Variation of tolerance to manganese toxicity in Australian hexaploid wheat

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Abstract

High concentrations of manganese (Mn), iron (Fe), and aluminium (Al) induced in waterlogged acid soils are a potential constraint for growing sensitive wheat cultivars in waterlogged-prone areas of Western Australian wheat-belt. Tackling induced ion toxicities by a genetic approach requires a good understanding of the existing variability in ion toxicity tolerance of the current wheat germplasm. A bioassay for tolerance to high concentration of Mn in wheat was developed using Norquay (Mn-tolerant), Columbus (Mn-intolerant), and Cascades (moderately tolerant) as control genotypes and a range of $MnCl_2$ concentrations (2, 250, 500, 750, 1000, 2000, and 3000 μM Mn) at pH 4.8 in a nutrient solution. Increasing solution Mn concentration decreased shoot and root dry weight and intensified the development of toxicity symptoms more in the Mn-intolerant cv. Columbus than in Norquay and Cascades. The genotypic discrimination based on relative shoot (54% to 79%) and root dry weight (17% to 76%), the development of toxicity symptoms (scores 2 to 4) and the shoot Mn concentration (1428 to 2960 mg kg^{-1}) was most pronounced at 750 μM Mn. Using this concentration to screen 60 Australian and 6 wheat genotypes from other sources, a wide variation in relative root dry weight (11% to 95%), relative shoot dry weight (31% to 91%), toxicity symptoms (1.5 to 4.5), and shoot Mn concentration (901 to 2695 mg kg^{-1}) were observed. Evidence suggests that Mn tolerance has been introduced into Australian wheat through CIMMYT germplasm having "LERMO-ROJO" within their parentage, preserved either through a co-tolerance to Mn deficiency or a process of passive selection for Mn tolerance. Cultivars Westonia and Krichauff expressed a high level of tolerance to both Mn toxicity and deficiency, whereas Trident and Janz (reputed to be tolerant to Mn deficiency) were intolerant to Mn toxicity, suggesting that tolerance to excess and shortage of Mn are different, but not mutually exclusive traits. The co-tolerance for Mn and Al in ET8 (an Al-tolerant near-isogenic line) and the absence of Mn tolerance in BH1146 (an Al-tolerant genotype from Brazil) limits the effectiveness of these indicator genotypes to environments where only one constraint is induced. Wide variation of Mn tolerance in Australian wheat cultivars will enable breeding genotypes for the genetic solution to the Mn toxicity problem.

Key words: genotypic variation / manganese tolerance / screening / waterlogging

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1 Introduction

Soils with excess in Mn, causing toxicity to plants are widespread. After Al, excess in Mn is the most growth-limiting factor in acid soils worldwide (*Manyowa* and *Miller*, 1991). High concentrations of Mn in the soil solution also occur in poorly drained and reduced environments (*Sparrow* and *Uren*, 1987) associated with waterlogged soils. Some environmental factors (*e.g.*, high temperature; *Marsh* et al., 1989) can also increase the availability of Mn and consequently affect crop growth.

High concentrations of Mn in soil (attributed to mild acidification) are a widespread nutritional problem in southeastern Australia (*Culvenor* et al., 1986; *Weir*, 1988). Waterlogging was identified as a cause of increased soil Mn concentrations in northeastern Victoria (*Sparrow* and *Uren*, 1987). Similarly in Western Australia, high to toxic concentrations of Mn were observed in shoot tissues of wheat after waterlogging of an acidic soil (*Khabaz-Saberi* et al., 2006), giving the first indication of a potential Mn toxicity problem. Further evidence was provided from a pot study using acidic soils from waterlogging-prone areas of the Western Australian wheat-belt (*Setter* et al. 2008).

There are large areas of waterlogging-prone agricultural land in Australia, including 1.8 million ha in Western Australia (with >400 mm annual rainfall) and further 4.1 million ha (mostly duplex soils) in Victoria (*Setter* and *Waters*, 2003). There are proven engineering solutions (raised beds and drainage) to tackle waterlogging. However, they are not only expensive (*Dennis* et al., 2000), but also frequently unfeasible because of problems with discharging drainage water (especially if acidic) in the Australian agricultural landscape. The alterna-

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tive solutions to drainage would be to select genotypes tolerant to Mn toxicity as an important consequence of waterlogging. Indeed, in some plant species (Mn-tolerant *Trifolium subterraneum* cultivar *vs* Mn-sensitive *Medicago truncatula*), tolerance to excess Mn coincided with tolerance to waterlogging (*Robson* and *Loneragan*, 1970) raising a possibility that in other agricultural species (*e.g.*, wheat as the most important crop in Australia) genotypes tolerant to Mn toxicity might also be tolerant to waterlogging. This raises a potential in exploiting the variability of Mn tolerance in current wheat genotypes to be used in breeding for Mn tolerance.

