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The Effects of Pregermination and Ageing on Germinability under Different Salt Concentrations of Soybean (*Glycine max* (L.) Merrill) Seeds

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ABSTRACT

Seeds of 2 soybean varieties namely SJ5 and CM60 were pregerminated by allowing the seeds to slowly absrop moisture vapour at high relative humidity and low temperature (3°C-100%RH and 25°C-100%RH, 24 hours) and were artificially aged at 42°C-100%RH for 2 and 3 days before being subjected to germination tests under different salt concentrations. The results revealed that pregerminated seeds by both temperatures showed a higher germination percentage under salt stress than untreated seeds when tested in the between paper method but in the sand emergence test this effect was not found. In the latter test, the improper moisture status affected by the low relative himidity (50-55%) of the surrounding atmosphere may result in a loss of effectiveness of pregermination. Aged seeds at 3 days were found to be low in germination even though in non-saline media but 2-day aged seeds were not significantly different from unaged seeds. Increasing NaCl concentrations reduced seed germinability and between the two varieties studied, CM60 tended to be more susceptible to salinity than SJ5. It can be concluded that pregermination improved the ability of soybean seeds to germinate under salinity stress when the moisture status of the media during seed gemination was suitable.

Key words : soybean, salinity stress, germination, pre-sowing seed treatment

INTRODUCTION

For many crop species, salt stress is more inhibitory during seed germination than at any other stage of growth (Bewley and Black, 1982). Salinity may affect the germination of seeds in two ways: (a) osmotically, by decreasing the ease with which seeds may take up water; and (b) ionically, by facilitating the uptake of ions in sufficient amounts to be toxic (Ayers, 1952). Germination can also be influenced by the type of salts involved (Ryan *et al.*, 1975). Mohamed and Hamada (1988) found that Na_2SO_4 had a greater inhibitory effect on germinating wheat seeds than NaCl. Although, there hvae been many reports suggesting that there is no relationship between tolerance to salinity at the germination stage and tolerance to salinity at later growth stages (Miyamoto *et al.*, 1982; Pearce-Pinto *et al.*, 1990), the plant of a seed that possesses an ability to germinate under salt stress may have survived if management to reduce salt stress is done in time after seedling stage. Therefore, techniques for improving salt tolerance of seed, at least at the early stage may facilitate crop management under saline soil conditions.

Recent research interest in pre-sowing treatments for improving field emergence under stress has shown considerable benefits (Heydecker and Coolbear, 1977; Bradford, 1986). Several seed treatments such as osmotic conditioning or priming, hardening (imbibing and drying) and pregermination have been developed and used to enhance germination under unfavourable conditions. Generally, treated seeds germinate more quickly and more uniformly. In this study, pregermination was defined as a technique whereby seeds are placed in a high relative humidity condition at low temperature for a period of time. The effect of raising the initial seed moisture before planting was reported to increase germination percentage, germination rate and plant stand and prevented imbibitional injury in many crop species (e.g. Pollock and Toole, 1966; Obendorf and Hobbs, 1970; Cal and Obendorf, 1972). The main objective of this study was to determine the effect of pregermination on tolerance to salinity during seed germination. Artificial ageing technique was also used to study the susceptibility of low vigour seeds to salinity stress.

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MATERIALS AND METHODS

Seeds of 2 soybean varieties namely SJ5 and CM60, grown and harvested at Kasetsart University, Kamphaeng Saen campus, in dry season 1990 were used in this study. Before transfering to germination tests under salt stress, the seeds were pretreated in 2 ways : (a) pregermination; placing the seeds under 3°C-100%RH and 25°C-100%RH for 24 hours; and (b) accelerated ageing; placing the seeds at 42°C-100%RH for 2 and 3 days. Both pretreat methods were carried out by putting 200 seeds in a tripod wire basket 7.5 cm in diameter fitting in a hermetic jar 11 cm in height and 10 cm in diameter which containing 100 ml of water. For pregermination study, after incubating the seeds for 24 hours at 3°C, seed moisture content of SJ5 and CM60 was increased from 9.3 and 10.4% to 11.7 and 12.4%, while at 25°C seed moisture content became 12.5 and 13.2%, respectively. After subjecting the seeds to both pretreat conditions, the seeds were then taken to evaluate their performance under different concentrations of NaCl using the between paper germination test and the sand emergence test. The sand used was carefully washed by using 1 N HCl and was put in the 18.4x27x10 cm^3 plastic boxes with the sand height of 3.8 cm. Fifty seeds (per box) were sown at about 1 cm deep. Modified Hoagland's solutions (Asher, 1975) added with 0, 2, 4 and 8 gm NaCl per litre (0, 0.2, 0.4 and 0.8% NaCl, weight by volume; respectively) were used as moistening agent. The electrical conductivity of the Hoagland's solutions measured at 25°C were 1.99, 5.52, 8.70 and 14.00 mmho/cm, respectively. Two hundred millilitre of modified Hoagland's solution was applied to the sand in each box (10% v/v of sand) at sowing and 75 ml was daily added thereafter. The plastic boxes were left uncovered during the testing period. For the between paper test, 50 seeds were sown in a 3-ply rool of towel paper. Moistening agent (Hoagland's solution) was applied to the paper only once at sowing time. The rolls of paper were then placed in a germinator controlling temperature at 25°C and relative humidity at 100%. Seedlings were evaluated following the rules of ISTA (1985) at 5 and 8 days after sowing. Data were presented as percentages of the normal seedlings. Factorial in RCB with 4 replicates was employed in this study.

