Theor Appl Genet (2001) 103:277–281

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Inheritance of cell size in wheat (*Triticum aestivum* L.) and its relationship to the vernalization loci

Received: 17 August 2000 / Accepted: 20 November 2000

Abstract Reduced cell size is an important adaptive feature in plant response to environmental stresses. The objectives of the present study were to determine the inheritance and location of genes controlling cell size and to establish the relationship between cell size, low-temperature (LT) tolerance, and growth habit as determined by the *Vrn* loci in wheat. Guard cell length was measured in F_1, F_2, and F_2-derived F_3 populations from parents ranging widely in cell size and in the Chinese Spring/Cheyenne (CS/CNN) chromosome substitution series. The cell size of F_1 hybrids was similar to the parental midpoint and the F_2 frequency distribution was symmetrical about the mean indicating that cell size was determined by additive gene action with little or no dominance. It appears that there are several genes involved since none of the F_2 progeny had a cell size as large or as small as the parental mean range. The cell size of the homozygous spring and winter lines from F_2-derived F_3 populations fell into two distinct groups that were related to plant growth habit. Large cell size was associated with the spring-habit alleles (*Vrn-A1*) and small cell size was associated with the winter-habit alleles (*vrn-A1*) on chromosome 5A. Analyses of the CS/CNN chromosome substitution series showed that CNN chromosomes 5A and 5B both reduced cell size without changing the growth habit, indicating that growth habit per se does not determine cell size. The group-5 chromosomes therefore appear to carry homoeologous alleles with major effects on cell size in wheat. This places cell-size control and many other low-temperature (LT) tolerance associated characters in close proximity to the *vrn* region of the group-5 chromosomes.

Communicated by B.S. Gill

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Keywords Cell size · Low-temperature tolerance · Vernalization · *Vrn* genes · Wheat

Introduction

Studies involving a wide range of plant species have indicated a direct relationship between DNA content and cell volume in meristematic cells (Price et al. 1973). Within the Triticeae, increased ploidy levels or doubling of DNA content was found to increase the length of leaf guard cells (Kuspira et al. 1986). Large cell size in wheat and interspecific amphiploids has been associated with poor low-temperature (LT) tolerance (Limin and Fowler 1989, 1994) and small cells have been implicated as an adaptation to drought (Cutler et al. 1977; Ristic and Cass 1991). Environmental conditions greatly influence cell size in plants (Bennett 1973; Limin and Fowler 1989), but cell size differences are inherent in each cultivar and can be detected in both LT acclimated and non-acclimated material (Limin and Fowler 1989).

Differentiated cells of multicellular organisms range widely in size but the inheritance of size within these cell types has not been investigated in detail. Doney and Theurer (1983) determined the inheritance of cell diameter in sugarbeet (*Beta vulgaris* L.) root cells and found hybrid cell sizes tended to be near midparent means. Baer and Schrader (1985) found partial dominance for small volume in the leaf cells of one maize (*Zea mays* L.) cross. Both studies found cell size to be controlled by mainly additive genetic action.

Small cell size has been associated with low-temperature (LT) tolerance in wheat, and leaf length and guard cell size were found to be closely related in plants grown at LT (Limin and Fowler 1989, 2000). The short leaf length character has been associated with LT tolerance genes located on chromosome 5A of wheat (Roberts 1990) and there is an abundance of evidence associating LT tolerance with the *Vrn1* region on chromosome 5A (Brule-Babel and Fowler 1988; Storlie et al. 1998; Sutka and Snape 1989). The group-5 chromosomes have also

been shown to regulate the expression of many LT-induced genes (Limin et al. 1997) and other characters associated with LT tolerance (Fowler et al. 1999).

The studies reported above demonstrate that reduced cell size is an important adaptive feature in plant response to environmental stresses. Consequently, the objectives of the present study were to determine the inheritance of cell size, the relationship between cell size and growth habit as determined by the *Vrn* loci, and to determine the location of the genes controlling cell size in wheat.