However, given many competing objectives in wheat breeding programs, a breeding approach to tackle the constraint of Mn toxicity induced under waterlogged acid soils needs to be further justified. Such justification was considered previously, but did not receive support, with a view that Al tolerance alone (rather than in combination with Mn tolerance) is sufficient for plants growing on acidic soil (*Carver* and *Ownby*, 1995). In Australia, *Scott* et al. (2001) reported no yield advantage associated with Mn tolerance in six pairs of Al-tolerant lines with contrasting Mn tolerance growing on an acidic soil with high Mn in southern New South Wales. However, the study site (dry summer and below-average rainfall during the growing season) did not favour Mn toxicity during that particular growing season; moreover, the range of Mn tolerance was relatively narrow. Recognising these limitations, the authors concluded it was premature to consider that Mn tolerance in wheat does not impact on grain yield (*Scott* et al., 2001). Hence, for evaluation of the importance of Mn tolerance, it is critical to use genotypes with vastly different Mn tolerance grown in soil where reduced conditions exacerbate Mn toxicity.

Variation of Mn tolerance has been reported within Brazilian (*Camargo*, 1988), American (*Foy* et al., 1988), and Canadian wheat varieties (*Moroni* et al., 1991) as well as in seven Australian wheat varieties in a pot assay (*Scott* et al., 1998). Clearly, evaluation of a wider range of Australian locally adapted wheat germplasm is needed to assess potential presence of sufficient variation of Mn tolerance to justify the breeding efforts. Such knowledge would also represent a basis for further characterization of genetic and molecular aspects of Mn tolerance in wheat, the work that has already been done in some other crops such as rice (*Wang* et al., 2002) and soybean (*Kassem* et al., 2006). Therefore, the present study was designed to (1) develop a screening system for tolerance to high concentration of Mn in wheat using genotypes with previously known differential Mn tolerance, and (2) use that screening system to evaluate genotypic variation for Mn tolerance in Australian wheat varieties and advanced breeding lines.

2 Materials and methods

The experiment was carried out in naturally lit phytotron with a day/night temperature set at 20°C/15°C at the University of Western Australia (31.58° S, 115.49° E), in May. The mean (1994–2009) daily solar exposure in May was 11.6 MJ m^{-2} or 3.2 kWh m^{-2} (http://www.bom.gov.au/climate/averages/).

2.1 Development of a screening technique

Uniform seeds of the cultivars Norquay (Mn-tolerant), Columbus (Mn-intolerant) (*Foy* et al., 1988), and Cascades (moderately Mn-tolerant) (Tab. 1) were surface-sterilised using 0.5% (v/v) sodium hypochlorite and placed on wet filter papers in the dark at 4°C for 48 h. Seeds were then positioned with their crease down on a nylon mesh lined on a floating tray over 10 L of nutrient solution and grown initially for 7 d. The aerated standard nutrient solution (*Moroni* et al., 1991) contained (μM): Ca 1000; Mg 300; K 800; NO_3 3300–3600; NH_4 600; PO_4 100; SO_4 101; Cl 34; Na 20; Fe (as Fe-EDTA prepared from equimolar amounts of $FeCl_3$ and Na_2EDTA) 10; B 6; Mn 2; Zn 0.5; Cu 0.15, and Mo 0.01. The pH was adjusted to 4.8 using 1 M HCl and 1 M NaOH as appropriate. After seedling pre-treatment (standard solution as above, except 3300 μM NO_3 and 300 μM NH_4), 7 d old seedlings were mounted (using strips of polyurethane foam) into the lids of 4 L pots (six seedlings per pot) containing the aerated standard nutrient solution (as described above). Pots were shielded from light by aluminium foil. Nutrient solution in pots was controlled daily for pH, was changed weekly, and also adjusted periodically to 4 L with distilled water to compensate for water loss by evaporation and transpiration.

The concentration of Mn was 2 μM Mn (as $MnCl_2$) during pre-treatment (7 d), and the same concentration was also employed as a control. The cultivars Norquay, Columbus, and Cascades were exposed to seven treatment concentrations of Mn (2, 250, 500, 750, 1000, 2000, and 3000 μM Mn) for 14 d. Manganese toxicity symptoms were recorded on the scale of 1 (no symptoms), 2 (very tolerant), 3 (tolerant), 4 (moderately tolerant), 5 (intolerant), 6 (very intolerant) to 7 (the most severe symptoms). After 14 d of Mn treatments, plants were separated into shoots and roots and oven-dried at 75°C for 3 d for determination of dry weight. Manganese concentration of shoots was determined using Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES) as described by *Zarcinas* et al. (1987) after acid digestion. The relative shoot and root dry weights of seedlings grown in nutrient solution with variable concentrations of Mn was calculated with respect to control (2 μM Mn).

A completely randomised block design with four replicates was employed. Data were subjected to analysis of variance (with LSD5% for comparison among means) using GenStat Release 9.2.

2.2 Screening wheat genotypes for tolerance to high concentration of Mn

Growth conditions, seed preparation, and experimental set up were as described above for the development of screening technique. The screening was carried out in July, with a mean (1994–2009) daily solar exposure of 10 MJ m^{-2} or 2.8 kWh m^{-2} (http://www.bom.gov.au/climate/averages/).

