Effects of Adenosine Monophosphate on Germination of Forage Species in Salt Solutions

D.J. UNDERSANDER

Seed germination can be a limiting step in the establishment of plant species on saline soils. There are indications that the level of adenosine monophosphate (AMP) in the seed may be a limiting factor in seed germination under stress. The objective of this research was to determine if added AMP would improve germination of grass and legume seeds under saline conditions. The seeds of tall fescue (*Festuca arundinaceae* Schreb. 'K-31'), bluegrama [*Bouteloua gracilis* (H.B.K.) Lag. ex Steud.], crested wheatgrass [*Agropyron cristatum* (L) Goertn 'Nordan'], switchgrass (*Panicum virgatum* L. 'Blackwell'), perennial ryegrass (*Lolium perenne* L. 'Lynn'), tall wheatgrass [*Agropyron elongatum* (Host) uv. 'Platte'], Russian wildrye (*Elymus junceus* Fisch.), western wheatgrass (*Agropyron smithii* Rydb.), and alfalfa (*Medicago sativa* L. 'Dawson') were germinated in petri dishes at varying levels of salinity with and without AMP. Time required for germination was shortened for all species, except switchgrass and western wheatgrass, with added AMP. Percent germination of alfalfa was increased with AMP at 14 days in 0.068 M sodium chloride and of tall fescue in the same concentration of sodium sulfate (dibasic). Perennial ryegrass, Russian wildrye and alfalfa demonstrated similar responses at 0.102 M sodium chloride. The germination of alfalfa was improved with AMP at 14 days in 0.034 M sodium sulfate. Adenosine monophosphate tended to have little effect when severe germination depression occurred from high salt concentrations.

Many thousands of hectares of soil have salt accumulations to the extent that seedling germination and vigor are reduced. Stand establishment on these soils is frequently very difficult. Germination is both delayed and final germination percentage reduced (Berstein and Wayward 1958). Holm and Miller (1972) reported that adenosine monophosphate (AMP) promoted germination of several weed seeds and that seed treatment with AMP accelerated germination (McDaniel and Taylor 1976). Pretreatment of cotton seeds with AMP hastened germination under saline conditions but no more than soaking in distilled water (Shannon and Francois 1977). Pretreatment of seeds with salt solutions has been found to increase salt tolerance during germination of wheat (Chaudhuri and Wiebe 1968).

Seedling germination involves the syntheses of RNA and proteins. This synthesis is dependent on the prior synthesis of adenosine triphosphate (ATP) in the seed (Anderson 1977a) but AMP may limit formation of ATP in seeds (Anderson 1977b). If AMP is a limiting factor in seedling germination, pretreatment of the seed with AMP may enhance germination. Anderson (1977a) reported that exogenously applied adenosine was incorporated into the embryonic axes of soybeans.

Under conditions of stress, AMP may be limiting germination and therefore, externally applied AMP may enhance germination. The objective of this research was to determine if pretreatment of rangeland seeds with AMP would enhance germination under saline conditions.

Methods

Germination studies in petri dishes were conducted on the following species: tall fescue (*Festuca arundinaceae* Schreb. 'K-31'), bluegrama [*Bouteloua gracilis* (H.B.K.) Lag. ex Steud.], crested wheatgrass [*Agropyron cristatum* (L) Goertn 'Nordan'], switchgrass (*Panicum virgatum* L. 'Blackwell'), perennial ryegrass (*Lolium perenne* L. 'Lynn'), tall wheatgrass [*Agropyron elongatum* (Host) Beauv. 'Platte'], Russian wildrye (*Elymus junceus* Fisch.), western wheatgrass (*Agropyron smithii* Rydb.), and alfalfa (*Medicago sativa* L. 'Dawson').

Fifty seeds of each species in three replications were germinated in distilled water in the light (20 μe $S^{-1}m^{-2}$) at 25 °C to determine the rate and extent of germination under nonlimiting conditions. Seed pretreatment consisted of applying AMP (10^{-4}M in 10^{-2}M phosphate buffer pH 7) or an equal volume of distilled water to the seeds and immediately placing 50 seeds into petri dishes on filter paper to begin the trial. A preliminary study indicated that there was no effect on seed germination from inclusion of phosphate buffer alone in distilled water or saline solutions.

