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Long-term phytotoxic effects of aluminium on Al susceptible and Al-tolerant genotypes of wheat selected from microspores

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Aluminium toxicity is considered to be the most important factor limiting plant growth and production in acid soils. Thus, the selection of Al-resistant crops via plant breeding or biotechnology is of agronomic importance (Kochian 1995). Since Al tolerance is expressed in both the vegetative and generative life cycle of plants (Seary and Mulcachy 1990), unicellular microspores in anther culture are suitable for the selection of Al-resistant wheat genotypes, as reported by Kovacs et al. (1993). The spontaneous and induced diploidisation of uninucleate microspores may enhance the possibility of selecting aluminium-tolerant genotypes in homozygotic form. The present work compares the physiological responses of DH_2 lines of Al-sensitive and Al-tolerant wheat genotypes selected from microspores.

Materials and Methods

The experiments were performed on DH_2 (second generation of fertile dihaploid) plants of Al-resistant and sensitive genotypes of cv. Mv 16 wheat. The plants were grown in Hoagland solution containing 300 μM $AlCl_3$ at pH 4.0, with control experiments at pH 5.2 and at pH 4.0 without Al. Ten-day old plants were used for the investigations. Hematoxylin staining was carried out as decribed by Cancado et al. (1999). Morin staining was used for the detection of Al in plant tissue as described by Tice et al. (1992). Quenching analysis and CO_2 fixation measurements were performed as described by Schoefs et al. (2001) and Darkó et al. (1996), respectively.

Table 1. Root length of 10-day-old Al-sensitive (S) and Al-tolerant (R1, R2) genotypes of wheat grown in Hoagland solution at pH 5.2 and at pH 4.0 with or without 300 μM $AlCl_3$. Root regrowth after hematoxylin staining.

		S	R_1	R_2
Root length (cm)	pH 5.2	9.2±1.1	9.6±1.4	10.2±1.2
	pH 4.0	8.4±1	9.4±1.1	10.5±1.4
	pH 4.0 Al	6.8±1	8.9±1.2	9.2±1.2
Hem.test (mm/day)		4.12 ±0.4	7.55±0.5	8.5±0.6

Results and Discussion

The first symptom of Al toxicity is reduced root growth (Kochian 1995). This reduction was evident in the sensitive line but was limited in resistant genotypes of microspore-selected wheat (Table 1). This result was supported by the root regrowth after hematoxylin staining (Table 1) Aluminium accumulates especially in the apex region of root, as detected by morin staining (Fig. 1D). The presence of Al was significantly reduced in the elongation zone of the root and could not be observed in leaves (Fig. 1B,C). As presented in Figure 1D,E,F. Aluminium accumulation was significantly higher in Al-sensitive lines than in Al-resistant genotypes, suggesting an Al-exclusion mechanism operating in the resistant lines.

The phytotoxic effects of Al treatment were reduced in the shoot as indicated by the slight shoot growth inhibition in sensitive line and by the lack of inhibition in resistant genotypes.

Table 2. Shoot growth of 10-day- old Al-sensitive (S) and Al-tolerant (R1, R2) genotypes of wheat grown in Hoagland solution at pH 5.2 and at pH 4.0 with or without 300 μM $AlCl_3$. NPQ is the non-photochemical quenching parameter measured on leaves by quenching analysis at 600 mmol photon $m^{-2}s^{-1}$ light intensity. A_{max} is the maximal CO_2 assimilation rate measured at saturated light intensity. A (in dark) and O_2 uptake reflects the CO_2 evolution and O_2 consumption in the dark.

	S			R_1			R_2		
	pH 5.2	pH 4.0	pH 4.0 Al	pH 5.2	pH 4.0	pH 4.0 Al	pH 5.2	pH 4.0	pH 4.0 Al
Shoot length	18.7	19.7	17.2	19.4	20.5	20.2	19.1	20.6	19.8
(cm)	±1.2	±1.4	±1.1	±1.3	±1.4	±1.5	±1.2	±1.3	±1.2
NPQ	0.662	0.840	1.45	0.0624	0.887	1.11	0.0615	0.820	1.05
	±0.06	±0.07	±0.10	±0.06	±0.08	±0.09	±0.06	±0.075	±0.09
A max	7.8	5.9	3.2	7.2	5.9	5.4	8.8	8.2	7.3
(μmol CO_2/m^2s)	±0.5	±0.4	±0.3	±0.4	±0.4	±0.3	±0.6	±0.4	±0.4
A in dark	-0.430	-0.489	-1.429	-0.245	-0.325	-1.09	-0.41	-0.468	-0.794
(μmol CO_2/m^2s)	±0.05	±0.05	±0.07	±0.03	±0.035	±0.075	±0.04	±0.05	±0.4
O_2 uptake	1.12	1.42	1.84	1.18	1.22	1.15	1.04	1.11	0.98
μmolO_2/gr FW mn	±0.2	±0.15	±0.26	±0.21	±0.26	±0.16	±0.1	±0.2	.±0.2

