TECHNICAL NOTES

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"GERMINATION RESPONSES AND BORON ACCUMULATION IN GERMPLASM FROM CHILE AND USA GROWN WITH BORON ENRICHED WATER"

Attached is the above titled paper.

This paper provides and excellent source of new information and background for personnel involved with saline agriculture. It is an outstanding example of NRCS and ARS working together.

Prepared by David Dyer, Manager, Lockeford Plant Materials Center, Lockeford, California

GERMINATION RESPONSES AND BORON ACCUMULATION IN GERMPLASM FROM CHILE AND USA GROWN WITH BORON ENRICHED WATER

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ABSTRACT

Boron (B) is toxic to most plant species when accumulated in high concentrations. Differences in a plant's ability to adapt to high concentrations of B may depend upon the origin of the germplasm. Chilean and domestic (USA) germplasm; corn (*Zea mays* L.), carrots (*Daucus carotas*), tomato (*Lycopersicum esculentum* L.),and alfalfa (*Medicago sativa* L.) were evaluated for germination, emergence of cotyledonary leaves, and tissue B accumulation under high B conditions in both an environmental growth chamber and greenhouse. Increasing B levels (20-40 mg B L^{-1}) inhibited the percentage of germination for both the Chilean and domestic germplasm. Chilean germplasm exhibited generally a greater percentage of healthy cotyledonary leaves at the 20 mg B L^{-1} treatment than the domestic germplasm. Comparing B concentrations between both germplasm grown and irrigated with B-enriched water (10-20 mg B L^{-1}) under greenhouse conditions, leaves from domestic germplasm contained more B. Moreover, B toxicity symptoms were more severe for the tested plant species from the domestic germplasm. Apparent B tolerance by germplasm of different origin should be further tested under field conditions.

INTRODUCTION

California and Chile are major agricultural production areas in North and South America because of their favorable climate and their extensive irrigation networks. Chile occupies 75.6 million ha of land in South America, and California occupies 40 million ha of land in North America. Both regions contain prominent mountain ranges (the Andes in Chile and the Sierra Nevada and Coastal Ranges in California). Competition for limited water resources by the rapidly increasing urban population in both regions has forced agricultural communities to consider alternative sources of water. Ninety percent of the total surface water in California is consumed by agriculture (Aqueduct, 1986), and up to 75% of the total water in northern Chile is utilized by the mining industry (personal communication; Caceres, 1997).

Poor quality water that contains high levels of naturally-occurring salts, especially boron (B), is available to growers in arid regions of central California and in northern Chile (Caceres et al., 1992; Ayars et al., 1994; Shennan et al. 1995). In northern Chile, alluvial deposits are the source of high levels of B and other salts (i.e., chlorides and sulfates) in soils. Similarly, some soils of the San Joaquin Valley in central California contain high levels of B and other trace elements that originate from Cretaceous shales (Schroeder et al., 1988; Moore et al., 1989). For both regions, natural processes of weathering and geochemical transformations in the soil led to the solubilization of natural deposits of borate salts and their eventual migration into groundwater or rivers. It is the use of these B-laden waters or the land application of agricultural drainage water that contribute most significantly to the deposition of soluble B on agricultural soils.

Use of B-enriched Water in Irrigated Agriculture

The effects of using water containing high levels of B on agricultural soils have been reported for arid regions of central California and northern Chile (Rhoades et al., 1988; Thellier et al., 1990a, b; Ayars et al., 1994; Shennan et al., 1995). Reusing agricultural effluent with high levels of B may be suitable for certain crops (Rhoades et al., 1989; Ayars et al., 1986, 1990, 1994); however, continued irrigation with water containing concentrations of B greater than 4 mg B L^{-1} may pose a hazard for some B-sensitive crops, such as beans *(Phaseolus vulgaris* L.), carrots (*Daucus carota* L.), and lettuce *(Latuca sativa* L.)(Mass and Hoffman, 1977; Hoffman, 1986; Maas, 1986; Shennan et al., 1995).

