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# Physical Mapping of Micronutritional Genes in Wheat-rye Translocations

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## ABSTRACT

In rye (*Secale cereale* L.), there are loci on chromosome arm 5RL which give rise to increased copper (Cu)- and iron (Fe)-efficiency, respectively. Four different wheat-rye translocations each harboring a terminal segment of different size of the rye chromosome arm 5RL were identified by test crosses and Giemsa-banding: 'T29' (5AS.5RL), 'T63' (5BS.5BL-5RL), 'Vhn' (4BS.4BL-5RL) and 'Cor' (4BS.4BL-5RL). The translocation break points were detected by chromosome painting technique GISH and the sizes of the rye chromosome segments involved were determined by computer image analysis. The Cu-efficiency gene *Ce* was physically mapped to the terminal region of 5RL, and the genes for mugineic acid and for hydroxymugineic acid synthetases involved in the strategy II of Fe-efficiency control to two intercalary regions of 5RL. In all wheat-rye translocation lines the *Ce* gene is linked to the dominant hairy neck character (*Hal*) from rye. This morphological trait and the RFLP probe 'WG199' as well can serve as proper markers for a marker-based large-scale selection in wheat breeding.

## INTRODUCTION

Cereals differ considerably in their efficiency to acquire and/or metabolize micronutrients (Snowball and Robson 1984, Podlesak *et al.* 1990). Genes influencing the micronutritional system are clustered on the homoeologous chromosome groups 4 and 5 (Mori and Nishizawa 1989, Schlegel *et al.* 1991). In rye (*Secale cereale* L.), loci on chromosome arm 5RL were found to control the response to Cu- and Fe-shortage stress (Graham *et al.* 1987, Mori *et al.* 1990, Schlegel *et al.* 1993). These genes may be used as suitable sources for crop improvement by chromosome engineering in alien species, especially in

wheat for cultivation on marginal soils (Graham 1984). Here, we report the physical mapping of the Cu-efficiency gene *Ce* to the terminal and the genes for mugineic acid and for hydroxymugineic acid synthetases (*Mas* and *Hmas*) involved in Fe-efficiency control to the intercalary regions of 5RL. We also present the development of genetical and molecular markers as the main prerequisite for a marker-based large-scale selection of micronutritionally efficient genotypes in plant breeding programs.

## MATERIALS AND METHODS

Plant genotypes were obtained from a cytogenetic tester stock collection of the Cereals Cytogenetics Group in Gatersleben: The rye, *Secale cereale* L., 'PC361' is an inbred line and originated from a selected self-fertile mutant of 'Petkus Spring'. The wheat, *Triticum aestivum* L., 'Chinese Spring' came from the Gatersleben Germplasm Bank. The wheat-rye translocation line (WRT) 'T29' harbouring the 5AS.5RL chromosome was kindly provided by T. Miller (Norwich, UK). The WRTs 'T63' (5BS.5BL-5RL) and 'Cor' (4BS.4BL-5RL) were kindly provided by J. P. Gustafson (Columbia, USA). The WRT 'Vhn' arose from a single selection of the wheat 'Viking' and carries the 4BS.4BL-5RL chromosome.

Copper efficiency was analysed on 40 plants per line and variant grown in pots in the greenhouse as described by Schlegel *et al.* (1991), except that the copper treatments were modified to 3 mg Cu per pot (deficiency variant) and 60 mg Cu per pot (sufficiency variant). At maturity, the three main spikes of each plant were harvested for grain yield (GY) measurements. Statistical calculations were evaluated using the F- and the t-Test.

Iron efficiency was analysed on 35 plants per line and

variant germinated and grown for 7 days in deionized water followed by a hydroponic culture in nutrient solutions according Römheld and Marschner (1986) with either Fe-absence (deficiency variant) or presence of 100  $\mu$ M Fe-EDTA (sufficiency variant) for 21 days. The chlorophyll was extracted (Arnon 1949), and the contents of chlorophylls A and B per mg fresh matter were determined using a DU650 spectrophotometer (Beckman).

