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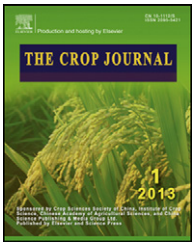


# Anatomical and chemical characteristics associated with lodging resistance in wheat

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ABSTRACT

Anatomical and chemical characteristics of stems affect lodging in wheat (*Triticum aestivum* L.) cultivars. Traits associated with lodging resistance, such as plant height, stem strength, culm wall thickness, pith diameter, and stem diameter, were extensively investigated in earlier studies. However, the solid stem trait was rarely considered. In this study, we measured a range of anatomical and chemical characteristics on solid and hollow stemmed wheat cultivars. Significant correlations were detected between resistance to lodging and several anatomical features, including width of mechanical tissue, weight of low internodes, and width of stem walls. Morphological features that gave the best indication of improved lodging resistance were increased stem width, width of mechanical tissue layer, and stem density. Multiple linear regression analysis showed that 99% of the variation in lodging resistance could be explained by the width of the mechanical tissue layer, suggesting that solid stemmed wheat has several anatomical features for increasing resistance to lodging. In addition, microsatellite markers GWM247 and GWM340 were linked to a single solid stem QTL on chromosome 3BL in a population derived from the cross Xinongshixin (solid stem)/Line 3159 (hollow stem). These markers should be valuable in breeding wheat for solid stem.



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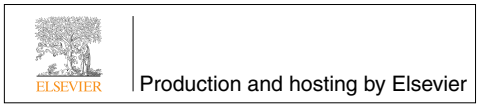


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## 1. Introduction

Lodging in cereal crops causes significant economic losses associated with reduced yields, quality, and harvesting efficiency. Previous studies showed that lodging resistance was significantly correlated with some morphological and chemical characteristics [1–5].

Solid stemmed wheat (*Triticum aestivum* L.) has thin but very hard stems, in which the stem pith is filled with solid materials. The morphological features of solid stemmed wheat suggest that it could be highly resistant to lodging. It is known that solid stemmed crop plants have increased resistance to damage from sawfly larvae, as the presence of solid pith impedes larval growth and migration [6]. Some wheat cultivars with high yield potential, such as Genou, Rampart, Choteau, Bynum, and Duclair, developed by Montana Agricultural Experimental Station, USA, have solid stems [7–10].

The hereditary characteristics of solid stem in durum wheat (*Triticum durum* Desf., $2n = 4x = 28$) were simple, dominant, recessive or complex, depending on the manner in which studies were carried out and/or the genetic characteristics of the parental plants [11]. Cook et al. [12] reported four microsatellite markers linked to Qss.msub-3BL for stem characteristics in a double haploid winter wheat population derived from a cross between 'Rampart' (solid stem) and "Jerry" (hollow stem). However, few studies have investigated the anatomical features and chemical composition of solid stemmed wheat varieties. Such characteristics are potentially important for stem strength at physiological and anatomical levels.

The aim of this study was to improve our understanding of the relationship between the anatomical features and chemical composition of stems in different wheat cultivars and their influence on resistance to lodging. In addition, the gene(s) controlling stem solidness was mapped based on an $F_2$ population derived from a cross between a solid stemmed variety and a hollow stemmed one. The result will be helpful for molecular marker assisted selection (MAS) for solid stem in wheat breeding.

## 2. Materials and methods

### 2.1. Plant materials

Solid stemmed wheat line Xinongshixin (XNSX), hollow stemmed Line 3159, the $F_1$ and $F_2$ populations from cross XNSX/Line 3159 and Chinese Spring (CS) were planted at Changping Experimental Station, CAS, Beijing, China. Plant samples were collected from early April (three-leaf stage) to late June (mature stage). To evaluate stem solidness, more than 10 stems were randomly selected at post-anthesis and were cross-sectionally cut at the center of each internode. The level of pith solidness was rated on a previously established score system [12] ranging from 1 to 5 (1 for hollow and 5 for solid). All samples were collected from main tillers.

### 2.2. Anatomical and chemical evaluation

The internodes on samples were numbered consecutively from the base to the top of the stem. Sections were cut at the center of each internode and stained with either phloroglucine-HCl or Calcoflour (Sigma) according to the procedure described in our previous study [13]. The following morphological characteristics were measured and analyzed using a statistical software package attached to fluorescence microscope (Axioskop 40 with UV excitation, ZEISS), i.e., outer and inner stem diameters, area of stem wall, radius of stem wall (RSW), width of stem wall (WOSW), area of vascular bundles (AOVB), area of transverse section (AOT), width of the mechanical tissue layer (WOMT), number of vascular bundles (NOVB), number of large and small vascular bundles (NLVB and NSVB), weight of the three lower internodes (WOL), and stem length.

Carbohydrate contents (lignin and cellulose) were assayed according to the methods described previously [13–15]. Three internodes from the bottom upwards collected from stems were ground to fine powder in liquid nitrogen using a mortar and a pestle. Lignin content was assayed using the methods described by Kirk and Obst [16] and histochemical detection (the Wiesner reaction) following established protocols [17]. For cellulose staining, polyethylene glycol (PEG)-embedded sections (10 μm) were treated with a 0.005% aqueous solution of Calcoflour (fluorescent brightener 28, Sigma) for 2 min and then observed with a fluorescence microscope (Axioskop 40, ZEISS). Lodging resistance was ranked according to the measured resistance of stems to pushing, which was carried out on the bottom part of the stem following Kashiwagi and Ishimaru [18].

### 2.3. Statistical analysis

The data were analyzed by multiple ANOVA with 95% confidence limits using mean values measured for each genotype. The relationships between morphological characteristics and lodging resistance were revealed by linear regression analysis following the procedure for stepwise forward regression analysis described in the SPSS 11.0 software package for Windows. Lodging resistance was used as the dependent variable, while lignin, cellulose, AOVB, NOVB, AOT and WOMT were used as independent variables.

### 2.4. Microsatellite markers linked to gene(s) conferring stem solidness

Potential microsatellite markers linked to stem solidness genes were identified by screening the $F_2$ population using bulked segregant analysis. DNA was extracted from young leaf tissues using the CTAB method. The solid and hollow stem DNA pools were composed of 5 solid and 5 hollow stemmed $F_2$ plants, respectively. Along with the parental DNA, the bulked DNA samples were used to screen 607 SSR markers (210 GWM [19] and 397 BARC [20]). The PCR mixture (20 μL) consisted of 2.0 μL of 10× buffer, 1.6 μL of $Mg^{2+}$ (25 mmol $L^{-1}$), 2.0 μL of dNTP (2 mmol $L^{-1}$), 2.0 μL of DNA (10–20 ng $\mu L^{-1}$), 2.0 μL of primer (2 μmol $L^{-1}$), 0.2 μL of *Taq* DNA polymerase (5 U $\mu L^{-1}$), and 10.2 μL of $ddH_2O$ and was subjected to a thermocycler program of 94 °C for 5 min; followed by 30 cycles at 94 °C for 1 min, 60, 55, or 50 °C for 1 min (depending on each primer set), and 72 °C for 1 min; with a final extension at 72 °C for 5 min. The PCR products were electrophoresed in 4% polyacrylamide gels and detected by silver staining [21]. Marker-trait associations were identified by