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Germination and Seedling Growth of Perennial Ryegrasses in Soluble Salts

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Abstract. **A laboratory experiment was conducted with seeds of perennial ryegrass (*Lolium perenne* L.) cultivars germinating and growing on floating mats in saline hydroponic solutions. This study was done to determine the relative intraspecific salt resistances of 48 perennial ryegrasses during germination and seedling growth in saline solutions. Total germination, germination rate, leaf blade length, root length, and total seedling fresh and dry weight were measured after 21 days. Test solutions prepared from deionized water and equal quantities of NaCl and $CaCl_2$ by weight consisted of 11.6 (low), 19.5 (medium), and 23.5 $dS \cdot m^{-1}$ (high) salinity. Cultivars had highly significant total germination and germination rate responses to salt stress. Seedling growth responses as measured by blade and root length and weights were also significant. A hydroponic medium with a salt concentration of 23.4 $dS \cdot m^{-1}$ should provide a suitable stress level for screening ryegrass genotypes for improved germination and seedling salt resistance. At the high salinity level, cultivars that average less than a 50% reduction in growth parameters relative to high-yielding cultivars should be considered. Broad-sense heritability estimates indicate that seedling dry and fresh weight and germination rate would be valuable criteria for use in selection of perennial ryegrasses for salt resistance.**

Perennial ryegrasses are widely used in the southern and southwestern United States for overseeding dormant warm-season turfgrasses. However, saline conditions often limit the establishment and quality of perennial ryegrasses for turf use. Salts concentrate in the soil from use of poor-quality water; soil solutions can reach harmful concentrations. Comparisons characterizing several turf and forage grasses (1, 2, 8, 9, 11) indicate that cool-season grasses are sensitive to salinity. A significant range of intraspecies responses to soluble salts on germination and seedling growth of Kentucky bluegrass (*Poa pratensis* L.) (6) and tall fescue (*Festuca arundinacea* Schreb.) cultivars (5) had been reported. Younger et al. (12) reported a wide range

Table 1. Mean squares for salt resistance sources of variation for perennial ryegrass, calculated as a percentage of the control for three salt levels and 48 cultivars.

		Mean squares					
		Germination		Weight		Growth	
Source of variation	df	Total	Rate	Dry	Wet	Blade	Root
Salt level	2	386**	11,007**	55,931**	63,017**	49,200**	161,210**
Cultivars	47	150	259**	2,084**	1,369**	428**	1,026**
Salt level × cultivar	94	49	92	301**	287**	68	206

**Significant at 0.01 level of probability.

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Table 2. Mean germination and blade and root length expressed as a percentage of the control for perennial ryegrass cultivars averaged across three salt solution concentrations.

Cultivar	Germination (%)	Blade length (%)	Root length (%)
282	100 a[z]	68 d	51 c
2ED	99 a	71 c	45 d
Acclaim	98 a	76 b	43 d
Allstar	97 b	71 c	45 d
Barry	97 b	76 b	50 c
Blazer	100 a	74 c	47 d
BT-I	96 b	73 c	48 d
Cigil	95 b	81 a	50 c
Cockade	98 a	77 b	53 c
Crown	102 a	71 c	44 d
Dasher	98 a	72 c	46 d
Delray	97 b	73 c	46 d
Diplomat	100 a	75 b	53 c
Elka	97 b	76 b	49 c
Fiesta	98 a	70 c	48 d
GT-II	100 a	75 b	57 b
HE 168	101 a	84 a	56 b
NK 79309	91 b	66 d	51 c
Omega	99 a	74 c	63 b
Pennant	99 a	71 c	51 c
Pennfine	97 b	84 a	61 b
Pippin	100 a	75 b	74 a
Prelude	98 a	71 c	52 c
Premier	98 a	73 c	55 c
Ranger	98 a	77 b	53 c
Regal	94 b	72 c	46 d
SWRC-1	101 a	79 a	63 b
WWE 19	101 a	72 c	47 d
Yorktown II	92 b	75 b	60 b
2EE	96 b	70 c	41 d
Citation	96 b	68 d	55 c
Cupido	97 b	66 d	54 c
Derby	96 b	71 c	53 c
Gator	101 a	76 b	59 c
HE 178	100 a	77 b	51 c
LP 210	100 a	76 b	54 c
LP 702	99 a	80 a	47 d
LP 736	101 a	77 b	47 d
LP 792	103 a	81 a	60 b
Manhattan	98 a	71 c	47 d
Manhattan II	98 a	77 b	53 c
HR-1	98 a	67 d	51 c
IA 728	100 a	73 c	51 c
Linn	94 b	71 c	51 c
M382	100 a	76 b	64 b
NK 79307	98 a	72 c	65 b
NK 80389	104 a	71 c	51 c
Palmer	100 a	80 a	51 c
Grand mean	98.3	74.0	52.5
Heritability	0.19	0.37	0.30

[z] Cultivars followed by the same letter are not significantly different at the 0.05 level of probability according to cluster analysis (10).

in resistance among creeping bentgrass (*Agrostis palustris* Huds.) cultivars.