Phytosiderophores (PSs) were analysed from root exudates of three successive collections by HPLC (Mori *et al.* 1987) and the portions of DMA (2'-deoxymugineic acid), MA (mugineic acid) and HMA (3-hydroxymugineic acid) determined. Exudates were collected after the Fe-deficiency chlorosis became visible in the plants cultivated in a continuously aerated nutrient solution as described by Marschner *et al.* (1987).

Chromosome painting was accomplished by GISH (genomic *in situ* hybridization) to squashed mitotic metaphases and interphase nuclei as well. Per slide, 0.1 g of labelled total genomic DNA of rye together with 3 g unlabelled wheat DNA as competitor were applied in 50 % formamide, 2xSSC, 0.1 % SDS, 10 % dextran sulfate. Slides were washed to a stringency greater than 85 %. Conditions of labelling, hybridization and detection were chosen as described in detail by Heslop-Harrison *et al.* (1991).

RFLP analyses were carried out using Southern blot hybridizations with the 3.3 kbp long, low copy, genomic probe WG199 (Heun *et al.* 1991) onto Pst I-, Dra I- and Sst I-digested total DNA of the plant genotypes mentioned above under the experimental conditions described by Anderson *et al.* (1992).

## RESULTS AND DISCUSSION

Although, in comparison to wheat, rye cultivars are preferably planted on light, sandy clay soils with bad nutrient supply, severe iron shortage induced a considerable decrease in fresh matter production of young rye shoots (Tab. 1). Whereas, the grain yields of rye (PC361) demonstrated a higher tolerance against copper shortage than those of wheat (CS) did. Moreover, the presence of rye chromatin of the 5RL arm improves the copper efficiency in each WRT (Tab. 1). That indicates the presence of the Ce gene in each WRT. The difference between the decrease in fresh matter production of rye (46 %) and the decrease in that of wheat (26 %) is highly significant. The wheat-rye translocations (WRTs) reduced their production of fresh matter more than the "translocation-free" 'Chinese Spring' wheat and also more than rye, except the 'T63' line which behaved in an intermediate way between wheat and rye (Tab. 1). Obviously, the decrease reaction to iron shortage stress in the WRTs was enhanced beyond the level of rye itself by either the presence of the rye and/or absence of the wheat chromatins. At sufficient iron supply, however, the translocated segments of the 5RL arms evidently accelerated the growth of the WRTs during the first weeks. Therefore, these WRT types can apparently be used to improve the seedling emergence in wheat.

Since the shoot fresh matter amount does not solely reflect iron efficiency, the symptom of mild chlorosis (Marschner *et al.* 1987) was substantiated by determining the chlorophyll contents (Tab. 1). The response to Fe-shortage varied among the WRTs (36 - 1 %). Their efficiencies were elevated by genes from the 5RL arm. This gradation fits nicely with the results from the analyses of

Table 1. Grain yield (GY), fresh matter (FM) and chlorophyll contents of shoots GY determined from mature plants; FM and chlorophyll (A+B) measured from 28 day old plants; [a] 60 mg Cu/pot; [b] 3 mg Cu/pot; [c] 100  $\mu$ M Fe-EDTA; [d] no iron.

|                      | Genotype |        |        |        |        |        |
|----------------------|----------|--------|--------|--------|--------|--------|
|                      | PC361    | CS     | T29    | T63    | Vhn    | Cor    |
| g GY/main spikes [a] | 3.11     | 4.41   | 4.54   | 3.93   | 5.84   | 4.27   |
| g GY/main spikes [b] | 2.67     | 0.79   | 2.72   | 2.71   | 4.32   | 2.86   |
| % GY decrease        | 14       | 82     | 40     | 31     | 26     | 33     |
| mg FM [c]            | 289      | 633    | 1202   | 1093   | 1030   | 930    |
| mg FM [d]            | 160      | 467    | 470    | 688    | 448    | 502    |
| % FM decrease        | 46       | 26     | 61     | 37     | 56     | 46     |
| ng(A+B)/mg FM [c]    | 729.9    | 650.53 | 405.4  | 408.41 | 547.12 | 569.01 |
| ng(A+B)/ mg FM [d]   | 271.71   | 510.82 | 348.97 | 405.24 | 497.75 | 363.92 |
| % (A+B) decrease     | 63       | 22     | 14     | 1      | 9      | 36     |
| A:B ratio [c]        | 4.1:1    | 2.7:1  | 1.8:1  | 2.1:1  | 1.4:1  | 1.7:1  |
| A:B ratio [d]        | 4.3:1    | 4.1:1  | 4.0:1  | 4.1:1  | 4.0:1  | 4.4:1  |