A total of 64 bread wheat (representing a subset of Australian cultivars and advanced breeding lines from Western Australia, plus two genotypes from Canada, two from CIMMYT, and one from Brazil) and two durum wheat genotypes (Tab. 1)

Table 1: Entry number (Ent), name, pedigree/parentage, and source of seed of Australian bread (61 entries) and durum (two entries, on positions #2 and #9) wheat genotypes.

Ent	Name	Pedigree/parentage	Source
1	**WAWHT 2713**	*Amery//(RAC777) VPM1/4*Dagger*	DAFWA
2	**Bellaroi (durum)**	*Yallaroi//TAM 1B-17/Kamilaroi/3/TAM1B-17/Kamilaroi/3/Durati 'S'/ Leeds//Guillemots/4/Wagtail 10//Shearwater/Mallard-DW*	NSWA
3	**Trident**	*VPM1/5*Cook//4*Spear*	RAC
4	**Columbus**	*RL-4352[1170][1318];BW-37[1170][1318];PGR-11858[1318];BN-37[1323]; BN-55[1323][2200];RL-4352.1[1323];BW-55[1956];*	CAN
5	**Camm**	*SPEAR*4//VPM-1/5*COOK[2854];*	DAFWA
6	**Fortune (WAWHT 2856)**	*Calingiri/(386372) Calingiri sib//Calingiri/Worrakatta*	DAFWA
7	**Amery**	*Lr21-SrX/2*Shortim//3*Bodallin*	DAFWA
8	**Tincurrin**	*Gluclub/3/Chile 1B//Insignia/Falcon*	DAFWA
9	**Arrivato (durum)**	*NA*	NZ
10	**EGA EagleRock**	*Sunelg*2/Blade*	DAFWA
11	**Tammarin Rock**	*Kalannie/3/(81Y:970)Skorospelka/4*Lance//3*Bodallin*	DAFWA
12	**Tasman**	*Torres/3/Gaboto/Siete Cerros 66//Bluebird/Ciano 67*	QWRI
13	**Datatine**	*3Ag3/3*Halberd//4*Tincurrin*	DAFWA
14	**Mitre**	*Janz/Beulah*	DPIVIC
15	**Cranbrook**	*Wren-Mex//Ciano,,S"//Noroeste 66/3/Zambezi*	DAFWA
16	**Machete**	*MEC3/2*Gabo(RAC177)//Madden*	RAC
17	**EGA Wentworth**	*Janz/Vulcan//Janz*	EGA
18	**Janz**	*3Ag3/4*Condor//Cook*	QWRI
19	**Qalbis**	*Tincurrin*4/3/Lance*2//Condor*4/3Ag14/4/Tatiara*3//Cook*5/VPM1*	QDPI&F
20	**Blade**	*MEC3/2*Gabo(RAC177)//Kite*	RAC
21	**Cadoux**	*Centrifen/Gamenya (F3)//Gamenya/3/Jacup*	DAFWA
22	**Spear**	*Sabre/MEC3 (RAC111)//Insignia*	RAC
23	**Calingiri**	*Chino/Kulin//Reeves*	DAFWA
24	**BH1146**	*Ponta Grossa I//Fronteira/Mentana*	BR
25	**ClearfdJNZ**	*Janz*4/Fidel Selection 3*	DAFWA
26	**GBA Sapphire**	*GBA008/Janz*	GBA
27	**EGA2248**	*Madden/Bokal (70W18–14–2 Starchy)/3/Lance//Eradu (79W:793)/4/(83W:1087)Matong*2/ IRN 75–560*	EGA
28	**Karlgarin**	*Spear//(79W:781) Bodallin/Eradu*	DAFWA
29	**Gutha**	*Gamenya//Gabo*3/Khapstein(M146)/3/Falcon*3/Chile 1B*	DAFWA
30	**Gamenya**	*Gabo*5/Mentana (W1124)//(W1347)Gabo*2/Kenya 117*	USPBI
31	**WAWHT2036**	*Cotipora/Gamenya//Eradu/3/(77W660) Complex pedigree*	DAFWA
32	**Kulin**	*Bodallin sib//(Hyden sib) Gamenya/Inia66*	DAFWA
33	**Carnamah**	*Bolsena-1CH/(77W:660) Complex pedigree*	DAFWA
34	**EGABonnieRock**	*Sr9e/3*Warigal//3*Aroona (83Z:1048)/4/(82W:1097) 3Ag3/4*Condor//3*Millewa/3/Bodallin*	EGA
35	**Perenjori**	*Bodallin/Hyden*	DAFWA
36	**Schomburg**	*W3589/Oxley//2*Warigal/3/2*Aroona*	UA
37	**ES8**	*Colonista/Frontana (Carazinho)//Egret*	CSIRO
38	**Wyalkatchem**	*Machete/4/(W84–129*504) Gutha/3/Jacup*2//(11thISEPTON135) Iassul/H567–71*	DAFWA
39	**Frame**	*Molineux/3*Dagger*	UA
40	**Cunderdin**	*Cranbrook sib(9thIBWSN322,Flicker,,S")/(SUN95H)Sunfield sib*	DAFWA
41	**Cascades**	*Aroona*3//(AUSENVII-95,Qualset 601–20)Tadorna/Inia66*	DAFWA
42	**EGA-Hume**	*Pelsart/2*Batavia*	EGA

Table 1: continued.