Materials and methods

The lengths of guard cells were measured in the following populations. (1) Parental and F_1 generation progeny from ten crosses involving both winter and spring growth habit. (2) The Chinese Spring/Cheyenne (CS/CNN) chromosome substitution series. (3) F_1 and F_2 generation progeny from a cross between parents with extremes in guard cell size. (4) F_2-derived F_3 populations that were selected for homozygosity at the *Vrn-A1* locus determining spring (*Vrn-A1*) or winter (*vrn-A1*) habit. Twenty guard cell pairs were measured midway along the abaxial side of the fully developed leaves of each plant evaluated. Epidermal peels were mounted in water on a microscope slide with a coverglass and measured with an eyepiece micrometer.

Parental, F_1, and F_2 populations

Seed was imbibed and held at 4°C for 2 days then transferred to 20°C for 3 days. Uniformly germinating seed was placed in flats containing a soil-peat-vermiculite mixture in a 2:1:1 ratio and grown for 5 weeks under a 16 h, 17°C day and 8 h, 15°C night regime. The parental lines and F_1s that were evaluated are listed in Table 1. All parental lines were winter-habit cultivars of *Triticum aestivum* L. em. Thell., with the exception of Manitou, which is a spring-habit cultivar. The experimental design for parental and F_1 populations was a randomized complete block design (RCBD) with two replicates and 12 sub-samples in each replicate. Twenty guard cell pairs were measured on the second fully developed leaves of each plant evaluated. Two hundred and two F_2 generation plants from the Norstar×Manitou cross were grown in flats along with 20 plants of each of the parental cultivars and their F_1 hybrids. To obtain a good estimation of cell size in these individual plants 20 guard cell pairs were measured on the first three fully expanded leaves of each individual F_2 plant.

F_2-derived F_3 populations and chromosome substitution lines

Growth habit was determined for F_2 plants grown in individual pots at 20°C with a 16 h day/8 h night cycle. Plants flowering before the F_1 hybrids were considered spring-habit types. F_2 plants that showed no sign of heading after 93 days (14 days after F_1 hybrid anthesis) were considered to be winter types and were vernalized for 8 weeks at 5°C. Spring-winter growth habit in the Norstar×Manitou cross is determined by the *Vrn-A1* locus with the spring *Vrn-A1* allele being dominant to the winter *vrn-A1* allele (Brule-Babel and Fowler 1988). Therefore, to ensure homozygosity, 20 F_2-derived F_3 plants representing each F_2 plant were grown hydroponically (Limin and Fowler 1984) and 50 lines were selected based on 100% indication of heading, or of not heading, after 52 days.

Guard cell size was determined on homozygous F_2-derived F_3 lines grown from residual seed of the 50 lines selected for growth habit and the chromosome substitution lines. Seed was pre-germinated then planted in trays that were wrapped in foil to prevent radiant heat absorption by the soil surface. Preliminary experiments have shown that there is a better resolution of genotypic differences in cell size when plants are grown at low temperatures (Limin and Fowler, unpublished data). Therefore, growth conditions were adjusted to a constant temperature of 4°C at plant height with a 16 h day/8 h night. Twenty cells were measured on each of the second and third fully expanded leaves. The F_2-derived F_3 lines selected for spring (*Vrn-A1*) or winter (*vrn-A1*) homozygosity from the Norstar×Manitou cross were grown in a three-replicate RCBD. The 'Cheyenne' chromosome substitution series into 'Chinese Spring' wheat (CS/CNN) was grown as a two-replicate RCBD experiment.

Results and discussion

Cell size of the ten F_1 hybrid combinations produced in this study did not differ significantly from the parental midpoint (Table 1). Reciprocals were produced in four crosses but significant differences were not detected and the results for each of these crosses were pooled. The absence of reciprocal differences indicates that maternal inheritance was not involved in the determination of cell size. Eight out of ten hybrids had cell sizes that were significantly ($P<0.05$) different from both parents (Table 1). In one cross (Norwin×Norstar), the hybrid was not significantly different from the parental midpoint or the larger-celled parent (Norwin) suggesting partial dominance for larger cell size. However, the difference in cell size of the parental cultivars was relatively small in this instance and dominance was not indicated in any of the three other crosses involving Norwin, although it was always the parent with the smaller cell size in these crosses. There was only a small difference (1.98 µm) between the cell size of the parents in the Kharkov 22MC×Cappelle Desprez cross, making significant differences difficult to detect for this combination.