This study was initiated to identify the most salt-resistant cultivars of perennial ryegrass for turfgrass use. The relative importance of germination, germination rate, leaf blade length, root length, and total seedling fresh and dry weight as evaluation criteria was studied. Our goal was to provide information for selection and breeding of salt-resistant perennial ryegrasses.

Materials and Methods

Perennial ryegrass seeds of 48 cultivars and experimental cultivars were germinated in aerated solutions. Styrofoam rings with plastic window screen attached to the bottom were used to float the seed on the salt solution surface. Milk filters were split into two disks and one-half was placed on top of the screen. Each cultivar replicate of 25 seeds was placed on a separate floating mat. As roots developed, they grew through the milk filter and window screen into the salt solutions (5, 6). The experimental design was a factorial arrangement of a randomized, complete block with eight replications.

Solutions were prepared using deionized water and equal parts of NaCl and $CaCl_2$, resulting in EC values of 0.0 (control), 11.6 (low), 19.5 (medium), and 23.4 (high) $dS\cdot m^{-1}$. Compressed air was constantly bubbled through the solutions. Seedlings were grown at a constant temperature of 21 ± 3C. Supplementary lighting was supplied at a flux of 800 $\mu mol\cdot s^{-1}\cdot m^{-2}$.

Germination rate was calculated by dividing the number of seedlings in each experimental unit at each counting by the number of days the seeds had been in the salt solutions and then summing (7). Seedlings were counted and all growth (top growth and root growth) was harvested and weighed after 21 days. Numbers of plants, fresh weight, and length of six random leaf blades (from the crown node to blade tip) were recorded. The plant samples were then oven-dried at 55C for 48 hr and weighed. Differences attributable to variable seed size or plant vigor were reduced by calculating each growth measurement as a percentage of the control value for that cultivar. Means were separated by the clustering procedure of Scott and Knott (10). Regression calculations of each selection variable on salt level were based on unequal intervals between salt levels. Broad-sense heritability estimates were calculated on a cultivar basis from the analysis of variance by using the component of variance method described by Gardner (3).

Results and Discussion

No significant salt level × cultivar interactions for germination, blade length, and root length was recorded (Table 1). Mean germination (i.e., seedling survival) at the end of 21 days averaged over the three salt levels was 98% (Tables 2 and 3). This indicates little influence on germination at salt levels up to 23.4 $dS\cdot m^{-1}$. However, it was possible to separate the cultivars into two significantly different groups. Differences in mean percent germination relative to the control ranged from 104% for 'NK 80389' to 91% for 'NK 79309'. Two-thirds of the cultivars had significantly higher germination in salt solutions than the bottom third of the cultivars. Even though there was significantly different germination among the cultivars, the range was too narrow to be a useful selection criterion for salt resistance.

Blade length was reduced to an average of 67% at 19.5 $dS\cdot m^{-1}$, with no additional reduction at the 23.4 $dS\cdot m^{-1}$ salt level (Table 3). Averaged over three salt concentrations, blade length compared to the control ranged from 66% to 84% among the cultivars and root length ranged from 43% to 74%. The tested cultivars fell into four distinct groups using blade and root lengths as separation criteria. There were seven and five cultivars at the top and bottom extremes of blade length and one and 15 cultivars at the top and bottom extremes of root length, respectively. The limited number of groups and range of difference for both blade and root lengths suggested a limited amount of genetic variation in the population evaluated and associated with these two criteria for salt resistance.

Table 3. Maximum, minimum, mean germination, and seedling blade and root growth expressed as a percentage of the control (0.0 dS·m⁻¹) for three salt concentrations with indicated electrical conductivity and 48 cultivars.

