Aluminum Resistance among Seeded Bermudagrasses

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Additional index words. aluminum toxicity, acid soil, aluminum tolerance

Abstract. Aluminum toxicity is a major limiting factor for turfgrass establishment and growth when soil pH is <5.0. Limited information on aluminum resistance is available among warm-season turfgrasses and these turfgrasses often grow in the areas with acid soil conditions. The objectives of this study were 1) to evaluate seeded bermudagrass (*Cynodon dactylon* L.) cultivars for the ability to tolerate a high level of aluminum and 2) to measure the extent of aluminum damage to the root systems. In total, 16 bermudagrass cultivars were evaluated under greenhouse conditions using a solution culture and an acid Tatum soil (Clayey, mixed, thermic, typic, Hapludult). The soil had pH 4.4% and 69% exchangeable aluminum. A concentration of 640 μM aluminum and a pH 4.0 was used for solution culture. The grasses were grown for 28 days in solution culture; 28 days in the acid Tatum soil; and 78 days in the acid Tatum soil before harvesting. Aluminum resistance was determined by measuring the longest root length, the longest shoot length, dry root weight, dry shoot weight, and shoot to root ratio in comparing the control to obtain the relative Al resistance among the cultivars. The results indicate that seeded bermudagrass cultivars differ in their aluminum resistance.

Bermudagrass is the most popular warm-season turfgrass in the world. Soil acidity is one of the problems associated with some southern areas and the transition zone where bermudagrass grows. Under acid soil conditions, warm-season turfgrass will face more stresses such as nutrient deficiency, poor drought resistance, and winter-kill in the transition zone (Duncan, 2000; Duncan and Carrow, 1999; Duncan and Shuman, 1993; Liu, 2001). Seeded bermudagrass has been a new turfgrass for medium to low maintenance turf in the southern and transitional areas. The new breakthroughs of transgenic Al resistant plants or organisms (Andersson, 1988; De la Fuente et al., 1997; De la Fuente-Martinez and Herrera-Estrella, 1999; Ezaki et al., 1999, 2000; Taylor 1988, 1991) provide the promising future for turfgrass enhancement to acid soil stress by using genetic engineering techniques. Aluminum toxicity has been reported in perennial crops and turfgrasses (Duncan, 2000; Foy, 1988, 1992; Kochian, 1995; Kochian and Jones, 1999; Kochian et al., 2004; Murray and Foy, 1978). The genetic diversity of bermudagrass may be another positive aspect for breeding bermudagrass for Al resistance (Taliaferro, 1995). However, the information of acid resistance among bermudagrass cultivars is limited.

Received for publication 14 Jan. 2004. Accepted for publication 14 June 2004. Many thanks to Joseph Heckman, James Murphy, C.R. Funk, B.B. Clarke, and the New Jersey Agricultural Experiment Station for the support and encouragement during this study; the Center of Turfgrass Science at Rutgers for providing the facilities and funding for this project; the seed supplies from the NTEP program and Simplot (Jacklin Seed); Dave Starner (Agricultural Experiment Station of Virginia Polytech. & State University) for providing the acid Tatum subsoil; and David Stiles and David Coffey from Western Kentucky University for reviewing the manuscript.

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The objectives of this study were 1) to evaluate 16 seeded bermudagrass (*Cynodon dactylon* L.) cultivars for the ability to tolerate a high level of aluminum by using different culture methods with variable time length and 2) to measure the extent of aluminum damage to root and shoot growth.

Materials and Methods

Three growth media (solution culture, acid Tatum subsoil, and atop-dressing sand) were used to screen the relative Al resistance among the bermudagrass cultivars. The seeds were from the NETP program and Simplot (Jacklin Seed). The experiments were conducted in two different locations with similar greenhouse conditions. Greenhouse day/night temperatures were set for about 28/22 °C. The experimental design was a randomized complete block with three replications. The screenings of 16 bermudagrass cultivars using the solution culture and the acid Tatum subsoil culture were conducted at, Cook College, Rutgers University, New Brunswick, N.J.