Table 2. Phytosiderophore (PS) exudation after Fe-shortage in root dry matter (DM) DMA: 2'-deoxymugineic acid; MA: mugineic acid; HMA: 3-hydroxymugineic acid.

|                              | Genotype |         |       |         |         |         |
|------------------------------|----------|---------|-------|---------|---------|---------|
|                              | PC361    | CS      | T29   | T63     | Vhn     | Cor     |
| g root DM                    | 0.479    | 1.351   | 1.198 | 0.817   | 3.670   | 1.046   |
| $\mu\text{mol PS/g root DM}$ | 6.71     | 32.84   | 28.72 | 26.20   | 42.10   | 34.21   |
| $\mu\text{M DMA}$            | 5.62     | 1154.48 | *     | 1352.40 | 2843.38 | 2408.82 |
| $\mu\text{M MA}$             | 23.26    | 0       | *     | 133.62  | 44.77   | 0       |
| $\mu\text{M HMA}$            | 234.73   | 0       | *     | 33.00   | 0       | 0       |

exudated Phytosiderophores (Tab.2). 'T63' shows the highest HMA and MA exudation of the WRTs investigated and the lowest decrease of chlorophyll content (Tab. 1), while 'Cor' reacts like the control 'Chinese Spring' with respect to MA and HMA, but with more than a doubled production of DMA (Tab. 2) connected with the highest decrease of chlorophyll content after rye (Tab. 1).

PSs are essential parts of the strategy II system for the mobilization of Fe<sup>III</sup> ions via chelating in the rhizosphere and transmission into the apical root zones (Römheld and Marschner 1986, Marschner *et al.* 1986, 1987, Treeby *et al.* 1989). The genes of the synthetases for MA and HMA ('Mas' and 'Hmas') were localized together on chromosome 5R by Mori *et al.* (1990). The translocation points of WRTs 'T63', 'Vhn' and 'Cor' "break" this linkage group and enable the genes to be physically mapped to two defined interstitial regions. GISH was used to paint the different 5RL chromosome segments of rye in the alien genomic background of the wheat in interphases (Fig. 1) and metaphases (Fig. 2) of the WRTs. The wheat translocation segments were identified by test crosses and Giemsa-banding. The translocation break points were confirmed by computer image analysis and fixed the sizes of the 5RL-segments as follows: 'T29' = 1 Plu (=100 cPlu), 'T63' = 13.5 cPlu, 'Vhn' = 8.4 cPlu, and 'Cor' = 8.2 cPlu of the 5RL arm.

Assuming that the full length of a 5RL chromosome arm as the reference comprises 1 Plu (Physical length unit), the size of the region accounts for 0.2 cPlu (centiPlu) for *Hmas*, 8.2 cPlu proximal to the chromosome end. For *Mas* the region size is 5.1 cPlu, 8.4 cPlu proximal to the

chromosome end (Fig. 3 and 4).

