CHAPTER 4

SEEDLING GROWTH AND CULTIVAR DIFFERENCES

In this chapter the results of the glasshouse experiment to determine the tolerance of crops to actual mine waters in the seedling growth stage, are presented. The aims of these water culture experiments were firstly to determine the seedling growth response of crops and cultivars to two types of actual 'worst case' mine waters relative to a one third Hoagland control; and secondly, to determine whether the recommended cultivars differed in their tolerance to these waters. The study focusses on $CaSO_4$-dominated mine water with a more traditional NaCl-dominated mine water included for comparison. The crops have been subdivided into two groups: the subtropical or summer annual crops and the temperate or winter annual crops and lucerne.

4.1 INTRODUCTION

Numerous studies have investigated why and how species and cultivars differ with NaCl-dominated salinity. Only in a few cases, however, have crops been evaluated for growth with water where $CaSO_4$ is the predominant salt, but to the knowledge of the writer no literature on seedling growth response or cultivar differences with $CaSO_4$ water exist.

Shannon (1997) suggests that cultivar differences should be seen more clearly in the most sensitive growth stage or stages of a particular crop. The seedling stage has been identified as the most sensitive for cereals and grass forages (Francois & Maas, 1994) (2.6.1). This stage is, however, crucial to all crops as the salinity of the top layer of the soil is subject to rapid concentration changes due to evaporation from the soil surface.

It has been concluded that the main adverse mechanism by which salinity retards the growth of *seedlings* is that of a decreased osmotic potential (Munns, Schachtman & Condon, 1995; Neumann, 1997). Munns (1993) suggested that cultivars reacted similarly to salinity in the seedling stage and that selections for salt tolerance based on this growth stage would be inappropriate. By reviewing work done on seedling varietal differences, Neumann (1997) agrees that early growth inhibition has often been due to the decreased osmotic potential (presumably of NaCl-dominated conditions) and not to toxic or osmotic effects of salt accumulation in the plant. However, he disagrees with the conclusion that there is a lack of genotypic diversity in the early growth response (10 days) to osmotic stress and presents clear evidence for such diversity to salt or poly-ethylene-glycol (PEG) induced osmotic stress. He suggests that genotypic differences during seedling growth could be the result of metabolically regulated responses to osmotic potential. Such mechanisms could include the effect on cell wall plasticity and thus growth (Neumann 1995a, 1997), K-deficiency variations associated with NaCl salinity (Wu, Ding & Zhu, 1996) and the ability to compartmentalise Na (Kingsbury, Epstein & Pearcy, 1984).

In the above arguments the possible influence of nutrient interactions in the seedling stage was, however, not really addressed. The biphasic model of growth response to salinity suggested by Munns (1993) is based on the adverse mechanisms either being decreased osmotic potential in the seedling stage, or of the toxic influences of the accumulation of salts in the plants at later growth stages. The above mentioned metabolic effects are not considered. With a $CaSO_4$ water, additional nutrient effects could be due to the interaction between SO_4 and other anions and/or of Ca with other ions. If Mg is also present in appreciable quantities, detrimental ratios of Mg to Ca may develop by the precipitation of $CaSO_4$ while $MgSO_4$ remains soluble; this may be a nutrient effect that must be considered in irrigation studies with this water, especially in arid areas.

The aim of the experiments reported in this chapter is twofold: to determine firstly, the relative salt tolerance of selected subtropical and temperate crops and cultivars in the seedling growth stage on an actual ‘worst case’ $CaSO_4$-dominated water; and secondly, whether cultivars of the respective crops differ in their tolerance to this water in the seedling growth stage (sowing to Day 18). A NaCl-dominated mine water was also included for comparison to a more traditional type of NaCl salinity.

The method used is described in Chapter 3 (3.2.2.1).

4.2 RESULTS AND DISCUSSION

4.2.1 SUBTROPICAL ANNUAL CROPS

The subtropical annual crops evaluated were maize, sorghum, pearl millet (babala), sunflower, soybean, cowpea and dry bean (*Phaseolus vulgaris* L.).

Cereals and pastures

The seedling growth of several hybrids of **maize** and cultivars of **sorghum** and **pearl millet** were suppressed by the high sulphate mine waters: seedling growth of six of the 18 maize hybrids was significantly decreased by *ca* 30 %; four of the 14 sorghum cultivars by 32-42 %, and the growth of pearl millet cv. SA Standard was greatly decreased in contrast to the high forage cultivar, PAN 911, which grew very well on the sulphate water (Tables 4.1 and 4.2).

There were some significant cultivar differences in all three of these crops: With **maize** the relative growth of the two most tolerant hybrids (CRN 4403 and CRN 3631) was significantly higher than that of the four most sensitive ones (SNK 2151, SNK 2665, PAN 6552 and PAN 6549). This was in contrast to the seedling growth on the NaCl-dominated water where no significant cultivar differences occurred and the growth was decreased to a greater extent (Table 4.1). For **sorghum** only the growth of the most tolerant cultivar (CRN 7686) was significantly higher than the least tolerant (SNK 3860) (Table 4.2). The two **pearl millet** cultivars responded very differently to the high $CaSO_4$ waters; PAN 911 grew very well while the seedling growth of SA Standard was decreased by 68 % (Table 4.2).

