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# Gibberellic Acid Regulates Cell Wall Extensibility in Wheat (*Triticum aestivum* L.)[1]

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## ABSTRACT

**Mutations (*Rht* genes) blocking sensitivity to gibberellic acid (GA) were used to examine phytohormone mediated cell wall expansion in wheat (*Triticum aestivum* L.). Irreversible extensibility of immature leaf segments, as determined by stress/strain (instron) measurements, declined with *Rht* gene dose. Exogenous $GA_3$ significantly increased wall extensibility in the nonmutant controls but had no effect on the near-isogenic GA-insensitive genotypes. Furthermore, ancymidol, an inhibitor of gibberellin biosynthesis, diminished wall extensibility in the nonmutant control. Extensibility of immature segments was highly correlated with mature leaf sheath length ($R = +0.95$). The results indicate that wall yielding properties of expanding wheat leaves are associated with leaf cell expansion potential and that GA is involved in the determination of those properties.**

The plane of cell division together with the rate and orientation of irreversible expansion determine cell size and shape. The rate of plant cell expansion may be further reduced to a few physical components including: (a) cell wall extensibility, which is the capacity of the wall to undergo irreversible (plastic) extension in response to a load; (b) the yield threshold of the wall, which is the minimum stress required to initiate extension; (c) hydraulic conductance, the capacity of the tissue to transport water to the expanding cell; and (d) the difference in water potential between the cell and extracellular space (16). Lockhart's (16) equations show that a change in the growth rate can take place with changes in wall structural/mechanical properties which determine extensibility and/or changes in hydraulic processes and/or turgor changes.

Heyn (9) reported that IAA promoted cell enlargement by altering physical properties of the cell wall, specifically, by increasing wall extensibility. Now a substantial body of literature reports that in cases where auxin has increased growth rate it has caused a correlated increase in wall extensibility as measured by creep, stress relaxation, or instron technique (4).

Evidence substantiating a role for GA in changing wall extensibility is less abundant. Cell wall plasticity of *Avena* internode segments, measured by instron extension and microextension of living internode segments, was increased 150% by treatment with GA, with a lag of about 1 h (1). Excised hypocotyls of lettuce, incubated in the light, elongated in response to GA (21). The addition of 10 mM KCl doubled elongation for segments preincubated with GA but had no effect on the controls. Uptake of KCl was not modified by GA (24). Stuart and Jones (24) concluded that GA-induced increases in elongation were regulated by changes in wall extensibility, and that in the absence of increased extensibility, changes in turgor pressure had little direct effect. In another report (7), etiolated pea stems treated with an inhibitor of GA synthesis showed a significant threefold decline in relative growth rate that was reversed by addition of GA. Measurements on individual cortical cells showed that turgor pressure and yield threshold was greater for inhibitor-treated cells than for inhibitor + GA-treated cells but the differences were not large enough to explain the differences in intact growth. The authors attributed this to the effects of excision which was found to reduce growth rate also. Plastic compliance of inhibitor treated cells was not different from controls. Stems treated with both GA and the inhibitor exhibited about 50% greater plastic compliance. These investigators concluded that the major effect of GA was to affect chemorheological properties, rather than cell turgor or cell osmoticum accumulation.

While these reports support a role for GA in promoting increased wall extensibility, some contradictory evidence also exists. Katsumi and Kazama (11) used a stress relaxation technique to show that GA does not increase extensibility in cucumber hypocotyls. They suggested that GA promoted elongation via a more negative osmotic potential in the target tissue.

The immature sheath of wheat seedlings is highly sensitive to exogenous GA treatment (13). However, mutant plants having the Norin 10 genes, *Rht1* and *Rht2*, are insensitive to exogenously supplied GA (18). Elongation growth of leaves and stems was retarded in these mutants (8), and the effect could not be overcome by GA treatment. When *Rht* mutants were treated with an inhibitor of GA synthesis, growth was reduced; however, the original growth rate of the mutant was restored with added GA (3, 13). This suggests that *Rht* mutants are saturated at endogenous levels of GA and are therefore insensitive to additional GA, even though exogenously supplied hormone is readily taken up (10). Increasing the dose of *Rht* alleles lowered the level at which GA is saturating (13). This is consistent with the observation that neither the double-dwarf (four copies of *Rht*) nor the semidwarf (two copies of *Rht*) respond to added GA, even though they differ significantly for GA-mediated effects (13). Furthermore, these mutants are known to have normal rates of GA synthesis (10). Genetically regulated insensitivity to GA conferred by these genes results in significantly elevated levels of endogenous

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$GA_1$, but the mutants are unable to respond normally to GA (8, 19). The current study was designed to test the hypothesis that the mechanism of GA-mediated wall extension in immature wheat leaves involves altered cell wall extensibility.