Solution culture. Sixteen bermudagrass cultivars (Tables 1, 2, and 3) were seeded at a rate of 2.0 g·m⁻² and grown for 28 d in a modified 1/4-strength Hoagland's nutrient solution (Hoagland and Arnon, 1950). A floating mesh was used to support the plants as described by Liu et al. (1995). Aluminum was added to solution as Al₂(SO₄)₃·18H₂O at a concentration of 0 (the control) or 640 μM . The pH of both the control and the 640 μM Al solutions was adjusted daily to 4.0 (± 0.1) using 0.1 M HCl or 0.1 M NaOH. All solutions were changed every four days. Deionised distilled water was used to make all solutions. The solution was aerated except for the first week after seeding. All the treatments including the control were replicated three times.

The acid Tatum subsoil culture. Two different time lengths were used for the acid

Tatum subsoil screening: a 28-d culture using conetainers containing about 250 g of the soil and a 78-d culture using pots containing about 4 kg of soil. The acid Tatum subsoil (Clayey, mixed, thermic, typic, Hapludult) from Orange, Va., had a pH of 4.4 (1 soil : 1 water ratio), a cation exchange capacity of 13.0 cmol·kg⁻¹, and 8.82 cmol·kg⁻¹ of KCl-extractable Al. Other cations present in the soil included Na at 0.36 cmol·kg⁻¹; Mg at 0.64 cmol·kg⁻¹; Ca at 0.21 cmol·kg⁻¹; and K at 0.32 cmol·kg⁻¹, as determined by extraction with 1 N NH₄OAc at pH 7.0. The soil was treated with either 0 or 3,500 mg·kg⁻¹ of CaCO₃; liming the soil increasing the pH to 6.3 with lime used as the control. Seeds were planted 3 weeks after liming the soil. Fertilization with NH₄NO₃ and KH₂PO₄ added N, P, and K at 100, 109, and 137 mg·kg⁻¹ of the soil, respectively for both limed and nonlimed soils. Tap water (pH 6.9) was added as needed to avoid drought stress. All the treatments including the control were replicated three times.

Data collection and analysis. All treatments in the three methods described were replicated three times. The measurement of root length and shoot length were conducted immediately after harvesting. The dry weight of roots and shoots were obtained after dried at 65 °C for 48 h washed off soil.

Relative root length and relative shoot length were obtained by using such a formula: length with Al/length without Al $\times$ 100 (Liu et al., 1995). The relative root mass and relative shoot mass were obtained by using a such formula: [(root or shoot mass/number of plants in a single culture) with Al/(root or shoot mass/number of plants in a single culture) without Al] $\times$ 100. In this case, the relative mass was based on one single plant instead of a culture unit with different number of plants. However, for the 78-d acid Tatum soil culture, it was impossible to separate individual plants. Each pot was seeded with 30 seeds and the relative mass was obtained based on individual pots.

The main reason to have such relative values for measurement of Al toxicity is because that the control pH is at 4.0, which is a stressful condition for most plants. The use of such relative values will separate the stressful condition of a lower pH (4.0) and Al toxicity in this study.

The values of the screening parameters were subjected to ANOVA procedures for each medium, and cultivar means were separated by Duncan's multiple range test as a randomized complete block design. All statistical computations were conducted by using ANOVA procedures within the Statistical Analysis System (SAS), a PC version.

Results and Discussion

Solution culture. The growth of all cultivars screened was significantly reduced as affected by Al based on all parameters measured. However, 'FMC 3-91' exhibited a greater resistance among the 16 cultivars, while 'Guymon' and 'OKS 91-11' even failed to germinate (Table 1). The parameters used for the screening were

Table 1. Relative growth of 16 bermudagrass cultivars grown for 28 d in solution culture containing 640 μM Al at pH 4.0

