

all salinity used resulted in a higher content of soluble carbohydrates than found in the control. Moreover, this increase was more pronounced under the moderate levels of salinity (Table 3).

There is a general trend towards an increase in the content of soluble protein in seedlings of anise and coriander seedlings with increasing salinity levels. With caraway and cumin seedlings the soluble protein content remained unchanged up to 120 mM NaCl and then progessively decreased with increasing salinity levels. This decrease was more pronounced in case of cumin than in caraway (Table 3). A higher proline content was associated with a small increase in the other free amino acids in anis and coriander, but with a marked decreased in amino acids in caraway and cumin seedlings (Table 3).

Tab. 3: Effect of various concentrations of NaCl salinity on carbohydrate and protein (mg per g dry wt), proline and other amino acids (m moles per g dry wt)

Salinity			mM				
Species	0.0	40	80	120	160	200	L.S.D. (1%)
Anis							
Carbohydrate	120.4	118.4	119.2	105.4*	98.2*	91.4*	7.22
Protein	115.5	118.3	120.4	124.2*	125.4*	125.6*	8.21
Amino Acids	16.6	16.8	17.5	18.5	18.9*	20.4*	2.01
Proline	5.8	6.8	7.3*	8.5*	12.9*	15.7*	1.21
Coriander							
Carbohydrate	90.7	88.4	91.2	85.4*	79.6*	72.8*	4.33
Protein	120.6	123.5	130.4*	135.4*	136.2*	136.8*	9.22
Amino Acids	18.9	20.2	20.1	20.8	21.3*	22.9*	2.05
Proline	6.7	7.1	8.5*	9.6	10.4*	15.1*	1.31
Caraway							
Carbohydrate	130.3	132.4	145.8*	140.2*	137.7	135.6	8.21
Protein	80.6	83.5	82.4	81.2	75.1*	70.4*	5.01
Amino Acids	30.9	28.7	25.3*	24.9*	21.7*	20.3*	3.41
Proline	3.2	3.80	5.20*	7.8*	8.60*	10.80*	1.22
Cumin							
Carbohydrate	100.2	121.5*	135.4*	130.5*	115.7*	109.8*	8.51
Protein	130.4	133.2	131.2	130.8	120.1*	110.7*	6.31
Amino Acids	20.9	18.8	16.4*	16.2*	15.8*	15.1*	2.72
Proline	7.6	8.2*	8.9*	9.1*	9.5*	10.3*	1.51

* Significant difference as compared with control seedlings.

This reduction was more pronounced in the case of caraway. The respiration capacity of the salinized anise and coriander seeds was unchanged up to 80 mM NaCl (Table 4). Thereafter, the rate of respiration increased sharply with increasing NaCl level.

This increase was more pronounced for anise than for coriander. Similarly, the respiration rate is unaffected up to 120 mM NaCl in the case of caraway seeds and up to 160 mM NaCl in the case of cumin seeds. There is a greater increase in the rate of respiration with the increase of salinity level (Table 4).

Tab. 4: Effect of various concentrations of NaCl salinity on the respiration rate (mM $O_2 \times 10^2$ / g dry wt/h

Species Salinity (mol m^{-3})	Anise	Coriander	Caraway	Cumin
0.0	1.45	2.35	2.55	2.95
40	1.63	2.51	2-68	3.01
80	1.51	2.26	2.45	2.85
120	3.45*	5.35*	2.69	3.15
160	5.53*	7.55*	4.35*	3.05*
200	8.91*	10.52*	7.45*	5.33*
LSD at 1%	0.85	1.05	0.97	0.65

