AJAB

Asian J Agric & Biol. xxxx(x).
DOI: 10.35495/ajab.2022.076

Original Article

Seed treatment with 24-epibrassinolide improves wheat germination under salinity stress

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Received:
April 29, 2022
Accepted:
January 28, 2023
Published Online:
April 02, 2023

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Abstract

Salt stress is a key ecological challenge to wheat establishment at the early stage of germination, especially in drylands. A germination experiment was conducted to determine whether an exogenous seed treatment with 24-epibrassinolide could mitigate salinity stress effects on wheat germination. Seeds of the Sudanese wheat cv. *Imam* were treated with 24-epibrassinolide (BR_1) or without (BR_0) at eleven concentrations of sodium chloride (NaCl) (0.00, 1.56, 3.13, 4.69, 6.25, 7.81, 9.38, 10.94, 12.50, 14.06 and 15.63 dSm^{-1}), in a 2 x 11 factorial experiment arranged into a completely randomized design. Seed germination was progressively delayed with increasing salinity and the daily germination was reduced significantly. The germination average time and relative injury rate increased considerably ($p \leq 0.05$) at salt levels of 7.81 dSm^{-1} or more. The inhibitory effects of salinity on germination were significantly ($p \leq 0.05$) reversed by seed treatment with BR_1. Wheat cv. *Imam* tolerated salt stress up to 6.25 dSm^{-1} at BR_0 with respect to velocity of germination, germination rate, final germination rate, germination percentage and germination index, relative to no salt (0.00 dSm^{-1}). An early uniform establishment of wheat in saline media could be enhanced by seed treatment with BR.

Keywords: Arid region, Plant growth hormone, *Triticum aestivum* (L), Abiotic stress

How to cite this:

Victoria O, Idorenyin U, Asana M, Jia L, Shuoshuo L, Yang S, Okoi IM, Ping A and Egrinya EA. Seed treatment with 24-epibrassinolide improves wheat germination under salinity stress. Asian J. Agric. Biol. xxxx(x): 2022076 DOI: https://doi.org/10.35495/ajab.2022.076

Introduction

Salinity stress is one of the most critical environmental hazards that limits crop production. Its adverse effect reflects on poor seed germination, reduced plant vigour and yield as a result of sodium and chloride ions that contribute to increased salt level in soil solution (Auge et al., 2018). Crop yield begins to decline when electrical conductivity (EC) of the saturated soil extract exceeds 4 dSm^{-1} within the plant's rhizophere (Jamil et al., 2011). Globally, arable lands of over 45 million hectares have been damaged by salt, causing about 1.5 million hectares to be out of production yearly (Munns and Tester, 2008). Salinity delays the onset of germination by creating high osmotic potential through a reduction in water uptake and ion toxicity on the germinating seeds (Tian et al., 2015), thereby reducing growth and grain yield (Ashraf and Harris, 2004). Salinity stress inhibits wheat growth from seed germination to grain filling stage (Otu et al., 2018). The mitigating role of some plant growth regulators, including 24-epibrassinolide by inducing tolerance in plants under environmental stresses have been documented fairly recently (Vardhini et al., 2012; Otie, et al., 2021; 2022).

Brassinosteroids, 24-epibrassinolides (BR) are plant growth hormones that regulate many physiological processes in plants, including seed germination, growth and responses to different biotic and abiotic stresses (Vardhini and Anjum, 2015; Otie et al., 2019b, c). They play vital roles in enhancing salinity tolerance of plants under saline stress (Ozedemir et al., 2004; Otie et al., 2021).

Wheat (*Triticum aestivum* L.) is an important cereal crop that is widely grown for food. Its wider adaptability tendency, higher nutritive value, and significant roles on national economy across the globe make it outstanding, compared with other cereals (Yildirim et al., 2018; Noor et al., 2023). Although, its demand increases on daily basis, its production is short changed by many abiotic factors, salinity being the most limiting, especially in arid and semi-arid regions (Otu et al., 2018; Jahan et al., 2019). For the purpose of this research, a popular Sudanese wheat cultivar (*cv*.) known as *Imam* was used. It is salt sensitive wheat mostly grown in arid and semi-arid regions. Although some studies demonstrated that BR could mitigate salinity stress on wheat (Dong et al., 2017), the effects of its exogenous application on seed germination under salinity stress is little studied. The experiment examined the effects of BR on various germination parameters of wheat cv. *Imam* across a range of simulated salinity concentrations.

Material and Methods

The experiment was conducted in a growth chamber of the Arid Land Research Center (ALRC), Tottori University, Tottori, Japan. It involved a germination test of wheat seeds (*Triticum aestivum* L. cv. *Imam*) treated with or without 24-epibrassinolide (BR) at various concentrations of saline water. The wheat *cv.* was originally produced by the International Maize Improvement Center (CIMMYT), Mexico City. The seeds were sterilized with 5 % sodium hypochlorite solution by soaking for 5 minutes and thoroughly rinsing with distilled water to remove traces of chlorine. One litre (1 L) of deionized water (0.00 dSm^{-1}) was used to dissolve 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 g of salt (NaCl) to achieve salinity rates of 1.56, 3.13, 4.69, 6.25, 7.81, 9.38, 10.94, 12.50, 14.06 and 15.63 dSm^{-1}, respectively. Deionized water with no salt served as the control. Three layers of Whatman No. 1 filter paper were placed on 10 cm diameter Petri dishes and moistened with 10 mL of the respective saline water. The sterilized seeds were then placed in lots of 10 on the soaked filter papers in each Petri dish. The BR was procured from Zhejiang Laiyi Biology Technique Company Ltd, Shengzhou, China. Following the manufacturer's recommended rate of 1 mL to 1L deionized water, 5 mL of BR solution was applied to the seeds. Seeds that received no BR (5.00 mL deionized water) served as the control. Treatments were arranged into a 2 x 11 factorial combination, using a completely randomized design (CRD) and replicated three times to give 22 treatment combinations and 66 experimental units. The Petri dishes were placed in the growth chamber-2 (SANYO, MLT-350HT, Japan) at a relative humidity of 80 %, in the dark at 30°C. The germinants were counted daily throughout the germination test for 10 days with the first count of germinated seeds occurring on the second day of the experiment. The investigation was performed in two experiments by similar procedures, to ascertain its validity and reliability of resulting data.

Germination variables

Counting of seed sprouts began from the second day after sowing until the final count (tenth day).

Dormancy phase (DP- days)

These were the number of days before the emergence

of sprouts. They were observed according to treatment combinations.

Daily germination (DG-%):
The DG was recorded daily based on the number of newly sprouted seeds at each observation from day of germination per total number of seeds sown:

DG = Daily germinated seeds/Total number of seeds sown x 100

Germination average time (GAT- days)
The germination average time was calculated according to Scott et al. (1984):

$$GAT = \frac{\Sigma T1N1}{S}$$

Where:
T1 = Number of days from the beginning of the experiment
N1 = Number of seeds germinated per day
S = Total number of seeds germinated

Velocity of germination (VG)
The VG was estimated according to Hartmann et al. (1997) thus:

$$VG = \frac{A_1T_1 + A_2T_2 + \text{-----}A_xT_x}{A_1 + A_2 + \text{-----}A_x}$$

Germination rate (GR- %)
The germination rate was calculated as the sum of the values obtained when dividing the percentage of partial germination in each count by the elapsed time from the beginning of the test (Maguire, 1962):

$$GR = \frac{\text{Number of normal seedlings}}{\text{Days to final count}} + \ldots\ldots\ldots + \frac{\text{Number of normal seedlings}}{\text{Days to final count}}$$

Final germination rate (FGR- %)
The final germination rate was determined by adding the daily rates of germinated seeds from beginning to the end of germination, based on the treatment combinations.

