

Molecular cytogenetic characterization of a new leaf rolling triticale

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ABSTRACT. Leaf rolling occurs in some cereal genotypes in response to drought. We identified and made a phenotypic, cytological and physiological analysis of a leaf-rolling genotype (CMH83) of hexaploid triticale (*X Triticosecale* Wittmack) that exhibited reduced plant height, rolled and narrow leaves. Gliadin electrophoresis of seed protein showed that CMH83 was genetically stable. Sequential Giemsa-C-banding and genomic *in situ* hybridization showed that CMH83 contains 12 rye chromosomes; two pairs of these chromosomes have reduced telomeric heterochromatin bands. Tests of relative water content and water loss rate of leaves of CMH83 compared with those of wheat cultivars indicated that rapid water loss after drought stress in CMH83 is associated with the leaf rolling phenotypes. Leaf rolling in CMH83 was a dominant trait in our inheritance studies. Triticale line CMH83 could be used to study drought resistance mechanisms in triticale.

Key words: C-banding; *In situ* hybridization; Leaf rolling; Triticale; *X Triticosecale* Wittmack

INTRODUCTION

Triticale (*X Triticosecale* Wittmack) is a man-made species with a short history of just over 100 years since the first attempt was made to produce an artificial hybrid (Wilson, 1876). It is a chromosome-doubled inter-generic hybrid between various wheat species (*Triticum* ssp, AA, AABB and AABBDD) and rye (*Secale cereale*, RR). Triticale has two forms: primary and secondary. The primary triticale is an amphiploid derived from the hybridization of wheat and rye. Secondary triticale lines are the stable hexaploid derivatives obtained by intercrossing an octoploid triticale (AABBDDRR) and/or hexaploid wheat with a hexaploid triticale (Lukaszewski and Gustafson, 1987). Dou et al. (2006) reported that stable hexaploid triticale can spontaneously appear in octaploid triticales.

Although hexaploid triticale has been extensively cultivated in marginal areas in many countries, there are few examples of the understanding of the physiology and genetics of the putative important drought-related traits that led to the improvement of drought resistance in improved triticale varieties (Richard et al., 2010). The phenotypic selection for traits associated with drought tolerance is likely to be the most attractive for crop improvement.

Leaf rolling may be associated with improved grain yield in some drought situations. Identification of different kinds of rolling leaf provides a theoretical basis and technical support for leaf shape improvement and also for crop drought tolerance. Several recessive genes have been characterized to control the rolling leaf character of rice (Zhou et al., 2010). Evidence of varietal differences for leaf rolling in wheat (*Triticum aestivum* L.) has been reported (Rebetzke et al., 2001; Sirault, 2007); studies investigating the triticale genotypes with leaf rolling characteristics remain unknown (Barary, 2005; Kadioglu and Terzi, 2007).

The aim of our study was to determine the genetic constitution and the physiological traits governing leaf rolling in triticale using molecular cytogenetic identification and leaf water loss for further studies on drought tolerance mechanisms in triticale.

MATERIAL AND METHODS

Plant materials

The secondary hexaploid triticale CMH83 was obtained from CIMMYT. *S. cereale* cv. Jinzhouheimai, and control wheat lines Chinese Spring (CS), triticale lines Currency, Chuannong 19 (CN19) and R59 were provided by the University of Electronic Science and Technology of China. Wheat cultivars Chuanmai42 (CM42), Chuanmai 45 (CM45), Chuanmai 107 (CM107), Chuanmai 55 (CM55), Chuanyu 20 (CY20), M06-367, Yumai 49 (YM49), and triticale Guangmai76 (GM75) were developed by the Sichuan Academy of Agricultural Sciences.

Seed storage protein electrophoresis

Acid polyacrylamide gel electrophoresis (APAGE) was used to separate endosperm gliadin proteins from seeds. The procedures were described by Yang et al. (2001).