* Significant difference as compared with control seedlings

4 Discussion

The observed tolerance in the rate of germination and the final germination percentage and consequently seedling growth of the 4 test plants subjected to lower and moderate salinization were found to be associated with more or less constant values of water content. However, HEIKAL et al. (1982) and DELL'AQUILA (1992) pointed out that the decrease in final germination percentage was always associated with a decrease in water absorption. This behaviour of the water content was found to be linked with a pronounced accumulation of organic solutes (soluble protein in anise and coriander and soluble carbohydrates in caraway and cumin), which might play an important role in increasing the internal osmotic pressure (compatible solutes). This conclusion is in accordance with FLOWERS et al. (1977) and DROSSOPOULOS et al. (1987). In the case of anise and coriander the losses in protein were accompanied by increases in soluble carbohydrates, but in caraway and cumin, the opposite effect was recorded. This leads to the conclusion that salt tolerance seems to be linked with an equilibrium and interconversion between carbohydrates and nitrogen metabolism, whereas saline injury leads to metabolic disturbance in both components. In accordance with this THAKUR and RAI (1982) have shown that in drought-resistant maize cultivars exposed to osmotic stress more protein was accumulated than in susceptible ones. Similar results were obtained by SING and RAI (1982) with chickpea cultivars. Further evidence was found of the role played by the NaCl supply in modifying some other facts of metabolism to withstand the proline and other free amino acid accumulation in salt-stressed plants. With caraway and cumin the pattern of changes in proline was opposite to that of other amino acids, indicating that the increase in proline is at the expence of other amino acids through an effect of salinity in promoting their conversion (BOGGESS et al. 1976, HANSON et al. 1977). However, in the case of anise and coriander, it appeared probable that the pronounced accumulation of proline was not at the expence of other amino acids. The significance of proline accumulation in response to salt has been contentious. Proline has been suggested to act as a compatible cytoplasmic osmoticum (STEWART and LEE 1974). Moreover, it has been suggested that proline may either enhance salt tolerance or be a symptom of cell damage during salt/water stress (CHANDLER and THOPE 1987). Also SHAH et al. (1990) suggested that proline accumulation is important for cell growth only when a certain level of salt stress is attained, and this level will depend on the presence or absence of other protectant mechanisms in the tissue. The dry matter production of anise and coriander plants remained more or less unaffected up to 80 mM NaCl, while in caraway and cumin this tolerance was observed up to 120 mM NaCl. Moreover, 40 and 80 mM NaCl stimulated a pronounced increase in seedling growth (root and shoot lengths) and dry matter production of caraway and cumin seedlings as compared with the control plants. In support of this increase in dry matter production in response to salinity, AHMED et al. (1980), HEIKAL et al. (1981), and SHADDAD and ZIDAN (1989) recorded a promotion in the dry weight of some salinized plants. Also HOPPER (1982) found an increase in the yield of shoots and roots of *Lolium perenne* L. with increasing NaCl in the solution during the early growth stages. Here it was also found that at salinity levels insufficient to affect the production of dry matter, respiration remained unchanged, whereas at NaCl levels which caused reductions in dry matter, the respiration rate considerably increased (Table 4) Thus the increase in respiration seems to be linked to the suppression of growth. This may be due to a greater energy cost of maintenance under salt stress as a result of requirements for compartmentation and secretion and for repair of cellular damage (SCHWARZ and GAIE 1981, SCHWARZ 1985). Finally it can be said that the tolerance of these 4 experimental plants might be linked to the accumulation of soluble carbohydrates, soluble proteins, and proline, which in turn increased their ability for water absorption under moderate salinity.

Zusammenfassung

Untersucht wurde die Wirkung verschiedener Salzstufen (bis 280 mM NaCl) auf die Keimung, das Sämlingswachstum, die Trockenmasseproduktion und einige Stoffwechselparameter bei Anis (*Pimpinella anisum* L.), Kümmel (*Carum carvi* L.), Koriander (*Coriandrum sativum* L.) und Kreuzkümmel (*Cuminum cuminum* L.). Im Keimstadium tolerierte Anis eine Versalzung bis 160 mM NaCl, Koriander bis 200 mM, Kümmel