Germination percentage (GP- %)
Germination percentage was estimated using the formula:
GP (%) = Total number of seeds germinated/Total number of seeds x 100

Germination index (GI- %)
The germination index was determined according to Kader and Jutzi (2004) as:
GI = ΣTiNi/Ti
Where, Ti is the number of days after sowing and Ni is number of germinated seeds in the observation days.

Relative injury rate (RIR- %)
The RIR of NaCl was calculated as the difference between germination percentage in control (with and without BR) and germination percentage in salt treated seeds, divided by the germination percentage in the untreated seeds:

$$RIR = \frac{GP\ \%\ (control) - GP\ \%\ (salt\text{-}treated\ seed) \times 100}{GP\ \%\ (control)}$$

Statistical analysis
The data were subjected to analysis of variance using GenStat software 15.1 Edition to partition the effects of the two factors and their interactions. Data on FGR and GP were transformed using the arc sine ($Sin^{-1}\sqrt{(x + 0.5)}$) method as their ranges were more than 40 % and could have violated one of the assumptions of analysis of variance. Treatment means were compared using Duncan's New Multiple Range Test at the 5 % level of probability. The data from the two experimental repeats were pooled for analysis since their statistical comparison showed no significant difference.

Results

The analysis of variance (ANOVA) showing sources of variation, degrees of freedom, mean squares, variance ratio and their respective significance level for all the variables measured are presented in Tables 1-3. Table 1 shows ANOVA of daily germination (DG) from day (D) 2 to 10. For D2, salinity rates (SR), 24-epibrassinolide (BR) and SR x BR (interaction) significantly ($p \leq 0.001$) affected DG. For D3, only SR and BR had significant ($p \leq 0.001$) effect on DG. For days 4 and 5, SR, BR and their interactions had no significant ($p > 0.05$) effect on DG. Meanwhile, for days 6 to 10, only BR significantly ($p \leq 0.001$) affected DG. The ANOVA for germination percentage (GP) are presented in Table 2. There was a significant ($p \leq 0.001$) effect of SR, BR and (SR x BR) on GP in

all the days except D3, D6 and D7 in which SR x BR was not significant ($p>0.05$). Table 3 shows ANOVA of dormancy phase (DP), germination average time (GAT), velocity of germination (VG), germination rate (GR), and final germination rate (FGR). Salinity rates (SR), BR and SR x BR all had significant ($p\leq0.001$) effect on all the germination variables listed above except DP.

Dormancy phase (DP)

The BR had no significant effect on the DP of the seeds across salinity concentrations (Figure 1). However, at salt concentrations of 7.81 to 15.63 dSm^{-1}, it reduced the number of days to first sprout.

Table-1: Analysis of variance (mean squares and significant level) of daily germination (DG) of wheat seeds from day (D) 2 to 10

Sources of variance	d.f.	D2		D3		D4		D5		D6		D7		D8		D9		D10	
		m.s.	v.r.	m.s.	v.r.	m.s.	v.r.	m.s.	v.r.	m.s.	v.r.	m.s.	v.r.	m.s.	v.r.	m.s.	v.r.	m.s.	v.r.
Salinity rate (SR)	10	314.04	11.20 ***	79.39	3.08 ***	31.52	0.83 ns	30.00	0.76 ns	37.58	1.38 ns	40.30	1.40 ns	90.91	1.54 ns	43.64	1.69 ns	14.24	1.18 ns
24-epirassinolide (BR)	1	14892.03	531.11 ***	183.33	7.12 ***	54.55	1.44 ns	37.88	0.96 ns	256.06	9.39 ***	340.91	11.84 ***	801.52	13.56 ***	183.33	7.12 ***	340.19	28.13 ***
SR x BR	10	67.74	2.42 ***	33.33	1.29 ns	41.21	1.09 ns	37.88	0.96 ns	19.39	0.71 ns	54.24	1.88 ns	44.85	0.76 ns	40.00	1.55 ns	14.24	1.18 ns
Experimental error	44	28.04		25.76		37.88		39.39		27.27		28.79		59.09		25.76		12.12	
Total	65																		

*** = $p<0.001$, ns = not significant; m.s. = mean square; v.r. = variance ratio

Table-2: Analysis of variance (mean squares and significant level) of germination percentage (GP) of wheat seeds from day (D) 2 to 10

Sources of variance	d.f.	D2		D3		D4		D5		D6		D7		D8		D9		D10	
		m.s.	v.r.	m.s.	v.r.	m.s.	v.r.	m.s.	v.r.	m.s.	v.r.	m.s.	v.r.	m.s.	v.r.	m.s.	v.r.	m.s.	v.r.
Salinity rate (SR)	10	299.30	12.10 ***	474.91	33.85 ***	464.52	13.17 ***	431.04	12.37 ***	473.54	16.31 ***	725.66	12.37 ***	1378.14	57.96 ***	1297.86	75.98 ***	1409.91	85.60 ***
24-epibrassinolide (BR)	1	14964.22	604.99 ***	15965.19	1137.90 ***	11754.68	333.20 ***	10655.30	305.67 ***	12212.80	420.61 ***	16679.10	284.29 ***	30289.59	1273.78 ***	26862.00	1572.67 ***	23323.28	1416.04 ***
SR x BR	10	67.36	2.72 ***	78.31	5.58 ***	60.66	1.72 ns	67.78	1.94 ***	43.56	1.50 ns	61.63	1.05 ns	220.02	9.25 ***	167.54	9.81 ***	152.21	9.24 ***
Experimental error	44	24.73		14.03		35.28		34.86		29.04		58.67		23.78		17.08		16.47	
Total	65																		

*** = $p<0.001$, ns = not significant; m.s. = mean square; v.r. = variance ratio

Table-3: Analysis of variance (mean squares and significant level) of dormancy phase (DP), germination average time (GAT), velocity of germination (VG), germination rate (GR) and final germination rate (FGR) of wheat seeds.

