Leaf biomechanical properties in *Arabidopsis thaliana* polysaccharide mutants affect drought survival¹

ORIGINAL ARTICLE

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2 ABSTRACT

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4 Individual sugars are the building blocks of cell wall polysaccharides, which in turn
5 comprise a plant's overall architectural structure. But which sugars play the most
6 prominent role in maintaining a plant's mechanical stability during large cellular
7 deformations induced by drought? We investigated the individual contributions of several
8 genes that are involved in the synthesis of monosaccharides which are important for cell
9 wall structure. We then measured drought tolerance and mechanical integrity during
10 simulated drought in *Arabidopsis thaliana*. To assess mechanical properties, we designed
11 a small-scale tensile tester for measuring failure strain, ultimate tensile stress, work to
12 failure, toughness, and elastic modulus of 6-week-old leaves in both hydrated and
13 drought-simulated states. Col-0 mutants used in this study include those deficient in
14 lignin, cellulose, components of hemicellulose such as xylose and fucose, the pectic
15 components arabinose and rhamnose, as well as mutants with enhanced arabinose and
16 total pectin content. We found that drought tolerance is correlated to the mechanical and
17 architectural stability of leaves as they experience dehydration. Of the mutants, S096418
18 with mutations for reduced xylose and galactose was the least drought tolerant, while the
19 arabinose-altered CS8578 mutants were the least affected by water loss. There were also
20 notable correlations between drought tolerance and mechanical properties in the
21 diminished rhamnose mutant, CS8575 and the dehydrogenase-disrupted S120106. Our
22 findings suggest that components of hemicellulose and pectins affect leaf biomechanical

- 1 properties and may play an important role in the ability of this model system to survive
- 2 drought.
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INTRODUCTION

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4 In this study, we measured several biomechanical properties as well as electrolyte
5 leakage in several *Arabidopsis thaliana* strains with known mutations in genes related to
6 polysaccharide synthesis that affect cell wall structural integrity

7 Leaf mechanical properties are an indicator of survival during environmentally
8 induced water loss. This cell mechanics – dehydration relationship has been confirmed in
9 drought tolerant species such as *Ceanothus* (Vincent 1983; Balsamo et al. 2003), drought
10 and desiccation-tolerant *Eragrostis* grasses (Balsamo et al. 2003; Balsamo et al. 2005;
11 Balsamo et al. 2006) and desiccation-tolerant *Polypodium* ferns (Layton et al. 2010), but
12 has yet to be studied in *Arabidopsis thaliana* (Pigliucci 2002).

13 The mechanical integrity of plant leaves that have experienced environmentally
14 induced water loss also show changes at the cellular and subcellular level (Burgert 2006;
15 Milwich et al. 2006; Popper 2008; Layton et al. 2010). Thus, the architecture of cell walls
16 and cell-to-cell adhesions via pectins have a direct impact on leaf mechanical properties
17 and consequently overall organ mechanical stability (Balsamo et al. 2003).

18 While cellulose structure is relatively simple (1,4-linked β -D-glucose residues
19 forming microfibrils) (Zykwinska et al. 2005), there are multiple types of hemicelluloses
20 (e.g. xylan and xyloglucan) (Peña et al. 2004; Polizeli et al. 2005; Park and Cosgrove
21 2012) as well as pectins, HGA, RG I, and RG II (Zabackis et al. 1995). The primary
22 monosaccharides present in cell walls are D-glucose, D-galactose, D-mannose, D-xylose,
23 L-arabinose, L-fucose, L-rhamnose and D-galacturonic acid (Popper 2008).

1 Cell wall mechanical properties are also impacted by saccharide components of
2 cellulose, hemicelluloses and pectin that may contribute directly to mechanical strength
3 (Zabackis et al. 1995; Polizeli et al. 2005). For example, galactose residues of
4 xyloglucan impact the effectiveness of hemicellular binding of cellulose microfibrils, and
5 subsequently mechanical strength in cell walls (Peña et al. 2004). Additionally, a variety
6 of neutral sugar sidechains (including arabinose and galactose) allow pectin components
7 to crosslink to cellulose microfibrils, enhancing mechanical strength, while pectins
8 without these sidechains are unable to bind (Zykwinska et al. 2005). Mechanical strength
9 may also be impacted by the galactosinated sidechains of xyloglucan, while the
10 fucosylated sidechains may be less important (Ryden et al. 2003).