TABLE 4.1 The influence of $CaSO_4$- and NaCl-dominated mine waters on the seedling growth of maize hybrids

Maize Hybrids	Dry mass of top growth per 10 plants (g)			c.v. %	Relative growth %	
	Control	Mine A	Mine B		Mine A	Mine B
SNK 2042	3.99	3.19	3.08 *	13.9	bc	a
SNK 2888	4.26	3.62	2.76 *	16.3	abc	ab
SNK 2266	4.43	3.45 *	2.81 *	10.3	bc	ab
SNK 2151	3.87	2.59 *	2.14 *	20.8	c	ab
SNK 2665	4.32	3.11 *	2.21 *	13.9	c	ab
PAN 6480	3.09	2.48	2.3	12.9	abc	ab
PAN 6364	4.14	3.76	2.37 *	20.1	abc	ab
PAN 6552	3.23	2.25 *	1.84 *	13.0	c	ab
PAN 6363	3.65	3.03	1.78 *	7.1	abc	b
PAN 6549	3.11	2.21 *	1.77 *	13.2	c	ab
PAN 6479	2.98	2.38	1.91 *	11.2	abc	ab
CRN 3816	2.43	2.17	1.57 *	8.8	abc	ab
CRN 3414	2.00	1.71	1.37	12.1	abc	ab
CRN 3818	2.37	2.21	1.55	13.0	abc	ab
CRN 3631	3.25	3.59	1.93 *	18.6	110 ab	ab
CRN 4403	4.16	4.66	2.35 *	14.7	a	ab
CRN 4523	3.91	3.54	2.07 *	7.3	abc	ab
SNK 2340[1.]	2.30	1.73 *	1.33	13.7	-	-
c.v. %	14.6				18.7	20.6
LSD_F					33	26

* Significant difference from control ($P < 0.05$)

Mine A 7/94 Mine B 7/94

[1.] This hybrid was not included with Mine A water, but was evaluated with the sorghums on more concentrated water: Mine C water (10/94) EC 402 mS m^{-1}; 2533 mg L^{-1} sulphate and Mine B (11/94) EC 590 mS m^{-1}, 52 mmol L^{-1} Na, 35 mmol L^{-1} Cl and 1135 mg L^{-1} sulphate (Table 3.1).

TABLE 4.2 The influence of $CaSO_4$- and NaCl-dominated mine waters on the seedling growth of sorghum **and** pearl millet **cultivars**

Cultivars	**Dry mass of top growth/10 plants g**			**c.v. %**	**Relative growth %**	
	Control	**Mine C**	**Mine B**		**Mine C**	**Mine B**
SORGHUM						
SNK 3860	1.11	0.64 *	0.37 *	9.5	58 cd	33 cd
SNK 3939	0.79	0.70	0.37 *	14.3	81 abc	43 abc
SENFOR	0.71	0.57	0.33 *	16.2	82 abc	47 abc
SENTOP	0.97	0.66 *	0.47 *	8.8	68 bcd	48 abc
SNK 3000	0.75	0.59	0.36 *	25.6	83 abc	51 abc
PAN 8494	0.67	0.44 *	0.36 *	14.7	66 bcd	55 ab
PAN 8501	0.83	0.55 *	0.32 *	6.8	66 bcd	38 bcd
PAN 8522	0.58	0.56	0.32 *	13.5	97 abc	54 ab
PAN 8564	0.73	0.69	0.36 *	5.0	95 abc	50 abc
PAN 8591	0.89	0.82	0.47 *	6.5	92 abc	52 abc
NK 283	0.90	0.87	0.50 *	12.5	99 abc	55 ab
PAN 888	0.45	0.47	0.23 *	10.7	104 abc	52 abc
CRN 776W	0.73	0.72	0.34 *	12.0	98 abc	47 abc
CRN 7686	0.61	0.62	0.32 *	19.0	105 ab	48 abc
PEARL MILLET						
PAN 911	0.51	0.61	0.11 *	31.5	120 a	22 d
Common	0.82	0.26 *	0.32 *	23.4	32 d	39 abcd
c.v. %	15.7				26.6	19.8
LSD_F					47	19

* Significant difference from control ($P < 0.05$)

Mine B 11/94.

Mine C 10/94.

The decrease of the osmotic potential by salinity has been shown to be the major growth suppressing mechanism for these three crops (Cramer, 1994, maize; Ashraf & Idrees, 1995, pearl millet; Shannon, 1997, sorghum).

Cramer (1994) concluded that the reduction of growth in **maize** by salinity appears to be caused by a reduced leaf area, which seems to be primarily due to an osmotic potential effect. Specific ion effects apparently play a minor role for most saline conditions, but for soil types or irrigation waters with unusual ion ratios it could be a more important growth inhibitory mechanism. Seedling growth up to 21 days was found to be the most sensitive stage for maize (Maas, Hoffman, Chaba, Poss & Shannon, 1983). The decrease of seedling growth for the affected cultivars may therefore be due to the decreased osmotic potential of the lime-treated acid mine drainage water (EC 278 mS m^{-1}). The same could be true for **sorghum** with the Kleinkopje mine water (EC *ca* 400 mS m^{-1}), as osmotic adaptation has also been found to be responsible for differences in the tolerance of sorghum cultivars (Shannon, 1997).

Cultivar differences in **pearl millet** have been attributed to the ability to synthesize organic osmotica and thus also to osmotic adaptation (Ashraf & Idrees, 1995). However, in SA Standard growth decrease could be partly due to the water culture method of screening, as the relative growth in the sand culture experiment (Chapter 5) with water of similar composition was 84 % as opposed to the 32 % in the water culture.The coefficient of variation for the pearl millet cultivars in the water culture was also unacceptably high (Table 4.2).