RESULTS AND DISCUSSION

Results from the between paper germination test (Table 1) indicated that seeds of both pregermination temperatures were more significantly tolerant to salt stress than the untreated seeds. There were no significant differences between the low (3°C) and the high (25°C) temperature used in the pregermination treatments of both varieties. By contrast, this response of germinating seeds tested under sand emergence condition was quite different from that of the paper germination test (Table 2). In this case, pregerminated seeds at 25°C-100%RH performed poorer than pregenminated seeds at 3°C-100%RH and untreated seeds. The failure of pre-sowing treatment to enhance salt tolerance in the sand emergence test may be due to the improper sand moisture status during germination. Seeds which were sown about 1-cm deep seemed to dry out before the time of watering each day because of the low relative humidity of the surrounding atmosphere (50-55%RH). This caused the pregerminated seeds to lost their ability to germinate and

Table 1 Effects of pregermination on percentages of the between paper germination under different NaCl concentrations of SJ5 and CM60 soybean seeds

Soybean variety	NaCl (% w/v)	Untreated seed	Pregerminated seed at 3°C-100%RH	Pregerminated seed at 25°C-100%RH	Average
SJ5	0.0	B* 83 a**	A 88 a	A 94 a	88 a
	0.2	B 79 a	A 91 a	A 91 a	87 a
	0.4	B 68 b	A 86 a	A 82 b	79 b
	0.8	B 12 c	A 47 b	A 47 c	35 c
	Average	B 61	A 78	A 78	
	CV(%)				6.3
CM60	0.0	A 83 a	A 80 a	A 85 a	83 a
	0.2	B 57 b	A 76 a	A 74 a	69 b
	0.4	A 56 b	B 44 b	A 55 c	52 c
	0.8	B 10 c	A 25 c	A 27 d	21 d
	Average	B 51	A 56	A 60	
	CV(%)				10.7

* Mean values preceded by the same capital letters in each row are not significantly different at probability level .05

** Mean values of each variety followed by the same lower cases in each column are not significantly different at probability level .05

performed a poorer germinability comparing to untreated seeds. Pregerminated seeds of pepper were also reported to be particularly sensitive to drought conditions (Irwin and Price, 1981). Therefore, although the effect of pregermination was encouraging, this limitation may affect practical application under field conditions.

Increasing salt concentrations resulted in reduction in germination percentages in both varieties and in both tests, the between paper germination (Table 1) and the sand emregence (Table 2). Srinives *et al.* (1989) also reported with 50 soybean lines that salt stress suppressed laboratory seed germination and soybean seeds were unable to germinate when the NaCl concentration exceeded 1%. They also found that there were different responses in terms of salt tolerance between the soybean lines studied. In the present study, seeds of CM60 tended to be more sensitive to salinity than SJ5. Under sand emergence test, the effect of salt stress was more severe comparing to the between paper test (compare Table 1 vs Table 2 and Table 3 vs Table 4). This may be explained that there was an accumulation of salt on the sand surface because the moistening agent (with salt added) was daily applied to the germinating seeds in the containers.