	Germination (%)			Growth					
				Blade (%)			Root (%)		
	Electrical conductivity (dS·m⁻¹)								
Statistic	11.6	19.5	23.4	11.6	19.5	23.4	11.6	19.5	23.4
Maximum	106	105	106	100	78	79	106	51	68
Minimum	89	89	89	74	59	59	64	31	27
Mean	99	98	97	87	67	67	76	40	41
Heritability	0.06	0.01	0.01	0.61	0.44	0.63	0.53	−0.26	0.56

Significant salt level × cultivar interaction for germination rate may be attributed to the 12 cultivars that did not exhibit a steady decline in germination rate as salt level increased (last 12 cultivars listed in Table 4). 'Linn' exhibited the greatest increase in germination rate, increasing 8 percentage points as the salt level increased from 19.5 to 23.4 dS·m⁻¹. 'NK 79307' exhibited the greatest decline in germination rate between 11.6 and 19.5 dS·m⁻¹ salts. 'Acclaim' and 'Cigil' had the greatest reduction in germination rates as salt levels increased from 11.6 to 23.4 dS·m⁻¹. Germination rate of 'Premier' was least influenced by increasing salt levels. Increased salt levels separated the population into three, four, and four groups, respectively, for solutions of 11.6, 19.5, and 23.4 dS·m⁻¹.

There was a significant salt level × cultivar dry weight interaction. However, 'Crown' was the only cultivar that exhibited an increase in dry weight as the salt level increased from 19.5 to 23.4 dS·m⁻¹ (Table 5). Five cultivars increased dry weight over the control at 11.6 dS·m⁻¹ salt. Average dry weight reductions were 14%, 21%, and 10% between the control and each increase in salt level. Eleven cultivars, or 23% of this population, had a 50% or greater reduction in dry weight at 23.4 dS·m⁻¹ salt. Differences in dry weight separated the population into four, two, and four groups within the three salt levels, respectively. 'LP 702' declined 52% in dry weight as the salt level increased from 11.6 to 23.4 dS·m⁻¹, while 'Diplomat' declined only 18% over the same levels.

Seedling fresh weights exhibited almost equal amounts (≈30%) of reduction between control and 11.6 dS·m⁻¹ and the next salt increment of 19.5 dS·m⁻¹ (Table 6). However, average fresh weight increased by 7% between 19.5 and 23.4 dS·m⁻¹ salts. This reversal could account for the significant salt level × cultivar interaction. 'Allstar' was the only cultivar that had <50% reduction in fresh weight as salt levels were increased to 19.5 dS·m⁻¹. As the salt level was increased to 23.4 dS·m⁻¹, there were four cultivars that had <50% reduction in fresh weight after 21 days of growth. Average fresh weight reductions were 31% and 32% between the control and each increase in salt level up to 19.5 dS·m⁻¹. Significant group separations for fresh weight were three, four, and three within the three salt levels, respectively. This fluctuation in fresh weights as the salt concentration increased may have been due to a reduction in the ability of root cell membranes to exclude salts. If the membrane semi-permeability was decreased and salts entered the cells, an increase in water content with the corresponding osmotic adjustment may have occurred (4).

Root growth and seedling fresh and dry weights appear to be useful characteristics for selecting salt-resistant perennial ryegrass cultivars because some cultivars exhibited >50% reduction in these traits compared to controls. Germination and germination rate were not reduced below 50%, even at the 23.4 dS·m⁻¹ salt concentration. Leaf blade growth reduction was >50% at all salt concentrations. However, root growth was severely reduced as salt concentrations reached 19.5 dS·m⁻¹. Other growth characteristics (seedling dry and fresh weights) also showed reductions of 50% or more at the 19.5 dS·m⁻¹ salt concentration.

Assignment of equal weight to all six measured traits and using the mean of all cultivars over the three salt concentrations makes it possible to choose arbitrary salt levels required to screen a particular population or group of cultivars. If an existing cultivar is considered for use under saline conditions, it would be best to maintain the medium below 19.5 dS·m⁻¹ due to salts and use a cultivar that averages 50% or less reduction in growth indeces at that salt level. A salinity level where 75% reduction in growth is obtained would be a good cut-off point for identifying individual genotypes of perennial ryegrass for salt resistance. This procedure would eliminate a large percentage of the population and exert considerable selection pressures on the germplasm base in a recurrent selection program.