Cultivar	Relative root length (%)	Relative shoot length (%)	Relative root mass (%)	Relative shoot mass (%)	Shoot to root ratio with Al	Shoot to root ratio without Al
Cheyenne	23.7 b ^z	26.8ab	13.8 bc	24.3 ac	23. b	5.8
OKS 91-1	2.1 cd	5.7 ef	18.97 ab	29.64 abc	370.7 a	6.37
FMC 3-91	43.62 a	34.82 a	26.64 a	43.76 a	7.8 b	5.97
90173	6.42 bcd	11.88 b-f	6.68 bc	13.28 bcd	153.5 ab	4.96
A. common ^y	18.27 bc	22.14 a-d	15.40 ab	24.37 abc	235.2 ab	6.23
FMC 1-90	13.86 bcd	23.72 abc	9.81 bc	16.51 bcd	102.7 ab	5.66
Sonesta	14.00 bcd	17.79 b-e	13.16 abc	23.10 a-d	178.5 ab	5.97
Sundevil	20.00 bc	25.64 abc	10.04 bc	16.16 bcd	141.2 ab	6.49
J-192	15.75 bcd	12.37 b-f	14.55 ab	24.95 abc	134.3 ab	5.93
FMC 2-90	11.55 bcd	15.59 b-e	13.00 abc	20.59 a-d	123.2 ab	5.70
Jackpot	9.55 bcd	10.86 c-f	18.59 ab	35.07 ab	74.7 ab	5.82
FMC 5-91	8.08 bcd	13.99 b-f	11.43 bc	19.63 bcd	42.4 b	5.77
FMC 6-91	19.36 bc	16.54 b-e	11.43 bc	24.51 abc	46.7 b	6.49
Guymon	0.00 d	0.00 f	0.00 c	0.00 d	---	6.26
J-27	6.62 bcd	8.65 ef	6.10 bc	9.02 cd	78.0 ab	6.78
OKS 91-11	0.00 d	0.00 f	0.00 c	0.00 d	---	5.90

^zValues within a column followed by the same letter are not different at the 5% level of probability based on Duncan's multiple range test.^yA. common = Arizona common.

Table 2. Relative growth of 16 bermudagrass cultivars grown for 28 d in nonlimed acid Tatum subsoil at a pH of 4.4

Cultivar	Relative root length (%)	Relative shoot length (%)	Relative root mass (%)	Relative shoot mass (%)	Shoot to root ratio with Al	Shoot to root ratio without Al
Cheyenne	80.08 a ^z	67.72 ab	69.10 ab	48.24 a-d	1.85 b	2.68 c-f
OKS 91-1	68.69 a-d	62.70 a-d	44.29 bc	32.02 ef	2.07 b	2.90 c-f
FMC 3-91	75.92 abc	64.01 abc	55.99 abc	46.63 a-d	2.31 b	2.65 c-f
90173	36.43 e	38.51 ef	33.79 cd	28.97 fg	3.90 b	3.35 b-d
A. common ^y	83.92 a	76.79 ab	66.08 ab	54.69 ab	2.37 b	2.90 c-f
FMC 1-90	79.69 ab	77.23 ab	63.50 ab	47.94 a-d	1.97 b	2.56 ef
Sonesta	76.05 abc	78.23 a	71.58 a	58.23 a	2.10 b	2.53 d-f
Sundevil	71.11 a-d	76.22 ab	71.41 a	55.23 ab	1.98 b	2.55 ef
J-192	56.83 d	51.08 cde	48.53 abc	39.86 c-f	3.26 b	3.59 abc
FMC 2-90	67.13 a-d	62.15 bcd	67.12 ab	49.69 abc	2.53 b	3.56 b-d
Jackpot	61.41 bcd	47.50 de	49.27 abc	45.94 a-d	4.26 b	4.18 ab
FMC 5-91	59.57 cd	48.79 cde	44.27 bc	35.98 def	2.57 b	3.04 c-f
FMC 6-91	59.69 cd	72.22 ab	63.88 ab	53.40 ab	2.83 b	3.21 b-f
Guymon	21.42 ef	27.08 fg	8.04 e	42.34 b-e	113.90 a	4.57 a
J-27	29.14 e	38.84 ef	10.34 de	29.64 efg	7.03 b	2.28 f
OKS 91-11	10.50 f	16.18 g	1.61 e	17.25 g	30.33 b	2.85 c-f

^zValues within a column followed by the same letter are not different at the 5% level of probability based on Duncan's multiple range test.^yA. common = Arizona common.