This phenomenon, that the water culture screening method was more severe than sand culture screening, has also been found with dry bean (Zaiter & Mahfouz, 1993).

The growth of all the **sunflower** cultivars was also severely decreased in the seedling stage with the water culture (Table 4.3). However, SNK 43 sunflower seedlings grew very well on sand culture with a water of similar composition (Chapter 5) – 106% compared to the 58% in the water culture. This could be due to a possible aeration effect, as it was mostly encountered in typical dryland crops.

TABLE 4.3 The influence of $CaSO_4$- and NaCl-dominated mine waters on the seedling growth of sunflower **cultivars**

Cultivars	Mass of top growth/10 plants (g)			c.v. %	Relative growth %	
	Control	Mine C	Mine B		Mine C	Mine B
SNK 43[1]	4.78	2.77 **	2.38 *	9.5	58 abc	50 ab
SNK 34	3.44	2.13 **	1.86 **	2.4	62 ab	54 ab
SNK 37	2.35	1.38 **	1.26 **	12.7	59 abc	54 ab
PAN 7392	3.36	1.82 **	1.52 **	13.5	54 abc	45 ab
PAN 7411	3.94	1.76 **	1.86 **	5.3	45 abc	47 b
PAN 7369	3.39	2.18 **	2.00 **	9.5	64 a	59 a
CRN 1445	3.32	2.03 **	1.56 **	5.2	61 ab	47 b
CRN 543	2.84	1.32 **	1.29 **	17.2	47 abc	46 b
A 1006	4.18	1.81 **	2.08 **	4.6	43 c	50 ab
c.v. %					14.36	9.70
LSD_F	9.2				17	11

* Tendency to differ from control ($P < 0.1$)

** Significant difference from control ($P < 0.05$)

Mine B 7/94

Mine C 10/94

1. SNK 43 seeds were infected with a fungus.

Legumes

Nine recommended **soybean**, one **cowpea** and four **dry bean** cultivars were screened for their tolerance to the actual mine waters in the seedling growth stage. The results are presented in Table 4.4.

In contrast to the cereals discussed above, the $CaSO_4$-dominated water did not significantly affect the seedling growth of the **soybean** cultivars and there were no significant differences between cultivars. The **dry bean** cultivars grew exceptionally well on the high SO_4 water; the seedling growth of three dry bean cultivars, PAN 127, Mkusi and Nandi, were significantly higher than the control with this water while PAN122 was not significantly affected. The relative seedling growth of the most tolerant cultivar (PAN 127) was significantly higher than that of the most sensitive (PAN 122). The seedling growth of **cowpea**, Dr Saunders, seemed to be sensitive and was significantly suppressed by the SO_4-dominated water in these water culture experiments (relative growth 65 %) (Table 4.3).These results are, however, unreliable as the variation was unacceptably high for cowpea. As in the case of pearl millet and sunflower seedlings, growth of cowpea in the sand culture experiment (Chapter 5) with a similar high SO_4 water was not significantly affected, although it did tend to decrease (Figure 5.2).

Soybean has been classified as a moderately tolerant crop with a relatively high threshold value of EC_e 500 mS m^{-1} ($\approx EC_{sw}$ 1000 mS m^{-1}) for yield decrease (Maas & Hoffman, 1977). Sensitivity of soybean is also mainly related to Cl toxicity in the shoots (Abel & McKenzie, 1964; Parker, Gascho & Gaines, 1983). The tolerance of the soybean may therefore be related to these two attributes. It has been possible to breed tolerant cultivars that prevent or restrict the transport of Cl to the shoots; as the seedling growth was not suppressed by the high NaCl-dominated water (52 mmol L^{-1}), these cultivars, that were partly selected because of good performance under irrigation, have probably been bred for tolerance to NaCl salinity.

Meiri & Poljakoff-Mayber (1970) studied the effect of NaCl salinity on the growth, leaf expansion and transpiration of **dry bean**. Retardation of bean growth was found to be dependent on the rate, the ultimate level and the duration of salinity. Growth is mainly suppressed through a smaller leaf area and number of leaves. The adverse effect was due mainly to a reduction in transpiration. However, under constant salinity, beans showed a slight adaptation to saline conditions. Dry bean could also have a low capacity of discrimination of the K-uptake system in the presence of high Na levels, which could account for their sensitivity to NaCl (Benlloch, Ojeda, Ramos & Rodriquez-Navarro, 1994). It has also been found that bean plants adjusted osmotically to salt stress resulting in increased leaf water content and it was suggested that "two major physiological traits enable plants to tolerate salinity: (a) compensatory growth following adjustment to salinity, and (b) the ability to increase both leaf area ratio (LAR) and net assimilation rate (NAR) to achieve this increased growth" (Wignarajah, 1990).