To assess the potential toxicity of B-enriched irrigation water, the physical and chemical characteristics of the soil must be considered (Goldberg, 1993; 1997). A soil that has a high adsorption capacity would be expected to maintain a lower solution B over a longer period of time than a soil with a low adsorption capacity. Plant response to B in the soil depends on the B concentration of the soil solution under field conditions (Ryan et al., 1977). Early work by Eaton (1935), Scofield (1935), Bingham et al (1985), and Maas (1986) resulted in criteria for determining B toxicity in irrigation waters. These criteria were adopted with Eaton's rankings of North American crop species for B tolerance by the U.S. Salinity Laboratory staff (1954) for USDA Handbook 60. Maas (1984) correlated yields with the concentration of B in solution to determine the threshold concentration range, which is defined as the maximum concentration that a given plant species tolerates without manifesting visual injury symptoms and/or a decrease in yield Maas (1986) assumed that the principal factor controlling plants responses to high B concentrations is the solution bathing the root system

Boron tolerance has been observed in non-horticultural plants growing in Chile which include salt grass (*Distichlis spicata*), bermuda (*Cynodon dactylon)*, mesquite *(Prosopis chilensis),* saltbush (*Atriplex sp., Azorella compacta*), as well as in horticultural crops [i.e., tomato (*Lycopersicum esculentum*), carrot and corn (*Zea mays)].* Genetic variation/diversity or physiological adaptation by the plant may contribute to this apparent B-tolerance in such plant species. In this regard, evidence shows that B-susceptible genotypes have higher B concentrations in leaves and shoots than do tolerant genotypes (Nable, 1988). Considerable genotypic variation in susceptibility to B toxicity has been identified for agronomic species like wheat (*Triticum aestivum*) and barley (*Hordeum vulgare*) (Nable et al., 1990; Paull et al., 1990). Successful use of B-enriched waters on certain agronomic crops [e.g., corn, alfalfa *(Medicago sativa*) and tomato] in some areas of northern Chile has generated world-wide interest and led to collaborative effort between United States and Chilean agencies [Natural Resources Conservation Service (NRCS), Agricultural Research Service (ARS), and the Office of International Cooperation and Development (OICD)] and the Chilean FONDEF Programs, respectively. Seed was collected from crops which were successfully growing with high B water in northern Chile. The objective of the study was to compare the responses of collected germplasm from northern Chile with domestic germplasm commonly used in California under high-B conditions at germination and during growth.

METHODS AND MATERIALS

Germination Test

Chilean and domestic (USA) germplasm were initially evaluated for germination and appearance of cotyledonary leaves or first shoots under high B conditions. Seeds of the following were evaluated in this study: corn (*Zea mays* L.; PI #9068919 from Chile and Golden Jubilee, USA), carrots (*Daucus carotas* L.; PI #9068933 from Chile and Imperator, USA), and tomato (*Lycopersicum esculentum* L.; PI #9068924 from Chile and Cal Ace, USA). Seed germplasm from Chile was collected in high B regions of northern Chile, i.e., San Pedro de Atacarna, Quillaque, and Calama. Seed, petri-dishes, and filter-paper were sterilized with a 10% hypochlorite solution before setting up the experiment. Twenty five seeds of each cultivar were imbibed on one layer of Whatman No. 1 filter paper moistened with **4** ml of one of the following treatments: 1) deionized water, control (K^0); 2) 20 mg B L^{-1}; or 3) 40 mg B L^{-1}. Boron solutions were made with boric acid. Petri-dishes were sealed with parafilm and placed in an environmental growth chamber at 22°/18° day/night temperatures for 16 and 8 hrs with a photosynthetic photo flux density (PPFD) of 200^{-1} μmol $m^{-1}s^{-2}$ (measured with a Li-cor Quantum Porameter). The experiment followed a complete randomized design which was replicated three times for germplasm from both Chile and California. After 8 days of imbibition, seeds were evaluated for both germination (radical emergence through the seed coat) and for appearance of cotyledonary or first leaves

