Small Leaf Mid-Rib Xylem Related to Leaf Freeze Tolerance Trait in Bahia (*Paspalum Notatum* Flugge) Grass Lines

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Keywords: cold tolerance**,** Bahia grass, *Paspalum notatum* Flugge, xylem diameter, xylem area

Introduction Controlled freeze (-60^{o} C) trials of 31 bahiagrass selections from a breeding program for cold tolerance by Blount *et al.* (2001) showed diverse genotype Leaf Tissue Cold Damage (LTCD). Breman *et al.* (2003) defined LTCD on a rating scale (1 = no damage to 9 = 100% leaf damage). Unique midrib damage was observed as part of LTCD in bahiagrass under transpiration stress after a freeze trial. Small xylem conduit diameter and area has been strongly correlated with reduced cavitation caused by freeze thaw cycles which maintain leaf tissue in evergreen temperate woody plants; further shown in twelve woody species by Davis *et al*. (1999). Air bubbles in vessel ice columns prevent normal refill and function upon thawing. The purpose of this study was to test whether genotype xylem diameter and area could be used to predict LTCD.

Materials and methods Permanent stained (crystal violet) slides of four cold sensitive and four cold tolerant genotype lamina cross sections (I.E.C. CTF Microtome Cryostat) taken at the first, second and third emerged leaf 2 cm above the leaf collar from four different plants per genotype, five reps per plant in February. Olympus BH2 microscope at 400X with an ocular micrometer calibrated with a stage micrometer was used to measure midrib abaxial vessel diameters from which vessel area was calculated.

Results Xylem area produced the most consistent determination of whether a genotype was cold tolerant. Simple effects of xylem diameter and xylem area were significant ($P<0.001$) for LTCD regardless of leaf position and predicted whether a genotype was freeze sensitive or tolerant.

Table 1 Mean Midrib Abaxial Xylem Diameters and Areas

Cold Tolerance Category	LTCD Rating	Genotype	Mean Xylem Diameter (μ)	Mean Xylem Area (μ2)
Sensitive	**9.0**	**1-30-4**	**242 a**	**46260 a**
Sensitive	**9.0**	**1-30-3**	**223 b**	**39364 b**
Sensitive	**6.2**	**FL9**	**209 c**	**35911 c**
Sensitive	**9.0**	**2-22-1**	**209 c**	**34735 c**
Tolerant	2.6	OK1	187 d	27570 d
Tolerant	2.2	OK2	170 e	22971 e
Tolerant	2.0	FL67	157 f	22024 e
Tolerant	2.0	CO6	158 f	20109 e

Conclusions Critical values for xylem diameter must lie between 187 and 209 μ and for xylem area between 27,570 and 34,735 μ^2, determining whether a genotype is susceptible or tolerant to freezing. This method has the potential for screening bahiagrass breeding lines for low LTCD (leaf freeze tolerance) without cold chamber trials or natural freeze events in the field with small amounts of plant leaf tissue. This data also suggests a mode of action by which some bahiagrass genotypes tolerate freeze events. Davis et al. (1999) postulated smaller ice volumes in frozen vessels with small diameters maintain dissolved air in the ice column. No gas bubbles form to disrupt vessel transport function upon thawing. This might explain why bahiagrass lines with small leaf xylem diameters or areas maintain a green and functioning lamina under transpiration demand after a freeze event.

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