Ent	Name	Pedigree/parentage	Source
43	**Yandanooka (WAWHT2773)**	*Calingiri/(81W:1137)Tammin sib/3/(WAWHT2029, 386443) 13IBWSN397(IW:725)/ Hyden bulk*	DAFWA
44	**Siete Cerros**	*Penjama 62 sib/Gabo 55*	CIMMYT
45	**Kalannie**	*Falcon-EMS/Shabarti Sonora 64(70Y71–315)/6/(71W15–7)Madden sister(M146)/4/ (P10522B) Ciano/8156B/3/ Ciano//Sonora64/Klein Rendidor(76W:591)/5/Aroona*	DAFWA
46	**WAWHT 2884**	*Sunelg/2*Westonia*	DAFWA
47	**Yanac**	*Jabiru/M5392–1//M5392/3/Cook*	AV
48	**Aroona**	*WW15/Raven*	SARDI
49	**BT-Schomburgk**	*Halberd/Aroona//3*Schomburgk*	SARDI
50	**Brookton**	*Torres/Cranbrook/4/(76W596)Emblem/1640//Nuri70/3/Cranbrook*	DAFWA
51	**Arrino**	*Complex pedigree (77W:660)/Eradu*	DAFWA
52	**Magenta (WAWHT2726)**	*Carnamah/Tammin18*	DAFWA
53	**H46**	*H45*3/Sunbri*	DAFWA
54	**Westonia**	*Spica/Timgalen(QT2085–20)//Tosca(81R:1052,CO1190–203)/3/(84W127–501)Cranbrook// Jacup*2/Bobwhite*	DAFWA
55	**Eradu**	*Ciano67/Gamenya*	DAFWA
56	**WAWHT 2772**	*Calingiri/(81W:1137)Tammin sib/3/(WAWHT2029, 386443) 13IBWSN397(IW:725)/ Hyden bulk*	DAFWA
57	**WAWHT 2750**	*Westonia/2*Perenjori26*	DAFWA
58	**Norquay**	*LERMA-ROJO-64/SONORA-64//JUSTIN[39][1323];*	CAN
59	**Kauz-s**	*Jupateco73,,s"/Bluejay//Ures81*	CIMMYT
60	**GBA-Hunter**	*Attila/3/Altar84/Aros//Attila*	NSWA
61	**Nyabing**	*3Ag3/Aroona (WT329)/3/(IW753,WD194) 3Ag14/4*Condor//Jabiru*	DAFWA
62	**Egret**	*Heron/2*WW15*	NSWA
63	**WAWHT 2734**	*Arrino//(Y89–4034) Eradu*4/VPM1*	DAFWA
64	**ET8**	*Colonista/Frontana (Carazinho)//Egret*	CSIRO
65	**Krichauff**	*Wariquam//Kloka/Pitic 62/3/Warimek/Halberd/4/3Ag3/Aroona*	UA
66	**Warigal**	*WW15/Raven*	SARDI

AG: Access Genetics**, AGT**: Australian Grain Technology, **BR**: Brazil**, AV**: Agriculture Victoria, **CAN**: Canada**, CIMMYT**: International Maize and Wheat Improvement Center**, CRC**: Value Added Wheat CRC**, DAFWA**: Department of Agriculture and Food Western Australia, **EGA:** Enterprise Grains Australia, **GBA:** Grain Bio-tech Australia Pty Ltd, **HS**: Heritage Seeds Pty Ltd, **NSWA**: New South Whales Agriculture**, NZ:** New Zealand, **QDPI&F**: Queensland Department of Primary Industry and Fishery, **QWRI**: Queensland Wheat Research Institute**, RAC**: Roseworthy Agricultural Campus, The University of Adelaide **SARDI:** South Australian Research & Development Institute, **UA**: The University of Adelaide, **USPBI**: The University of Sydney Plant Breeding Institute, **VAWCRC**: Value Added Wheat CRC, **VIDA:** Victorian Institute for Dryland Agriculture

were screened using 2 and 750 μM Mn ($MnCl_2$) as control and toxic concentrations, respectively. Norquay, Columbus, and Cascades were used as reference genotypes.

A completely randomised block design with three replicates was employed. Data were analysed as described above.

3 Results and discussion

3.1 Development of a screening technique

Increasing the concentration of Mn in nutrient solution up to 3000 μM decreased the shoot dry weight by 56% in Norquay, 78% in Columbus, and 62% in Cascades (Fig. 1). Genotypic discrimination between tolerant and intolerant genotypes was observed across almost all applied concentrations of Mn, but especially at 500, 750, and 1000 μM Mn, whereby Cascades was shown to be moderately tolerant and also distinct from the tolerant (Norqway) and intolerant (Columbus) genotype (Fig. 1).

