Mapping of loci influencing the copper tolerance and shoot Cu, Fe, Mn and Zn concentrations of wheat

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Main points of the thesis

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1. BACKGROUND AND AIMS

In small quantities copper is an essential micronutrient for all living organisms, but in larger quantities it is toxic, inhibiting the growth and development of plants. On soils polluted with heavy metals, a decline in yield can be achieved either by reducing the heavy metal content of the soil or by developing varieties tolerant of heavy metals. Tolerant plants that accumulate heavy metals in the shoots could be of significance in the cheap phytoremediation of polluted soils.

Some 50% of all foodstuffs are made from the grain yield of cereals. However, the grain of common wheat contains far below the optimum quantity of the essential elements Fe and Zn, and also little Cu and Mn. Thus, although the heavy metal content of plant parts intended for consumption must be kept at a low level in plants grown on polluted soils, on non-polluted soils it may be necessary to increase the mineral element concentration of the grain for certain elements (Fe, Mn, Cu, Zn).

Much work has already been done on the physiological analysis of the mechanisms of copper tolerance and toxicity in cereals, but very little is yet known on the location of genes responsible for tolerance. If these genes were mapped and linked markers were identified, copper-tolerant varieties could be developed using molecular marker-assisted selection. The present work was thus aimed at determining chromosomes and quantitative trait loci (QTLs) involved in the copper tolerance of wheat. It was also hoped to identify loci influencing shoot Cu, Fe, Mn and Zn concentrations and to investigate ways of utilising copper-tolerant wheats in practice. To this end, the following studies were undertaken:

1. Identification of wheat genotypes tolerant of or sensitive to copper, and the comparison of the copper tolerance of wheat with that of closely related cereal species.

2. Elaboration of an analytical system for the rapid testing of large numbers of genotypes to determine the stress tolerance of mapping populations and gene bank accessions.

3. Determination of the chromosomal localisation of copper tolerance by testing the copper tolerance of cytogenetic materials (e.g. substitution series), followed by the use of genetic materials such as deletions, introgressions and recombinant inbred lines to identify loci influencing copper tolerance.
4. Identification of loci influencing Cu translocation into the shoots by analysing the shoot Cu content of recombinant lines grown on control and copper-treated soil, and identification of loci influencing the shoot concentrations of microelements important for human nutrition (Fe, Mn, Zn) in plants grown on control and copper-treated soil.

5. Analysis of shoot Cu concentrations in copper-treated plants in order to determine whether wheat counters the toxic effect of copper by restricting heavy metal uptake (avoidance strategy) or by neutralising the surplus heavy metal absorbed (tolerance strategy).

6. Evaluation of the agronomic traits of tolerant and sensitive wheat varieties grown to maturity to investigate possible practical applications, including the feasibility of using wheat for the phyto remediation of Cu-polluted soils.

2. MATERIALS AND METHODS

2.1. Plant material

In order to identify copper-sensitive and -tolerant wheat varieties and to compare the copper tolerance of wheat and related species, tests were made on the copper tolerance of 27 genotypes belonging to the Triticinae subtribe (8 Aegilops, 5 einkorn, 6 bread wheat, 5 other wheat, 2 rye and 1 triticale) stored in the Martonvásár Cereal Gene Bank.

‘Chinese Spring’/‘Cappelle Desprez’ substitution lines, selected in the course of preliminary experiments from the available substitution series, were used to determine the chromosomal localisation of genes responsible for copper tolerance.

QTLs influencing copper tolerance were determined by analysing three mapping populations [the ‘ITMI’ wheat mapping population, ‘Chinese Spring(Cheyenne5A)’ × ‘CS(Tspelta5A)’ and ‘CS’ × ‘CS(Ch5B)’] and ‘CS’ – Aegilops tauschii 3D chromosome introgression lines.

The physical mapping of genes influencing copper tolerance was achieved by analysing deletion lines for the long arm of chromosomes 5A and 5D of ‘CS’.
2.2. Plant growth

Tests to compare the copper tolerance of wild and cultivated wheat species were carried out in hydroponics, while for the other analyses the plants were grown in soil. In the hydroponic system the plants were grown in Hoagland nutrient solution with Cu concentrations of $10^{-7}$ M (control) and $10^{-4}$ M (Cu treatment). These levels were chosen on the basis of preliminary experiments. In the soil system, no copper was added to the control, while concentrations of 1000, 1250 and 1500 mg/kg Cu were used for the treatments, again chosen on the basis of preliminary experiments. After germination the plants were grown to the 2- to 3-week stage in a phytotron chamber (Agricultural Research Institute of the Hungarian Academy of Sciences, Martonvásár) or in a greenhouse (IPK-Gatersleben, Germany), after which measurements were made on the root and shoot lengths and on the dry mass. A Tolerance Index (TI), consisting of the ratio of the given parameter (e.g. dry mass) under treated and control conditions, was used to determine the degree of tolerance. In the greenhouse (IPK-Gatersleben) varieties found in earlier experiments to be sensitive or tolerant were also grown to maturity for the determination of yield levels. Each experiment was carried out in three replications, with at least 4–6 plants per genotype in each replication.