Table 1 Parents (P1 and P2), parental midpoint cell size (MP), and planned contrasts between parental and F_1 populations

P1[a]×P2[a]	MP	F_1-MP	F_1-P1	F_1-P2	P1-P2
Manitou×Norstar	88.49	1.35	–5.12**	7.83**	12.95**
Manitou×Norwin	90.47	–0.18	–4.67**	4.50**	9.17**
Sprague×Norwin	90.47	–1.08	–5.57*	3.60**	9.17**
Sprague×Norstar[b]	88.49	0.63	–5.84**	7.11*	12.95**
Gaines×Norwin	90.11	0.90	–3.23**	5.22**	8.45**
Gaines×Ulianovka[b]	90.11	–1.44	–5.57**	2.88**	8.45**
Yorkstar×Norstar[b]	85.25	1.08	–2.16*	4.32**	6.48**
Kharkov 22MC×Capelle	87.41	0.72	–0.18	1.80	1.98*
Kharkov 22MC×Norstar	85.25	0.72	–2.34*	3.96**	6.30**
Norwin×Norstar	83.9	0.99	–0.72	2.70**	3.42**

*, ** Significant at the 0.05 and 0.01 probability levels, respectively
[a] P1=larger parent, P2=smaller parent
[b] Reciprocals pooled

This predominantly intermediate type of inheritance in the F_1 hybrids suggests that additive gene action determines guard cell length. The F_2 frequency distribution (Fig. 1) was symmetrical about the mean, again suggesting additive gene action and no dominance. These observations are in agreement with the *B. vulgaris* L. root cell size results reported by Doney and Theurer (1983). It appears that there are several genes involved since very few of the F_2 progeny had cell sizes approaching the parental means.

In the Chinese Spring/Cheyenne (CS/CNN) chromosome substitution series, CNN had a much smaller cell size than CS. These differences were expected based on LT tolerance and the cell size measurements reported in a previous study (Limin and Fowler 1989). The CNN group-5 chromosomes 5A, 5B and 5D caused the greatest reduction in the cell size of CS while chromosomes 6A and 1D of CNN increased the cell size of CS (Fig. 2). These observations indicate that several genes control cell size in wheat, but the group-5 chromosomes appear to carry homoeologous alleles with major effects.

CNN, being of winter habit, carries all recessive winter-habit alleles (Pugsley 1971) while CS carries winter-habit *vrn-A1* and *vrn-B1* alleles on chromosome 5A and 5B and the dominant spring-habit allele *Vrn-D1* on chromosome 5D (Pugsley 1972). CNN 5A and 5B chromosome-substitutions are therefore spring habit but when the winter-habit allele *vrn-D1* replaces the spring-habit allele *Vrn-D1* it makes the CNN 5D-substitution a winter-habit type. Spring growth habit has been associated with larger cell size (Limin and Fowler 1989). However, since all CNN group-5 chromosomes reduced cell size, the winter or spring habit per se does not in itself determine cell size. Rather, it appears that there are multiple alleles for this character at each chromosomal locus. In fact, it is likely that the additive action of homoeoalleles on chromosomes 5A, 5B, and 5D account for most of the differences in cell size between CS and CNN.

The cross between the large-celled spring-wheat cultivar Manitou and the small-celled winter-wheat cultivar Norstar produced an F_1 hybrid that was intermediate in guard cell size relative to the parents. The cell size of the homozygous spring and winter lines fell into two distinct groups where large cell size was associated with the spring-habit alleles (*Vrn-A1*) and small cell size was associated with the winter-habit alleles (*vrn-A1*) on chromosome 5A (Fig. 3). The mean cell size of homozygous spring (*Vrn-A1*) lines (73.6 µm) was larger than the mean cell size of the homozygous winter (*vrn-A1*) lines (67.8 µm) in this F_2-derived F_3 population, indicating a strong association between the different alleles at the *Vrn-A1* locus on chromosome 5A and guard cell size. Since the CS/CNN substitution lines indicated that chromosomes 5B and 5D were also involved, much of the variation observed within the F_2-derived F_3 spring- and winter-habit populations was most likely due to the segregation of alleles for cell size associated with the *vrn-B1* and *vrn-D1* regions on these chromosomes.