A significant decrease in root dry weight upon increasing concentration of Mn in nutrient solution was observed for all three genotypes and was more pronounced than for relative shoot dry weight (Fig. 2). Manganese toxicity is likely to affect root growth indirectly through the shoot injury caused by excess accumulation of Mn (*Zhang* et al., 1999). The relatively high sensitivity of wheat root growth to other ion toxicities, including Fe^{2+} (Khabaz-Saberi et al., in preparation), B (*Cartwright*

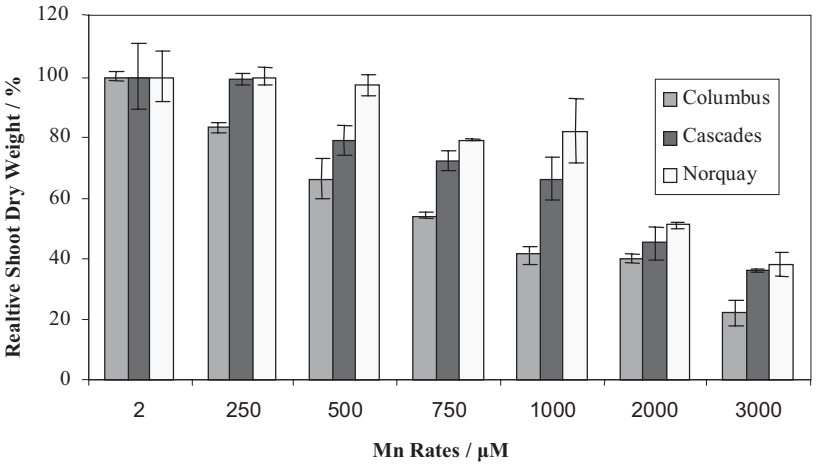

Figure 1: Relative shoot weight (100% at 2 µM Mn) of wheat genotypes Columbus (Mn-intolerant), Cascades (moderately Mn-tolerant), and Norquay (Mn-tolerant). The Mn rates (as MnCl) were applied to 9 d old plants for 14 d. Values are means ± SE (n = 4).

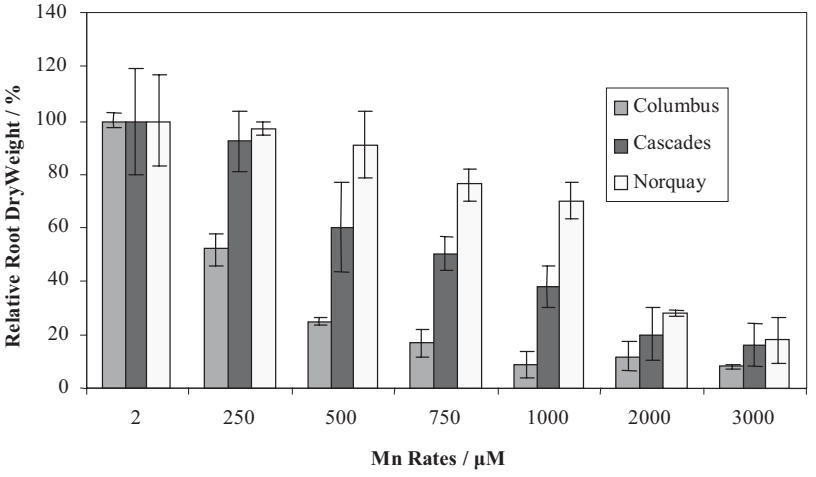

Figure 2: Relative root dry weight (100% at 2 µM Mn) of wheat genotypes Columbus (Mn-intolerant), Cascades (moderately Mn-tolerant), and Norquay (Mn-tolerant). The Mn rates (as $MnCl_2$) were applied to 9 d old plants for 14 d. Values are means ± SE (n = 4).

et al., 1987), and Al (*Tang* et al., 2003), is notable, and is in contrast to deficiencies of Zn (*Rengel* and *Graham,* 1995, 1996) and Mn (*Khabaz-Saberi* et al., 2002) that affect both root and shoot growth in wheat to a similar extent.

Increasing Mn concentrations in the nutrient solution intensified the development of Mn toxicity symptoms on leaves (Fig. 3, r = 0.92**). These symptoms developed as brown spots on older leaves followed by chlorosis, necrosis, yellowing of the leaf tip, and withered leaf margins as described in rice (*Wang* et al., 2002) and other plants (*Wissemeier* and *Horst*, 1992). The necrotic brown spots may be caused by an accumulation of oxidised Mn and oxidised phenols in the cell wall (*Horiguchi*, 1987; *Wissemeier* and *Horst*, 1992). The