2.3. Fluorescence induction and the measurement of element contents

Fluorescence induction measurements were carried out using a PAM-2000 instrument (Waltz, Effeltrich, Germany) after 2 h dark adaptation on the youngest fully developed leaf. The $F_v/F_m$ parameter was used to calculate the Tolerance Index. The Cu, Fe, Mn and Zn concentrations were determined by atomic absorption spectrophotometry and flame atomisation.

2.4. Microsatellite analysis

The genotypes of recombinant lines for a single chromosome from the populations ‘CS(Ch5A)’ × ‘CS(Tspelta5A)’ and ‘CS’ × ‘CS(Ch5B)’, and 5AL and 5DL deletion lines of ‘CS’ were identified using microsatellite (SSR) markers. Complete genomic DNA was isolated for this analysis as described by Anderson et al. (1992). The primer pairs used for SSR analysis were either developed by Röder et al.
(1995) or localised on the chromosomes by these authors (Röder et al., 1998). The SSR analysis was carried out using the method of Röder (1998).

2.5. Linkage map preparation and QTL analysis

The MAPMAKER program (chromosome 5B) and the JoinMap 3.0 program (chromosomes 5A, 5B) were used to prepare linkage maps.

The first step in QTL analysis was to determine the distribution of phenotypic data using the Statistica 6.0 software, after which the linkage between individual marker alleles and a given phenotype was determined using the single marker ANOVA method. This was followed by interval mapping with the regression and maximum likelihood methods, using QTLCafe and MapQTL 5 software.

3. RESULTS AND CONCLUSIONS

3.1. Identification of copper-tolerant and -sensitive wheat genotypes, and the comparison of wheat copper tolerance with that of closely related cereal species

On the basis of Cu-induced growth inhibition or of the reduction in the fluorescence induction parameter (Fv/Fm), the most tolerant of the 27 genotypes in the Triticinae subtribe were the rye (Secale) species, while the most sensitive were found among the diploid A genome species (T. monococcum – einkorn). Although the previously demonstrated metal tolerance of the rye genotypes was manifested in this case too, it was only slightly better than that of the most tolerant wheat varieties. No exceptionally tolerant genotypes were found among the Aegilops species. However, most of the hexaploid genotypes examined could be regarded as tolerant, the most tolerant of all being ‘Chinese Spring’, though ‘Bánkúti 1201’ proved to have pronounced sensitivity to the damage caused by excess copper. In later tests, ‘Chinese Spring’ continued to be the most tolerant genotype, while ‘Bánkúti 1201’ was used as the sensitive control variety in later experiments.
3.2. Elaboration of an analytical system suitable for the testing of large numbers of genotypes

Hydroponic tests are generally used for the physiological testing of copper tolerance, but the method is not suitable for the testing of large numbers of genotypes, such as mapping populations or gene bank accessions. For this reason a soil testing system was elaborated in which solid pulverised CuSO$_4$·5 H$_2$O was mixed into the soil. Copper sulphate was chosen because it has been used for centuries in plant protection (Bordeaux mixture), thus being present in the soil of vineyards and hop plantations, where it often causes Cu toxicity problems if these plantations are replaced by annual crops. Although data in the literature suggest that toxicity is observed at soil Cu concentrations of 60–125 mg/kg, in the present work no symptoms of Cu toxicity were observed on 2-month-old wheat plants even at a soil Cu concentration of 375 mg/kg. The results suggest that a Cu concentration of 1000–1500 mg/kg is required to test the copper tolerance of wheat. Over a three-year period, a concentration of 1250 mg/kg proved to be the most informative, as it revealed differences in tolerance between the genotypes by the age of two weeks, but plants grown to maturity were still capable of producing grain, depending on the genotype. The results obtained with the soil testing system for six genotypes exhibited a close correlation with those obtained in hydroponic experiments.

3.3. Determination of the chromosomal localisation of genes responsible for copper tolerance, and identification of loci influencing copper tolerance

Testing of the ‘Chinese Spring’ (recipient) / ‘Cappelle Desprez’ (donor) substitution series indicated that chromosomes 7D, 5A, 3D and 5D had the greatest effect on copper tolerance in wheat, while chromosomes 5B and 6B had a small but significant influence on the copper tolerance of the recipient parent. The role of homoeologous group 5 was confirmed by tests on wheat/rye substitutions.

In the course of QTL and deletion analysis, a major QTL for Cu tolerance was identified on the long arm of chromosome 5D, while loci with minor effects were found on chromosomes 1AL, 2DS, 4AL, 5AL, 5BL and 7DS. Tests on ‘CS’–*Aegilops tauschii* introgression lines also revealed a locus influencing Cu tolerance on
chromosome 3DS. These results, together with those reported by other teams, all confirm the polygenic control of Cu tolerance.