11 While wild type *Arabidopsis*, while not drought-tolerant, has been sequenced and
12 ~10% of its genes affect cell wall physiology and development (Geitmann 2006; Taiz and
13 Zeiger 2006). *Arabidopsis* also offers a variety of single- and multiple-gene mutants with
14 altered cell wall monosaccharide compositions, making it a useful model for drought
15 studies.

16 This original study reports the differences among mechanical properties of
17 *Arabidopsis* leaves resulting from dehydration. It is the first attempt to relate these
18 measurements to drought tolerance in eight mutants that overexpress, underexpress or
19 otherwise disrupt specific cell-wall monosaccharides. Control and experimental groups
20 were grown under fully hydrating and partially dehydrating conditions then destructively
21 tensile-tested to reveal how drought affects mechanical properties and survivability.

22 23 METHODS & MATERIALS

1 **Overview**—We grew an assortment of Arabidopsis mutant lines with known
2 deficiencies in specific monosaccharides. These are described under methods of growth
3 conditions and drought simulations and summarized in Table 1.

1 **Table 1.** Mutants selected for their role in monosaccharide-related alterations. CS=Chris
2 Sommerville; JV=John Vogel, SS= Shauna Somerville; CC=Clint Chapple, WDR= Wolf-Dieter
3 Reiter, DC=David Cavalier, KK=Ken Keegstra, JE=Joseph Ecker; EMS=ethyl
4 methanesulfonate.

mutant	polymorph/ locus	donor/ origin	Phenotype	mutagen	ref
CS3094	At5g48300	CS	↓ maltose, starch ↓ xylose and fucose	EMS	(Lin et al. 1988)
CS6354	At3g54920	JV, SS	↑ pectin altered cellulose H-bonding	EMS	(Vogel et al. 2002)
CS8575	mur8-1	CC, CS, WDR	65% ↓ leaf rhamnose ↑ arabinose in cell wall; ↑ arabinan:galactan ratio	EMS	(Reiter et al. 1997)
CS8578	At5g17420 (MUR10-2)	CC, CS, WDR	↓ fucose, xylose, arabinose, mannose and galactan ↓ rhamnose, fucose,	EMS	(Reiter et al. 1997)
CS8579	mur11	CC, CS, WDR	xylose ↑ mannose disrupts xylose transferase	EMS	(Reiter et al. 1997)
CS16349	At3g62720 At4g02500	DC, KK	which decreases UDP-xylose	T-DNA	(Faik et al. 2002)
S038620	At5g54690	JE	50% ↓ xylose	T-DNA	(Alonso et al. 2003)
S096418	At5g15630	JE	40% ↓ galactose	T-DNA	(Alonso et al. 2003)

S120106	At2g40410	JE	possible mannitol	T-DNA (Alonso et al. 2003)
	At2g40400		dehydrogenase disruption ⁱⁱ	
S144144	At1g78860	JE	possible mannose binding disruption	T-DNA (Alonso et al. 2003)

Mutant selections were based upon literature review and availability, with the majority provided by The Arabidopsis Information Resource, Ohio State University (TAIR 2009). Plant lines selected were Col-0 controls, monosaccharide mutants (arabinose, fucose, galactose, glucose, mannose, rhamnose, or xylose), or complex plant cell-wall mutants (Zabackis et al. 1995; Reiter 2002; Peña et al. 2004; Zykwiniska et al. 2005; Rösti et al. 2007; Popper 2008). All seeds purchased from TAIR were homozygous to t-DNA insertions. An extensive search of available cell-wall related mutants found that a number of the key monosaccharides (galactose, mannose, and xylose) were not represented in altered concentration mutant lines, but were instead available only as t-DNA insertion lines created at the Salk Institute, which have not been as extensively tested and thus have monosaccharide alterations that are less well understood (Alonso et al. 2003). The Reiter laboratory had generated a number of altered cell wall monosaccharide mutants, most of which were utilized in this project, as were altered starch concentration mutants (as a proxy for glucose) from the Somerville laboratory (Caspar et al. 1988; Lin et al. 1991; Reiter et al. 1997).