Sources of variance	d.f.	DP		GAT		VG		GR		FGR	
		m.s.	v.r.	m.s.	v.r.	m.s.	v.r.	m.s.	v.r.	m.s.	v.r.
Salinity rate (SR)	10	1.64	ns	0.747	962.29 ***	1.152	1005.99 ***	171.247	29.520 ***	1385.08	25.47 ***
24-epibrassinolide (BR)	1	19.64	ns	7.049	9087.21 ***	6.299	5499.37 ***	2004.318	345.510 ***	18782.72	345.34 ***
SR x BR	10	1.64	ns	0.682	878.86 ***	0.256	223.37 ***	16.358	2.820 **	119.64	2.20 ***
Experimental error	44	0.00		0.001		0.001		5.801		54.39	
Total	65										

*** = $p<0.001$, ** = $p<0.01$, ns = not significant; m.s. = mean square; v.r. = variance ratio

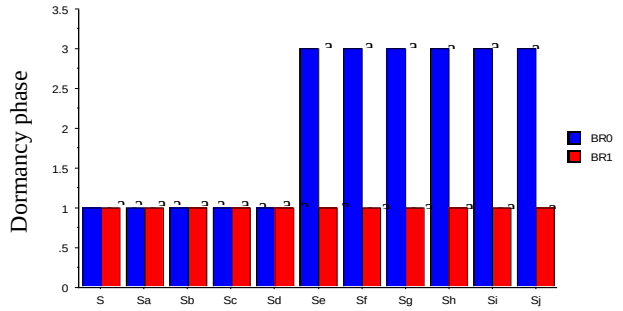

Figure-1: Salinity rates and BR interactions on the dormancy phase (DP) of wheat seeds.
BR= 24-epibrassinolide; BR_0= no BR application (0 mL); BR_1= 5 mL; S= 0.00 dSm^{-1}, (control); Sa= 1.56 dSm^{-1}; Sb= 3.13 dSm^{-1}; Sc= 4.69 dSm^{-1}; Sd= 6.25 dSm^{-1}; Se= 7.81 dSm^{-1}; Sf= 9.38 dSm^{-1}; Sg= 10.94 dSm^{-1}; Sh= 12.50 dSm^{-1}; Si= 14.06 dSm^{-1}; Sj= 15.63 dSm^{-1}. Mean pairs with different letters are significantly different at the 5 % probability level according to Duncan's New Multiple Range Test.

Daily germination (DG)

On day 2, higher salt concentrations (7.81 - 15.63 dSm^{-1}) significantly reduced the DG (Figure 2b), but the effects of salinity were not consistent on day 3 (Figure 2b) as no seed germinated from day 2 to 10 under 7.81 dSm^{-1}. However, treatment with BR significantly ($p \leq 0.05$) increased the DG (Figure 2c); on day 2, it significantly ($p \leq 0.05$) improved the DG across salinity levels (Figure 3). Without BR, no seed germinated at salt concentrations ≥ 7.81 dSm^{-1}.

Germination average time (GAT), velocity of germination (VG), germination rate (GR) and final germination rate (FGR)

There was no consistent trend in the GAT across salinity levels (Table 4). However, the lowest GAT was observed under Sc = 4.69 dSm^{-1}, while higher salt concentrations (12.50 to 15.63 dSm^{-1}) significantly reduced the GAT relative to the control (S= 0.00 dSm^{-1}). At all salinity levels, BR application significantly reduced the GAT and BR-treated seeds under 14.06 or 15.63 dSm^{-1} salinity had the lowest GAT (Table 4).

The velocity of germination (VG) also showed no consistent trend across salinity levels (Table 4). However, it improved significantly at 7.81 to 15.63 dSm^{-1} with a peak at 7.81 dSm^{-1}. 24-epibrassinolide application significantly increased the VG across salinity levels (except at 4.69 dSm^{-1}) (Table 4).
The germination rate decreased progressively ($p \leq 0.05$) with increasing salinity (Table 4) and the decrease was significant beyond 6.25 dSm^{-1}, especially at 10.94 - 15.63 dSm^{-1}. Application of BR significantly ($p \leq 0.05$) increased the GR and the response to BR was similar at the salinity range of 1.56 to 6.25 dSm^{-1} (Table 4).
The final germination rate (FGR) decreased with increasing salt concentration (Table 4), especially at salinity exceeding 6.25 dSm^{-1}. The decrease was significantly reversed by BR application (Table 1) at ≤ 7.81 dSm^{-1} salinity.

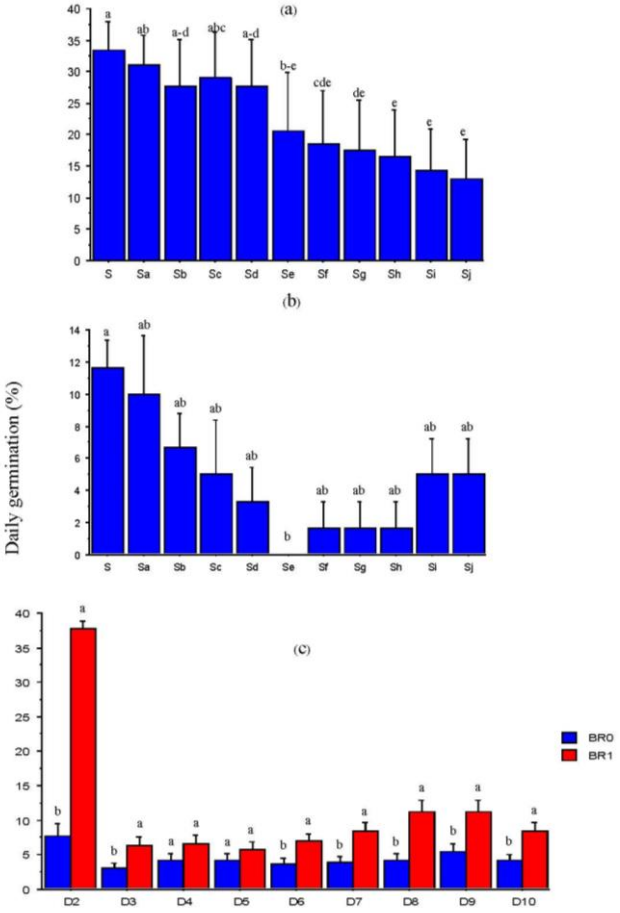

Figure-2: Salinity and BR effects on daily germination of wheat seeds on days 2 (a), 3 (b) and 2 to 10 (c).
SR= salinity rates; BR= 24-epibrassinolide; BR_0= no BR application (0 mL); BR_1= 5.00 mL; D2= day 2; D3= day 3; D4= day 4; D5= day 5; D6= day 6; D7= day 7; D8= day 8; D9= day 9; D10= day 10; S= 0.00 dSm^{-1}, (control); Sa= 1.56 dSm^{-1}; Sb= 3.13 dSm^{-1}; Sc= 4.69 dSm^{-1}; Sd= 6.25 dSm^{-1}; Se= 7.81 dSm^{-1}; Sf= 9.38 dSm^{-1}; Sg= 10.94 dSm^{-1}; Sh= 12.50 dSm^{-1}; Si= 14.06 dSm^{-1}; Sj= 15.63 dSm^{-1}. Mean pairs with different letters are significantly different at the 5 % probability level according to Duncan's New Multiple Range Test.