und Kreuzkümmel bis 240 mM NaCl. Sämlingswachstum und Trockenmasseproduktion verminderten sich bei Anis und Koriander bei einer Versalzung über 80 mM, bei Kümmel und Kreuzkümmel über 120 mM NaCl. Unverändert blieben bei niedriger und mittlerer NaCl-Versalzung bei Anis und Koriander die löslichen Proteine und bei Kümmel und Kreuzkümmel die löslichen Kohlenhydrate. Höhere Salzstufen ergaben etwas andere Effekte. Versalzung induzierte einen deutlich progressiven Anstieg von Prolin und anderen Aminosäuren bei Anis und Koriander. Bei Kümmel und Kreuzkümmel wurde die Prolinzunahme vom Verlust anderer Aminosäuren begleitet. Die Atmung blieb bei Anis und Koriander bis 80 mM NaCl, bei Kümmel und Kreuzkümmel bis 120 mM NaCl unverändert. Bei höherer Versalzung nahm sie progressiv zu.

M.A. Zidan et M.A. Elewa: L'influence d'une salification de NaCl sur la germination, la croissance des semis et quelques paramètre de métabolisme chez quatre variétés de plante (Umbelliferae)

A été étudiée l'influence de différents stades de salification (jusqu'à 280 mM NaCl) sur la germination, la croissance des semis, la production de matière sèche et sur quelques paramètre de métabolisme chez l'anis (*Pimpinella anisum* L.), le cumin (*Carum carvi* L.), la coriandre (*Coriandrum sativum* L.) et le cumin romain (*Cuminum cuminum* L.). Pendant le stade de germination l'anis a toléré une salification jusqu'à 160 mM NaCl, la coriandre jusqu'à 200 mM, le cumin et le cumin romain jusqu'à 240 mM NaCl. La croissance des semis et la production de matière sèche ont diminué quant à l'anis et la coriandre en cas d'une salification de plus de 80 mM, Quant au cumin et le cumin romain de plus de 120 mM NaCl. Restaient invariées, avec une salification de NaCl basse et moyenne, les protéines soluble chez l'anis et la coriandre, et les hydrates de carbone soluble chez le cumin et le cumin romain. Les stades plus élevés de salification avaient pour résultat des effects un peu différents. La salification induisait une croissance nettement progressive de proline et d'autres aminoacids chez l'anis et la coriandre. Chez le cumin et le cumin romain la croissance de proline était accompagnée de la perte d'autres aminoacids. La respiration restait invariée chez l'anis et la coriandre jusqu'à 80 mM NaCl, chez le cumin et le cumin romain jusqu'à 120 mM NaCl. En cas d'une salification plus haute, elle augmentait progessivement.

M.A. Zidan y M.A. Elewa: Efecto dela salinificación con NaCl sobre la germinación, el crecimiento temprano y algunos parámetros metabólicos en cuatro especies gegetales (Umbelliferae)

Se investigó el efecto de diferentes niveles salinos (hasta 280 mM NaCl) sobre la germinación, el crecimiento temprano, la producción de materia seca y sobre algunos parámetros metabólicos del anis (*Pimpinella anisum* L.), de la alcaravea (*Carum carvi* L.), del cilantro (*Coriandrum sativum* L.) y del comono (*Cumin cuminum* L.). En estado de germinación resistieron el anis una salinificación de hasta 100 mM NaCl, el cilantro hasta 200 mM, la alcaravea y al comino hasta 240 mM NaCl. El anis y el cilantro disminuyeron el crecimiento temprano y la producción de materia seca con una salinificación mayor de 80 mM y la alcaravea y el comino con valores mayores a 120 mM NaCl. Con niveles bajos y medios de salinificación, las proteinas solubles permanecieron invariables en el caso del anis y del cilantro y en el caso de la alcaravea y del comino ocurrió lo mismo con los hidratos de carbono. Mayores niveles de salinidad ocasionaron otros efectos de la prolina y otros aminoácidos en caso del anis y del cilantro. En el caso de la alcaravea y del comino, el aumento de la prolina fué acompañado de una disminución de otros aminoácidos. La respiración permaneció en el caso del anis y del cilantro invariable hasta 80 mM NaCl y en el caso de la alcaravea y del comino hasta 120 mM NaCl. Esta aumentó en forma progresiva cuando se aumentó también la salinidad.

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