## MATERIALS AND METHODS

### Cultivars and GA-Related Treatments

Isogenic wheat (*Triticum aestivum* L.) lines carrying zero, two, or four mutant alleles, which were developed by backcrossing and selection for five (Marfed series) or seven (Burt series) generations, were generously provided by R. E. Allan, Washington State University, Pullman, WA (2). Chemical treatments were 1 mM gibberellic acid ($GA_3$, Sigma Chemical Co., St. Louis, MO) formulated as the sodium salt, and 2% (w/v) A-REST containing 22 $\mu$M ancymidol, $\alpha$-cyclopropyl-a[p-methoxyphenyl]-5-pyrimidine methyl alcohol (Elanco).

### Instron Extensibility Measurement

Immature second leaf blade and sheath tissue was secured between two clamps of the stress/strain device. The lower clamp was fixed to a plexiglass platform which rested on a top loading electronic balance (model 1213 MP, Sartorius). The upper clamp was fixed to a trolley driven by a reversible-direction, constant-speed motor (Bendix P/N 31008-4004, TRW, Dayton, OH). An LVDT[4] (type E-200 LVDT, Schaevitz Engineering, Pennsauken, NJ) was used to record changes in distance between the two clamps. As the trolley was driven upward, sheath tissue between the clamps was stretched. The load on the tissue was observed as a loss of mass on the balance. Balance and LVDT output were converted from analog to digital by a ± 1 V DC, 12 bit converter (model ADALAB, Interactive Microware Inc., State College, PA). A BASIC program, running on an Apple IIe computer, controlled data acquisition. The regression of clamp displacement on LVDT output showed that displacement was linear (constant rate), at 104 $\mu$m/s over the range of displacement used in this study (data not shown). The rate of clamp displacement was independent of load within the range examined. Balance and LVDT output were measured with resolutions of 18 mg and 4.9 $\mu$m, respectively.

### Sampling for Sheath Length and Instron Extensibility

Seeds of Marfed and Burt isolines were planted to a depth of approximately 3 cm in moist vermiculite in 207 mL perforated styrofoam cups, 5 seeds per cup. These were subirrigated with 80 mL of one-quarter strength Hoagland solution or one-quarter strength Hoagland supplemented with 1 mM $GA_3$ formulated as the sodium salt, or 2% (w/v) A-REST. Seeds were germinated and grown at a constant 22°C and 95% RH in a growth cabinet with 15:9 (day:night) photoperiod providing 50 to 60 $\mu$mol photons (400–700 nm) $m^{-2}s^{-1}$. Cups were subirrigated with 75 mL of one-quarter strength Hoagland 3 and 6 d after planting.

Plants were sampled 9 d after planting when the lamina of the second leaf had just emerged. We measured instron extensibility on 8 mm segments of immature second leaf blade and sheath tissue. Length of the first leaf sheath was measured for each sample and the immature second leaf blade and sheath was dissected out, sealed in aluminum foil, frozen in liquid nitrogen, and then thawed to disrupt the plasma membrane and eliminate turgor stresses on the walls. Samples were secured between two instron clamps, spaced approximately 7 mm apart, and extended twice to a load of at least 8 g. The true initial length of the sample was determined by LVDT output at the point where extension began to pull a load. The first extension contained both plastic and elastic components. Since the second extension had approximately the same force vectors as the first it is expected to contain only the elastic component of extensibility (26). Plastic extensibility was determined by the difference in slope between the first and second extensions.

### Statistical Methods

The instron procedure was repeated on 2 to 4 replications of 16 treatment combinations. Missing replications for some treatments were the result of tissue damage during excision. The slopes of first and second extension as a function of load were determined by regression analysis. Plastic extensibility was calculated as the difference between plastic + elastic (first extension) and elastic only (second extension). Simple linear correlation was calculated between plastic extensibility of the second leaf and length of the fully expanded first leaf sheath for each plant, in order to relate plasticity in a developmentally immature tissue to mature leaf length.

The experiment was a completely randomized unbalanced design (some missing plots) with four replicates. The analysis was executed using the SAS GLM procedure for unbalanced data (22). Since the *Rht* isolines consist of equally spaced levels of the Norin 10 genes with zero, two, or four copies, the orthogonal contrast for a linear effect of gene number on plastic extensibility was determined.

## RESULTS

Significant differences in plastic extensibility of immature leaf blade and sheath tissue resulted from treatment effects consisting of (a) mutations which altered sensitivity to GA and (b) hormone or inhibitor applications which altered the level of GA available to the growing sheath (Fig. 1). Plastic extensibility was a highly significant, inverse, linear function of *Rht* gene dose. Tall genotypes (*Rht* = 0 alleles) possessed 57% greater extensibility than semidwarfs (*Rht* = 2 alleles); which possessed 57% greater extensibility than double-dwarfs (*Rht* = 4 alleles).