For artificial aged seeds, it was found that aged seeds at 42°C-100%RH for 3 days were seriously low in germinability, especially seeds of CM60 soybean were more susceptible to ageing than SJ5 (Table 3 and 4). It should be noted that aged seeds accelerated by artificial ageing at 42°C-100%RH for 2 days which has been reported to be comparable with natural ageing for 3 months (Chanprasert, 1990) showed the same performance under salt stress as unaged seeds. In this study, it was intended to use accelerated ageing technique to test the idea that low vigour seeds perform poorer than high vigour seeds under stressful conditions. In general, low vigour seeds are more sensitive to environmental stresses than high vigour seeds. However, the results from this study showed that the 3-day aged seeds (the lowest vigour seeds) were initially low in germination percentage and salt stress caused reduced germination in the same manner as unaged seeds and 2-day aged seeds. Therefore, artificial ageing may not be a proper treatment to diverge seed vigour to meet the prupose of this study.

CONCLUSION

It is evident that pregermination improved salt tolerance of germinating soybean seeds when sown under favourable moisture supply in the between paper test. But under the sand emergence test, the pregerminated seeds performed in a different way due to the unfavourable moisture status and probably salt accumulation on the sand surface during germination period. Germination percentage decreased as salt concentration increased and CM60 soybean variety seemed to be more susceptible to salt stress than SJ5. From the results of this study, it is possible for scientists to develop further a technique in pre-sowing seed treatment to enhance salt tolerance during seed germination.

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Table 2 Effects of pregermination on percentages of the sand emergence under different NaCl concentrations of SJ5 and CM60 soybean seeds

Soybean variety	NaCl (% w/v)	Untreated seed	Pregerminated seed at 3°C-100%RH	Pregerminated seed at 25°C-100%RH	Average
SJ5	0.0	A* 87 a**	A 85 a	B 61 a	78 a
	0.2	A 60 b	A 64 b	B 50 b	58 b
	0.4	A 3 c	A 1 c	A 0 c	1 c
	0.8	A 0 c	A 0 c	A 0 c	0 c
	Average	A 37	A 37	B 28	
	CV(%)				9.0
CM60	0.0	A 79 a	B 70 a	C 53 a	68 a
	0.2	A 39 b	A 38 b	B 22 B	33 b
	0.4	A 9 c	B 2 c	B 0 c	3 c
	0.8	A 0 d	A 0 c	A 0 c	0 c
	Average	A 32	B 27	C 19	
	CV(%)				14.7

* Mean values preceded by the same capital letters in each row are not significantly different at probability level .05

** Mean values of each variety followed by the same lower cases in each column are not significantly different at probability level .05

Table 3 Effects of artificial ageing on percentages of the between paper germination under different NaCl concentrations of SJ5 and CM60 soybean seeds

Soybean variety	NaCl (% w/v)	Unaged seed	Aged seed (2 days)	Aged seed (3 days)	Average
SJ5	0.0	A* 83 a**	A 90 a	B 66 a	80 a
	0.2	A 65 b	A 81 a	B 56 a	67 b
	0.4	A 68 b	A 57 b	B 28 b	51 c
	0.8	AB 12 c	A 21 b	B 0 c	11 d
	Average	A 57	A 62	B 37	
	CV(%)				18.3
CM60	0.0	A 83 a	A 81 a	B 19 a	61 a
	0.2	A 48 b	A 55 b	B 21 a	41 b
	0.4	A 60 b	A 46 b	B 4 b	37 b
	0.8	A 10 c	A 11 c	A 0 b	7 c
	Average	A 50	A 48	B 11	
	CV(%)				27.8

* Mean values preceded by the same capital letters in each row are not significantly different at probability level .05

** Mean values of each variety followed by the same lower cases in each column are not significantly different at probability level .05

Table 4 Effects of artificial ageing on percentages of the sand emergence under different NaCl concentrations of SJ5 and CM60 soybean seeds

Soybean variety	NaCl (% w/v)	Unaged seed	Aged seed (2 days)	Aged seed (3 days)	Average
SJ5	0.0	A* 85 a**	A 88 a	B 23 a	65 a
	0.2	A 64 b	A 64 b	B 14 b	47 b
	0.4	B 1 c	A 11 c	B 0 c	4 c
	0.8	A 0 c	A 0 b	A 0 c	0 d
	Average	B 37	A 41	C 9	
	CV(%)				12.8
CM60	0.0	A 79 a	A 78 a	B 9 a	56 a
	0.2	A 39 b	A 41 b	B 5 ab	28 b
	0.4	A 9 c	AB 5 c	B 0 b	5 c
	0.8	A 0 d	A 0 c	A 0 b	0 d
	Average	A 32	A 31	B 4	
	CV(%)				16.8

* Mean values preceded by the same capital letters in each row are not significantly different at probability level .05

** Mean values of each variety followed by the same lower cases in each column are not significantly different at probability level .05

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