Greenhouse Study

Boron accumulation by the germplasm already described, as well as alfalfa (*Medicago sativa* L.; #9068931 from Chile and Southern Special, USA) was investigated under greenhouse conditions in Fresno, California between April 1996 and October 1996. Eight kilograms of a low-B-containing Hanford sandy loam (*Typic durixeralfs*) were placed in 18 L growing pots. Ten seeds from each germplasm (except alfalfa; for which approximately one gram was used) were sown at depths between 1 and 3 cm depending on the seed size of each species. After 10 d, seedlings were thinned to the three healthiest plants (except alfalfa). Plants were grown under controlled greenhouse conditions at 24±2 °C with an average PPFD of approximately 500 μmol $m^{-1}s^{-2}$. The experimental design was completely randomized with seven replicates for each germplasm. The initial B concentration in irrigation water was 10 mg L^{-1} at planting and increased to 20 mg L^{-1} [(the target B concentrations have been reported up to 16 mg L^{-1} (Figueroa et al., 1994)] after 65 d. This modification of the B concentration in the water allowed for the observation of any visual toxicity symptoms at lower B concentrations of 10 mg B L^{-1}. Plants were irrigated with the amount of water estimated to be lost (determined by weight) by evapotranspiration (ET) and maintained at about 65-70% field capacity to minimize leaching. Both domestic and Chilean germplasm received the same quantity of B-enriched water for each species (the mean ET losses between domestic and Chilean germplasm were used for calculating the amount of water to apply to each species). Care was taken to not make contact with the plant when applying water and thus prevent foliar absorption of B.

Corn and tomato plants were harvested approximately 135 d after planting, while carrots and alfalfa were harvested 155 d after planting. Dates of harvest for each species were determined based on the severity of B toxicity observed on the foliage and not on the stage of plant maturity. Depending on the species, plants were separated into leaves, stems, fruit, and roots, respectively. Plant parts were then rinsed carefully with deionized water, oven-dried at 50°C for 7 d, ground in a stainless steel Wiley mill equipped with a 1-mm screen, and stored for B analyses. After plant harvest, soil from each pot was thoroughly mixed, and a 500 g sample was taken, oven-dried at 50°C for 7 d, and ground to pass a 850-μm sieve. Extractable soil B was collected from a 1:1 saturated paste. Both plant and total soil B were determined by inductively coupled plasma spectroscopy (Perkin-Elmer 2000) after wet-acid digestion as described by Bañuelos and Akohoue (1994) . The NIST Apple Leaf Standard (27±1 mg B kg^{-1} DM, 99% recovery) was used as an external quality control for B analysis of plant samples. There were no available soil B standards at the time of the study.

All data were subjected to t-test analysis using the Statistical Analysis System **(SAS** Institute, 1987). Percentage data were transformed before analysis; actual percentages are, however, shown.

RESULTS

Germination Evaluation

Increasing B levels inhibited the percentage of germination for both the Chilean and domestic germplasm of each species (Table 1). Among the three species, germination of all tomato germplasm was least affected by the increasing B concentrations. Germplasm collected from Chile exhibited a greater percentage of healthy cotyledonary or first leaves emerged at the 20 mg

B L^{-1} treatment than the domestic germplasm for tomatoes and corn (Table 1). Differences between both germplasm for each species was, however, not discernible at the 40 mg B L^{-1} treatment. Roots were generally shorter and whiter for all germplasm with increasing B concentrations. Lateral roots were visibly missing in all high B treatments (40 mg B L^{-1}) for all germplasm.

Greenhouse-Grown Plants

The accumulation of B in both the Chilean and domestic germplasm of four plant species irrigated with B-enriched water is shown in Table 2 for the different organs. Only selected dry matter yields are reported due to the manner in which harvest dates were determined for each species (based on severity of B toxicity observed on foliage). In general, dry matter production was lower in domestic germplasm compared to Chilean germplasm after irrigation with the same volume of B-enriched water (Table 3). Control plants (receiving B-free water) from both types of germplasm were not used for dry matter comparison in the study. Compared to the Chilean germplasm, leaves from the domestic germplasm contained the greatest B concentration for corn, alfalfa, and tomatoes. Final soil B concentrations are presented for all species in Table 2 after irrigation with B-enriched water.

DISCUSSION

Differences in tolerance to B at germination were not clearly observed atnong the tested species for both Chilean and domestic germplasm. Boron's inhibiting effect on seed germination was, however, similar, irrespective of origin of germplasm. For both germplasm the results suggest that the seed embryo may have been damaged at the high B levels, thus resulting in defective seedling growth, i.e , root development. **A** lack of lateral root growth may eventually lead to a reduced growth, because of the inability of the plant to take up sufficient nutrients and water. Relationships between seed boron concentration and germination and growth have been reported on soybean [(*Glycine max*) (Perkasem et al 1997)], however, B concentrations were not measured in the seed roots or cotyledons/leaves in the present study. Although differences at germination or cotyledonary stages were not apparent among the species tested, the tomato germplasm appeared to be the most tolerant to increasing B levels.