In each of the experimental groups (Table 1), either ethyl methanesulfonate or t-DNA insertion created the original lines, with t-DNA being the lesser preferred because of the increased potential for additional unrelated mutations (Radhamony et al. 2005).

ⁱⁱ Personal communication with George Lindsay.

Growth conditions—Seeds in 20- to 30-seed batches were treated in three 30-second baths of 400 µl 5% Clorox solution (vortexing each bath) to remove mold or fungus, followed by three 30-second baths in 400 µl 100% ethanol to remove Clorox residue, and three 30-second baths in deionized water to remove ethanol residue. Each batch of seeds was transferred to a 250 ml culture plate (4.4 g/L) Murashige and Skoog salts (GIBCO/BRL) with Gamborg's vitamins (Sigma-Aldrich, St. Louis, Missouri) 6 g/L agar, pH 5.8. (Sigma-Aldrich) and deionized water (Taylor et al. 1999; Vogel et al. 2002; Cavalier et al. 2008; Lindberg et al. 2012). Labware was autoclaved at 121 °C for 30 min to ensure an aseptic germination environment. Plates were taped, covered, and cold-treated at 4° Celsius ~72 hours (NASC 2009; Purdue 2009). Plants were germinated on culture plates. After five days, plates were uncovered, and transferred to enclosed growth chamber for two weeks, after which seedlings were transferred to soil (Salinas and Sanchez-Serrano 2006).

Seedlings were grown in Pro-Mix PGX or MetroMix Redi-earth (perlite/vermiculite topsoil/peat mixes). This was augmented with 25% 0.2 to 0.5 cm diameter calcined clay granules (porous ceramic) to regulate water retention, a Miracle Grow slow-release fertilizer pellet (15-9-12), and imidacloprid (Marathon 1G, an insecticide) at 4.2 kg m⁻³ (about 0.25 teaspoon per pot). The soil mixture was autoclaved twice prior to use. 6" × 3" × 3" plastic containers with drainage openings (three 2" × 3" compartments) were filled with the augmented soil mixture, which was thoroughly moistened and allowed to drain. Two seedlings were transferred to each compartment, such that each seedling had approximately 1" × 1.5" × 3", with a total of 18 seedlings per plant type transplanted. Pots were maintained in 21 to 23° C growth chambers during March to December, with

1 24-hour continuous exposure to 100 to 120 $\mu\text{mol m}^{-2} \text{s}^{-1}$ using Convion A1000
2 (Pembina, SD) 50% humidity. As plants developed they were bottom-watered (placed in
3 one inch of standing water and left for no longer than 30 min) every two days, to prevent
4 damage to aboveground tissue and root stress. Water was also applied via a spray bottle
5 daily. Plants were covered with a non-airtight clear plastic lid for the first two weeks
6 following transplantation to promote survival rates. At the end of five weeks of
7 greenhouse conditions, drought simulations began. This period lasted one week and
8 plants were harvested.

9 ***Drought Simulation***—After harvest, mechanical and conductivity experiments were
10 conducted. The measure of electrolyte leakage is an established technique for assessing
11 cellular damage and thus drought tolerance for each experimental group (Tinus 2002;
12 Rolny et al. 2011).