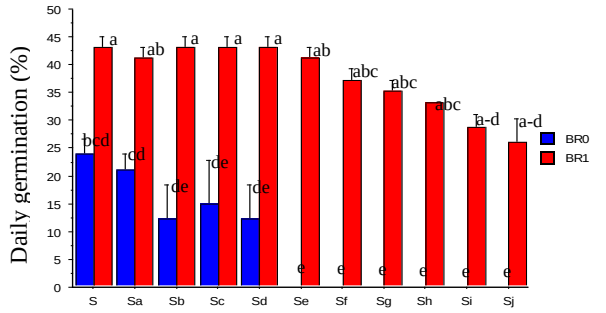

Figure-3: Interaction effects of SR x BR on daily germination of wheat on day 2

SR= salinity rates; BR= 24-epibrassinolide; BR_0= no BR application (0 mL); BR_1= 5.00 mL; ; D2= day 2; S= 0.00 dSm^{-1}, (control); Sa= 1.56 dSm^{-1}; Sb= 3.13 dSm^{-1}; Sc= 4.69 dSm^{-1}; Sd= 6.25 dSm^{-1}; Se= 7.81 dSm^{-1}; Sf= 9.38 dSm^{-1}; Sg= 10.94 dSm^{-1}; Sh= 12.50 dSm^{-1}; Si= 14.06 dSm^{-1}; Sj= 15.63 dSm^{-1}. Mean pairs with different letters are significantly different at the 5 % probability level according to Duncan's New Multiple

Table-4: Effects of 24-epibrassinolide (BR) and sodium chloride (NaCl) concentrations on germination average time (GAT), velocity of germination (VG), germination rate (GR) and final germination rate (FGR) of wheat seeds

Sources of variance	G. Ave. Time (days)	VG	GR (%)	FGR (%)
Salinity rate (SR)				
S (0.00 dSm^{-1})	4.56 ^{b}	6.81 ^{g}	20.33^{a}	(75.97^{a}) 88.34
S_a (1.56 dSm^{-1})	4.45 ^{c}	6.79 ^{g}	16.63 ^{b}	(71.53^{ab}) 85.00
S_b (3.13 dSm^{-1})	4.36 ^{d}	6.86 ^{f}	14.82^{bc}	(68.47^{ab}) 76.67
S_c (4.69 dSm^{-1})	3.91 ^{f}	6.69 ^{h}	14.07^{bc}	(64.47^{ab}) 73.34
S_d (6.25 dSm^{-1})	4.29 ^{d}	6.80 ^{g}	12.85 ^{cd}	(64.57^{ab}) 70.00
S_e (7.81 dSm^{-1})	4.60 ^{b}	9.29 ^{a}	10.96^{de}	(59.13^{bc}) 65.00
S_f (9.38 dSm^{-1})	4.27 ^{d}	6.96 ^{e}	8.34^{ef}	(47.10^{cd}) 53.34
S_g (10.94 dSm^{-1})	4.99 ^{a}	7.30 ^{c}	6.92^{fg}	(46.00^{cd}) 51.67
S_h (12.50 dSm^{-1})	4.01 ^{e}	7.35 ^{b}	6.69^{fg}	(43.90^{de}) 48.34
S_i (14.06 dSm^{-1})	4.07 ^{e}	7.02^{d}	4.88 ^{gh}	(36.97^{de})38.33
S_j (15.63 dSm^{-1})	4.09 ^{e}	7.01 ^{d}	3.28^{h}	(30.00^{e}) 30.00
BR rate				
BR_0 (0.00 mL)	5.03 ^{a}	6.62 ^{b}	5.38^{b}	(38.41^{b}) 40.00
BR_1 (5.00 mL)	3.63 ^{b}	7.72 ^{a}	16.40^{a}	(72.15^{a}) 83.64
SR x BR				
SBR_0	5.12^{de}	6.68^{i}	13.66 ^{de}	(61.93^{bcd}) 76.67
S_a BR_0	5.37^{b}	6.52^{kl}	10.93^{efg}	(59.20^{cde}) 73.33
S_b BR_0	5.12^{de}	6.56^{jk}	7.86^{ghij}	(46.93^{c-d}) 53.33
S_c BR_0	4.01^{h}	6.67^{i}	7.08^{ghijk}	(45.07^{d-h}) 50.00
S_d BR_0	5.28^{bc}	6.48^{l}	4.87^{ijkl}	(39.13^{d-i}) 40.00
S_e BR_0	5.20^{cd}	6.70^{i}	4.36^{jklm}	(37.13^{e-i}) 36.67
S_f BR_0	5.00^{e}	6.59^{j}	3.42^{jklm}	(33.00^{f-i}) 30.00
S_g BR_0	5.67^{a}	6.90^{h}	3.02^{klm}	(33.00 $^{f-i}$) 30.00
S_h BR_0	4.50^{f}	6.67^{i}	2.94^{klm}	(31.00 ^{ghj}) 26.67
S_i BR_0	5.01 ^{e}	6.51^{kl}	0.67^{lm}	(21.13 ^{hi}) 13.33
S_j BR_0	5.03 ^{e}	6.51^{kl}	0.33^{m}	(15.00 ^{i}) 10.00
SBR_1	4.01 ^{h}	6.94^{h}	27.00^{a}	(90.00 ^{a}) 100.00
S_a BR_1	3.53 ^{j}	7.06^{g}	22.33^{b}	(83.87 ^{ab}) 96.67
S_b BR_1	3.59 ^{j}	7.16^{f}	21.78^{bc}	(90.00 ^{a}) 100.00
S_c BR_1	3.80 ^{i}	6.71^{i}	21.05^{bc}	(83.87 ^{ab}) 96.67
S_d BR_1	3.29 ^{k}	7.13^{f}	20.83^{bc}	(90.00 ^{a})100.00
S_e BR_1	4.00 ^{h}	11.87 ^{a}	17.56^{cd}	(81.13 ^{abc}) 93.33
S_f BR_1	3.55 ^{j}	7.34^{e}	13.25^{ef}	(61.20 $^{b-e}$) 76.67
S_g BR_1	4.32 ^{g}	7.69^{c}	10.81^{efg}	(59.00 ^{cde}) 73.33
S_h BR_1	3.52 ^{j}	8.04^{b}	10.43^{efgh}	(56.80 ^{def}) 70.00
S_i BR_1	3.14 ^{l}	7.52^{d}	9.10^{fghi}	(52.80 $^{d-g}$) 63.33
S_j BR_1	3.15 ^{l}	7.50^{d}	6.22^{hijk}	(45.00^{d-h}) 50.00

Mean pairs within a column with different letters are significantly ($p \leq 0.05$) different at the 5 % probability level according to Duncan's New Multiple Range Test

Values in parenthesis are transformed data, using the arcsine $(\text{Sin}^{-1}\sqrt{(x + 0.5)}$, where x = the original data

Values outside parenthesis are original data (n = 3)

BR = 24-epibrassinolide; BR_1 = with 24-epibrassinolide; BR_0 = without 24-epibrassinolide; SR = Salt rate

Germination percentage (GP)

The GP decreased gradually from day 2 to day 10 after sowing with increase in salt concentration (Table 5). During the early days (2 and 3), the salinity concentration of 7.81 dSm^{-1} or more significantly ($p \leq 0.05$) reduced the GP. On days 5 to 8, the salt concentration of either 14.06 or 15.63 dSm^{-1} showed the lowest ($p \leq 0.05$) GP, while the same was obtained on days 9 to 10 at 15.63 dSm^{-1} salt concentrations. Across the germination days, BR application considerably ($p \leq 0.05$) enhanced the GP (Table 5). In the early days (2 and 3), no seed germinated at salt concentrations of 7.81 to 15.63 dSm^{-1} with no BR application (Table 5). On day 2, there was no significant change in GP for BR-treated seeds at 0 - 10.94 dSm^{-1}. On days 8 to 10, BR-treated seeds under 7.81 dSm^{-1} had the highest ($p \leq 0.05$) GP (100 %). Across the various days of germination, there was little difference between BR-treated seeds at 15.63 dSm^{-1} and untreated seeds at 0.00 dSm^{-1} (Table 5). Generally, there was an improved germination at all salt concentrations following BR application (Table 5).