Table 3. Relative growth of 16 bermudagrass cultivars grown for 78 d in nonlimed acid Tatum subsoil at a pH of 4.4

Cultivar	Root dry wt without Al (g)	Shoot dry wt without Al (g)	Relative root wt with Al (%)	Relative shoot wt with Al (%)	Shoot to root ratio with Al	Shoot to root ratio without Al
Cheyenne	2.32	18.61 b-e ^z	124.52 a	60.69 a	3.56 b	8.38
OKS 91-1	2.37	19.33 bc	102.54 ab	47.96 ab	3.97 b	8.56
FMC 3-91	2.78	19.01 bcd	98.13 ab	51.90 ab	3.13 b	7.54
90173	2.33	16.38 b-g	89.48 ab	48.56 ab	3.86 b	7.47
A. common ^y	3.52	20.17 ab	79.59 bcd	43.40 ab	2.63 b	5.98
FMC 1-90	2.72	19.42 abc	78.51 bcd	42.15 ab	3.54 b	7.71
Sonesta	2.66	20.03 ab	69.83 b-e	38.68 ab	3.90 b	7.88
Sundevil	3.77	23.29 a	53.89 b-e	29.74 ab	3.35 b	6.78
J-192	2.22	15.00 efg	48.68 c-f	28.29 ab	3.75 b	7.61
FMC 2-90	2.35	13.44 fg	43.83 c-f	32.33 ab	4.18 b	5.86
Jackpot	2.80	15.78 c-g	42.17 def	20.92 ab	2.58 b	6.23
FMC 5-91	3.87	14.19 fg	38.43 efg	39.65 ab	3.74 b	5.73
FMC 6-91	3.65	17.01 b-f	33.87 efg	33.75 ab	4.53 b	5.21
Guymon	3.26	15.08 d-g	8.34 g	0.52 b	3.13 b	5.39
J-27	1.97	13.91 fg	4.79 g	1.60 b	3.05 b	7.08
OKS 91-11	2.37	19.33 bc	0.09 g	0.08 b	70.92 a	7.65

^zValues within a column followed by the same letter are not different at the 5% level of probability based on Duncan's multiple range test.^yA. common = Arizona common.

adequate and similar to other findings (Liu et al., 1995, 1996, 1997a, 1997b), both the roots and shoots were significantly reduced (Foy and Murray, 1998a, 1998b; Liu et al., 1995, 1996, 1997a, 1997b; Murray and Foy, 1978).

Root and shoot ratio is another parameter to compare the relative reduction of root or shoot. Significant changes were found among all cultivars except 'FMC 3-91' with 5- to 50-fold increase of shoot/root ratios compared with the control. The pH of the control was 4.0 and that is a stressful condition for most plants even without Al existence. Significant differences found were obviously due to the Al toxic effect along on the roots and shoots.

The acid Tatum soil culture. Significant differences were found either through 28 day screening and 78 day screening (Table 2 and 3). Similar to the results of the solution screening, 'Guymon' and 'OKS 91-11' were sensitive cultivars compared to 'Cheyenne', 'OKS 91-1', and several others. The correlations between different screening methods were significant ($r = 0.46$ to 0.89 at $p < 0.05$ to 0.0001).

The shoot/root ratio was not the same as the results of the solution culture and most the cultivars exhibited a reduced shoot/root ratio. It clearly indicates that shoot growth was significantly reduced as well. What caused the decreased shoot/ratio compared with the control remains unclear. However, based on the 78 day acid Tatum soil screening, the relative root mass and relative shoot mass was highly correlated ($r = 0.89$ at $p < 0.0001$). Short, thick, and swollen roots were observed with most cultivars after growing in Al treated media.

Slightly increased root growth was found only with 'Cheyenne' and 'OKS 91-1' and similar slight increase was found among kentucky bluegrass and hard fescue cultivars (Liu et al., 1995, 1996). Even some results indicate the beneficial effects of lower concentration of Al among other plants (Marschner, 1995), there was not enough evidence among the turfgrasses studied to support the beneficial effects of Al. The study by Vinceli and Williams (1998) indicates the beneficial lower soil pH (5.3 to 5.6) for reducing spring dead spot (*Ophiopharella herpotricha* and *O. korre*) among of bermudagrass and future investigations are needed.

Conclusions

Seeded bermudagrasses differ in Al resistance based on the greenhouse screenings using different screening media. Root and shoot growth reductions prove the Al toxicity to sensitive seeded bermudagrass cultivars. The results indicate that the genetic diversity in Al resistance exists among seeded bermudagrass and the future enhancement of bermudagrass for Al resistance is promising.

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