13 ***Mechanical Measurements***—Five mechanical properties of rosette leaves were
14 measured. Whole leaves were excised for tensile strength measurements. The mass of
15 each leaf (± 0.001 gram) was recorded on Sartorius GCA2502 Milligram Balance
16 (Goettingen, Germany). Fowler ProMax digital caliper (Newton, Massachusetts) was
17 used to measure the length (along the midrib), the width (across the widest point of the
18 leaf), and the thickness (at the base of the leaf, across the petiole), ± 0.01 millimeter.
19 Although the width varied, rather than attempt to invent an arbitrary and complicated
20 metric, we chose the widest point as a standard. The length was used to determine the
21 displacement rate in $\mu\text{m s}^{-1}$ to maintain strain rate of 0.004 to 0.005 s^{-1} for viscoelastic
22 composite materials (ASTM D 3039 and ASTM D 412) (Davis 2004), resulting in
23 displacement rates 20 to 80 $\mu\text{m s}^{-1}$. To minimize boundary effects, we clamped each leaf

1 such that half of the total length served as the gage length (Figure 1). For example, a 25-
2 mm leaf has a gage length of 12.5 mm. Thus, $12.5 \text{ mm} \cdot 0.005 \text{ s}^{-1} = 62.5 \text{ } \mu\text{m s}^{-1}$. This
3 number was then rounded to the nearest 10 μm , in this case, 60 $\mu\text{m s}^{-1}$. A Hewlett
4 Packard 34401-A multimeter was attached to a Mastech HY5002 DC power supply, a
5 Dell Optiplex GX240 computer, and a custom-built tensile testing apparatus with an
6 Omega 113 gram sensor and an Omegabus D1521 transmitter. The leaf was placed in a
7 padded clamp perpendicular to the midrib, which was screwed into the top of the load
8 sensor attached to a platform. A second clamp was attached at the opposite tip of the leaf
9 such that the chord lengths within the clamps were approximately equal and one-half of
10 the leaf served as the staple length. The second clamp was attached to a vertical pull bar,
11 which was rotated upwards at a precalculated rate (in the example above, 60 μm per
12 second), applying force to the leaf (gravitational forces were neglected). The force was
13 registered by the load sensor, translated through the multimeter, and sent to the computer,
14 where it was recorded by a custom-written Labview VI (National Instruments LabVIEW
15 v7, Austin, Texas) and translated from microvolts to mN. Measurements were taken
16 every 0.3 s as force was applied until failure, after which the largest recorded
17 measurement was divided by the average cross-sectional area of the leaf between the
18 clamps in order to generate the tensile strength measurement (Balsamo et al. 2003;
19 Layton and Sastry 2006). Leaf width and thickness prior to testing were used to calculate
20 the surface area, which was then divided by the maximum failure load in millinewtons
21 (mN) generated during the test to provide the tensile strength for that leaf in megapascals
22 (MPa). Work to failure, W was found by numerical integration under generated force-
23 displacement curves. Toughness or volume-specific energy density, W_V was calculated

1 by dividing the work to failure in mJ by the gage volume in mm³. Elastic modulus was
2 determined heuristically by individually inspecting every stress-strain curve and selecting
3 a low datapoint where the upward curvature of the stress-strain curve became negligible,
4 and a high datapoint where downward curvature became prevalent and calculated as
5 $E = (\sigma_H - \sigma_L) / (\epsilon_H - \epsilon_L)$ (Figure 2).

6 ***Drought Tolerance Conductivity Measurements***—Levels of dehydrative insult were
7 assessed by electrolyte leakage as a measure of irreversible tissue damage (Tinus 2002;
8 Peng et al. 2010). Conductivity measurements were performed using an EP11/pH pDS
9 meter (Myron L. Company, Carlsbad, California). After calibrating the meter with a
10 standard conductivity solution (Myron L. Company), the sensor and sensor well were
11 rinsed three times with deionized water. Rosette leaf samples were excised with a 6.4 mm
12 diameter cork borer for leaf samples (Sigma-Aldrich). 10 ml of Milli-Q water added to a
13 25 ml glass sample bottle with one leaf sample, and the bottle gently agitated for 10
14 minutes. The water and leaf sample were transferred to the meter's sensor well with care
15 to ensuring that the liquid covered the sensor. The sensor well was rinsed and dried twice
16 between each reading with deionized water.