Germination index (GI)

On day 1, GI was zero as no seed germinated, but from days 2 to 10, there was a gradual decrease in GI with increasing salt concentration without BR application (Table 6). On days 2 and 3, seeds not treated with BR had zero GI at salt concentrations from 7.81 to 15.63 dSm^{-1}. In contrast, on the early day of germination (day 2), BR-treated seeds had higher GI at 0 - 7.81 dSm^{-1}, but with a gradual decrease as the salinity increased up to 15.63 dSm^{-1}. A similar trend was observed for other germination days (3 to 9). On day 10, seeds treated with BR, irrespective of salinity level had GI of zero (Table 6).

Relative injury rate (RIR)

The data for RIR from day 1 to day 10 after sowing are summarized in Table 7. On days 2 and 3, with no BR application (BR_0), salt concentrations $\leq$ 7.81 dSm^{-1} caused a 100 % RIR. However, on days 4, 5, 6 and 8,

salinity at 4.69 dSm^{-1} enhanced germination relative to control (0.00 dSm^{-1}) as indicated by negative values of RIR (Table 7). From day 6 to 10, an increase in salinity from 7.81 to 15.63 dSm^{-1} led to a gradual increase in RIR. On days 2, 4, 5 and 6, the germination improved up to the salinity of 9.38 dSm^{-1} following BR application. On days 7 to 10, there was no injury up to 9.38 dSm^{-1} following BR application. However, at 10.94 - 15.63 dSm^{-1} salinity level, the RIR was 50 % on days 2 and 3 while there was a gradual increase on days 7 through 10 (Table 7).

Table-5: Main and interaction effects of 24-epibrassinolide (BR) and sodium chloride (NaCl) concentrations on germination percentage (GP) of wheat seeds at 2 to 10 days after sowing

Sources of variance	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10
Salinity rates (SR)									
S(0.00 dSm^{-1})	(33.47^{a}) 31.67	(38.43^{a}) 40.00	(42.90^{a}) 46.67	(47.10^{a}) 53.33	(48.20^{a}) 57.67	(55.00^{a}) 65.00	(66.57^{a}) 73.34	(69.43^{a}) 78.34	(71.47^{a}) 81.65
S_a (1.56 dSm^{-1})	(31.13^{a}) 28.33	(37.73^{a}) 38.34	(39.43^{ab}) 41.67	(45.10^{ab}) 50.00	(49.57^{a}) 53.33	(51.67^{a}) 60.00	(63.63^{a}) 68.34	(66.57^{abc}) 73.34	(69.48^{ab}) 78.34
S_b (3.13 dSm^{-1})	(27.67^{ab}) 26.67	(34.03^{a}) 33.33	(38.83^{ab}) 40.00	(40.93^{abc}) 43.34	(47.10^{a}) 56.67	(54.73^{a}) 61.67	(66.53^{a}) 73.34	(67.50^{ab}) 75.00	(68.50^{ab}) 76.67
S_c (4.69 dSm^{-1})	(26.70^{abc}) 25.00	(31.70^{a}) 30.00	(35.53^{ab}) 35.00	(38.83^{bcd}) 40.00	(43,90^{ab}) 50.00	(55.10^{a}) 61.67	(63.63^{a}) 68.34	(65.52^{abc}) 71.67	(67.57^{ab}) 75.00
S_d (6.25 dSm^{-1})	(27.67^{ab}) 26.67	(32.10^{a}) 30.00	(34.43^{bc}) 33.34	(38.47^{b-e}) 40.00	(42.90^{abc}) 46.67	(50.53^{a}) 55.00	(61.50^{a}) 65.00	(63.60^{bc}) 68.34	(64.57^{bc}) 70.00
S_e (7.81 dSm^{-1})	(20.57^{bcd}) 21.67	(20.57^{b}) 21.67	(33.07^{bc}) 28.34	(34.43^{c-f}) 33.34	(38.47^{bcd}) 45.00	(45.27^{ab}) 50.00	(60.50^{a}) 63.34	(61.57^{c}) 65.00	(63.63^{c}) 68.34
S_f (9.38 dSm^{-1})	(18.60^{bcd}) 18.34	(20.53^{b}) 21.67	(26.63^{cd}) 21.67	(30.00^{f}) 30.00	(36.37^{cd}) 38.34	(40.43^{bc}) 43.34	(43.62^{b}) 48.34	(46.77^{d}) 53.34	(48.17^{d}) 55.00
S_g(10.94 dSm^{-1})	(17.60^{bcd}) 16.67	(18.60^{b}) 18.34	(22.63^{de}) 20.00	(32.07^{def}) 30.00	(34.43^{d}) 36.67	(37.73^{bc}) 38.34	(42.80^{b}) 46.67	(43.90^{d}) 48.32	(45.98^{d}) 51.67
S_h(12.50 dSm^{-1})	(16,60^{cd}) 15.00	(17.60^{b}) 16.67	(23.73^{de}) 20.00	(31.13^{ef}) 28.33	(32.10^{d}) 30.00	(34.43^{dc}) 33.34	(36.37^{c}) 36.67	(42.73^{d}) 46.67	(43.85^{d}) 48.34
S_i (14.06 dSm^{-1})	(14.40^{d}) 11.67	(17.62^{b}) 16.67	(18.60^{e}) 18.34	(22.67^{g}) 21.67	(23.63^{e}) 23.33	(27.67^{d}) 26.67	(29.60^{d}) 30.00	(34.50^{e}) 35.00	(34.53^{e}) 35.00
S_j (15.63 dSm^{-1})	(13.03^{d}) 10.00	(15.50^{b}) 13.34	(17.60^{e}) 16.67	(20.57^{g}) 21.67	(25.57^{e}) 26.67	(25.57^{d}) 26.67	(28.63^{d}) 18.89	(28.63^{f}) 28.34	(28.70^{f}) 28.34
BR rates									
BR_0 (0.00 mL)	(7.44^{b}) 4.55	(10.30^{b}) 6.97	(17.05^{b}) 12.73	(21.96^{b}) 17.27	(24.78^{b}) 20.61	(27.57^{b}) 23.94	(29.79^{b}) 26.97	(33.54^{b}) 32.42	(36.33^{b}) 36.97
BR_1 (5.00 mL)	(37.55^{b}) 37.58	(41.41^{a}) 43.97	(43.75^{a}) 47.88	(47.37^{a}) 53.94	(51.99^{a}) 63.64	(59.36^{a}) 70.91	(72.64^{a}) 82.42	(73.88^{a}) 84.55	(73.93^{a}) 84.55
SR x BR									
SBR_0	(23.87^{e})16.67	(26.07^{gh}) 20.00	(31.00^{a}) 26.67	(35.20^{d-h}) 33.33	(35.20^{a}) 36.67	(41.13^{a}) 43.33	(43.07^{de}) 46.67	(48.87^{de}) 56.67	(52.80^{cd}) 63.33
S_a BR_0	(21.13^{ef}) 13.33	(26.60^{gh}) 20.00	(26.07^{a}) 20.00	(33.20^{e-i}) 30.00	(35.20^{a}) 33.33	(37.20^{a}) 36.67	(37.13^{ef}) 36.67	(43.07^{efg}) 46.67	(48.87^{de}) 56.67
S_b BR_0	(12.27^{f}) 6.67	(21.13^{hi}) 13.33	(28.80^{a}) 23.33	(31.00^{f-j}) 26.67	(35.20^{a}) 33.33	(37.20^{a}) 36.67	(43.07^{de}) 46.67	(45.00^{def}) 50.00	(46.93^{de}) 53.33
S_c BR_0	(12.27^{f}) 6.67	(18.40^{i}) 10.00	(26.07^{a}) 20.00	(28.80^{g-j}) 23.33	(31.00^{a}) 26.67	(35.20^{a}) 33.33	(37.20^{ef}) 36.67	(41.13^{fg}) 43.33	(45.00^{ef}) 50.00
S_d BR_0	(12.27^{f}) 6.67	(21.13^{hi}) 13.33	(23.87^{a}) 16.67	(26.07^{hij}) 20.00	(28.80^{a}) 23.33	(31.00^{a}) 26.67	(33.00^{fg}) 30.00	(37.20^{gh}) 36.67	(39.13^{fg}) 40.00
S_e BR_0	(0.00^{g}) 0.00	(0.00^{j}) 0.00	(21.13^{a}) 13.33	(23.87^{ijk}) 16.67	(26.07^{a}) 20.00	(28.80^{a})23.33	(31.00^{fgh}) 26.63	(33.00^{hi}) 30.00	(37.13^{gh}) 36.67
S_f BR_0	(0.00^{g}) 0.00	(0.00^{j}) 0.00	(12.27^{a}) 10.00	(15.00^{kl}) 10.00	(23.87^{a}) 16.67	(23.87^{a}) 16.67	(26.07^{gh}) 20.00	(30.27^{hi}) 26.67	(33.00^{gh}) 30.00
S_g BR_0	(0.00^{g}) 0.00	(0.00^{j}) 0.00	(6.13^{a}) 3.33	(21.13^{jk}) 13.33	(23.87^{a}) 16.67	(26.60^{a}) 20.00	(28.80^{fgh}) 23.33	(31.00^{hi}) 26.63	(35.20^{gh}) 33.33
S_h BR_0	(0.00^{g}) 0.00	(0.00^{j}) 0.00	(12.27^{a}) 6.67	(21.13^{jk}) 13.33	(21.13^{a}) 13.33	(23.87^{a}) 16.67	(23.87^{h}) 16.67	(28.80^{i}) 23.63	(31.00^{h}) 26.67
S_i BR_0	(0.00^{g}) 0.00	(0.00^{j}) 0.00	(0.00^{a}) 0.00	(6.13^{lm})3.33	(6.13^{a})3.33	(12.27^{a}) 6.67	(12.27^{i}) 6.67	(18.30^{j}) 10.00	(18.33^{i}) 10.00
S_j BR_0	(0.00^{g}) 0.00	(0.00^{j}) 0.00	(0.00^{a}) 0.00	(0.00^{m}) 0.00	(6.13^{a}) 3.33	(6.13^{a}) 3.33	(12.27^{i}) 6.67	(12.27^{j}) 6.67	(12.27^{i}) 6.67
SBR_1	(43.07^{a}) 46.67	(50,80^{a}) 60.00	(54.80^{a}) 66.67	(59.00^{a}) 73.33	(61.20^{a}) 76.67	(68.87^{a}) 86.67	(90.07^{a}) 100.00	(90.00^{a}) 100.00	(90.13^{a}) 100.00