The salt treatments consisted of either sodium chloride or sodium sulfate (dibasic) at 0.034, 0.068, and 0.102 M. The salt solutions were added to the petri dishes. All treatments were replicated 3 times. Filter papers and salt solutions were changed every 3 days to reduce changes in salt concentration during the study. Treatments were incubated at 25 °C. Germination counts were taken every 2 to 3 days to determine rate of germination. Final germination was considered to be total germination at day 14.

Data were analyzed as a completely randomized design for the factors: species, salt type, salt concentration, and seed treatment and the results are presented in Table 1. Individual means of treated and untreated seeds for each day-salt type-salt concentration combination were separated with a *t*-test. The effect of days was analyzed by separated measures analysis.

Table 1. Analysis of variance of forage species germination in two salts at three concentrations with or without adenosine monophosphate (AMP).

Source	DF	Sum or squares	F	PR>F
Species	8	294332.26	212.84	0.0001
Salt type	1	110254.70	637.82	0.0001
Treatment (with/without AMP)	1	14495.30	93.85	0.0001
Salt concentration	2	367159.92	1062.00	0.0001
Days	6	179462.55	173.03	0.0001
Species * Salt type	8	39842.93	28.81	0.0001
Species * Treatment	3	9699.94	7.01	0.0001
Species * salt concentration	16	99252.76	35.89	0.0001
Salt type * treatment	1	455.15	2.63	0.1049
Salt type * salt concentration	2	31729.81	91.78	0.0001
Error	1460	252378.53		

Results and Discussion

The germination of all species increased with time. There was no germination of any grass species on the second day but, by day four, most live seeds of the tall fescue, bluegrama, crested wheatgrass, perennial ryegrass, and the tall wheatgrass had germinated in distilled water (Table 2). The germination of switchgrass was somewhat slower, being nearly complete by day six of the trial. Germination of Russian wildrye and western wheatgrass was largely completed by day eight of the trial. Germination of Dawson alfalfa was faster than that of any of the grasses and was largely completed by day two of the trial. The final germination of the

Author is assistant professor, Texas Agricultural Experiment Station, P.O. Drawer 10, Bushland 79012.
Manuscript accepted 11 May 1985.

Table 2. Germination of forage species in distilled water.

Species	Germination Day 2	Day 4	Day 6	Day 8	Day 11	Day 14
	----	----	----	%	----	----
Tall fescue	0	93	93	—*	93	93
Bluegrama	0	79	83	—	85	88
Crested wheatgrass	0	87	89	—	90	91
Switchgrass	0	23	41	—	48	53
Perennial ryegrass	0	87	89	—	91	91
Tall wheatgrass	0	87	91	—	92	92
Russian wildrye	0	56	—	85	85	85
Western wheatgrass	0	9	—	43	50	51
Alfalfa	93	95	—	95	95	95

$S_{\bar{x}} = 2.6$
*Data not collected.

species ranged from 85 to 95% with the exception of switchgrass and western wheatgrass, which had significantly lower ($P = 0.01$) final germinations of 53 and 51%, respectively.

Several generalizations can be made concerning the effect of salinity on germination of the species studied. The germination of tall fescue, perennial ryegrass, tall wheatgrass, and Russian wildrye was not affected by either sodium chloride or sodium sulfate at the 0.034 M (Tables 3 and 4). The results agree with the report by Mass and Hoffman (1977) that these grass species are more salt tolerant than most grass species. Other species were more sensitive to salt concentration and showed varying reductions in germination in the presence of 0.034 M salt concentration. Switchgrass and western wheatgrass appeared particularly sensitive to salt concentration, having 50% or greater reductions in germination at the lowest salt level. All species showed considerable reduction in germination at the 0.102 M salt concentration.

Sodium sulfate appeared to have a greater effect on germination than did sodium chloride. The germination of crested wheatgrass was reduced by the 0.034 and 0.068 M sodium sulfate treatments but not by equivalent levels of sodium chloride. Similarly, germi-

Table 3. Percentage germination of seeds of forage species with or without adenosine monophosphate (AMP).