TABLE 4.4 The influence of $CaSO_4$- and NaCl-dominated mine waters on the seedling growth of soybean, dry bean **and** cowpea **cultivars**

Cultivar	**Dry mass top growth/10 plants (g)**			**c.v.**	**Relative growth %**	
	Control	**Mine C**	**Mine B**	**%**	**Mine C**	**Mine B**
SOYBEAN						
1. Bakgat	3.01	2.40	*	8.3	abc	cd
2. Ibis	3.00	2.47	2.42	10.7	abc	abcd
3. PAN 494	2.79	2.53	*	14.2	ab	d
4. PAN 577 G	2.98	2.62	*	5.1	ab	bcd
5. Prima	2.61	2.29	*	14.2	ab	d
6. Hutcheson[1]	(2.15)	(1.78)	(1.44)	21.4	ab	bcd
7. A2233[1, 2]	(3.24)	(3.05)	(3.31)	10.6	ab	a
8. A5409	3.90	3.21	*	7.2	abc	d
9. A7119	2.73	2.11	*	13.2	bc	cd
COWPEA						
1. Dr Saunders[4]	2.87	*	2.13	26.5	65	80
DRY BEAN[5]						
1. PAN 122	6.16	5.12	*	13.4	c	c
2. PAN 127	7.26	*	6.54	5.3	a	a
3. Mkusi	6.78	*	*	5.3	ab	ab
4. Nandi	7.22	*	*	7.5	ab	b

*Significant difference from control ($P < 0.05$)

Mine C 10/94 (soybean & cowpea); 3/95 (dry bean)
Mine B 11/94 (soybean & cowpea); 3/95 (dry bean)

1. Germination affected in all treatments by infections. The seedlings planted were very weak.
2. Fewer plants survived, especially in the control, probably due to infection; more plants survived with the salt treatments.
3. Brackets indicate that growth could have been influenced by infection of the seeds and young seedlings.
4. The number of surviving plants , as well as growth of individual plants, varied. This is probably an indication of sensitivity in the seedling stage
5. Dry beans were evaluated with 15 plants per replicate.

This osmotic compensation followed by compensatory growth may partly be an explanation for the increased growth of bean seedlings on the $CaSO_4$ water. Furthermore, if bean sensitivity to NaCl is mainly due to “a low capacity of discrimination of the K-uptake system”

(Benlloch et al., 1994) in competition to Na, this could further explain the tolerance to a $CaSO_4$-dominated water where NaCl was present at very low concentrations. Another contributing factor to the increased growth compared to that on the one-third Hoagland of the control, could be a positive nutrient effect, as legumes have a high S-requirement (Mengel & Kirkby, 1987).

The relatively good growth of three dry bean cultivars on the NaCl-dominated water may be an indication that these cultivars also have genetic characteristics for salt tolerance such as an increased K-uptake efficiency and/or an increased ability for osmotic adaptation.There have been indications of cultivar differences in the salt tolerance of seedling growth of dry bean (Zaiter & Mahfouz, 1993).

There has been some indication that Cl content of shoots and not that of Na, may be related to salt sensitivity of **cowpea** (Keating, 1986; West & Francois, 1982); if this is the case, tolerance of cowpea seedlings for a $CaSO_4$ water, as is seen in the sand culture experiment (Table 5.9), may be similar to that of soybean. The results for the NaCl actual mine water (Table 4.4) coincide better with the seedling growth with the simulated NaCl mine water on sand culture (Table 5.10), probably because the Cl was the main limiting factor.

4.2.2 TEMPERATE ANNUAL CROPS

The temperate annual crops evaluated in the seedling growth stage were wheat, triticale, rye,ryegrass, oats and barley. Seven cultivars of wheat, seven triticale, four rye, four ryegrass and six of oats were evaluated. One barley cultivar was included for comparison.

Wheat, triticale and rye

The high sulphate water did not significantly affect the seedling growth in 6 of the 7 **wheat** cultivars evaluated; SST 822 was the most sensitive with the growth suppressed by 31 % (Table 4.5). With **triticale** only Kiewiet was significantly reduced by 14 % (Table 4.6), and none of the **rye** cultivars was influenced (Table 4.7). The NaCl mine water significantly suppressed the seedling growth of all the wheat cultivars significantly, ranging from a relative growth of 42 % for SST 822 to 61 % for Marico. The same was true for triticale cultivars (59 to 85 %) and for rye (43 to 54 %).

There were few significant *cultivar differences* with the sulphate water. In the case of wheat, only SST 822, the most sensitive, differed significantly from Inia, the most tolerant; similarly in the case of triticale, Rex was significantly higher than Kiewiet. With the NaCl salinity, the wheat cultivar SST 822 was also the most sensitive, although not differing significantly from the others, while triticale Rex was again significantly higher than Kiewiet (Tables 4.5 and 4.6). Rye showed no cultivar differences with both waters (Table 4.7). During the selection of these cultivars, a pre-screening for salinity tolerance had, however, already been conducted in a sense, as the cultivars were selected for the geographical area where winter cereals are mostly irrigated, which usually presupposes the possibility of salinization.