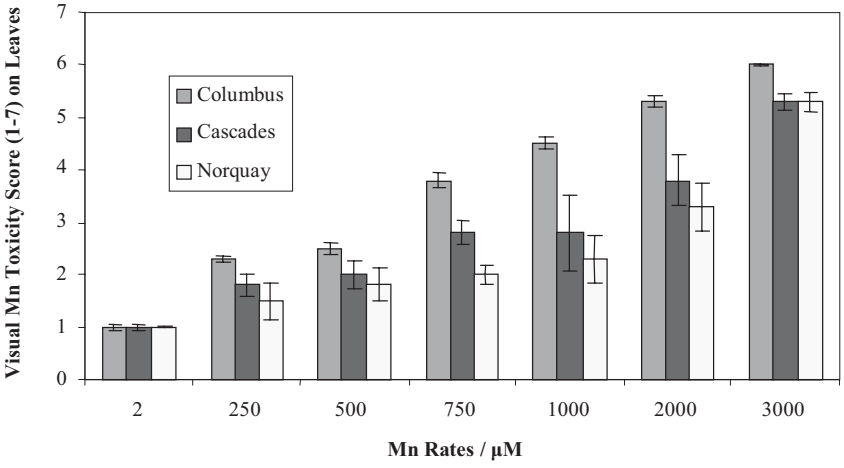

Figure 3: Development of Mn toxicity symptoms (1 = no symptoms; 7 = severe symptoms) of wheat genotypes Columbus (Mn-intolerant), Cascades (moderately Mn-tolerant), and Norquay (Mn-tolerant). The Mn rates (as $MnCl_2$) were applied to 9 d old plants for 14 d. Values are means ± SE (n = 4).

severity of toxicity symptoms on wheat inversely correlated with relative root dry weight (r = –0.83*), showing potential of this parameter as a selection tool. In the present study, Mn-intolerant cv. Columbus showed more severe Mn toxicity symptoms than Mn-tolerant Norquay in all Mn-toxicity treatments. The best discrimination into tolerant, moderately tolerant, and intolerant genotype was achieved at 750 μM Mn (Fig. 1).

Compared with the 2 μM Mn control, shoot Mn concentrations increased similarly for all three genotypes at 250 μM Mn (Fig. 4). Manganese-tolerant Norquay had higher shoot concentrations of Mn at a wider range of applied Mn (500 to 2000 μM) compared with Columbus and Cascades. The same pattern was also observed in shoot Mn content (data not shown). In other studies, Mn-tolerant genotypes of wheat (*Burke* et al., 1990) and rice (*Wang* et al., 2002) also transported more Mn from roots to leaves than Mn-intolerant genotypes, indicating the presence of internal tolerance mechanisms.

The differential responses of Norquay, Columbus, and Cascades to Mn toxicity confirmed the findings of an earlier study (*Macfie* et al., 1989). A significant separation of these three genotypes for all criteria (root and shoot relative dry weight, symptoms score, and shoot Mn concentration and content) was observed only at 750 μM Mn in the growth medium, and this concentration was chosen for the subsequent screening of 64 bread and two durum genotypes (Tab. 1) under temperature-controlled conditions. The selected concentration (750 μM Mn) was higher than previously used for wheat (500 μM Mn) by *Moroni* et al. (1991), probably due to a different experimental set-up. Also, *Moroni* et al. (1991) used only root and shoot growth to determine severity of Mn toxicity compared with our study in which toxicity symptoms and shoot Mn concentration and content were also taken into consideration.

3.2 Screening wheat germplasm for tolerance to high concentration of Mn

In the screening experiment, the indicator genotypes Norquay, Columbus, and Cascades differed significantly in relative root and shoot dry weights (Tab. 2) as in the optimisation study (Fig. 1). Norquay had significantly higher shoot Mn concentration than Columbus, which was consistent with the optimisation study. There was also a significant positive correlation (r = 0.85*) between relative shoot dry weight and shoot Mn concentration for the range of tested genotypes grown at 750 μM Mn.

Wide genotypic variation was observed among the 66 wheat genotypes for relative root (11%–95%) and shoot dry weights (31%–94%). A wider variation in the relative root than in the shoot dry weight is in agreement with the optimisation study (Figs. 1 and 2). Hence, limited root growth of Mn-intolerant genotypes can be used as a selection criterion in screening studies. Manganese-tolerant Norquay developed significantly milder Mn toxicity symptoms (score 2.1) than Mn-intolerant Columbus (score 4.2, Tab. 2), which is in agreement with an earlier study using different genotypes (*Burke* et al., 1990). In the present study, there was a significant correlation (r = –0.81*) between relative root dry weight and symptoms score for all genotypes tested, suggesting that the relative root dry weight can be the main criterion for distinguishing genotypes in terms of their tolerance to Mn toxicity.

Trident and Warigal showed two extremes (12% and 95%, respectively) of Mn tolerance based on relative root dry weight (Tab. 2). Genotypes WAWHT2734 (an advanced breeding line from Western Australia), Krichauff, and Warigal had significantly higher relative root dry weight than Mn-tolerant Norquay (Tab. 1). In terms of relative shoot dry weight, no entry was better than Norquay, but ET8, Warigal, and Nyabing were similar to it. Genotype ET8, an isogenic Al-tolerant line (*Delhaize* et al., 1993; *Tang* et al., 2003), showed relatively high Mn tolerance in contrast to Mn-intolerant but Al-tolerant BH1146 (see also *Tang* et al., 2003), suggesting that Mn tolerance may or may not co-exist with Al tolerance. The co-tolerance to ion toxicities is a desirable trait in locally adapted germplasm and a major requirement for pyramiding multiple tolerances.