Species	Salt concentration	AMP	NaCl Day 4	NaCl Day 6	NaCl Day 11	NaCl Day 14	Na2SO4 Day 4	Na2SO4 Day 6	Na2SO4 Day 11	Na2SO4 Day 14
	----M----		----	----	----	----	%	----	----	----
Crested wheatgrass	.034	No	70	88	86	87	25	45	60	63
		Yes	89*	90	91	91	69*	77*	78	83
	.068	No	23	65	78	81	13	27	27	38
		Yes	71*	77	81	84	16	40	41	60
	.102	No	13	31	29	30	3	9	10	12
		Yes	27*	43	46	45	5	20	16	17
Tall fescue	.034	No	81	81	83	89	78	81	80	87
		Yes	93	94	91	91	83	83	83	83
	.068	No	64	64	61	76	10	19	17	19
		Yes	76*	77*	77	78	9	12	13	40*
	.102	No	10	12	21	25	0	0	0	3
		Yes	1	15	17	19	0	0	0	0
Bluegrama	.034	No	13	31	45	58	4	16	3	43
		Yes	13	31	45	58	19*	33*	45	49
	.068	No	1	7	27	40	0	0	3	4
		Yes	2	16*	34	57	0	7	20*	19
	.102	No	0	0	23	31	0	1	1	1
		Yes	0	7	21	23	0	1	1	2
Perennial ryegrass	.034	No	47	83	92	91	38	83	77	78
		Yes	87*	91*	92	93	84*	85*	88	89
	.068	No	28	85	86	86	11	39	60	60
		Yes	37	85	85	87	27*	53	55	57
	.102	No	11	60	57	55	3	6	33	36
		Yes	19	77*	77*	79*	12*	24*	25	21
Tall wheatgrass	.034	No	69	83	83	83	37	78	81	81
		Yes	71	85	89	88	53*	81	83	84
	.068	No	17	52	59	60	3	47	60	60
		Yes	23	72*	72	73	14	68*	72	73
	.102	No	5	48	54	54	0	20	37	38
		Yes	11*	53	55	57	5	33*	37	39
Switchgrass	.034	No	3	25	31	31	2	18	26	25
		Yes	11	22	25	23	4	19	23	25
	.068	No	2	13	23	23	0	5	10	12
		Yes	5	14	18	19	1	7	13	14
	.102	No	0	4	9	10	0	1	3	3
		Yes	0	5	11	11	0	1	3	4

*Significantly higher germination ($P < .05$) than untreated, t-test.

Table 4. Percentage germination of seeds of forage species with or without adenosine monophosphate (AMP).

Species	Salt Concentration	AMP	NaCl					Na_2SO_4				
			Day 2	Day 4	Day 8	Day 11	Day 14	Day 2	Day 4	Day 8	Day 11	Day 14
	---M---		--------%									--------%
Russian wildrye	.034	No	0	45	76	77	78	0	33	75	76	77
		Yes	0	64*	75	83	83	0	47	68*	70	70
	.068	No	0	35	72	73	74	0	7	29	39	39
		Yes	0	45	73	73	74	0	13	39	41	42
	.102	No	0	5	37	38	38	0	0	0	13	21
		Yes	0	31*	51	61*	63*	0	1	23*	25	25
Western wheatgrass	.034	No	0	2	17	22	23	0	0	7	9	11
		Yes	0	4	13	15	16	0	1	12	13	13
	.068	No	0	0	9	11	13	0	0	1	1	1
		Yes	0	0	12	11	13	0	0	1	2	2
	.102	No	0	0	0	1	1	0	0	0	0	0
		Yes	0	0	5	6	6	0	0	0	0	0
Alfalfa	.034	No	31	76	90	92	92	19	56	67	69	66
		Yes	83*	92	93	93	93	27	79	90	91	91*
	.068	No	11	39	79	84	85	0	0	5	6	25
		Yes	49*	79*	93*	92*	91*	0	5	23*	22*	22
	.102	No	2	6	27	29	33	0	0	0	0	0
		Yes	3	23*	61*	62*	63*	0	0	0	0	0

*Significantly higher germination ($P<.05$) than untreated, t-test.

nation of tall fescue, bluegrama, perennial ryegrass, Russian wildrye, and alfalfa was reduced to a greater extent by 0.068 M sodium sulfate than by the same concentration of sodium chloride. The difference between salts was probably due either to the higher concentration of sodium ions or to the slightly lower osmotic potential of sodium sulfate treatments but not by equivalent levels of sodium chloride.

The 0.034 M salt concentration did not reduce germination of tall fescue (Table 3). Thus, no effect of AMP would be anticipated. AMP treatments did have significantly (P=0.05) higher early germination for the 0.06 and 0.102 M concentrations of sodium chloride where the salt concentrations reduced germination. In both cases the rate of germination was enhanced while there was no significant (P=0.05) difference in the final germination at day 14. The AMP treatment did increase the final germination of tall fescue in sodium sulfate at 0.068 M where the final germination was 19% without AMP and 49% with AMP.