17 ***Statistical analyses***—Conductivity readings (one per leaf) were performed to assess
18 membrane stability as an indicator of cell damage and thus drought tolerance on leaves
19 from both of the experimental groups: mutant hydrated (MH) and mutant dehydrated
20 (MD). The highest and lowest readings of the 17 taken were discarded, and the average
21 and standard error of the remaining 15 was taken for as the trimmed mean for each
22 treatment of each of the four experimental groups. This mechanical testing described
23 above was also repeated on a total of 17 leaves per plant type, with the highest and lowest

1 measurements being excluded, and the remaining 15 being averaged for each of the five
2 mechanical properties measured to give a value for each experimental group according to
3 previously established methods (Balsamo et al. 2005; Balsamo et al. 2006; MacMillan et
4 al. 2010). All data were then tested via ANOVA and followed with a post hoc test
5 (Tukey's HSD) using SPSS 21.0.0 software.

6 **RESULTS**

7 In mutant specimens the work to failure in the hydrated state was diminished
8 compared to controls. With two exceptions, CS8575 and CS6354, this was also true of
9 the dehydrated state. This indicates that mutants had diminished the total cell wall mass.
10 In contrast, most mutants had toughness values that were either greater in the hydrated
11 state or dehydrated state, but rarely both, indicating that what structural material is
12 present is still intact and capable of sustaining mechanical loads. Notably, all but one of
13 the mutants selected for this study had tensile strengths greater than controls in the
14 dehydrated state, but comparable tensile strengths in the hydrated state. This was likely
15 caused by the relatively diminished leaf cross-sectional areas. As expected the two
16 mechanical properties that were affected most by drying were tensile strength and
17 toughness since both use cross sectional area in the calculation, which was significantly
18 diminished in the dehydrated state.

19 *Statistical analyses*—The post hoc test (Tukey's HSD) yielded a set of groupings for
20 the conductivity tests and each of the five mechanical tests (Table S1). Notable is that the
21 *W* in the wildtype specimens was greatest. Each of the results from the mutants are
22 described in more detail below.

1 Mutant CS6354 is a xylose- and fucose-deficient mutant, with increased pectin
2 (Vogel et al. 2002). Our results revealed substantially worse drought resistance
3 ($P < 0.0001$) (Fig S1A). This mutant, unlike the controls, had enhanced W in the dry state
4 (Fig 3D). Overall, since fucose appears to have a general stiffening nature and xylose
5 tends to have a generally compliant nature, the deficiency of both had a neutral effect.

6 Mutant CS8575 has been recorded to express reduced rhamnose (65% of wild-type)
7 (Reiter et al. 1997). In the present study its leaves exhibited the greatest tensile modulus
8 of any hydrated plant and an elevated tensile strength (Fig 3A). While its survivability as
9 measured by conductance was statistically equivalent to the controls, its leaves in the
10 hydrated state had the lowest average failure strain value (Fig 3C). Perhaps most notable
11 was that it had the greatest W under drought conditions, but significantly diminished W in
12 the hydrated state. Finally, W_V was enhanced in the dry state (Fig 3E). CS8575's E and ϵ_f
13 were relatively unaffected (Fig 3A, 3C).

14 Mutant CS8578 expresses increased arabinose in cell walls (Bosca et al. 2006). This
15 mutant had the lowest conductance (greatest survivability) of all mutants and sugar-
16 treated plants (Fig S1A) ($P < 0.010$). It also had the greatest failure strain, and toughness
17 in both the hydrated and dehydrated states of any of the mutants (Fig S1D, S1F). Its
18 modulus was second lowest among mutants, whereas its tensile strength was elevated.

19 Mutant CS8579 is a rhamnose, fucose, and xylose-deficient line with enhanced
20 mannose and decreased xyloglucan. These specimens exhibited among the lowest
21 conductances (greatest survivability) and the greatest tensile strength in the dry state,
22 which is consistent with our hypothesis that enhanced survivability correlates with
23 enhanced mechanical properties. It had the second greatest tensile modulus in the

1 hydrated state, whereas the failure strain was similar to that in control plants. This
2 mutant's W was among the smallest for both the hydrated and dehydrated states. Its
3 toughness in both the hydrated and dehydrates states was greater than those of control
4 values ($P < 0.05$). Morphologically, it exhibited notably large leaves with purple abaxial
5 surfaces.