The source of Mn tolerance in hexaploid Canadian wheat has been suggested to be Brazilian landraces introduced either directly or indirectly through CIMMYT's germplasm containing Brazilian parentage (*Moroni* et al., 1991). Similarly, the Brazilian Al-tolerant germplasm with or without co-tolerance

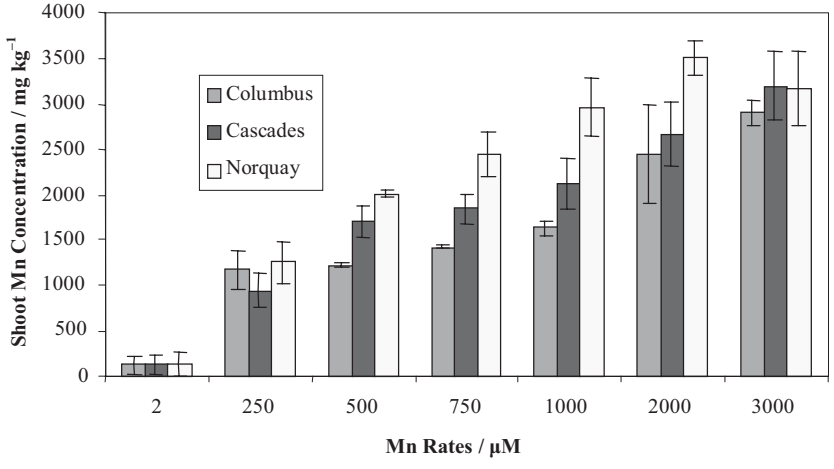

Figure 4: Shoot Mn concentrations of wheat genotypes Columbus (Mn-intolerant), Cascades (moderately Mn-tolerant), and Norquay (Mn-tolerant). The Mn rates (as $MnCl_2$) were applied to 9 d old plants for 14 d. Values are means ± SE (n = 4).

Table 2: Relative root and shoot dry weights (dry matter at 750 μM Mn relative to that at 2 μM Mn), root and shoot dry weight in the control treatment (mg seedling^{-1}), shoot Mn concentration (mg kg^{-1}), toxicity symptom score (1 = no symptoms; 7 = severe symptoms), and Mn content (μmol Mn seedling^{-1}) in 23 d old plants of Australian bread wheat genotypes. Entry number codes correspond to the genotypes presented in Tab. 1. Genotypes were ordered by increasing relative root dry weight. Values are means of three replicates with LSD 5% for comparison among means. Nd = not determined.

Entry Number	Relative root dry weight / %	Relative shoot dry weight / %	Root dry weight at 2 μM Mn / mg	Shoot dry weight at 2 μM Mn / mg	Mn concentr. at 750 μM Mn / mg kg^{-1}	Symptom score at 750 μM Mn (1–7)	Mn content at 75 μM Mn / μmol Mn
1	11	31	162	442	901	4.5	194
2	12	35	179	444	1096	4.2	295
3	12	33	143	382	884	3.8	208
4	14	43	141	337	1255	4.2	332
5	16	40	141	395	883	4.1	262
6	16	35	155	392	905	3.9	235
7	18	38	159	406	1118	3.8	319
8	20	43	131	303	942	4.3	225
9	22	60	139	323	1373	3.8	438
10	22	52	156	414	1229	3.8	308
11	22	42	171	459		3.2	
12	22	51	184	456		3.3	
13	23	57	129	259		4.0	
14	26	51	162	406		3.2	
15	27	59	142	347		3.5	
16	27	40	150	440		3.5	
17	28	49	174	386		3.6	
28	28	50	168	393		3.2	
19	28	61	158	373		3.6	
20	29	60	150	345		3.3	
21	29	55	129	339		3.5	
22	30	48	169	374		3.5	
23	30	52	136	377		3.2	
24	31	51	183	462		3.2	
25	31	50	176	426		3.6	
26	31	50	138	374		3.3	
27	32	53	171	459		3.4	
28	36	43	141	333		3.2	
29	37	48	134	440		3.1	
30	38	51	134	362		3.3	
31	38	64	162	443		2.8	
32	39	55	128	350		3.4	
33	41	56	180	422		3.1	
34	42	57	156	414		3.2	
35	42	53	137	349		3.2	
36	42	64	176	448		2.6	
37	43	65	183	390		3.0	1563
38	43	62	156	349		2.9	
39	44	79	138	358		3.0	
40	44	46	121	338		3.0	

Table 2: continued.

