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Genetics of tolerance to aluminium in wheat (*Triticum aestivum* L. Thell)

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Abstract

Preliminary studies indicated that aluminium-tolerance in wheat (*Triticum aestivum* L. Thell.) is a dominant character controlled by several genes. The present paper describes further work on localization and characterization of some of these genes in the genome of the medium Al tolerant wheat cultivar Chinese Spring (C.S.), using an aneuploid series (ditelosomics). Aluminium-tolerance of seedlings was assessed using the modified 'pulse' method; the aluminium concentration in the nutrient solution causing irreversible damage to the root apical meristems on exposure for 24 h at 25°C was the measure of Al-tolerance. At least three different factors controlling Al-tolerance in the C.S. cultivar were located on chromosomes 5As, 2Dl and 4Dl. Significant differences were found in Al-uptake and accumulation in roots of the respective ditelosomic lines and euploid seedlings of C.S. Genes controlling Al-tolerance located in the D genome (2Dl and 4Dl) were not expressed in solution culture when genes located on 5As were missing, whereas some tolerance was observed in aneuploid lines in which genes from 5As were present while genes from 2Dl and 4Dl were missing. It is concluded that Al-tolerance genes located in A genome control the expression of other Al-tolerance genes located in the D genome. The implications of the obtained results for chromosome and gene manipulations in cereals are discussed.

Introduction

Aluminium (Al) is regarded as one of the main toxic factors of acid mineral soils. A differential response of wheat cultivars to aluminium has been reported (Aniol and Kączkowski, 1979). Several attempts were made to determine the genetics of Al-tolerance in wheat. According to Kerridge and Kronstad (1968), a single dominant gene was responsible for Al-tolerance in a cross between the wheat varieties Druchamp and Brevor, but it was assumed that additional tolerance genes were present in 'Atlas 66'. The results of Iorczeski and Ohm (1974) indicated the occurrence of several different Al-tolerance genes in wheat cultivars 'IAS 58' and 'Norteno', which was consistent with Campbell and Lafever (1978, 1981) and Aniol (1984), who stated that Al-tolerance in wheat was not simply inherited and that the expression of Al-tolerance was additive with high values of heritability.

Polle *et al.* (1978) found that the substitution of chromosome 4D from the 'Thatcher' wheat cultivar into 'Chinese Spring' (C.S.) reduced the level of Al-tolerance in 'Chinese Spring' close to the level of 'Thatcher', suggesting that Al-tolerance in C.S. is located on chromosome 4D. Ditelosomic lines of C.S. were used by Aniol and Gustafson (1984a) for the localization of Al-tolerance genes in wheat. Several genes were located on chromosome arms of the A and D genomes. Takagi *et al.* (1983) found that the main genes controlling Al-tolerance in C.S. are located on the long arms of chromosomes 2D and 4D and a minor one on the long arm of chromosome 2B.

The results of further screening of C.S. aneuploid lines are presented together with the preliminary data on the biochemical expression of located genes in the 'Chinese Spring' genome.

Materials and methods

The 25 ditelosomic lines of C.S. and euploid seeds of this variety were obtained from Dr E R Sears. The Al-tolerance of tested seedlings was assessed using the nutrient culture, modified pulse method as described earlier (Aniol, 1983). The aneuploid lines were tested at three concentrations of aluminium in the nutrient medium; all concentrations were nontoxic to euploid C.S. seedlings. It was assumed that the ditelosomic lines showing no root regrowth after treatment with sublethal doses of Al, the missing chromosome arm carried a gene or genes controlling an Al-tolerance mechanism in C.S.

Selected aneuploid lines with missing chromosome segments, carrying Al-tolerance genes were tested in modified nutrient medium together with euploid C.S. seedlings. Cation concentration in the medium was increased fivefold as compared to a standard procedure in order to obtain wider differentiation of tested lines (Ali, 1973; Aniol, 1983). Aluminium concentrations in roots and root tips were estimated after ashing using the catechol violet method (Wilson, 1984).

Crossing of selected aneuploid lines with the euploid tolerant Brazilian variety BH1146 was performed in the greenhouse, the growth chamber, and the field. Chromosomes were counted in squash preparations from root meristems using the orcein method.