S_a BR_1	(41.13^{ab}) 43.33	(48.87^{ab}) 56.67	(52.80^{a}) 63.33	(57.00^{a}) 70.00	(63.93^{a}) 73.33	(66.13^{a}) 83.33	(90.13^{a}) 100.00	(90.07^{a}) 100.00	(90.10^{a}) 100.00
S_b BR_1	(43.07^{a}) 46.67	(46.93^{abc}) 53.33	(48.87^{a}) 56.67	(50.87^{ab}) 60.00	(59.00^{a}) 80.00	(72.27^{a})86.67	(90.00^{a}) 100.00	(90.00^{a}) 100.00	(90.07^{a}) 100.00
S_c BR_1	(41.13^{ab})43.33	(45.00^{abc}) 50.00	(45.00^{a}) 50.00	(48.87^{abc}) 56.67	(56.80^{a}) 73.33	(75.00^{a}) 90.00	(90.07^{a}) 100.00	(90.00^{a}) 100.00	(90.13^{a}) 100.00
S_d BR_1	(43.07^{a}) 46.67	(43.07^{bcd}) 46.67	(45.00^{a}) 50.00	(50.87^{ab}) 60.00	(57.00^{a}) 70.00	(70.07^{a})83.33	(90.00^{a}) 100.00	(90.00^{a}) 100.00	(90.00^{a}) 100.00
S_e BR_1	(41.13^{ab}) 43.33	(41.13^{cde}) 43.33	(45.00^{a}) 50.00	(45.00^{bcd}) 50.00	(50.87^{a}) 70.00	(61.73^{a}) 76.67	(90.00^{a}) 100.00	(90.13^{a}) 100.00	(90.13^{a}) 100.00
S_f BR_1	(37.20^{abc}) 36.67	(41.07^{cde}) 43.33	(43.00^{a}) 46.67	(45.00^{bcd}) 50.00	(48.87^{a}) 60.00	(57.00^{a}) 70.00	(61.17^{b}) 76.67	(63.27^{b}) 80.00	(66.33^{b}) 80.00
S_g BR_1	(35.20^{abc}) 33.33	(37.20^{def}) 36.67	(39.13^{a}) 40.00	(43.00^{b-e}) 46.67	(45.00^{a}) 56.67	(48.87^{a}) 56.67	(56.80^{bc}) 70.00	(56.80^{bc}) 70.00	(56.77^{bc}) 70.00
S_h BR_1	(33.20^{bcd}) 30.00	(35.20^{ef}) 33.33	(35.20^{a}) 33.33	(41.13^{b-f}) 43.33	(43.07^{a}) 46.67	(45.00^{a}) 50.00	(48.87^{cd}) 56.67	(56.67^{bc}) 70.00	(56.70^{bc}) 70.00
S_i BR_1	(28.80^{cde}) 23.33	(35.23^{ef}) 33.33	(37.20^{a}) 36.67	(39.20^{c-g}) 40.00	(41.13^{a}) 43.33	(43.07^{a}) 46.67	(46.93^{d})53.33	(50.70^{cd}) 60.00	(50.73^{cde}) 60.00
S_j BR_1	(26.07^{de}) 20.00	(31.00^{fg}) 26.67	(35.20^{a}) 33.33	(41.13^{b-f}) 43.33	(45.00^{a}) 50.00	(45.00^{a}) 50.00	(45.00^{de}) 50.00	(45.10^{def}) 50.00	(45.13^{ef})50.00

Mean pairs within a column in parenthesis with different letters are significantly different at the 5 % probability level according to Duncan's New Multiple Range Test

Values in parenthesis are transformed data, using the arc sine ($Sin^{-1}\sqrt{(x + 0.5)}$, where x = the original data

Values outside parenthesis are original data (n = 3); BR = 24-epibrassinolide; BR_1 = with 24-epibrassinolide; BR_0 = without 24-epibrassinolide; SR = Salt rate

Table-6: Effects of salinity and 24-epibrassinolide on the germination index (GI) of wheat seeds from 1 to 10 days after sowing