Entry Number	Relative root dry weight / %	Relative shoot dry weight / %	Root dry weight at 2 µM Mn / mg	Shoot dry weight at 2 µM Mn / mg	Mn concentr. at 750 µM Mn / mg kg^{-1}	Symptom score at 750 µM Mn (1–7)	Mn content at 75 µM Mn / µmol Mn
41	46	60	192	476		2.7	
42	48	64	153	387		3.3	
43	50	51	155	428		2.8	
44	50	66	166	396		2.7	
45	51	61	128	353		2.7	
46	51	71	149	385		3.1	
47	51	51	110	313		3.3	
48	53	69	184	460		2.5	
49	54	71	156	337		3.0	
50	55	77	138	342	1297	2.7	691
51	56	59	112	302	2212	2.7	817
52	61	62	134	329		2.8	
53	61	69	142	281	2167	2.6	764
54	62	65	151	402	1690	2.6	779
55	62	54	125	369		2.9	
56	63	79	135	362	2121	2.6	943
57	64	72	162	418		2.7	
58	64	94	216	384		2.1	
59	66	63	125	315	1833	2.7	623
60	66	73	180	417	1668	2.2	889
61	68	85	188	454	1627	2.4	1144
62	69	70	162	388	2715	2.2	1287
63	73	68	110	329	2056	2.9	846
64	77	89	170	350	2887	1.9	
65	80	70	130	419	2006	2.5	1009
66	95	91	173	432	2695	1.5	1867
Mean	**40**	**58**	**183**	**354**	**1635**	**3.2**	**646**
LSD 5%	**9**	**11**	**35**	**61**	**730**	**0.6**	**172**

to Mn was also introduced into Australia in an attempt to improve Al tolerance, resulting in Al-tolerant varieties without (Tammarin Rock) or with Mn tolerance (Westonia, for Al tolerance: *Tang* et al., 2003; for Mn tolerance: Tab. 2) developed by the major Western Australian breeding program with an active selection for Al tolerance (*Carver* et al., 1995; *Tang* et al., 2003).

The top five Australian Mn-tolerant varieties (Nyabing, Egret, ET8, Krichauf, and Warigal, Tabs. 1 and 2) share a common parent WW-15, *i.e.*, "WAGGA-WAGGA-15 (LERMA-ROJO-64//(SELECTION14)NORIN10/BREVOR/3/3*ANDES-ENANO) (http://genbank.vurv.cz/wheat/pedigree/pedigree.asp). It is therefore likely that LERMA-ROJO-64, a highly Mn-tolerant CIMMYT variety (*Moroni* et al., 1991), was the source of Mn tolerance in the top five Australian Mn-tolerant varieties. In Western Australia where selection pressure exists for tolerance to both Al and Mn toxicity (but only Al tolerance is being selected for), an indirect/passive selection for Mn tolerance is most likely and could explain the co-tolerance to Al and Mn toxicity in Arrino, Westonia, and some Western Australian advanced breeding lines (see also *Tang* et al., 2003).

Two durum wheat varieties Bellaroi and Arrivato represented the lower end of tolerance to Mn toxicity (Tab. 1 and 2). This finding is in agreement with durum being generally less tolerant to ion deficiencies and toxicities compared with bread wheat (*Cosic* et al., 1994; *Rengel* and *Graham*, 1995, 1996; *Erenoglu* et al., 1999; *Khabaz-Saberi* et al., 2000). However, some durum genotypes may have excellent tolerance to toxicity of Fe (Khabaz-Saberi et al., in preparation) and Al (*Foy*, 1996). A wider range of durum genotypes would need to be tested to ascertain the extent of their tolerance to Mn toxicity.

Some bread-wheat genotypes tolerant to Mn deficiency (Trident, Janz, and Frame: *McDonald* et al., 2001) showed

poor tolerance to Mn toxicity. In contrast, other genotypes tolerant to Mn deficiency (Westonia, Yitpi, and Krichauff: *McDonald* et al., 2001) expressed high tolerance to Mn toxicity, suggesting that tolerance to Mn deficiency and Mn toxicity are not mutually exclusive traits. Such combined tolerance broadens the adaptation of genotypes to agricultural areas where both constraints may occur over time and/or space due to soil heterogeneity and differential rainfall patterns, possibly explaining the observed broad adaptation of Westonia and Krichauff. Similar evidence of co-tolerance to Mn excess and Mn deficiency was observed in two Indian barley cultivars (*Manyowa* and *Miller*, 1991).

Wheat genotypes tolerant to Mn toxicity (Tab. 2) as well as Mn deficiency (*Khabaz-Saberi* et al., 2002, *e.g.*, Westonia and Krichauff) have an enhanced capability to accumulate Mn in shoots when grown in either Mn-toxic or Mn-deficient environments; however, shoot Mn accumulation in Mn-toxic environments should be accompanied in these genotypes by an improved internal tolerance to Mn. Further work is necessary to ascertain which mechanisms underlie increased shoot accumulation of Mn in wheat genotypes co-tolerating Mn excess and Mn deficiency.

4 Conclusions

A solution-based screening assay for Mn tolerance in hexaploid wheat was developed and used to identify wide variation in a subset of Australian wheat germplasm. The developed assay and the selection criteria could be used for further exploitation of genetic variability for Mn toxicity in wheat. The identified genotypic variation (upon validation in soil) may be used in further genetic and physiological characterisation of Mn toxicity or as a source of Mn tolerance in breeding programs.

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