TABLE 4.5 The influence of $CaSO_4$- and NaCl-dominated mine waters on the seedling growth of wheat **cultivars**

Cultivars	Top growth mass/10 plants (g)			c.v. %	Relative growth %	
	Control	Mine C[1]	Mine B[2]		Mine C	Mine B
1. SST 822	1.56	*	*	23.2	69 b	42 b
2. SST 825	1.63	1.68	*	10.20	103 a	48 ab
3. Palmiet	1.58	1.77	*	12.03	113 a	57 ab
4. Marico	1.27	1.20	*	10.9	95 ab	61 ab
5. Kariega	1.53	1.45	*	8.4	94 ab	55 ab
6. Inia	1.56	1.82	*	15.0	115 a	56 ab
7. Nursecrop	1.46	1.56	*	3.11	107 a	70 a
c.v. %					13.7	20.0
LSD_F	11.5				31	25

TABLE 4.6 The influence of $CaSO_4$- and NaCl-dominated mine waters on the seedling growth of triticale **cultivars**

	Top growth mass/10 plants (g)			c.v. %	Relative growth %	
Cultivars	Control	Mine C	Mine B		Mine C	Mine B
1. Kiewiet	1.66	*	*	9.9	86 a	59 b
2. SShR1	1.43	1.37	*	6.7	97 ab	64 ab
3. Rex	1.40	1.51	*	8.3	108 a	85 a
4. PAN 299	1.33	1.23	*	4.9	92 ab	67 ab
5. SSKR 626	0.97	0.99	*	14.5	103 ab	62 ab
6. SSKR 628	1.15	1.05	*	6.1	91 ab	61 ab
7. Cloc 1	1.10	1.07	*	3.5	97 ab	66 ab
c.v. %	8.2				9.1	14.72
LSD$_F$					19	23

* Significant difference from control ($P < 0.05$)

Mine C 3/95

Mine B 3/95

TABLE 4.7 The influence of $CaSO_4$- and NaCl-dominated mine waters on the seedling growth of rye **cultivars**

	Top growth mass/10 plants (g)			c.v. %	Relative growth %	
Cultivars	Control	Mine C	Mine B		Mine C	Mine B
RYE						
1. SSR 727	0.82	0.75	*	4.9	91 a	42 b
2. SSR 729	0.70	0.68	*	14.1	98 a	54 a
3. SSR 1	0.65	0.65	*	9.8	100 a	42 b
4. Henoch	0.61	0.61	*	19.3	104 a	42 b
c.v.%	13.4				19.1	17.2
LSD$_F$					40	16

* Significant difference from control ($P < 0.5$)

Mine B 3/95 Mine C 3/95

Growth observations of the wheat seedlings, however, indicated a possible toxic NH_4 effect on the control plants (NO_3:NH_4, 2:1). Especially the first emerging leaf of some cultivars was bronze coloured. Growth on the SO_4 water was healthy and showed no signs of bronzing or chlorosis. The ‘apparent’ salt tolerance (2.4.1) may therefore be higher due to possibly suppressed growth of the controls. The wheat cultivars were subsequently rescreened with half the NH_4 and an equivalent increase in NO_3-N, with the same mine water. In this case the controls were a healthy green and the top growth dry masses generally higher than with the higher NH_4 (except for SST 822) (Table 4.8). It was, however, very notable that with the lower NH_4, the seedlings on the $CaSO_4$-dominated mine water were generally very chlorotic in contrast to the healthy green seedlings of the previous evaluation.

TABLE 4.8 The influence of the $CaSO_4$-dominated mine water with different ratios of NH_4 and NO_3 **on the seedling top growth of** wheat **cultivars**

Cultivars	**First screening** **NO_3:NH_4 = 2:1**				**Second screening** **NO_3:NH_4 = 4:1**			
	Top growth mass /10 plants g		**c.v. %**	**Relative growth %**	**Top growth mass /10 plants g**		**c.v. %**	**Relative growth %**
	Control	**Mine C**			**Control**	**Mine C**		
1. SST 822	1.56	1.05	23.2	69	1.48	1.16	1.0	78
2. SST 825	1.63	1.68	10.2	103	1.94	1.23	10.4	63
3. Palmiet	1.58	1.77	12.0	113	1.81	1.21	3.3	67
4. Marico	1.27	1.20	10.9	95	1.65	1.26	0.9	76
5. Kariega	1.53	1.45	8.4	94	1.57	1.21	2.3	77
6. Inia	1.56	1.82	15.0	115	1.78	1.44	3.2	81
7. Nursecrop	1.46	1.56	3.1	107	1.77	1.59	5.0	90
Means	1.56	1.60	LSD_F 0.163		1.71	1.30	LSD_F 0.154	

It thus seems possible that N- uptake/assimilation was inhibited with the high sulphate water, despite the increased NO_3 concentration, which seems to be remedied by the higher NH_4 in the first screening. The effect of higher NH_4 was confirmed by a follow-up nutrient culture solution trial with wheat, where interactive effects of SO_4 salinity at varying levels of N (NO_3 and NH_4), P and K were determined (Ströhmenger et al., 1999). A similar effect of NH_4-nutrition was also previously found for wheat (Shaviv, Hazan, Neumann & Hagin, 1990).

The only cultivar that did not follow this response was SST 822 where the absolute growth of the control was depressed with less NH_4. This cultivar is sensitive to water stress and also responds very well to increasing N applications (P. Van der Merwe, SENSAKO, personal communication, 1996), which may explain the response to the saline waters.

Although N x S interaction has generally been found to be positive or additive (Tandon, 1992), the large difference in SO_4 and NO_3 concentrations (SO_4:NO_3 *ca.* 47: 4 as $\text{mmol}_c\ L^{-1}$) may possibly result in a N-deficiency due to competition between these anions. Ammonium could therefore have provided additional N where a ratio of 1:2 was used (*cf.* Ströhmenger, et al., 1999). In practice this could mean that when irrigating wheat with high sulphate water during the seedling growth stage, the inclusion of NH_4 for N-fertilization could be advantageous for most cultivars during establishment. Nitrification would probably cancel such an effect during later growth stages.