Salt concentrations of 0.034 M reduced germination of bluegrama to a greater extent than that of tall fescue (Table 3). Early germination of bluegrama was enhanced by AMP in the 0.034 M sodium sulfate and the 0.068 M sodium chloride. The AMP treatment had significantly (P=0.05) higher germination in the 0.068 M sodium sulfate solution by day 11. A similar trend continued for day 14 but the difference was not significant. There was no effect of AMP at the 0.102 M salt concentration where the salt concentration severely reduced germination.

The germination of crested wheatgrass was generally less effected at each salt level than the germination of other species (Table 3). AMP increased the early germination of the seeds germinated in sodium chloride at every salt concentration. Rate of germination of crested wheatgrass was also increased by AMP in 0.034 M sodium sulfate. In no case was there a significant increase in final germination due to the AMP treatments.

Similarly, perennial ryegrass was fairly tolerant of salinity during germination. Early germination of this species was increased by AMP at every level of sodium sulfate and at the 0.034 M sodium chloride treatment. Additionally, the final germination of perennial ryegrass was increased in 0.102 M sodium chloride with AMP (79 vs 55%).

The early germination of tall wheatgrass was increased with AMP at every level of sodium sulfate. Further, at the 0.068 and 0.102 M sodium chloride concentrations, early germination was enhanced with AMP.

The early germination of Russian wildrye was enhanced with the AMP treatment in sodium chloride at 0.034 M and 0.102 M. A similar trend was noted with sodium chloride at 0.068 M; however, the difference was not significant. Additionally, AMP enhanced the early germination of Russian wildrye in 0.102 M sodium sulfate. The final germination at 15 days of Russian wildrye was significantly increased from 38 to 63% in the 0.102 M sodium chloride.

As with crested wheatgrass, the early germination of alfalfa was increased by AMP at every concentration of sodium chloride. Also the final germination at 14 days was significantly increased by AMP in sodium chloride at 0.068 and 0.102 M salt concentration and in the sodium sulfate at 0.034 M.

As lack of significant salt type by treatment interaction (Table 1) indicates, treatment with AMP caused similar germination responses in both sodium chloride and sodium sulfate. The AMP seed treatment at no time had any effect on the seeds of switchgrass and western wheatgrass. The lack of response could be due to the lots of seed chosen for the study. As noted in Table 2, both species had low germinations in distilled water. Another possibility is that a metabolic difference may exist between these 2 species from the other species tested. Monocotyledons tend to have less salt tolerance than dicotyledons. However, differences occur in much more closely related species due to differences in membrane permeability, enzyme activity, or ability to synchronize compartmentalization (Greenway and Munns 1980). Both species showed a rapid decline in germination with increasing salinity even at the lowest levels in the experiment. As noted previously, few of the species responded when the salt concentration had greatly reduced germination.

Possibly, responses to AMP may be observed only at salt levels that moderately reduce overall germination.

Literature Cited

Anderson, J.D. 1977a. Adenylate metabolism of embryronic axes from deteriorated soybean seeds. Plant Phys. 101. 59:610-614.

Anderson, J.D. 1977b. Responses of adenine nucleotides in germinating soybean embryonic axes to exogenously applied adenine and adenosine. Plant Phys. 101. 60:689-692.

Berstein, L., and H.W. Wayward. 1958. Physiology of salt tolerance. Annu. Rev. Plant Phys. 101. 19:25-46.

Chaudhuri, I.I. and H.H. Wiebe. 1968. Influence of calcium pretreatment on wheat germination on saline medium. Plant and Soil 28:208-216.

Greenway, W., and R. Munns. 1980. Mechanisms of salt tolerance in nonhalophytes. Annu. Rev. Plant Phys. 101. 31:149-190.

Holm, R.E., and M.R. Miller. 1972. Hormonal sequence of weed seed germination. Weed Sci. 20:209-212.

Maas, E.V., and G.J. Hoffman. 1977. Crop salt tolerance-current assessment. ASCE J. Irrig. Drain. Div. 103:115-134.

McDaniel, R.G., and B.B. Taylor. 1976. AMP treatments improve emergence under cold stress. p. 9-10. Univ. Ariz. Agr. Exp. Sta. Series P-37.

Shannon, M.C., and L.E. Francois. 1977. Influence of seed pretreatments on salt tolerance of cotton during germination. Agron. J. 69:619-622.