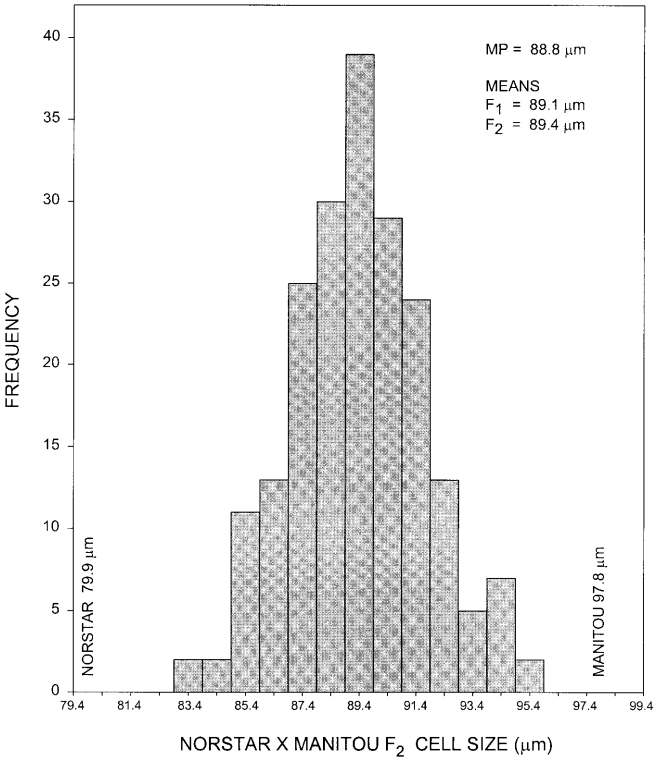

Fig. 1 Frequency distribution of the guard cell length of 202 F_2-generation plants and the means of the parental cultivars Norstar and Manitou

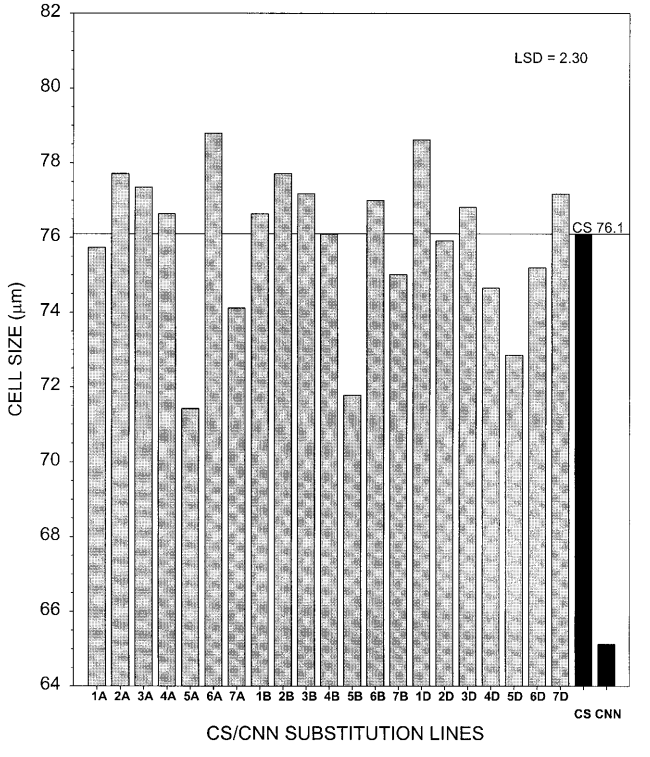

Fig. 2 Guard cell length (cell size) of the Chinese Spring/Cheyenne chromosome substitution series and the parent cultivars