3.4. Identification of loci influencing shoot Cu, Fe, Mn and Zn contents and shoot Cu accumulation

Loci influencing the heavy metal contents in the shoot were identified on an ‘Opata85’ × ‘Synthetic’ (‘ITMI’) wheat mapping population: QTLs influencing shoot Mn and Zn concentrations were localised on chromosomes 3BL and 3AL. Under Cu-treated conditions the Zn concentration was controlled by a locus on chromosome 7A. A QTL influencing the Fe concentration of Cu-treated plants was found in the centromere region of chromosome 3B, while a locus influencing the Cu concentration was identified on chromosome 1BL. The locus found on chromosome 5AL influenced shoot Cu accumulation.

3.5. Use of element content analyses to determine the defence strategy of wheat against Cu toxicity

An analysis of the element contents in recombinant lines of the ‘Opata85’ × ‘Synthetic’ (‘ITMI’) mapping population demonstrated that tolerant lines accumulated lower quantities of copper in their shoots from Cu-polluted soil. This indicates that Cu tolerance in wheat is based on the restriction of heavy metal uptake or of its translocation from the roots to the shoots (avoidance or exclusion strategy).

3.6. Practical utilisation of copper-tolerant wheats

Analyses on species related to wheat and on the ‘ITMI’ wheat mapping population revealed that genotypes that accumulate large quantities of copper in their shoots have poor tolerance and low biomass yield, so they are not suitable for phytoremediation. However, tests on tolerant and sensitive wheat genotypes grown to maturity on Cu-treated soil demonstrated that Cu-tolerant wheat, unlike the sensitive genotypes, is capable of producing an acceptable yield even on soil with a copper concentration of 1500 mg/kg. This indicates that yield losses could be reduced on copper-polluted soils by growing tolerant wheat varieties.
3.7. **New genetic map of wheat chromosome 5B and the physical mapping of microsatellite markers**

A new genetic map with better resolution was prepared for wheat chromosome 5B by genotyping the ‘Chinese Spring’ × ‘Chinese Spring(Cheyenne5B)’ mapping population using microsatellite markers. The new map contains 23 markers, with an average distance of 7.50 cM between markers. The map is in good agreement with the microsatellite consensus map for wheat (Somers et al., 2004). The approximate physical position of 11 microsatellite markers was determined using 5AL deletion lines of ‘Chinese Spring’, and a further 18 using 5DL deletion lines. The new mapping data could be of great assistance in the mapping of other traits in the future.

4. **LIST OF NEW SCIENTIFIC RESULTS**

1. Identification of a copper-tolerant wheat genotype on the basis of hydroponics and soil experiments (Bálint et al., 2002, 2003b).
2. Elaboration of a soil testing system suitable for testing the copper tolerance of large numbers of genotypes, the results of which are comparable with those obtained in hydroponic experiments (Bálint et al., 2003b).
3. Identification of chromosomes (Bálint et al., 2003a) and loci (results published in part: Bálint et al., 2003c, 2004) influencing copper tolerance in wheat.
4. Identification of loci influencing the shoot Cu, Fe, Mn and Zn concentrations in wheat (results published in part: Bálint et al., 2003, 2004).
5. Preparation of a new genetic map for wheat chromosome 5A and the mapping of microsatellite markers on chromosomes 5A and 5D (results not yet published).

5. **PRACTICAL APPLICATION OF THE RESULTS**

The testing system elaborated in this work is suitable for the rapid determination of the copper tolerance of a large number of genotypes, thus accelerating the selection of tolerant lines from gene bank accessions and promoting
the development of varieties tolerant to heavy metals. The identification of tolerant genotypes will also be promoted by the use of markers linked to major QTLs for the marker-assisted selection of genotypes with unknown copper tolerance. The determination of markers linked to loci influencing shoot mineral composition could be utilised in the marker-assisted selection of genotypes with higher shoot mineral composition, and thus higher grain yield. Although wheat does not appear to be suitable for the phytoremediation of severely copper-polluted soils, the cultivation of tolerant genotypes could make it possible to utilise soils with a Cu content of as much as 1500 mg/kg.

6. CITED LITERATURE


### 7. OWN PUBLICATIONS ON THE SUBJECT OF THE THESIS

#### I. SCI journals


#### II. Journals


#### III International conference proceedings

IV International conference abstracts


V. Hungarian conference proceedings


VI. Hungarian conference abstracts


Bálint A. (1999) Réz hatása búzafajták ásványielem akkumulációjára és a réztoxicitást jellemző fiziológiai paraméterekre/Effect of copper on the mineral element accumulation of wheat varieties and on physiological parameters characteristic of
copper toxicity. *XXIV. Országos Tudományos Diákköri Konferencia*, Debrecen, p. 117.


**VII. Other publications**


**VIII. Accepted conference participation**