6 Mutant CS16349, with disruptions in xylose transferase and diminished UDP-xylose,
7 exhibited increased conductance (Fig S1A). It was one of two to exhibit a greater tensile
8 modulus in hydrated versus dehydrated state (Fig S1B), which might be related to
9 hydrophilicity of specific sugars. Failure strain was significantly less in hydrated samples
10 versus dehydrated samples (Fig S1D). Mutant 16349 also had the lowest tensile strengths,
11 as well as modest work to failure and toughness (Fig S1E, S1F).

12 Mutant S038620 has been observed to be xylose deficient by 50% (Alonso et al.
13 2003). This mutant had the greatest tensile modulus ($P < 0.05$) in the dehydrated state and
14 the lowest work to failure and failure strain ($p < 0.01$ for dehydrated) (Fig S1). This
15 brittle behavior was as anticipated, based upon the biomechanical role that xylose is
16 known to play. Toughness values were similar to those of the wildtype (Fig S1F).

17 Mutant S096418 exhibits a reduction in galactose of 60% (Alonso et al. 2003). It had
18 the worst drought tolerance as indicated by its highest ranking conductance value
19 ($p < 0.005$) (Fig S1A). Its tensile modulus in the hydrated state was the lowest overall
20 ($p < 0.005$) (Fig S1B), as was its toughness value in the dehydrated state ($p < 0.005$)
21 (Fig S1F). Failure strain was greater in the hydrated ($p < 0.005$) versus dehydrated plants
22 ($p < 0.05$) (Fig S1D). Similarly to the effects of enhanced galactose, this galactose-

1 deficient mutant had very poor survivability but relatively unaffected mechanical
2 properties, indicating a plausible role in metabolism and signaling.

3 Mutant S120106 is a mannitol dehydrogenase mutant line from the Salk laboratory
4 (Alonso et al. 2003). Hydroxyl groups are replaced with hydrogen and mannose
5 intercalates into lignin milieu. Conductance measures were similar to those of controls
6 (Fig S1A). This mutant had the lowest tensile modulus with the second greatest failure
7 strain and a relatively high *W* (Figs S1B, S1D, S1E). Enhanced mannose in lignin should
8 stiffen tissue, whereas the altered mannose of this line should reduce the modulus.
9 However, since lignin is not a major component in leaves, the modest changes we
10 observed were expected.

11 **DISCUSSION**

12 Cell walls, which are composed of the polysaccharide components cellulose,
13 hemicelluloses, and pectins, provide mechanical support for plants (Reiter et al. 1993).
14 Our previous studies have shown that species or specimens that exhibit drought or
15 desiccation tolerance typically also exhibit enhanced biomechanical properties (Balsamo
16 et al. 2003; Balsamo et al. 2003; Balsamo et al. 2005). However, this is the first study of
17 its kind to seek the individual mechanical properties of failure strain, tensile strength,
18 modulus of elasticity, work to failure, and toughness in a genetically altered organism
19 undergoing drought.

20 Rhamnose features prominently as a component in two of the three primary pectic
21 polysaccharides: rhamnogalacturonan I and II and CS8575 mutants with reduced
22 rhamnose fared better under drought conditions.

1 Galactose is part of the hemicellular matrix. It is represented in a variety of cell wall
2 structures, including galacturonic acid in pectin, assorted other pectic structures, as a
3 component of arabinogalactan proteins, and as a possible side chain on mannans or
4 xylans. Quantification of cell wall components suggests that galactose is the second most
5 common neutral monosaccharide regardless of leaf age or Arabidopsis ecotype.
6 Furthermore, galactose-containing sidechains of xyloglucan make a major contribution to
7 wall strength (Ryden et al. 2003). This may help explain the brittle failure behavior of
8 S096418 in the hydrated state. Galactose is also very similar structurally to glucose and
9 thus might have also been in close competition for signaling as well as uptake during
10 polysaccharide assembly.