Rapid germination, germination rate, and root growth are important factors during establishment that enable turfgrass plants to compete aggressively with weeds and aid in increased resistance to drought and other environmental extremes. Correlation coefficients across three salt concentrations, between germination and the other five factors (germination rate, seedling dry and fresh weight, root length, and shoot length) were $r = 0.71$, 0.49, 0.25, 0.18, and 0.13 (df = 46), respectively. These values indicate germination would give a fair estimation of germination rate as the $r = 0.71$ value was highly significant. However, germination is probably of little usefulness as a selection criterion in the overall evaluation of perennial ryegrass cultivars for salt resistance because of poor correlation with the other characteristics. Seedling dry weight was moderately correlated with seedling fresh weight ($r = 0.72$, df = 46) and shoot growth ($r = 0.60$, df = 46) at 11.6 dS·m⁻¹ salt concentration. As the salt concentrations increased, even these relationships became erratic. The overall poor and erratic relationships of the traits indicate that each one would have to be selected on an individual basis under salt stress conditions. These results differ from previous reports on Kentucky bluegrass and tall fescue (5, 6).

Broad-sense heritability values for the six measured traits, calculated from the combined percent of control data, were 0.17, 0.19, 0.25, 0.30, 0.33, to 0.37 for germination rate, germination, seedling fresh weight, root length, seedling dry weight, and blade length (Tables 2–6). All but the estimates for seedling

Table 4. Mean germination rate expressed as a percentage of the control (0.0 dS·m^{-1}) for perennial ryegrass cultivars grown at salt solution concentrations with indicated electrical conductivity (EC).

	Germination rate (%)		
	EC (dS·m^{-1})		
Cultivar	11.6	19.5	23.4
282	97 a[z]	90 a	88 a
2ED	94 b	87 b	84 b
Acclaim	102 a	96 a	80 c
Allstar	91 c	85 b	78 c
Barry	95 b	82 c	74 d
Blazer	93 b	90 a	78 c
BT-I	90 c	82 c	78 c
Cigil	100 a	89 a	78 c
Cockade	93 b	88 a	79 c
Crown	95 b	93 a	81 c
Dasher	93 b	86 b	86 b
Delray	91 c	91 a	82 c
Diplomat	99 a	92 a	84 b
Elka	93 b	86 b	83 b
NK 79309	98 c	87 b	80 c
Omega	99 a	89 a	88 a
Pennant	94 b	91 a	87 a
Pennfine	92 b	88 a	86 b
Pippin	94 b	93 a	88 a
Prelude	95 b	87 b	84 b
Premier	94 b	93 a	92 a
Ranger	97 a	90 a	87 a
Regal	88 c	86 b	84 b
SWRC-1	95 b	91 a	90 a
WWE 19	97 a	92 a	85 b
Yorktown II	87 c	77 d	71 d
2EE	89 c	91 a	79 c
Citation	95 b	88 a	92 a
Fiesta	95 b	89 a	75 d
GT-II	93 b	86 b	86 b
HE 168	101 a	92 a	90 a
HE 178	97 a	91 a	86 b
LP 210	97 a	93 a	82 c
LP 702	94 b	85 b	79 c
LP 736	96 a	93 a	87 a
LP 792	97 a	93 a	87 a
Manhattan	93 b	87 b	83 b
Manhattan II	91 c	89 a	79 c
Cupido	93 b	82 c	83 b
Derby	93 b	88 a	89 a
Gator	100 a	89 a	90 a
HR-1	95 b	84 b	84 b
IA 728	90 c	85 b	87 a
Linn	94 b	78 d	86 b
M382	93 b	94 a	90 a
NK 79307	106 a	86 b	89 a
NK 80389	99 a	90 a	91 a
Palmer	97 a	83 b	84 b
Grand mean	94.6	88.2	84.0
Heritability	0.71	0.78	0.86
Combined heritability			0.17

[z] Cultivars followed by the same letter are not significantly different at the 0.05 level of probability according to cluster analysis (10).

Table 5. Mean seedling dry weight expressed as a percentage of the control for perennial ryegrass cultivars grown at three salt solution levels.