Sequential C-banding and genomic *in situ* hybridization (GISH)

Seedling root tips were collected, pretreated in water at 0°C for 24 h, and fixed

in ethanol-acetic acid (3:1) for 1 week. Root-tip squashes were stained using the conventional Feulgen method for chromosome counting. C-banding was done according to Gill et al. (1991). For GISH-banding analysis (Zhou et al., 2008), the genomic DNA of Chinese rye cv. Jinzhouheimai was labeled with digoxigenin-11-dUTP by nick translation following manufacturer instructions (Roche Diagnostics, Indianapolis, IN, USA). Sheared genomic DNA of CS wheat (genomes ABD, $2n = 42$) was used as blocking DNA. The hybridization mixture was prepared as described by Mukai et al. (1993). The GISH signal was detected with fluorescein-conjugated antidigoxigenin antibody (Roche Diagnostics), and the slide was mounted in propidium iodide dissolved in Vectrashield® antifade solution (Vector Laboratories, Burlingame, CA, USA). Microphotographs of C-banded and GISH chromosomes were taken with an Olympus BX-51 microscope using a DP-70 CCD camera.

Relative water content (RWC) and leaf drying test

The RWC was estimated according to Turner (1981) and was evaluated from the equation given below:

$$\text{RWC} = [(\text{FW} - \text{DW}) / (\text{TW} - \text{DW})] \times 100$$

where FW is the fresh weight of the leaves, TW is the weight at full turgid, measured after floating the leaves for 24 h in distilled water in the light at room temperature, and DW is the weight estimated after drying the leaves at 80°C until a constant weight is achieved.

The leaf drying test for CMH83 and control lines was conducted on the first fully expanded leaves of post-germinated seedlings. The leaves were cut into pieces of approximately 6 cm in length and the basal parts of the leaf blades were dried abaxial side up on tissue paper under laboratory conditions. The weight of the leaf fragments was measured at the designated time. Leaf water loss rate (WLR) was expressed as a percentage of the initial weight.

The determination of the WLR and RWC of the different genotypes was studied three times.

RESULTS AND DISCUSSION

Morphology of CMH83

Phenotypically, adult CMH83 plants were 50-60 cm high and produced 3-5 spikes per plant (Figure 1). Spikes had 15-18 spikelets on average. Leaf rolling was clearly observed from the seedling to the adult plants of CMH83. In comparison with the released wheat line CM55, the CMH83 displayed lower ability of tillering and plant height.

Seed protein electrophoresis

APAGE of seed gliadin storage proteins revealed that CS, R59 and CMH83 produced distinctive bands in the ω , γ , β , and α zones (Figure 2). CMH83 displayed identical APAGE band patterns suggesting that it is stable at the homologous group 1 chromosomes. Two strong bands in the w-gliadin mobility zone encoded by Gli-D1 were absent in CMH83. CMH83 contained the secalin bands, which proved that it contained the 1R chromosomes.



Figure 1. The seedling (A) and adult plant (B) of CMH83 (right) in comparison with a normal wheat line CM55 (left).

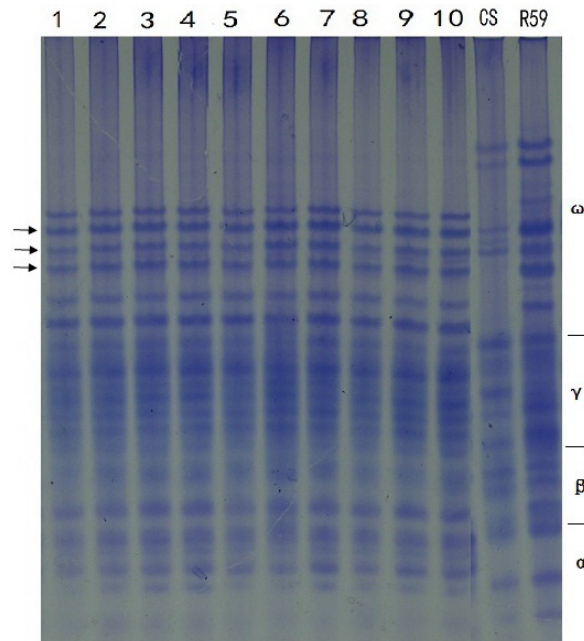


Figure 2. Gliadin proteins separated by APAGE of CMH83 (lanes 1-10), CS (Chinese Spring) and R59. Arrows indicate the ω -secalins.