Significant differences in B accumulation were measured in species between Chilean and domestic germplasm. Overall, the domestic species accumulated more tissue B than did the Chilean species. Leaves from domestic species irrigated with B-enriched water had ≈20% greater B concentrations than tissues of Chilean species. Boron tends to concentrate in parts of the plant, e.g., leaves, where B presumably migrates via the transpiration stream (Able et al., 1990). Boron concentrations were greatest in leaves, irrespective of origin of germplasm. Because of the high leaf B concentrations, it was not evident that the roots of the domestic germplasm excluded B and prevented B from translocating to the shoots. Moreover, the domestic species exhibited more severe B toxicity symptoms, which included leaf burn, chlorotic and/or necrotic patches, often at the margins and tips of older leaves (Eaton, 1944; Gupta, 1979; Gupta et al., 1985). Both tomato fruit and carrots of the domestic germplasm showed cell breakdown, which appeared to be blossom end rot (Bangerth, 1979) and brown cavity, respectively (Shear, 1975) Both of these disorders are reported to be related to calcium deficiency (Marschner, 1995) Some researchers have hypothesized that there may be a relationship between B and calcium concentrations (Ma

Tang, 1983), although Ca/B interactions are often inclusive (Bergmann, 1992). Compared to calcium, boron is bound less firmly to the cell wall matrix (Teasdale and Richardo, 1990). However, both Ca and B may have similar structural functions in the cell walls and at the cell wall-plasma membrane interface. These common features may explain similarities in Ca and B-deficiency symptoms (Crisp et al., 1976).!

Based on the parameters used for evaluating the responses of both germplasm under greenhouse conditions, it appears that the tested Chilean germplasm was better adapted to high B conditions than the domestic germplasm. Chilean germplasm may have developed exclusion or avoidance strategies that result in a lower accumulation of B in the shoot and roots. It is not known whether Chilean germplasm adapted physically or genetically to a high B conditions over time, which may have included reducing the influx of B or altering the distribution of accumulated B at the cellular, tissue, or organ level (Nable et al., 1997)

Adaption strategies by plants may be more difficult to distinguish under field conditions, where high B conditions are compounded by other environmental factors, i.e., salinity, temperature. There are indications observed in northern Chile suggesting that high B concentrations in the irrigation water contribute to lower yields in some field-grown agronomic crops (see Table 3) de la Riva et al. (1987) and Caceres et al. (1992). If B was the predominant factor responsible for lower yields, then plant B tolerance may, however, not be a constant plant property; it may be modified by other environmental and climatic conditions during a plant's ontogeny The present studies indicate, however, that differences in some type of B tolerance may exist between agronomic germplasm commonly used in Chile and in California Differences may be more pronounced in plants grown under controlled and greenhouse conditions than under field conditions. Thus, one should be careful in extrapolating results or assuming apparent B tolerances to the same plants field-grown in soils from both Chile and California.

CONCLUSIONS

Elevated levels of B in irrigation water are detrimental to many crops grown in agricultural regions of the world, i.e., California, Chile, where increasingly-poor-quality water is used for irrigation. Typical agronomic crops (i.e., corn, tomatoes, alfalfa, carrots) from domestic and Chilean germplasm were evaluated for B tolerance at germination and after irrigation with B-enriched water. Differences between the two origins of germplasm were not as pronounced at germination with increasing B in the germination medium, although it appeared that the Chilean germplasm did slightly better. Differences in the accumulation of B between the two germplasm irrigated with B-enriched water were, however, noticeable. Chilean germplasm consistently accumulated less B than the **USA** domestic germplasm. Apparently the Chilean germplasm has moderately adapted to the high B in the irrigation water. Although the evaluation of germination and B accumulation are invaluable for quickly determining the ability of plants to tolerate high B conditions, growing the same crops under field conditions in both Chile and California is the remaining practical and accurate test.

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Table 1. Germination rates and appearance of cotyledonary or first leaves in germplasm collected from Chile and typical germplasm from California exposed to different concentrations of B under controlled conditions.