Results and discussion

Ditelosomic lines of 'Chinese Spring' (C.S.) wheat were screened at three sublethal Al-concentrations in nutrient solution (Table 1). Line 5Al (with missing short arm on both homologous 5A chromosomes) showed irreversible damage to

Table 1. Aluminium tolerance of Chinese Spring ditelosomic lines. Roots of 4-days-old seedlings were exposed in nutrient solution to three aluminium concentrations for 24 h at 25°C. After Al stress, seedlings were grown 48 h in the same medium without Al. The ability of roots to continue growth after Al stress was a measure of tolerance

Aneuploid line	Al-conc. in the medium in μM			Missing arm
	37	56	74	
01. Ditelo 1As	+	+	+	1Al
02. Ditelo 1Al	+	+	+	1As
03. Ditelo 2As	+	+	+	2Al
04. Ditelo 3Al	+	+	+	3As
05. Ditelo 3As	+	+	+	3Al
06. Ditelo 4Al	+	+	+	4As
07. Ditelo 5Al	−	−	−	5As*
08. Ditelo 5Al	+	+	+	6As
09. Ditelo 7As	+	+	+	7Al
10. Ditelo 7Al	+	+	+	7As
11. Ditelo 1Bl	+	+	+	1Bs
12. Ditelo 2Bl	+	+	+	2Bs
13. Ditelo 3Bl	+	+	+	3Bs
14. Ditelo 4Bl	+	+	+	4Bs
15. Ditelo 5Bl	+	+	+	5Bs
16. Ditelo 6Bs	+	+	+	6Bl
17. Ditelo 6Bl	+	+	+	6Bs
18. Ditelo 1Ds	+	+	+	1Dl
19. Ditelo 1Dl	+	+	+	1Ds
20. Ditelo 2Ds	+	+	−	2Dl*
21. Ditelo 3Dl	+	+	+	3Ds
22. Ditelo 4Ds	+	+	−	4Dl*
23. Ditelo 4Dl	+	+	+	4Ds
24. Ditelo 5Dl	+	+	+	5Ds
25. Ditelo 6Dl	+	+	+	6Ds
26. C.S.	+	+	+	-

+ = roots able to regrow after Al stress.
− = roots with irreversible damage to meristems.

root apical meristems after exposure to all three Al concentrations used. Root apical meristems of both other 'critical' aneuploid lines (2Ds and 4Ds) were damaged only at highest Al concentration in the medium, while roots of euploid C.S. seedlings were not damaged at all.

The above results indicate that genetic factors controlling Al-tolerance in C.S. wheat are located on the short arm of chromosome 5A and on the long arms of chromosomes 2D and 4D. However, genes located on the D genome were expressed only at higher Al-concentration in the medium, while genes located on 5A chromosome were expressed at all Al-concentrations used. It can be concluded that genetic factors located on the short arm of chromosome 5A are the major ones, since aneuploid lines without this chromosome fragment show a complete lack of Al-tolerance despite the fact that genes located on 2Dl and 4Dl chromosomes are present.

The critical aneuploid lines: 5Al, 2Ds and 4Ds and some other aneuploid lines of C.S. together with euploid C.S. were crossed with very tolerant Brazilian wheat BH 1146. Root apical meristems of BH 1146 seedlings were approximately 5-times more tolerant to aluminium ions than C.S.; irreversible damage of root meristems was observed after 24 h incubation in medium containing 111 μM Al and 593 μM Al in seedlings of C.S. and BH 1146, respectively (Aniol, 1983). F_2 seedlings were tested at 148 μM Al in the medium, *e.g.* slightly above the toxicity level for C.S. It was assumed that at this Al concentration, all degrees of Al-tolerance introduced from tolerant parent into a hybrid would be identified (Table 2). The frequency of Al tolerant seedlings in the F_2 population from crosses between ditelo 5Al and 4Ds aneuploid lines and BH 1146 markedly differ from the frequency obtained in crosses of other aneuploid lines and euploid C.S. with BH 1146; approximately 50% less tolerant seedlings were found in F_2 hybrids from C.S. 'critical' aneuploid × BH 1146. Surprisingly, segregation in F_2 population involving 2Ds ditelo line did not differ from the one obtained in the control F_2 populations. It can be concluded that the results obtained confirm the presence of genes controlling Al-tolerance in C.S. on short arm of chromosome 5A and on the long arm of chromosome 4D.

A significantly higher frequency of tolerant seedlings was found among F_2 seedlings from the cross ditelo 6Bl × BH 1146. This might suggest that some factors suppressing Al-tolerance are located on the short arm of 6B chromosome of C.S.

It can be concluded that besides the major genetic factors controlling Al-tolerance in C.S., located on short-arm of chromosome 5A and on the long arm of chromosome 4D some other genetic factors modifying the expression of this gene are present in the genome of C.S. and BH 1146.