The endosperm storage proteins have been considered useful genetic markers and were utilized for the evaluation of gene pools, chromosomes and cultivar identification. The seed storage proteins of wheat are usually encoded by homologous group 1 and 6 chromosomes. The gliadin bands of wheat and rye can therefore serve as genetic markers to detect corresponding chromosome regions (Amiour et al., 2002a,b).

In this study, we found that all plants of CMH83 had the identical gliadin and secalin patterns of APAGE bands. The results suggested that CMH83 had the stable chromosome constitution for at least the homologous group 1 and 6 chromosomes from wheat or rye genomes.

C-banding and GISH patterns

Cytological analysis of 36 CMH83 plants revealed that their chromosome numbers varied from 40 to 42, indicating high aneuploidy. As shown in Figure 3, sequential C-banding and GISH of a mitotic metaphase cell of CMH83 allowed identification and distinction of *S. cereale* chromosomes. The strengths and numbers of telomeric heterochromatin bands were comparatively less than the banding patterns of *S. cereale* (Lukaszewski and Gustafson, 1987). In particular, the chromosomes 4R and 7R lacked the strong mid-arm bands when compared to the characteristics of *S. cereale* chromosomes.

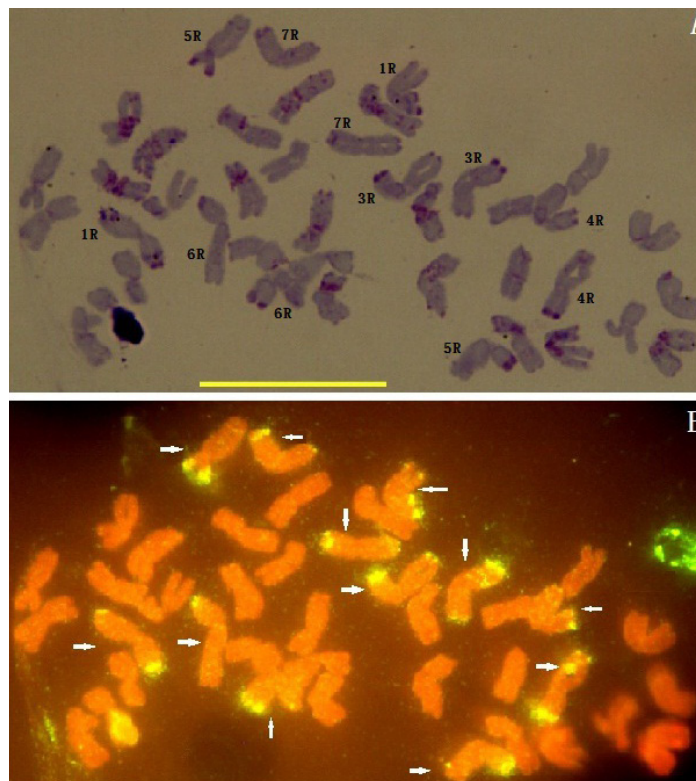


Figure 3. Sequential C-banding (A) and GISH (B) pattern of CMH83 mitotic metaphase chromosomes. Arrows indicate the rye chromosomes. Magnification bar = 20 μ m.

GISH, using genomic DNA from *S. cereale* as a probe on mitotic metaphase chromosomes of CMH83, is shown in Figure 3B. We found that 12 chromosomes were clearly hybridized, and the location of the hybridization signals was identical to the C-banding positions. The results confirmed that CMH83 contained 12 *S. cereale* chromosomes, and was therefore a secondary hexaploid triticales.

The Giemsa-C-banding technique was effective in identifying the chromosomes with strong bands; however, it is hard to distinguish the individual alien chromosomes through GISH. The secondary triticales appeared to be lower for heterochromatin in rye chromosomes and some translocation may have also occurred (Lukaszewski and Gustafson, 1987). Giemsa-C-banding can offer general features of rye chromosome constitution of the hexaploid Triticales by identifying the strong C-banded chromosomes; however, it is hard to distinguish the reduced C-banded chromosomes. Zhou et al. (2008) indicated that GISH-banding using the Chinese rye Jinzhouheimai as a probe can be used in studying evolutionary polymorphism in species or subspecies of *Secale*. The corresponding strong hybridized signal produced at the telomere or sub-telomere region is of adenine-thymine-rich heterochromatin. It also helps to confirm the rye chromosomes in the wheat background. In the present study, we found that CMH83 contains 10 chromosomes with strong bands, and the sequential GISH further confirmed that the reduced bands carrying chromosomes were also rye derived. It was therefore concluded that the CMH83 was a secondary hexaploid triticales with 12 rye chromosomes.