When nonmutants were grown continuously in the presence of 1 mM $GA_3$, plastic extensibility was significantly increased by 92% (Fig. 1). Similar treatment of the gibberellin insensitive double-dwarf mutants had no significant effect. Ancymidol, a potent inhibitor of gibberellin biosynthesis (5), decreased plastic extensibility of both the tall and semidwarf genotypes, by 71 and 69%, respectively, but had no significant effect on the double dwarf genotypes.

The effects of *Rht* genotype, GA, and ancymidol treatments on sheath elongation of the expanded first leaf sheath, paralleled the effects on cell wall extensibility of immature second

[4] Abbreviations: LVDT, linear variable displacement transducer; LEZ, length of expansion zone; LES, length of encircling sheath.

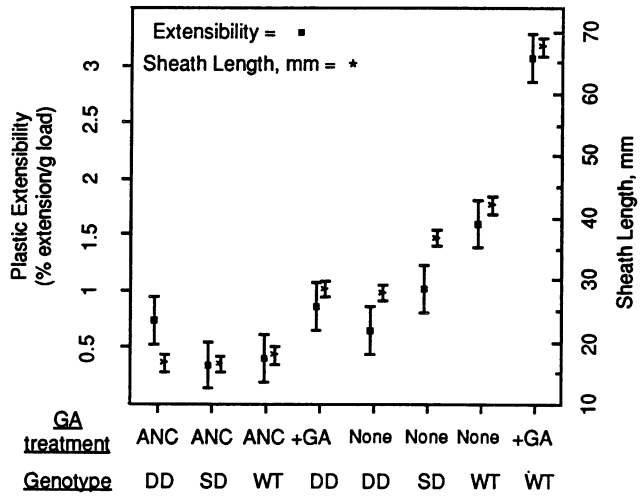


**Figure 1.** Plastic extensibility expressed as percent extension per gram load adjusted for initial segment length and length at maturity of the first leaf sheaths. Treatments are 1 mM GA or 22 μM ancymidol, an inhibitor of GA synthesis. Genotypes are *Rht* at three levels tall wild type (WT: *Rht* = 0 alleles), semi-dwarf (SD; *Rht* = 2 alleles), and double dwarf (DD; *Rht* = 4 alleles).

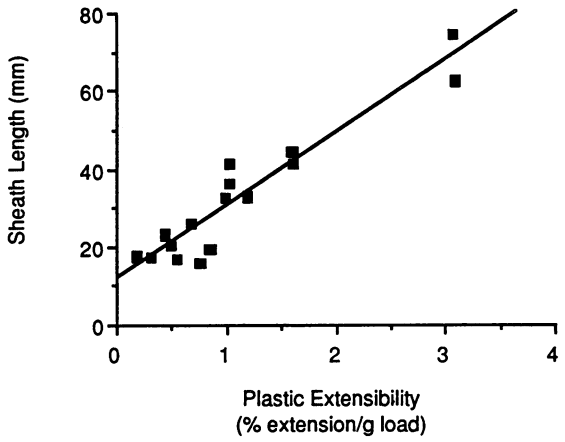


**Figure 2.** Length at maturity of the first leaf sheaths regressed on plastic extensibility for the immature second leaf. Extensibility expressed as the rate of instron-extension per gram load adjusted for initial segment length. Treatments are 1 mM GA or 22 μM ancymidol, an inhibitor of GA synthesis. Genotypes are *Rht* at three levels tall wild type (WT; *Rht* = 0 alleles), semi-dwarf (SD; *Rht* = 2 alleles), and double dwarf (DD; *Rht* = 4 alleles). Sheath length and plastic extensibility were positively and significantly ($P \leq 0.05$) correlated ($R = 0.95$).

leaf segments (Fig. 1). Sheath length significantly declined as a linear function of *Rht* gene dose. Sheath length of tall genotypes averaged 13 and 48% longer than semidwarfs and double dwarfs, respectively. Sheath length was increased by 60% when the nonmutant tall genotypes were treated with GA, whereas ancymidol produced a 56% decrease. GA treatment did not affect leaf sheath length of the double-dwarf. However, inhibition of GA synthesis with ancymidol resulted in significant decreases in sheath length for both semidwarf (55%) and double-dwarf isolines (40%).

Plastic extensibility and sheath length were positively and significantly correlated ($R = +0.95$) (Fig. 2) suggesting that the observed treatment effects on plastic extensibility (Fig. 1) were responsible, in part, for the differences in sheath growth (Fig. 1).