Absolute salt tolerance (based on absolute growth in saline conditions) of wheat and triticale was found to be largely dependent on the intrinsic growth rate of cultivars (Rawson, Richards & Munns, 1988). When selecting for salt tolerance this should first be taken into account, together with the physiological tolerance (relative salt tolerance). The main physiological mechanism for tolerance to NaCl salinity for wheat, triticale and rye seems to be the exclusion of mainly Cl, and also of Na (Gorham et al., 1986; Francois et al., 1988; Francois et al., 1989; Maas & Poss, 1989; Shannon, 1997). The influence of the Na ion on nutritional imbalances of the Na/K and Na/Ca ratios and the capacity of cultivars to maintain healthy ratios are major determinants of tolerance and cultivar differences of these crops to salinity (Grattan & Grieve, 1994; Chhipa & Lal, 1995; Ayala, Ashraf & O'Leary, 1997; Shannon, 1997).

Rye may be more tolerant to Cl than wheat and triticale (Francois et al., 1989) and there are indications that rye may be more sensitive to the Na/Ca ratio than wheat (Grattan & Grieve, 1994). Differences for osmoregulation also contribute to the salt tolerance but to a lesser extent (Shannon, 1997).

These three crops all fall into the moderately tolerant or tolerant category (threshold EC_e 300 - 600 mS m^{-1} or EC_{sw} 600-1000 mS m^{-1}) (Francois & Maas, 1994). This, together with the very low concentrations of Na and Cl in the $CaSO_4$-dominated water, probably explains the general tolerance of these crops to this type of water. The above mentioned mechanisms can also be ample reasons why these crop cultivars were sensitive to the NaCl-dominated water.

The greater sensitivity of these temperate crops to the NaCl-dominated salinity, despite the fact that the osmotic potential of the NaCl-dominated water was *higher* in this instance than that of the $CaSO_4$ water (Table 3.1), suggests that *also in the seedling growth stage* ionic effects are of greater importance than osmotic potential for the sensitivity of these crops and cultivars to salinity (*cf*. Munns, 1993 and Neumann, 1997).

Annual ryegrass

The seedling growth of annual ryegrass was generally not significantly influenced with the $CaSO_4$ water (from 75 % for Midmar to 100 % for Dargle).This was in contrast to the response to the NaCl-dominated water where growth was severely reduced (21 to 52 %) (Table 4.8). In both waters Dargle was the most tolerant and Midmar the most sensitive cultivar (by relative salt tolerance). The absolute tolerance of Midmar was, however, equal to that of Dargle, but the results of Midmar are unreliable due to an unacceptably high variation. There were no significant cultivar differences with the sulphate water, but with the NaCl-dominated water the relative growth of Dargle was significantly higher than the growth of the other three cultivars (Table 4.9).

TABLE 4.9 The influence of $CaSO_4$- and NaCl-dominated mine waters on the seedling growth of annual ryegrass **cultivars**

Cultivars	Top growth masses/10 plants (g)			c.v. %	Relative growth %	
	Control	Mine C	Mine B		Mine C	Mine B
RYEGRASS						
1. Macho	0.55	0.48	*	18.9	88 a	35 bc
2. Dargle	0.24	0.24	*	14.7	100 a	52 a
3. Apollo 64	0.40	0.36	*	9.9	89 a	30 bc
4. Midmar	0.33	0.25	*	33.1	75 a	21 c
c.v.	13.4				19.1	17.2
LSD_F					40	16

* Significant difference from control ($P < 0.5$) Mine C 3/95 Mine B 3/95

Annual ryegrass has been classified as moderately tolerant, the thresholds of which are possibly higher than the EC of the NaCl mine water used (534 mS m^{-1}). For seedlings, however, the threshold for a simulated NaCl mine water was found to be EC 240 mS m^{-1} (≈EC_e 120 mS m^{-1}) (Barnard et al., 1998), which could account for the sensitivity of seedling growth with the NaCl water. Yet on the sulphate water, which had an EC of 394 mS m^{-1}, that was also higher than the calculated threshold value, the growth was not decreased. This may be an indication that osmotic stress plays a lesser role in the salt tolerance of these annual ryegrass cultivars than Na and Cl ionic effects.

In an investigation of the ionic balance and biomass production in annual ryegrass with salinity it was found that the synthesis of organic acids in annual ryegrass was essential for osmoregulation under saline conditions (Sagi, Dovrat, Kipner & Lips, 1997). Tolerance in ryegrass was associated with osmotic adaptation by an increased plant tissue content of both inorganic ions and organic anions; Sagi et al.(1997) furthermore also found that biomass was correlated with the organic anion concentration in the plants, which in turn was in close relationship with the organic N content. The organic N concentrations were again highly correlated with the total inorganic cations in the plants. From this it could be concluded that an increase in cation-uptake could eventually lead to an increase in organic osmoregulation in annual ryegrass.

As osmotic influences are generally seen to be the adverse mechanism restricting growth in seedlings (Neumann, 1997), the high cation content of the sulphate water could be responsible for improved osmoregulation and thus growth of the ryegrass seedlings on this water. In Chapter 5 the influence of increasing gradients of this water (increasing Ca, Mg and SO_4) on seedling growth are reported. It is interesting that with these gradients the ryegrass seedlings showed unusual growth increases (up to 170%!) which could possibly confirm the inorganic cation relationship to osmoregulation. This is a metabolic salt tolerance mechanism related to the external osmotic potential, which manifested in the seedling growth stage and therefore supports the suggestions that cultivar differences may exist in the seedling growth stage due to differences in osmoregulation (Neumann, 1997). In Chapter 5 two ryegrass cultivars were tested with increasing concentrations of $CaSO_4$-dominated water. In both cases the seedling growth was increased but not to the same extent, showing possible cultivar differences at this growth stage.