Treatment combinations	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10
SBR_0	0.00	5.00	300	2.00	2.00	1.00	2.00	3.00	3.00	2.00
S_aBR_0	0.00	4.00	2.00	2.00	3.00	1.00	1.00	2.00	3.00	3.00
S_bBR_0	0.00	2.00	2.00	3.00	1.00	2.00	1.00	3.00	1.00	1.00
S_cBR_0	0.00	3.00	1.00	3.00	0.00	1.00	2.00	1.00	2.00	2.00
S_dBR_0	0.00	2.00	2.00	1.00	1.00	1.00	1.00	1.00	2.00	1.00
S_eBR_0	0.00	0.00	0.00	4.00	1.00	1.00	1.00	1.00	1.00	2.00
S_fBR_0	0.00	0.00	0.00	2.00	1.00	2.00	0.00	1.00	2.00	1.00
S_gBR_0	0.00	0.00	0.00	1.00	2.00	1.00	1.00	1.00	1.00	2.00
S_hBR_0	0.00	0.00	0.00	2.00	2.00	0.00	1.00	0.00	2.00	1.00
S_iBR_0	0.00	0.00	0.00	0.00	1.00	1.00	1.00	0.00	1.00	0.00
S_jBR_0	0.00	0.00	0.00	0.00	0.00	1.00	1.00	1.00	0.00	0.00
SBR_1	0.00	14.00	4.00	2.00	2.00	1.00	3.00	4.00	0.00	0.00
S_aBR_1	0.00	13.00	4.00	2.00	1.00	3.00	1.00	5.00	0.00	0.00
S_bBR_1	0.00	14.00	2.00	1.00	1.00	4.00	4.00	4.00	0.00	0.00
S_cBR_1	0.00	14.00	2.00	1.00	2.00	4.00	4.00	3.00	0.00	0.00
S_dBR_1	0.00	14.00	0.00	1.00	3.00	3.00	4.00	5.00	0.00	0.00
S_eBR_1	0.00	13.00	0.00	2.00	0.00	3.00	4.00	6.00	0.00	0.00
S_fBR_1	0.00	11.00	1.00	1.00	1.00	3.00	4.00	2.00	1.00	0.00
S_gBR_1	0.00	10.00	1.00	1.00	2.00	1.00	2.00	4.00	0.00	0.00
S_hBR_1	0.00	9.00	1.00	0.00	3.00	1.00	1.00	2.00	4.00	0.00
S_iBR_1	0.00	7.00	3.00	1.00	2.00	1.00	1.00	2.00	2.00	0.00
S_jBR_1	0.00	6.00	2.00	2.00	3.00	2.00	0.00	0.00	0.00	0.00

BR_0= without 24-epibrassinolide; BR_1= with 24-epibrassinolide; D = day; S= 0.00 dSm^{-1} (control); Sa= 1.56 dSm^{-1}; Sb= 3.13 dSm^{-1}; Sc= 4.69 dSm^{-1}; Sd= 6.25 dSm^{-1}; Se= 7.81 dSm^{-1}; Sf= 9.38 dSm^{-1}; Sg= 10.94 dSm^{-1}; Sh= 12.50 dSm^{-1}; Si= 14.06 dSm^{-1}; Sj= 15.63 dSm^{-1}.

Table-7: Effects of salinity and 24-epibrassinolide on the relative injury rate (RIR) of wheat seeds from 1 to 10 days after sowing

Treatment combinations	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10
S_aBR_0	0.00	0.00	33.33	0.00	20.00	0.00	16.67	-16.67	0.00	0.00
S_bBR_0	0.00	50.00	33.33	0.00	20.00	-20.00	0.00	-33.33	0.00	0.00
S_cBR_0	0.00	0.00	0.00	-25.00	-20.00	-20.00	0.00	-16.67	12.50	12.50
S_dBR_0	0.00	50.00	33.33	25.00	20.00	0.00	16.67	0.00	12.50	12.50
S_eBR_0	0.00	100.00	100.00	50.00	40.00	20.00	16.67	16.67	37.50	37.50
S_fBR_0	0.00	100.00	100.00	25.00	20.00	20.00	33.33	16.67	37.50	37.50
S_gBR_0	0.00	100.00	100.00	75.00	80.00	60.00	50.00	50.00	62.50	62.50
S_hBR_0	0.00	100.00	100.00	75.00	60.00	60.00	66.67	66.67	75.00	75.00
S_iBR_0	0.00	100.00	100.00	100.00	80.00	80.00	83.33	83.33	87.50	87.50
S_jBR_0	0.00	100.00	100.00	100.00	80.00	80.00	83.33	83.33	87.50	87.50
S_aBR_1	0.00	-25.00	0.00	-16.67	-33.33	0.00	0.00	0.00	0.00	0.00
S_bBR_1	0.00	-25.00	0.00	-16.67	-16.67	-12.50	0.00	0.00	0.00	0.00
S_cBR_1	0.00	0.00	16.67	0.00	-33.33	-12.50	0.00	0.00	0.00	0.00
S_dBR_1	0.00	-25.00	0.00	-33.33	-50.00	-12.50	0.00	0.00	0.00	0.00
S_eBR_1	0.00	0.00	16.67	0.00	16.67	0.00	0.00	0.00	0.00	0.00
S_fBR_1	0.00	0.00	0.00	-16.67	-33.33	-12.50	0.00	0.00	0.00	0.00
S_gBR_1	0.00	50.00	50.00	50.00	33.33	37.50	30.00	30.00	30.00	30.00
S_hBR_1	0.00	50.00	50.00	33.33	16.67	25.00	40.00	40.00	40.00	40.00
S_iBR_1	0.00	50.00	50.00	33.33	16.67	37.50	50.00	50.00	50.00	50.00
S_jBR_1	0.00	50.00	50.00	33.33	16.67	37.50	50.00	50.00	50.00	50.00

BR_0= without 24-epibrassinolide; BR_1= with 24-epibrassinolide; D = day; S= 0.00 dSm^{-1} (control); Sa= 1.56 dSm^{-1}; Sb= 3.13 dSm^{-1};Sc= 4.69 dSm^{-1};Sd= 6.25 dSm^{-1}; Se= 7.81 dSm^{-1};Sf= 9.38 dSm^{-1}; Sg= 10.94 dSm^{-1}; Sh= 12.50 dS/m; Si= 14.06 dSm^{-1}; Sj= 15.63 dSm^{-1}.

Discussion

Seed germination is a complex physiological and biochemical process where various crops exhibit their specific germination patterns, even under saline condition (Panuccio et al., 2014). The significant aspect of salinity tolerance is the ability of seeds to germinate in saline media, and the possibility to continue development. Germination and emergence of seedlings are the most informative stages of a plant's life cycle in evaluating salinity effect as the duration of this phase determines seedling establishment and future plant's growth (Ali et al., 2020). The germination of wheat (*Triticum aestivum* L.), like most other crops, is adversely affected by salinity stress. High salinity often results to osmotic stress that causes some specific ion toxicity which may retard seed germination (Luan et al., 2014). The pleiotropic role of BR against salt stress on seed germination and seedling growth have been widely documented (Zhen et al., 2010; Shu et al., 2016; Otie et al., 2021; 2022).

Seeds establish the next generation of a crop's life cycle and are influenced by the three critical phases of dormancy, development and germination. Dormancy of seeds may inhibit germination under optimal environmental conditions (Benech-Arnold et al., 2013). In the present study, increase in salinity concentration inhibited wheat seed germination, possibly due to loss of viability as a result of suppressed physiological changes in the seeds during germination processes (Hasan et al., 2018). Although, there was little effect of BR on the dormancy phase, the number of days to first sprout was reduced which could be attributed to its protective and increased tolerance effects on germinating seeds in response to saline stress (Zhou et al., 2013).