## DISCUSSION

### Stress/Strain Analysis of Cell Wall Extensibility

Cell division in cereal leaves is restricted to the intercalary meristem (20). Rapid cell expansion takes place in a zone distal to the meristem. The LEZ in wheat varies with the LES. The LEZ:LES ratio is greatest (0.63) for young leaves, when the lamina is just emerging from the encircling sheath (12). Based on this report, we extended 8 mm segments of the immature second leaf blade and sheath tissue, since we calculated that even the most extreme dwarfs would contain LEZ greater than 11 mm. Thus, all the tissue tested was in the region of elongation.

In this study, instron determination of leaf plastic extensibility is a measure of average latent (not yet expressed) wall extensibility in the leaf elongation zone at the time the sample is fixed (4). The application of a force vector in excess of turgor induces an irreversible expansion of the sample. Unlike turgor, which exerts a multiaxial force, instron applies force on a single axis. Therefore, the present data indicate that the *Rht* gene-dose affected the longitudinal component of wall extensibility. Further study will be required to determine whether multiaxial wall extensibility *per se* was affected. Although GA and GA synthesis inhibitor treatments caused only minor changes in stress/strain measurements of pea stems (7), pressure block measurements indicated that wall yielding properties were influenced by chemorheological processes. Cosgrove and Sovonick-Dunford (7) adjusted instron extensibility measurements for differences in wall cross-sectional area using a conversion based on dry weight per unit length. Their adjusted measure of plastic compliance was not closely related to growth rate. In our study, extensibility was not adjusted for cross-sectional area because cells in immature leaf tissue have undergone very little expansion and cells are similar in size for dwarf and tall genotypes.

The addition of $GA_3$ to the nonmutant isolines induced a significant increase in plastic extensibility, a result consistent with those obtained in other species (17, 23). Various mechanisms have been suggested to account for hormone-mediated changes in wall extensibility. These have included acid-catalyzed wall loosening, enzymatically catalyzed wall loosening, interactions with calcium, and new wall synthesis (25). Inhibition of wall extensibility, and sheath elongation with ancymidol treatments, further substantiates the role of GA in promoting plastic compliance in wheat. This compound inhibits conversion of ent-kaurene to ent-kaurenic acid via inhibition of sequential oxidations at the C-19 position (5). Ancymidol was associated with an inhibition of shoot growth in the lettuce hypocotyl bioassay for GA activity that was partly reversible with added GA (15).

Ancymidol-induced reduction of plasticity and sheath elongation in *Rht* mutant isolines is interpreted to result from a reduction of endogenous $GA_1$ below the saturation level for the GA 'receptor' mechanism (3, 13). If differences among *Rht* isolines reflect different saturation levels for response to

endogenous GA, then decreased GA concentration by ancymidol treatment should minimize differences in plasticity or elongation arising from the action of these genes. The data reported in the present study were consistent with this expectation.

### Correlation of Wall Plasticity with Sheath Expansion

Elongation of the first leaf sheath was regressed on extensibility of the immature second leaf. Since instron requires a destructive sampling technique it is not possible to measure extensibility and then subsequent elongation. Our study considers extensibility measurements of the second leaf as an assay for extensibility of the first leaf prior to its elongation. This assumption is warranted because (a) GA and ancymidol treatments were subirrigated continuously from germination, and (b) the effects of the *Rht* alleles are constitutive (8). The highly significant correlation between length and plastic compliance ($R = +0.95$) suggests that differences in plasticity are responsible, in part, for differences in elongation (Fig. 2). These conclusions are consistent with current interpretation of physical parameters regulating cell enlargement in *Pisum* (6, 7).

*Rht* genes have been reported to reduce organ length by reducing cell length, cell number, or both (8, 14). Cell depth was not affected by *Rht* gene dose while cell width was increased in dwarf types but not sufficiently to compensate for decreased length in determining projected surface area (14). Maximal leaf growth rates of tall types was sustained over a longer duration than corresponding double dwarf types; however, the total duration of leaf elongation was constant across all levels of *Rht* gene dose (14). While there is known to be a developmental gradient in the region of elongation, variation for extensibility in that zone is uncharacterized and the *Rht* genes would be expected to affect extensibility uniformly over the region. Our results of stress/strain analysis indicate that mechanical wall properties were strongly associated with leaf cell expansion potential in wheat but do not rule out that the underlying rate-limitation may be chemorheological processes which are responsible for wall loosening. In addition, the results of this study indicate that GA is involved in the determination of these mechanical wall properties. Further studies will be required to determine wall relaxation dynamics, turgor pressure, and osmotic pressure characteristic of dwarf and tall genotypes.

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