Relative water content and water loss rate

The line CMH83 together with 9 wheat lines or cultivars and a triticales line were tested for RWC of fresh leaf. As shown in Figure 4A, the clear difference in RWC was observed in these tested lines, and four lines (CY20, CMH83, M06-367, and CM55) were below 94.5%, and six lines (GM75, YM49, CM42, CN19, CM45, and CM107) were above 95.5%. CMH83 and CM55 had the lowest (93%), while CM45 had the highest RWC (97%).

We examined the WLR of CMH83 at 2, 4, 6, 8, and 24 h compared with 9 wheat and primary triticales genotypes (Figure 4B), and found that the WLR has no significant change after 2 h between these genotypes. However, after dehydration from 6 to 24 h, the average WLR of CMH83 was significantly higher than that of other 9 genotypes included in the study. The WLR of CMH83 reached 80% of the fresh weight, while the wheat line M06-367 and triticales (GM75) had a WLR of 40%. It suggested that the leaf rolling of CMH83 significantly accelerates the water evaporation, and was thus highly sensitive to drought stress.

RWC was used as a measure of drought. This index may be useful for determining the plant leaf water status (McCaig and Romagosa, 1991). Plants that are able to maintain high levels of RWC under drought stress should be less affected by the stress and be able to maintain better growth and yield. The RWCs were detected extensively on rice and barley, and the studies suggest that different genes are more expressed under stress conditions than under normal conditions and that the nature of the stress determines the genes involved (Price et al., 2002; Teulat et al., 2003; Kumar and Sharma, 2007). In the present study, we found that CMH83 exhibited lower RWC than other wheat and triticales lines, which may imply that the CMH83 was sensitive to drought stress. A spontaneous *eibi1* mutant was identified in wild barley (*Hordeum spontaneum* Koch), and is highly sensitive to drought stress (Chen et al., 2004). The defective *eibi1* cuticle not only enhances the plant's sensitivity to drought stress,

but is also associated with the rapid loss of water from detached leaves (Chen et al., 2009). In our study, the high WLR displayed by CMH83 is in agreement with the low RWC in leaf. We conclude that the hexaploid triticale CMH83 can be used in genetic analysis to investigate how the phenotypic or physiological characters are responsible for the high WLR.