The genes *Hal* and *Ce* are linked on the translocated chromosome segments from rye. In all WRTs the hairy neck character is expressed together with the improvement of copper efficiency (Tab. 1), though with different intensity. This can be attributed to the different biological origins of the 5RL segment donors. Nevertheless, the hairy neck character marks an increased Cu-efficiency in every WRT, even within the smallest segment of the 'Cor' line (Tab. 1 and Fig. 5b). To omit labour consuming pot experiments for direct Cu-efficiency selection and waiting till heading for hairy neck screening, a suitable RFLP probe was used as a molecular marker for both genes, *Ce* and *Hal*. Fig. 6 shows the polymorphic bands of the 'WG199' probe onto Pst I digested DNA of 'Chinese Spring' (W), 'PC361' (R), 'T29' (a), 'T63' (b), 'Vhn' (c) and 'Cor' (d). A 5.5 kb fragment in (b) marks the rye segment of 'T63'. In the other WRTs, however, the rye specific fragments were polymorphic (7.8 kb in (c) and (d)). An 8 kb fragment was observed in wheat and all WRTs except (d) and is therefore located at the distal end of 4BL. The presence of both fragments, the 7.8 kb rye specific and the 8.0 kb wheat specific fragment in (c) indicates, that the rearranged 4BS.4BL-5RL chromosome of 'Vhn' includes a duplication of an evolutionary modified fragment (Devos *et al.* 1993). Thus, the more than double of DMA production compared to Chinese Spring (Tab. 2) could be interpreted as dosage effect. Because of the cosegregation of the 'Vhn' specific fragment obtained with WG199 in an F<sub>2</sub> derived from (Vhn x wheat) with the *Hal* this probe is a molecular marker for Cu-efficiency as well.