Plant Species	Germplasm origin	B concentration in treatment --mg B L^{-1}--	Germination Rate --%[§]--	Cotyledons or Leaf growth --%[§]--
Corn	*Chile*	K°[†]	100(1)	100(2)
	(#9068919)[‡]	20	90(4)	84(4)
		40	72(8)	55(2)
corn	*California*	K"	100(1)	98
		20	80(8)	70(6)
		40	60(17)	50(12)
Carrot	*Chile*	K"	100(1)	NA
	(#9068933)[‡]	20	80(6)	NA
		40	60(13)	NA
Carrot	*California* (Imperator)	K°	100(1)	NA
		20	70(10)	NA
		40	60(20)	NA
Tomato	***Chile*** (#9068924)[‡]	K°	100(1)	100(1)
		20	90(10)	100(1)
		40	80(16)	90(7)
Tomato	*California* (Cal Ace)	K°	100(1)	100(1)
		20	90(14)	60(4)
		40	80(20)	0(0)

[†]K°=control B level is less than 1 mg B L^{-1}

[‡]Germplasm collected from Chile and stored at Plant Materials Center, Lockeford, CA

[§]Percentages are the mean presented from nine replicates with the standard error in parenthesis

[N/A]Not applicable.

Table 2. Accumulation and distribution of boron in different crops from Chile and California irrigated with B-enriched water under greenhouse conditions.+

Plant species	Germplasm origin	Plant organ	Plant tissue concentration of B --mg B kg^{-1} DM--	Dry mass --g--	Total B applied in water ---mg---	Final soil B: Ext. B --mg L^{-1}--	Final soil B: Total B - -mg kg^{-1} soil--
Corn (Golden Jubilee)	*California*						
		leaves*	2135(127)	225			
		root	159(6)	75	**324**	16	60
		kernal	53(5)	NA			
Corn (#9068919)	*Chile*						
		leaves'	676(88)	339			
		root	243(25)	125	324	19	58
		kemal	50(1)	NA			
Alfalfa (Southern special)	*California*						
		tops	1950(82)	79			
		roots	271(31)	44	213	30	85
Alfalfa (#9068931)	*Chile*						
		tops	1240(64)	95			
		roots	164(25)	65	213	31	87
Tomato (Cal Ace)	*California*						
		old leaves	1636(22)				
		young leaves	1201(144)	95§	286	25	63
		fruit (ripe)	120(25)	NA			
Tomato (#9068924)	***Chile***						
		old leaves	857(24)				
		young leaves	762(73)	150§	286	29	71
		fruit (ripe)	56(11)	NA			
Carrot' (Imperator)	*California*						
		root	214(22)	39#	391	28	81
		tops	2765(171)	108			
Carrot (#9068933)	*Chile*						
		root	507(49)	49"	391	30	79
		tops	2910(94)	212			

'Means are presented with standard error in parentheses. Irrigation water initially contained a B concentration of 10 mg B L^{-1} and was gradually increased to 20 mg B L^{-1} (see methods and materials).

*Fifth leaf from the growing top was taken for analysis.

§Total weight of both old and young leaves.

'Exhibited severe B toxicity symptoms; apparent brown-rot cavity.

#Dry mass is average for one carrot.

Table 3. Comparison of yields in crops grown under different B conditions in various regions of northern Chile.

Regions of Chile†	Soluble B concentrations in irrigation water ---------mg B L^{-1}---------	Cultivated crops	Typical yields ton ha^{-1}
Caspana	1-4	alfalfa	8.8
		carrot	14
Cupo	4-6	alfalfa	9.0
		corn	1.2
		wheat	1.2
San Pedro de Atacama	4-7	alfalfa	3.5
		corn	2.2
		wheat	1.5
Lasana	5-8	alfalfa	4.0
		corn	2.0
		wheat	10
		carrot	15
		onion	10
Chui-chui	6-8	alfalfa	8.7
		corn	3.5
		wheat	1.5
		carrot	23
		onion	13
Calama	10-16	alfalfa	1.7
		corn	0.8
		wheat	0.8
Quillaqua	18-42	alfalfa	1.5
		corn	0.5

Northern regions of Chile known for their high B conditions (Caceres et al., 1992).