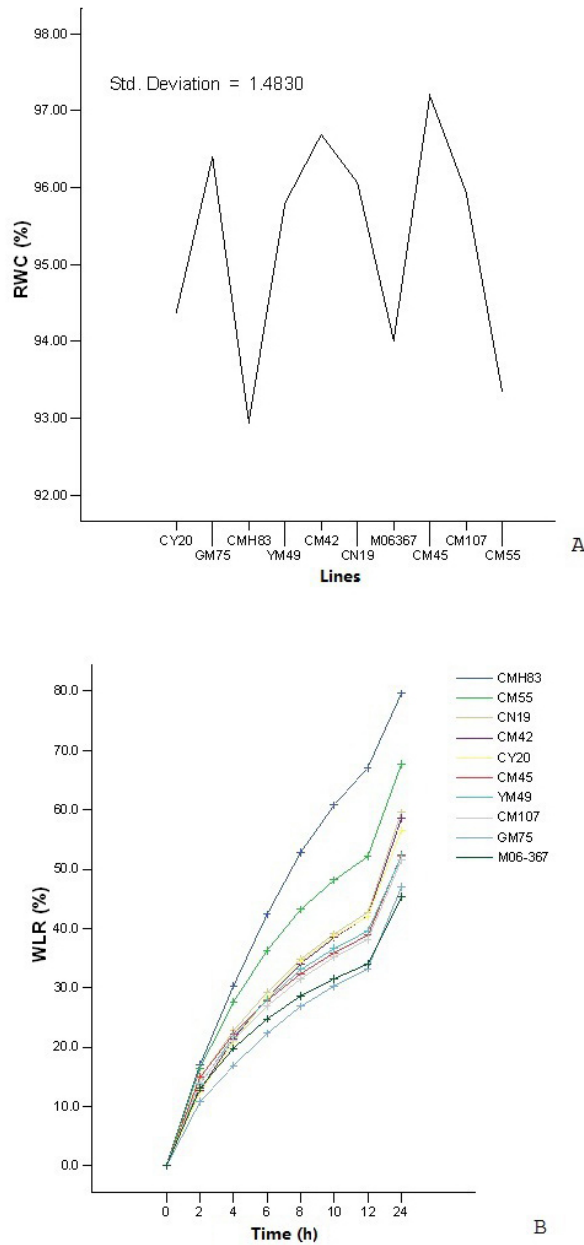


Figure 4. A. Relative water content (RWC) and **B.** water loss rate (WLR) tests of wheat and triticale lines.

Inheritance of leaf rolling

The F_1 plants from the crosses between CMH83 and wheat line R59, and between CMH83 and triticale line Currency were observed for the leaf rolling phenotype. Since line R59 is a 1RS/1BL translocation line, the 10 F_1 plants, detected by GISH, had six rye chromosomes and a 1RS/1BL translocation chromosome as expected (Figure 5A). Currency has 14 rye chromosomes, so the F_1 plants of CMH83 and Currency give rise to 13 rye chromosomes (Figure 5B). We found that all F_1 plants showed typical leaf rolling as adult plants, indicating that the gene(s) for controlling the leaf rolling were dominated in wheat chromosomes or in rye chromosomes.

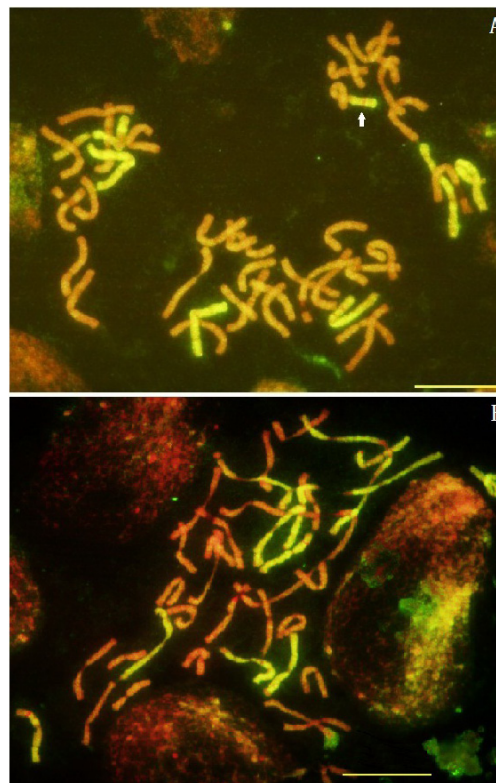


Figure 5. GISH of F_1 plants of CMH83 cross to CN18 (A) and Currency (B). Arrow indicates a wheat-rye 1RS/1BL translocation chromosome.

Several traits have been considered for drought tolerance screening to possibly eliminate the need for field testing in unpredictable environments. Some of these traits include osmotic adjustment, relative water content, water loss rate, and water use efficiency (Rampino et al., 2006). Recently, several recessive genes have been characterized to control the rolling leaf character of rice (Zhou et al., 2010). However, the leaf rolling phenotype of rice was not related to the water loss rate. Therefore, the present CMH83 with the dominant gene(s) conferring the leaf rolling, and possibly associated with higher WLR, may be a new mechanism to study phenotypic and physiological genes with respect to drought stresses.