An attempt was made to identify the physiological expression of Al-tolerance genes located in C.S. genome by analyzing Al accumulation in roots of seedlings exposed to Al ions (Table 3). Under the modified conditions of growth and Al-concentration, root apical meristems of seedlings ditelo 5Al

Table 2. Aluminium tolerance of F_2 seedlings from crosses of C.S. ditelosomics and tolerant BH 1146. Seedlings were screened at 148 μM of Al for 24 h at 25°C. Data are means of at least three independent experiments

Aneuploid line	# of tested F_2 seedlings	Percent of tolerant seedlings (± S.D.)
1Al	246	51 ± 13
2As	170	43 ± 12
3Al	467	53 ± 11
4Al	406	46 ± 5
5Al	597	20 ± 9*
6Al	725	52 ± 13
6As	202	47 ± 12
3Bl	440	50 ± 6
6Bs	489	51 ± 10
6Bl	154	75 ± 6*
2Ds	170	54 ± 12
4Ds	423	18 ± 12*
4Dl	681	57 ± 12
C.S.	425	42 ± 9

Table 3. Aluminium concentration in roots and root tips from tolerant and sensitive C.S. lines. Aluminium concentration is expressed in mg Al g dry wt^{-1}. Root tips constitute 20 ± 3.3% of total root dry wt

Line		Al-conc. in the medium in μM			
		74	148	222	296
C.S.	Roots	0.53	0.69	0.64	0.78
	Tips	1.90	1.89	3.77	5.17*
5Al	Roots	0.72	0.92	0.91	1.29
	Tips	3.20*	3.48*	4.20*	5.43*
2Ds	Roots	0.52	0.50	0.59	0.92
	Tips	1.35	2.21	2.59	5.47*

* = irreversible damage to apical meristems.

Table 4. Aluminium accumulation in roots and root tips of tested C.S. lines. Al accumulation expressed in micrograms of Al/100 seedlings

Line	Al-conc. in the medium in μM							
	74		148		222		296	
	Roots	Tips	Roots	Tips	Roots	Tips	Roots	Tips
C.S.	64.5	49.1	85.3	71.8	110.7	94.7	143.8	132.5
% in tips		76		84		86		92
5Al	99.6	95.1	112.3	102.4	126.2	120.9	173.5	161.1
% in tips		95		91		96		93
2Ds	59.7	37.3	75.6	64.2	91.5	79.8	144.1	130.3
% in tips		62		85		87		90

were damaged at all tested concentrations of Al in the medium, while roots of ditelo 2Ds and C.S. euploid seedlings were damaged only at highest Al concentration in the medium. These physiological differences were reflected in Al accumulation in the roots, particularly in the root tips, where 85–95% of total Al found in roots was accumulated (Table 4). Approximately 100% more aluminium was accumulated in root tips of ditelo 5Al seedlings at $75\,\mu M$ Al in the medium than in root tips of another ditelosomic line and euploid C.S., while root tips of 2Ds ditelo line accumulated less Al than euploid C.S. These differences in Al accumulation disappeared at highest external Al concentration ($296\,\mu M$) where roots of all tested genotypes were irreversibly damaged. It is important to note that root tips of tolerant euploid C.S. seedlings accumulated more Al at $222\,\mu M$ external Al (3.77 mg Al per g dry wt.) than root tips of ditelo 5Al line at $75\,\mu M$ Al in the medium, and despite this high Al content, roots of C.S. were able to grow while roots of ditelo 5Al were irreversibly damaged.

One can conclude that the absence of genes controlling Al tolerance located on 5As chromosome in C.S. is manifested by increased Al uptake by root tips at low external Al concentration. Consequently, Al accumulation in root tips is faster in sensitive than in tolerant lines, leading to destruction of root meristems at lower external Al concentrations, or shorter times of exposure to toxic ions. But also it is evident that root tips of tolerant lines can survive, at least temporary, higher internal concentration of aluminium than roots of sensitive genotypes. One can speculate that genes located on 5As chromosomes control the mechanism of Al-uptake, as do genes located on 2Dl chromosome, but additional genes from the 5A chromosome are also responsible for some mechanism of Al detoxification inside root tissue. Various mechanisms of Al detoxification have been postulated: binding of Al by mucilages produced by roots (Horst *et al.*, 1982), changes of pH in rhizosphere (Foy *et al.*, 1978) as well as chelation of Al by organic acids (Foy *et al.*, 1978) or metal binding proteins (Aniol, 1984). Further work on isolation and identification of the mechanism of located Al-tolerance genes in wheat is needed.

The results indicate that aluminium tolerance in wheat is a complex character, controlled by several major genes, minor modifying genes, and probably by genes controlling suppression of Al-tolerance genes. This information is important for breeders trying to incorporate Al-tolerance into wheat varieties as well as for programs aimed at breeding of Al tolerant varieties of wheat-rye hybrids (triticale). It can be concluded that incorporation of alien variation from related species into cereals would not be a simple process.

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