11 Mannose is present as a small percentage of a variety of cell wall and tissue types in
12 Arabidopsis, mostly in the form of mannans in lignified secondary xylem cell walls.
13 Mannose is recognized by hexokinase-II, which is a conserved eukaryotic glucose sensor
14 that phosphorylates the C6 on 6-carbon sugars. It is also a small constituent in lignified
15 tissue. Lignin has been measured to comprise approximately 3% of Arabidopsis leaves by
16 wet mass (Hedderon et al. 2009). As expected, there is also a positive correlation
17 between the lignin per unit cross-sectional area in leaves and tensile strength under fully
18 hydrated conditions (Hedderon et al., 2009).

19 Arabinose contributes to cell wall component structure as it is linked to pectic
20 components, hemicellulose side chain sugars, and in a variety of glycoproteins, including
21 extensions and extracellular arabinogalactan cell surface proteins, which mediate
22 discourse between the cell cytoplasm and cell wall (Cosgrove 2005). The
23 arabinose-enhanced 8578 mutant exhibited high survivability to drought. This apparent

1 causality of enhanced mechanical properties conferring enhanced survivability supports
2 our overall hypothesis.

3 Fucose features most prominently as a possible hemicellulose side chain or fucosyl
4 pectic component, and accounts for the smallest percentage of the neutral
5 monosaccharides in cell wall components. Mutants deficient in L-fucose were dwarfed in
6 growth and had fragile cell walls, consistent with the findings of (Reiter et al. 1993). One
7 explanation for this is that fucose redirects metabolic energy in its conversion to glucose,
8 with the overall effect of diminished fitness.

9 Xylose is one of the most prevalent of the neutral monosaccharide contributors to the
10 primary and secondary cell wall components, and is found most notably in the
11 hemicelluloses xyloglucan and xylan, and as a pectic component sidechain sugar.
12 Furthermore, plants that are desiccation tolerant make xylose as they are dehydrating. It
13 is a flexible X-linker xyloglucan that relaxes cell walls (Vicré et al. 2004). Overall we
14 observed this effect with the diminished moduli and UTS values, accompanied by large
15 failure strain values.

16 At 167 mM, glucose has been shown to maximally affect transcription of
17 ABA-related genes compared to control or higher glucose levels (Price et al. 2004).
18 Glucose affects a broad range of genes, from stress responses and cellular metabolism to
19 those involved in signaling and gene regulation, but its effects are relatively independent
20 of nitrogen status (Price et al., 2004).

21 In addition to the experimental conditions used in this study, other independent
22 factors that may have led to the differences among mechanical properties we measured
23 may include differing levels of expansin in the leaves or the distribution of turgor

1 pressure itself within the leaf structure (Cho and Cosgrove 2000). Our election to use the
2 widest portion of the leaf as our width measurement likely caused a minor underestimate
3 of stress, and thus an underestimate of modulus and toughness. Another independent
4 factor that may have led to differences in water loss may have been varying degrees of
5 evapotranspiration among the experimental groups that may have occurred during the
6 growing cycle.

7 These studies have demonstrated the importance of cellulose and non-cellulose
8 associated monosaccharides such as hemicellulose and pectic components to the
9 mechanical integrity of the plant cell wall during volumetric water loss. Both genetic
10 predisposition and environmental factors contribute to the ability survive episodic
11 drought in the model system *Arabidopsis thaliana*. Further work is needed to see if the
12 results found in this study are applicable to agriculturally and an ecologically relevant
13 species.

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16
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Figure 1: Example of leaf measurement and strategy for leaf clamping prior to mechanical testing.

Figure 2: Representative stress-strain plot of hydrated mutant S120106 indicating five mechanical properties measured. Work to failure, U and toughness, U_V are represented by the area under the curve. Ultimate tensile strength σ_{UTS} , failure strain ϵ_{fail} , and tensile modulus, E are also shown.

Figure 3

Figure 3: Mechanical properties plotted as a function of conductance for sugar-treated plants ■ untreated Col-0, ● 6354, △ 8575, ◇ 8578, ▲ 8579, □ 16349, ○ S08620, ◆ S096418, — S120106. Green represents hydrated specimens. Brown represents water-deprived specimens. Error bars represent ± 1 SEM. $n = 15$ for all measurements.