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REFERENCES

- Amiour N, Dardevet M, Khelifi D, Bouguennec A, et al. (2002a). Allelic variation of HMW and LMW glutenin subunits, HMW secalin subunits and 75K gamma-secalins of hexaploid triticale. *Euphytica* 123: 179-186.
- Amiour N, Bouguennec A, Marcoz C, Sourdil P, et al. (2002b). Diversity of seven glutenin and secalin loci within triticale cultivars grown in Europe. *Euphytica* 123: 295-305.
- Barary M (2005). An Investigation of Drought Tolerance Mechanisms in Triticale (*X Triticosecale* Wittmack) With Particular Reference to Osmotic Adjustment. PhD thesis, University of New England, Armidale.
- Chen G, Sagi M, Weining S, Krugman T, et al. (2004). Wild barley eibi1 mutation identifies a gene essential for leaf water conservation. *Planta* 219: 684-693.
- Chen G, Komatsuda T, Pourkheirandish M, Sameri M, et al. (2009). Mapping of the eibi1 gene responsible for the drought hypersensitive cuticle in wild barley (*Hordeum spontaneum*). *Breed. Sci.* 59: 21-26.
- Dou QW, Tanaka H, Nakata N and Tsujimoto H (2006). Molecular cytogenetic analyses of hexaploid lines spontaneously appearing in octoploid Triticale. *Theor. Appl. Genet.* 114: 41-47.
- Gill BS, Friebe B and Endo TR (1991). Standard karyotype and nomenclature system for description of chromosome bands and structural aberrations in wheat (*Triticum aestivum*). *Genome* 34: 830-839.
- Kadioglu A and Terzi RA (2007). Dehydration avoidance mechanism: leaf rolling. *Bot. Rev.* 73: 290-302.
- Kumar A and Sharma SC (2007). Genetics of excised-leaf water loss and relative water content in bread wheat (*Triticum aestivum* L.). *Cereal Res. Commun.* 35: 43-52.
- Lukaszewski AJ and Gustafson JP (1987). Cytogenetics of triticale. In: Plant Breeding Reviews (Janick J, ed.). Vol. 5. AVI Publishing, Nova York, 41-93.
- McCaig TN and Romagosa I (1991). Water status measurements of excised wheat leaves: position and age effects. *Crop Sci.* 31: 1583-1588.
- Mukai Y, Friebe B, Hatchett JH, Yamamoto M, et al. (1993). Molecular cytogenetic analysis of radiation-induced wheat-rye terminal and intercalary chromosomal translocations and the detection of rye chromatin specifying resistance to Hessian fly. *Chromosoma* 102: 88-95.
- Price AH, Townend J, Jones MP, Audebert A, et al. (2002). Mapping QTLs associated with drought avoidance in upland rice grown in the Philippines and West Africa. *Plant Mol. Biol.* 48: 683-695.
- Rampino P, Pataleo S, Gerardi C, Mita G, et al. (2006). Drought stress response in wheat: physiological and molecular analysis of resistant and sensitive genotypes. *Plant Cell Environ.* 29: 2143-2152.
- Rebetzke GJ, Morrison AD, Richards RA and Bonnett DG (2001). Genotypic Variation for Leaf Rolling in Wheat. Proceedings of the 10th Assembly Wheat Breeding Society of Australia, Mildura.
- Richards RA, Rebetzke GJ, Watt M, Condon AG, et al. (2010). Breeding for improved water productivity in temperate cereals: phenotyping, quantitative trait loci, markers and the selection environment. *Funct. Plant Biol.* 37: 85-97.
- Sirault XRR (2007). Leaf rolling in wheat. PhD thesis, ANU, Canberra.
- Teulat B, Zoumarou-Wallis N, Rotter B, Ben SM, et al. (2003). QTL for relative water content in field-grown barley and their stability across Mediterranean environments. *Theor. Appl. Genet.* 108: 181-188.
- Turner NC (1981). Techniques and experimental approaches for the measurement of plant water status. *Plant Soil* 58: 339-366.
- Wilson AS (1876). Wheat and rye hybrids. *Edinburgh Bot. Sac. Trans.* 12: 286-288.
- Yang ZJ, Li GR, Jiang HR and Ren ZL (2001). Expression of nucleolus, endosperm storage proteins and disease resistance in an amphiploid between *Aegilops tauschii* and *Secale silvestre*. *Euphytica* 119: 317-321.
- Zhou JP, Yang ZJ, Li GR, Liu C, et al. (2008). Discrimination of repetitive sequences polymorphism in *Secale cereale* by genomic *in situ* hybridization-banding. *J. Integr. Plant Biol.* 50: 452-456.
- Zhou Y, Fang YX, Zhu JY, Li SQ, et al. (2010). Genetic analysis and gene fine mapping of a rolling leaf mutant (rl1(t)) in rice (*Oryza sativa* L.). *Chin. Sci. Bull.* 55: 1763-1769.