Furthermore a restriction of Na transport from the roots in ryegrass (Sagi et al., 1997), could point to a possible detrimental ionic effect of high Na in the shoots. The low concentration of Na in the $CaSO_4$ water could therefore also have contributed to the tolerance with this water.

Oats & Barley

The seedling growth of **oats** was not influenced by either water, nor were there any cultivar differences (Table 4.10). Oats is classified as tolerant (threshold EC_e > 600 mS m^{-1}) and is sensitive in the early vegetative growth stage (Francois & Maas, 1994). It has been found to be sensitive to an Na/Ca imbalance (Maas & Grieve, unpublished data, 1984. In: Grattan & Grieve, 1994). These properties are again self explanatory for the tolerance of oats to the $CaSO_4$ water. In South Africa some oats is cultivated in areas adjacent to the sea, and it is also possible that these cultivars (e.g., Overberg) have already been bred for tolerance to NaCl.

Barley was not influenced by the SO_4 water but the NaCl-dominated water significantly suppressed growth by 21 % (Table 4.9). Salt tolerance of barley is related to osmoregulation (by glycine betaine production), the exclusion of Na and Cl and the ability to regulate Cl transport to the shoot (Shannon, 1997). Barley growth has also been found to be stimulated by

SO_4 (Hassan, Drew, Knudsen & Olsen, 1970). These mechanisms could possibly be responsible for the responses to these waters.

TABLE 4.10 The influence of $CaSO_4$- and NaCl-dominated mine waters on the seedling growth of oats **and** barley **cultivars**

Cultivars	**Top growth/10 plants (g)**			**c.v. %**	**Relative growth %**	
	Control	**Mine C**	**Mine B**		**Mine C**	**Mine B**
OATS						
1. SSH 421	1.12	1.10	1.13	17.6	98 abc	101 a
2. SSH 423	0.99	1.09	0.99	4.8	110 a	99 a
3. Witteberg	0.85	0.76	0.82	18.8	89 ab	96 ab
4. Perdeberg	1.26	1.26	1.16	9.2	100 abc	92 ab
5. Echidna	1.21	1.19	1.08	16.5	98 abc	89 ab
6. Overberg	1.36	1.45	1.28	16.7	107 ab	94 ab
BARLEY						
1. Stirling	2.32	2.04	*	19.0	88 c	79 ab
c.v. %	17.6				7.8	27.0
LSD_F					17	

*Significant difference from control ($P < 0.05$)

Mine B 3/95

Mine C 3/95

The annual temperate cereals all fall into the moderately salt tolerant (threshold EC_e 300 to 600 mS m^{-1}) or tolerant category (600 to 1000 mS m^{-1}) (Francois & Maas, 1994). This is possibly one reason why the growth of the seedlings of these temperate annuals was generally not affected by the $CaSO_4$ water (EC 394 mS $m^{-1} \approx EC_e$197 mS m^{-1}). The main mechanisms by which NaCl salinity suppresses the general (mature) growth of these crops are, however, associated in some way or another with the influence of Na and/or Cl on nutritional imbalances, and is also affected to a lesser or greater extent by osmotic influences. As the above results show a much greater suppression of seedling growth with NaCl salinity compared to that of the $CaSO_4$-dominated salinity, the low concentrations of Na and Cl in this water could once more account for the tolerance of most of these crops to this water.

In the tribe Triticeae salt tolerance has been found to be poligenic (Zong & Dvořák, 1995). Genotypic differences in salt tolerance are mainly associated with the ability for exclusion, the maintenance of a high K/Na ratio, sensitivity to a high Na/Ca ratio and in some species more than in others, to an ability for osmoregulation. Again the very low Na and Cl contents of this water, together with a high Ca content, probably explains the lack of salt tolerance differences of these crop cultivars to the particular $CaSO_4$ mine water evaluated.

Lucerne

Five cultivars of lucerne were compared on two types of mine water in the seedling growth stage. The seedling growth of all the cultivars was significantly reduced by the $CaSO_4$-dominated mine water, ranging from 55 % for PAN 4581 to 76 % for Diamond (Table 4.11). There was a significant difference in seedling growth between these two cultivars. Growth was severely restricted and chlorotic on the NaCl-dominated water and all cultivars responded in a similar way (21-25%).

Salinity affects *seedling* growth of lucerne through osmotic stress, whereas in the more mature stages tolerance is associated with exclusion of Cl or the level of Cl tolerated (Shannon, 1997), but contrasting data indicated that there is a “positive correlation between Na and Cl accumulation and growth” in lucerne (Ashraf & O’Leary, 1994). Salt tolerance differences in the seedling stage were not connected to differences in root and shoot Na, or shoot Cl but K versus Na selectivity was greater in the seedling stage of a more tolerant line (Ashraf & O’Leary, 1994).