	Seedling dry wt (%)		
	Salt (dS·m^{-1})		
Cultivar	11.6	19.5	23.4
282	77 d[z]	55 b	46 d
2ED	86 c	64 b	54 c
Acclaim	89 c	68 a	48 d
Allstar	86 c	59 b	51 c
Barry	71 d	49 b	41 d
Blazer	81 c	76 a	59 b
BT-I	85 c	67 a	52 c
Cigil	94 b	64 b	50 c
Cockade	83 c	64 b	56 b
Crown	86 c	57 b	59 b
Dasher	79 d	64 b	59 b
Delray	88 c	60 b	54 c
Diplomat	76 d	66 a	58 b
Elka	80 d	67 a	51 c
Fiesta	80 d	65 a	52 c
NK 79309	74 d	59 b	48 d
Omega	92 b	66 a	56 b
Pennant	79 d	63 b	53 c
Pennfine	83 c	63 b	54 c
Pippin	87 c	65 a	51 c
Prelude	81 c	66 a	54 c
Premier	87 c	64 b	62 a
Ranger	102 a	75 a	68 a
Regal	83 c	58 b	50 c
SWRC-1	94 b	68 a	63 a
WWE 19	83 c	62 b	59 b
Yorktown II	74 d	60 b	53 c
2EE	77 d	57 b	43 d
Citation	88 c	59 b	55 b
Cupido	75 d	61 b	47 d
GT-II	84 c	76 a	60 a
HE 168	103 a	67 a	57 b
HE 178	87 c	67 a	54 c
LP 210	84 c	63 b	49 c
LP 702	104 a	68 a	52 c
LP 736	91 b	72 a	56 b
LP 792	89 c	75 a	62 a
Manhattan	68 d	53 b	50 c
Manhattan II	98 a	78 a	62 a
Derby	87 c	70 a	58 b
Gator	102 a	77 a	65 a
HR-1	82 c	60 b	49 c
IA 728	82 c	68 a	54 c
Linn	79 d	64 b	57 b
M382	87 c	65 a	56 b
NK 79307	101 a	70 a	56 b
NK 80389	92 b	68 a	63 a
Palmer	88 c	64 b	62 a
Grand mean	85.6	64.9	54.7
Heritability	0.91	0.86	0.82
Combined heritability			0.33

[z] Cultivars followed by the same letter are not significantly different at the 0.05 level of probability according to cluster analysis (10).

dry weight and root and blade growth were 0.30 or above. The low heritability values for the combined data vs. the relatively high heritability ratios for the factors at the individual salt concentrations also indicate selection for salt resistance may have to be for prescribed salt stress conditions. Overall, there appears to be a limited, but useful, range of salt resistance in the population evaluated. Parent–progeny relationships and additional genetic variability are needed to best estimate genetic progress from selection and breeding.

Table 6. Mean seedling fresh weight expressed as a percentage of the control for perennial ryegrass cultivars grown at three salt solution levels.

	Seedling fresh wt (%)		
	Salt (dS·m^{-1})		
Cultivar	11.6	19.5	23.4
282	66 c[z]	31 d	36 c
2ED	66 c	33 c	41 b
Acclaim	76 b	36 c	49 a
Allstar	75 b	57 a	38 c
Barry	60 c	29 d	36 c
Blazer	70 b	41 b	45 a
BT-I	65 c	34 c	42 b
Cigil	71 b	31 d	44 b
Cockade	64 c	29 d	52 a
Crown	72 b	40 b	44 b
Dasher	60 c	34 c	37 c
Delray	68 c	34 c	41 b
Diplomat	63 c	38 b	45 a
Elka	75 b	48 b	55 a
Fiesta	68 c	37 c	44 b
NK 79309	59 c	34 c	39 c
Omega	72 b	37 c	41 b
Pennant	70 b	41 b	43 b
Pennfine	71 b	36 c	42 b
Pippin	69 b	34 c	42 b
Prelude	61 c	35 c	39 c
Premier	69 b	33 c	44 b
Ranger	78 a	41 b	43 b
Regal	66 c	31 d	41 b
SWRC-1	73 b	40 b	49 a
WWE 19	52 c	32 d	34 c
Yorktown II	70 b	37 c	48 a
2EE	56 c	29 d	39 c
Citation	68 c	30 d	38 c
Cupido	59 c	38 b	41 b
GT-II	71 b	42 b	49 a
HE 168	82 a	42 b	47 a
HE 178	76 b	39 b	44 b
LP 210	75 b	35 c	46 a
LP 702	84 a	34 c	52 a
LP 736	72 b	39 b	48 a
LP 792	60 c	35 c	37 c
Manhattan	59 c	36 c	48 a
Manhattan II	68 c	35 c	47 a
Derby	71 b	35 c	46 a
Gator	80 a	39 b	49 a
HR-1	64 c	36 c	40 b
IA 728	63 c	38 b	46 a
Linn	62 c	35 c	47 a
M382	71 b	36 c	40 b
NK 79307	82 a	45 b	52 a
NK 80389	74 b	41 b	47 a
Palmer	84 a[z]	42 b	47 a
Grant mean	69.0	36.7	43.9
Heritability	0.71	0.85	0.76
Combined heritability			0.25

[z] Cultivars followed by the same letter are not significantly different at the 0.05 level of probability according to cluster analysis (10).

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