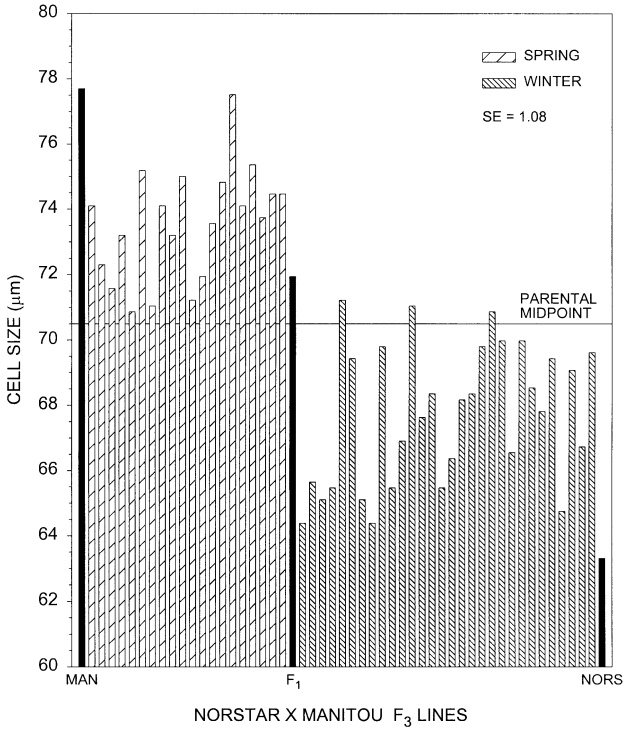

Fig. 3 Guard cell size of parental cultivars Manitou and Norstar, their F_1 hybrid, and F_2-derived F_3 lines homozygous for spring (*Vrn-A1*) or winter (*vrn-A1*) growth habit

Conclusions

The dominant spring alleles *Vrn-A1* and *Vrn-D1* were found to be associated with large cell size while the winter-habit *vrn-A1* and *vrn-D1* alleles have been associated with small cell size. Smaller cell size resulted when the winter-habit alleles of the LT-tolerant CNN replaced the winter-habit alleles of less LT-tolerant CS, indicating that multiple alleles exist at each locus determining cell size on the group-5 chromosomes in wheat. Cell size has been shown to have a high correlation with LT tolerance (Limin and Fowler 1989, 2000) and the present research localizes cell-size control to the homoeologous *Vrn* regions of the group-5 chromosomes. These observations are of additional significance because the genetic control of both LT tolerance (Brule-Babel and Fowler 1988; Storlie et al. 1998; Sutka and Snape 1989) and cell size have been traced to the *vrn* regions of the group-5 chromosomes. This places cell size, prostrate growth habit (Roberts 1990), unsaturated phospholipid synthesis (DeSilva 1978), and a LT tolerance gene, *Fr1* (Galiba et al. 1995), in very close proximity to the *vrn-A1* locus. Other LT tolerance-associated characters on the group-5 chromosomes of unknown linkage to the *vrn* loci include: leaf length (Roberts 1990), antifreeze protein accumulation (Griffith et al. 1997), sucrose accumulation (Galiba et al. 1997), and abscisic acid accumulation (Galiba et al. 1993).

Along with the diversity of genes whose location or control is apparently associated with this locus, the *Vrn* region is also critical to the plant because of its developmental influence through determination of vernalization requirement. This has wide-ranging pleiotropic effects because it determines the time of transition from the vegetative to the reproductive growth stages and also the regulation of LT-induced gene expression associated with LT tolerance. The general regulation of LT tolerance can be seen as it relates to the state of vernalization saturation (Fowler et al. 1996a, b). Specific regulation of the LT tolerance-associated *Wcs120* and *Wcor 410* gene families has also been localized to chromosome 5A of wheat (Limin et al.1997; Danyluk et al. 1998).

Acknowledgements The authors gratefully acknowledge the expert technical assistance of G. Schellhorn.

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