TABLE 4.11 The influence of $CaSO_4$- and NaCl-dominated mine waters on the seedling growth of lucerne cultivars

Cultivars	Top growth/10 plants (g)			c.v. %	Relative growth %	
	Control	Mine C	Mine B		Mine C	Mine B
1. PAN 4860	0.46	*	*	8.0	72 ab	22 a
2. PAN 4581	0.36	*	*	15.4	55 b	21 a
3. Baronet	0.48	*	*	2.3	71 ab	21 a
4. Topaz	0.45	*	*	5.7	71 ab	25 a
5. Diamond	0.52	*	*	2.6	76 a	22 a
c.v. %	6.9				10.9	15.1
LSD_F					19	8

* Significant difference from control ($P < 0.5$)

Mine C 3/95

Mine B 3/95

Lucerne is classified as moderately sensitive to salinity (threshold EC_e 200 mS m^{-1} ≈ EC_{sw} 400 mS m^{-1}) (Maas & Hoffman, 1977). Growth decreases with these two waters (EC 396 mS m^{-1} and 534 mS m^{-1}) are thus not unexpected. Salt tolerance in legumes has been associated with osmoregulators (Tramontana & Jouve, 1997), and in lucerne with an increase in proline content of the roots, where it may serve a protective function (Petrusa & Wincov, 1997). This mechanism may also be operative in the seedling growth stage and could possibly be responsible for cultivar differences.

The growth decrease with the $CaSO_4$ water found in this study could be due to sensitivity to a decreased osmotic potential. The threshold for shoot growth of lucerne has been determined at EC_e 200 mS m^{-1} (≈EC_{sw} 400 mS m^{-1}) (Bernstein, 1974; Maas & Hoffman, 1977) and as the seedling growth is more sensitive (Forsberg, 1953 as quoted in: Noble, Halloran & West, 1984; see also Figure 5.2), it can be deduced that the decrease in seedling growth was probably due to the decreased osmotic potential (EC 394 mS m^{-1}) and the cultivar differences were probably due to osmotic adaptive abilities (Neumann, 1997).

In the experiment with increasing concentrations of simulated NaCl mine water (Chapter 5), a threshold of EC 170 mS m^{-1} (≈EC$_e$ 85 mS m^{-1}) was determined for the seedling growth of PAN 4860 (Barnard et al., 1998). A decrease at EC 534 mS m^{-1} is thus inevitable on the NaCl mine water for these lucerne cultivars.

4.3 CONCLUSION

Seedling growth on the actual 'worst case' mine water showed that the *annual subtropical cereal* crops exhibited a greater sensitivity and more cultivar differences with the $CaSO_4$-dominated water than did the legumes. Although the seedling growth decreases of some crop cultivars were quite severe, there remains a relatively wide choice of cultivars that could be used for irrigation with $CaSO_4$ saline water in the sensitive seedling stage. Soybean and dry bean grew exceptionally well on the sulphate water, while cowpea seemed to be sensitive with the water culture in contrast to the response with sand culture where the growth was not severely affected.

Generally the seedling growth of the *annual temperate crops* was more tolerant to the sulphate water than that of the subtropicals, except for one sensitive wheat and one triticale cultivar. Wheat seedling growth was less sensitive to the sulphate water when N was partly supplied as NH_4. Lucerne cultivars were generally sensitive to the $CaSO_4$ mine water. With the NaCl-dominated water the seedling growth of all the temperate crops, with the exception of oats, was severely suppressed.

The presence and concentration of Na, Cl and Mg in $CaSO_4$-dominated waters could however influence the seedling growth, depending on the adverse and tolerance mechanisms operative in specific crops and cultivars. The general sensitivity/tolerance mechanisms that are known for specific crops may be an indication of the tolerance of the respective crops to this type of water. For instance, cultivars of crops sensitive to a decreasing osmotic potential - such as maize, sorghum and pearl millet - would be more sensitive to this water in the seedling growth stage; where tolerance is mainly connected to ionic effects of Na and Cl the crops may probably be more tolerant to this water.

The seedling growth of both the subtropical and temperate annual cereals was generally suppressed with the *NaCl-dominated* ‘worst case’, actual mine water. Again the subtropical annuals were influenced to a greater extent than the temperate annuals. The relative seedling growth of the *subtropical legumes* (soybean, dry bean and cowpea) was generally less suppressed than that of the *subtropical cereals* on the NaCl-dominated water. Oats seedling growth was exceptional in that none of the cultivars was sensitive to this particular concentration of NaCl-dominated water. As tolerance of soybean and oats is generally associated with the exclusion of Na and/or Cl, these cultivars may already have genetic properties for this purpose. All lucerne cultivars were very sensitive. Generally the choice of cultivars to be grown under irrigation with the NaCl-dominated mine waters is limited. There are, however, some cultivars that should be tolerant enough to bridge the sensitive seedling growth stage successfully.

There were significant *cultivar differences* in the seedling growth of maize, sorghum, pearl millet and dry bean with the $CaSO_4$-dominated water, whereas very few differences were found with the temperate cereals and lucerne. With the NaCl-dominated water some differences were manifested for wheat, triticale and ryegrass where significant differences mainly occurred between the most sensitive and tolerant cultivars.

In conclusion it can be said that although the seedling growth of some cultivars, especially of the subtropical cereals, was decreased by a saturated $CaSO_4$ water, there remains a wide choice of high yielding cultivars that can be successfully utilised for irrigation with this water.

Cultivar differences, especially among the cereals, should be considered when irrigating with these mine waters, as yield may be influenced by the effect of salinity on the primordial development of spikelets in the seedling growth stage.