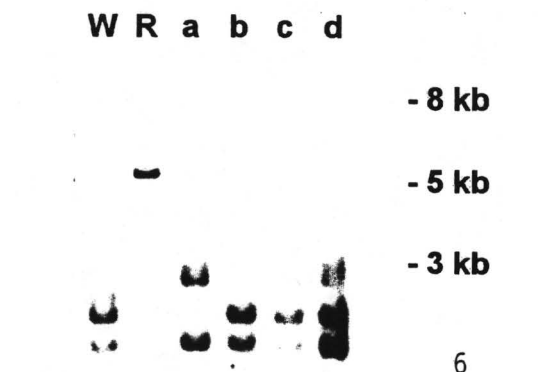
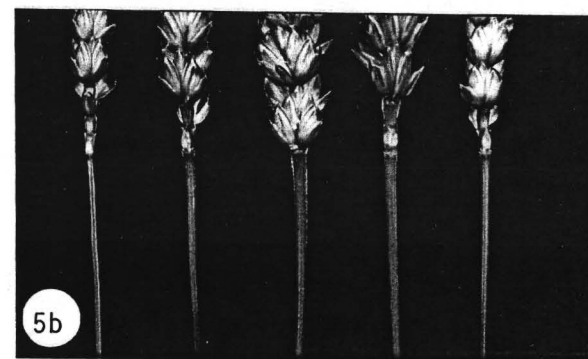
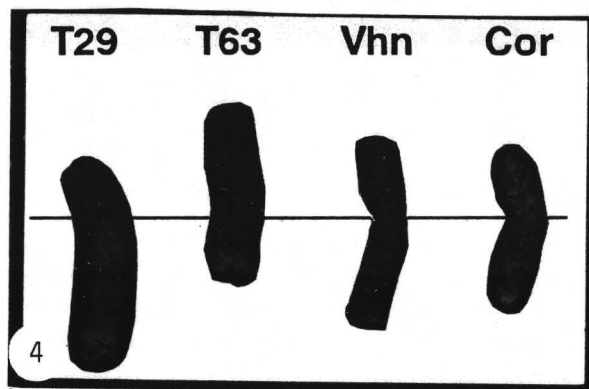
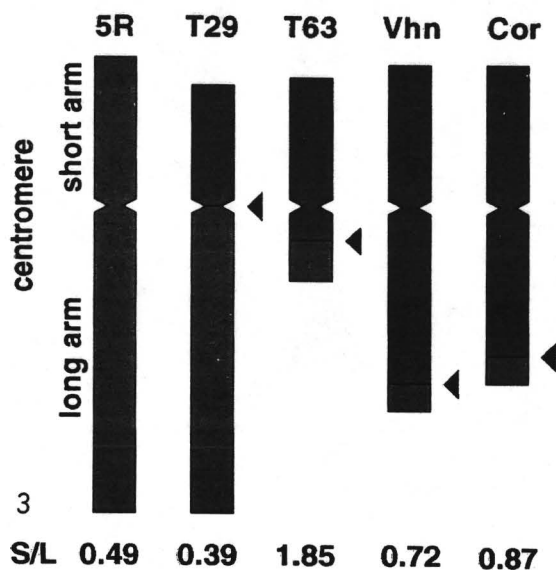
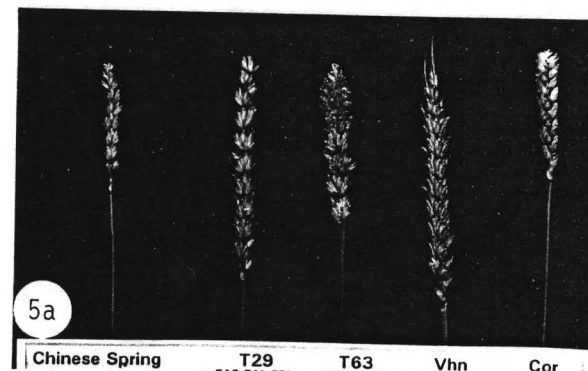
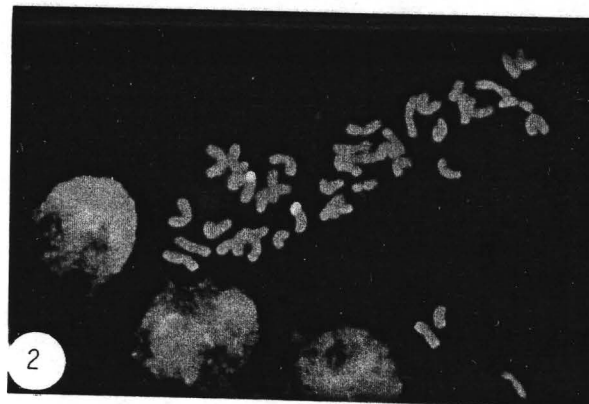
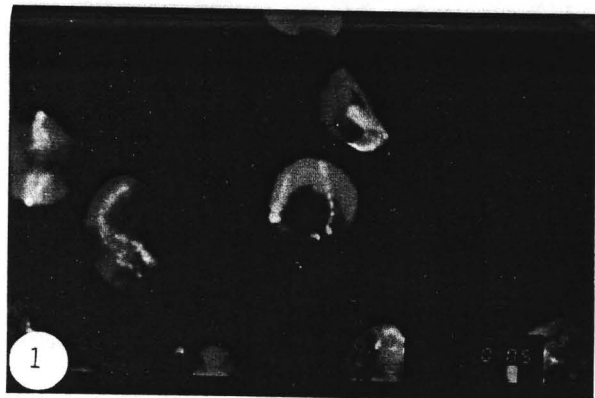


Fig. 1: bright yellow strings label the painted rye arm pairs in T29 interphases.

Fig. 2: T63 metaphase with brightly yellow painted terminal rye segments.

Fig. 3: Karyogramme of 5R and translocated chromosomes, wheat chromatin is labelled in red, while the rye segments involved are labelled green. Arrow heads mark break points.

Fig. 4: Examples of 'painted chromosomes' from the different WRTs.

Fig. 5a: Spike morphology of 'Chinese Spring' and WRTs. Notes: tip-awned ear of T29 indicates the lost of the awn inhibitor gene 'B1'. All WRTs show the hairy neck character from rye ('Ha1').

Fig. 5b: Magnification of the peduncles of the same plants.

Fig. 6: Southern blot of Pst I digested DNA of 'Chinese Sping' (W), 'PC361' (R), 'T29' (a), 'T63' (b) 'Vhn' (c) and 'Cor' (d) probed with 'WG199'.

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