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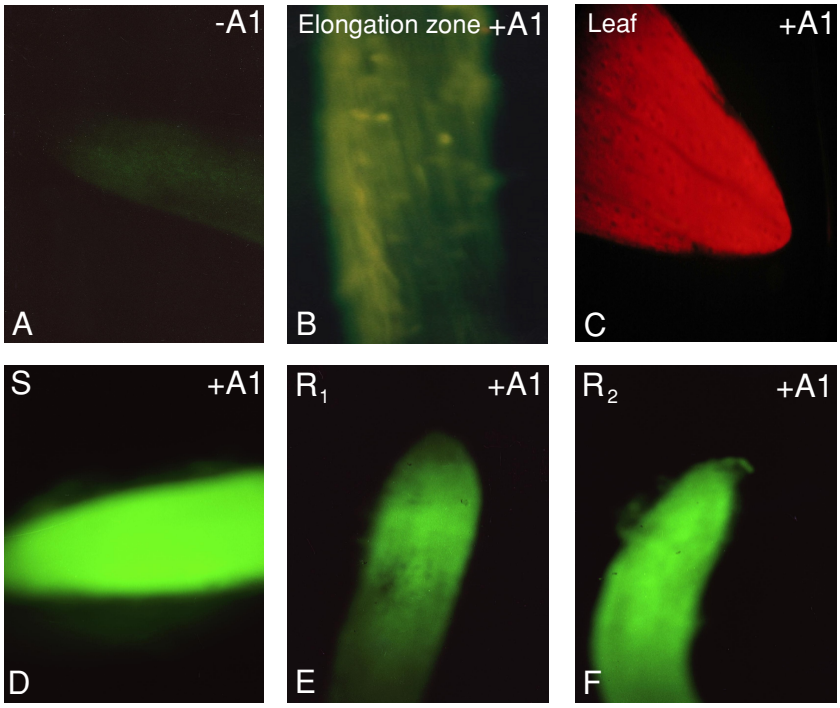

Figure 1. Determination of Al content by morin staining.
The autofluorescence of morin without Al is low (A). A low amount of Al can be detected in the elongation and absorption zones of roots but Al was not detectable in leaves by morin (C). Al accumulates in root apex (approx. 3-4 mm zone of the root tip), especially in the Al-sensitive genotype (D). Lower accumulation can be detected in AL-resistant lines (E,F).

Investigations were also made on the photosynthetic processes, which have a fundamental role in biomass production and thus in productivity, which is well-known to be limited under aluminium stress. No significant differences were found in the chlorophyll content of the leaves, in the PS II activity detected by the Fv/Fm, qP and DF/Fm' parameters of the fluorescence quenching analysis, either between the genotypes or between the different treatments (data not shown). These results suggest, that the photochemical processes of PS II are not limited by aluminium stress. However, the non-photochemical processes, reflecting the dissipation mechanism of the excitation energy, were slightly activated when the plants are grown at low pH and strongly activated in the presence of Al (Table 2.). When comparing the genotypes, this activation was found to be limited in the resistant lines. The photosynthetic capacity, measured as CO_2 fixation was also altered under stress conditions (at low pH with and without Al). Besides a reduction in the maximal CO_2 fixation rate measured at saturated light intensity (appr. 1400 mmol m^{-2} s^{-1}), increased CO_2 evolution and O_2 uptake during dark respiration were also observed (Table 2). These results suggest an alteration in the ATP and NADPH/NADH metabolisms under Al stress. Since Al accumulation in the leaves was not detectable, these effects must be indirect (Fig. 1F).

Acknowledgments

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