Seed germination ensures the continuity of plant's survival for successful crop production, and therefore, acts as the intermediate for genetic transfer between plant generations (He and Yang, 2013). Salinity, especially at 7.81 to 15.63 dSm^{-1} significantly reduced daily germination (DG) of seeds probably through the adverse effect of osmotic inhibition and ionic toxicity (Munns et al., 2006). This oxidative stress may have caused membrane peroxidation, ion leakage, and consequent damage to nucleic acids, cell membranes and cellular structures culminating to reduced seed quality (Plazek et al., 2013). Exogenous application of

BR may have activated the genes responsible for developmental processes to modulate the physiological and molecular mechanisms for enhanced DG of the seeds across the salinity levels (Bear et al., 2006).

Germination average time (GAT) is not a real indicator of salt tolerance because it is more or less dependent on the genetically inherited germination ability of a crop cultivar, as was the case for cv. *Imam*. Higher salinity (10.94 - 15.63 dSm^{-1}) significantly reduced the GAT relative to the control (S= 0.00 dSm^{-1}) (Table 4). Salinity may affect the germination time of wheat seeds through the toxic effects of excessive NaCl ions on the embryo viability (Daszkowska-Golec, 2011) that could severely damage cell organelles and seed's plasma membrane (Panda and Khan, 2009). High accumulation of Na^+ ions in a medium could delay water absorption and thus, reduce germination duration. Procházka et al. (2014) suggested that BR could counteract the effect of salinity-induced inhibition of seed germination. This study validates the claim of these authors. The result obtained showed that exogenous application of BR improved the GAT under high salt stress as recently reported (Otie et al., 2022).

Seed vitality is an important factor in stress conditions, which could also be attributed to seed tolerance to unfavourable conditions during germination and emergence. In this study, the highest velocity of germination (VG) was obtained at moderate salt concentration of 7.81 dSm^{-1}. This could be indicative that increasing osmotic potential could reduce water uptake and consequently, slow down germination speed (Hasan et al., 2018). For each salinity level, the use of BR increased the VG substantially (Table 4). Several experiments have shown salt inhibitory capacity of BR on seed germination and vigour (Procházka et al., 2014, 2016).

Increase in salt concentration adversely affected radicle development and coleoptile length due to osmotic imbalance and other physiological activities (Hasan et al., 2018). This may have impeded the germination rate (GR) of wheat seeds since higher salinity impairs water absorption, and consequently prevents nutrient assimilation in the saline media. There was a remarkable improvement in GR when seeds were treated with BR at all salt concentrations. 24-epibrassinolide facilitates seed embryonic development up to adult homeostasis (Procházka et al., 2016). This finding validates the claim that BR induces tolerance to crops under various kinds of stress, and modulates different physiological and molecular mechanisms to enhance seed and seedling growth (Anwar et al., 2018b). The role of BR in the regulation of a seed's developmental pathways to overcome abiotic stress, including salt-mediated inhibition of germination has been well established (Wang et al., 2016; Anwar et al., 2018a; Otie et al., 2022).

Increasing salinity concentration significantly decreased the final germination rate (FGR) of wheat seeds. Chauhan et al. (2018) attributed the reduction in FGR to alterations in metabolic processes of germination as a result of increased osmotic pressure from accumulated Na^+ and Cl^- in the seed's embryo, thereby causing toxic effects of ions on the embryo viability. Under salt stress, elongation rate of coleoptiles can be decreased by low soil water potential, and seedlings may not be adequately established due to weak coleoptile and root growth. Reduced germination and seedling growth has also been reported by Dehnavi et al. (2020) on sorghum, and Wu et al. (2022) on barnyard millet under salt stress conditions. However, BR have been reported to improve seed defence system to tolerate various stresses through its mitigating role of regulating final germination metabolism and preventing oxidative damage in the seed tissues (Anwar et al., 2018a).

The seed germination stage is more sensitive to salinity than later stages of plant growth. Thus, successful seed germination over a range of salinity is important for the establishment of plant population in diverse regions (Farooq et al., 2015). A significant reduction in germination percentage (GP) was generally observed at higher salt concentrations (7.81 to 15.63 dSm^{-1}), especially with no BR application (Table 5). The excessive salts or ions may be toxic to the embryo, and could possibly reduce the osmotic potential to an extent of preventing uptake of water necessary for mobilization of nutrients required for excellent germination (Mbinda and Kimtai, 2019). Seed tolerance to abiotic stresses during germination could be increased by different hydration methods. Interestingly, this research involved complete soaking of wheat seeds in solution of salt at different concentrations, deionized water without salt as control and treatment with, or without 24-epibrassinolide. It is possible that the BR could have boosted seeds' metabolic processes and activity of enzymes to support membrane stability, osmo-regulation and the foetal development, as confirmed in earlier reports (Procházka et al., 2014, 2016; Ali, 2017).

Germination index (GI) is an important tool for evaluating plant's salt tolerance during seed germination (Li et al., 2020). Data obtained indicated that high salinity levels negatively affected the GI (Table 6). Excessive salt concentration decreased GI when compared with the control. This could have hindered the metabolic process that oxidizes lipids and carbohydrates within seeds to break down stored proteins for energy and amino acids required for seedling growth. Additionally, salinity increases phenolic compounds which could reduce germination (Tsegay and Gebreslassie, 2014; Mbinda and Kimtai, 2019). Application of BR could have alleviated the suppression of seed germination caused by salinity by reducing the risk of poor stand establishment and improved seedling vigour for better performance (Zhang et al., 2007).

Relative injury rate (RIR) determines the degree of injury imposed on seeds by salinity during germination. In this study, the RIR increased across salt levels, especially on the early days of germination where higher salt concentrations caused a 100 % injury (Table 7). These results are consistent with those of Hadush and Gebreegziabher (2012) and Luan et al. (2014), who reported that a direct relationship could be established between salinity concentrations and injury rates, as seed injury increases with increasing concentrations of salinity during germination relative to other growth and developmental phases. Exogenous use of BR could counteract the effect of salinity-induced inhibition of seed germination. Results of this work showed that seed treatment with BR reduced the injury rate under high salt stress by probably regulating the seed developmental pathways (Jiang and Lin, 2013) for stress responses.

Conclusions

Exposure of wheat seeds cv. *Imam* to higher concentration of NaCl (7.81 to 15.63 dSm^{-1}) significantly inhibited their germination potential. The seeds were however tolerant of NaCl up to 6.25 dSm^{-1} with no BR application. Higher salt concentrations inhibited rapid and quality germination as depicted by the impairment in daily germination, velocity of germination, germination rate, final germination rate, germination percentage and germination index, but conversely increased the germination average time and relative injury rate. The positive effects of 24-epibrassinolide on the above germination indices at all salt levels further emphasized its potency for ensuring speedy and uniform wheat seedling establishment in agro-ecologies where salinity poses a major threat to wheat production.

Acknowledgement

The authors acknowledge the management of the Joint Research Associate Program of the Arid Land Research Center (ALRC), Tottori University, Tottori-Japan, for partly supporting this research.

Disclaimer: None
Conflict of Interest: None
Source of Funding: The Research was partly funded by the Arid Land Research Center (ALRC), Tottori University, Tottori, Japan.

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