

RESEARCH PAPER

Evaluation of exogenous application of 24-epibrassinolide and silicon on morphological characters of salt stressed wheat varieties

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ABSTRACT

Wheat is a major cereal crop in many parts of the world and it is commonly known as king of cereals. Brassinosteroids (BRs) are growth-promoting natural products found at low levels in pollen, seeds, and young vegetative tissues throughout the plant kingdom. An experiment was conducted to evaluate the effect of different combinations of Silicon (Si) and 24-epibrassinolide (EBL) on wheat varieties grown under 100 mM salt stress. The result showed that shoot length of plant increased with the application of EBL and Silicon and it was increased the maximum in salt tolerant varieties in comparison to non-tolerant. Whilst, reduction was observed in root length along with the increasing concentration of EBL. The effect of different combinations of silicon and 24-epibrassinolide also increased the germination percentage (%) in tolerant and non-tolerant wheat varieties.

Key Words : 24-epibrassinolide, Salt stress, Silicon, Seed germination, Shoot-root length

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Abiotic stress may either reduce the rate or delay seed germination and (Patade *et al.*, 2011; Ansari and Sharif-Zadeh, 2012). More than 45 million hectares (M ha) of irrigated land, account to 20 per cent of total land have been damaged by salt worldwide and 1.5 M ha are taken out of production each year due to high salinity levels in the soil (Pitman and Läuchli, 2002). Plants have been classified as glycophytes or halophytes as per their capacity to grow on different salt concentrations. Most plants are glycophytes and can't tolerate salt-stress. High salt concentrations decrease the osmotic potential of soil solution creating a water stress in plants. Secondly, they cause severe ion toxicity, since Na⁺ is not readily sequestered into

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vacuoles as in halophytes. Finally, the interactions of salts with mineral nutrition may result in nutrient imbalances and deficiencies. The consequence of all these can ultimately lead to plant death as a result of growth arrest and molecular damage (McCue and Hanson, 1990).

Brassinosteroids (BRs) are a group of plant steroidal hormones that regulate various aspects of plant growth and development, including cell elongation, photomorphogenesis, xylem differentiation, and seed germination (Sasse, 2003), as well as adaptation to abiotic and biotic environmental stresses (Krishna, 2003), (Divi and Krishna, 2009). Brassinosteroids were isolated from extract *Brassicanapus* pollen (Grove *et al.*, 1979) because of their growth promoting properties and their potential use for enhancing crop production. Recently (Bajguz, 2007) brassinosteroids represent a class of plant hormones, more than 70 compounds have been isolated from plants. The role of BRs in plant stress responses has been confirmed in several studies (Dhaubhadel *et al.*, 1999 and Koh *et al.*, 2007). Exogenous BR application can greatly stimulate cell elongation. For example, in dicots, the growth of an epicotyl – the shoot part above the cotyledons of a germinating seedling, or a hypocotyl – the shoot below the cotyledons, is stimulated by BRs; whereas in monocots, the elongation of a germinating seedling shoot part called coleoptile is also promoted by the application of exogenous BRs (Clouse and Sasse, 1998).

The use of Silicon in agriculture is cost effective and environmental friendly tool against salt stress. Silicon is well-known to enhance growth of plants subjected to saline stress, hence, is advantageous to ameliorate the salinity stress (Liang *et al.*, 1996). Although Si has not been considered as an essential element for higher plants, it has been proved to be beneficial for the healthy growth and development of many plant species particularly graminaceous plants such as rice and sugarcane and some cyperaceous plants (Epstein, 1994 and 1999; Liang, 1999; Ma *et al.*, 2001 and Liang *et al.*, 2005).

RESEARCH METHODOLOGY

The seeds of 8 wheat (*Triticum aestivum* L.) varieties including 4 salt tolerant (Kharchia, KRL119, KRL99, KRL19) and 4 non-tolerant (AAI W6, AAI W7, AAI W8, AAIW9) varieties were obtained from Directorate of Research, SHIATS. The experiment was conducted in the Department of Biological Sciences,

SHIATS. All the seed of different wheat varieties were surface sterilized with 0.01mM CoCl₂ and then sown in Petriplate with cotton beds wet by Hogland media. The seeds of all the eight wheat varieties were allowed to grow in different levels of NaCl, silicon and 24-epibrassinolide. Treatments were as follows: T₀- Control; T₁- 100 mM NaCl alone; T₂- 100 mM NaCl + 50 ppm Silicon; T₃-100 mM NaCl +100ppm Silicon; T₄-100 mM NaCl+0.1 μM EBL; T₅-100 mM NaCl+1.0μM EBL; T₆- 100 mM NaCl+50 ppm Silicon + 0.1μM EBL; T₇-100 mM NaCl+50 ppm Silicon+1.0μM EBL; T₈-100 mM NaCl+ 100ppm Silicon+0.1μM EBL; T₉-100 mM NaCl+ 100ppm Silicon+1.0μM EBL. These treatments given to plants at 3rd and 4th leaf stages and at the same time plants transfer to pots from Petriplates.

The different growth parameters were studied at the tillering stage (48 days of showing) and flowering stages (75 days after sowing). Morphological data *i.e.*, Root and Shoot length measured after 48 days of treatment. Germination percentage was estimated according to the formula outlined by (Krishnasamy and Seshu, 1990) :

$$\text{Germination (\%)} = \frac{\text{Number of normal seedlings}}{\text{Numbers of tested seed}} \times 100$$

RESULTS AND REMONSTRATION

The effect of Silicon alone and in combination with 24-EBL was studied on seed germination, shoot length and root length of different wheat varieties grown under salt stress (100 mM NaCl) condition. The result revealed that there was a non significant difference in seed germination (%) under controlled condition. Whereas, all the treatments (*viz.*, T₁, T₂, T₃, T₄, T₅, T₆, T₇, T₈, T₉) showed a significant variations in seed germination(%) (Table 1). Result showed that there was a reduction in seed germination due to NaCl treatment in tolerant as well as non tolerant varieties. Among the non tolerant varieties the decrease was more severe. Among salt tolerant varieties the maximum seed germination (98.3%) was recorded in tolerant variety Kharchia in treatment T₉. Whereas the minimum seed germination (66.6%) was recorded in non-tolerant variety AAI W9 in treatment T₂ in comparison to their control. Result showed that application of 24-EBL and silicon was able to overcome adverse effects as imposed by NaCl in tolerant and non-tolerant varieties.

Results further showed significant difference in

Table 1 : Effect of foliar application of 24-epibrassinolid (0.1µM, 1.0µM) on seed germination (%) of wheat (*Triticum aestivum* L.) varieties grown under salt stress (100mM) condition accompanied with two concentration of Si (50ppm, 100ppm)

Varieties	Seed germination(%)									
	T ₀	T ₁	T ₂	T ₃	T ₄	T ₅	T ₆	T ₇	T ₈	T ₉
KRL-19	99.7	78.1	79.2	80.5	81.8	83.5	85.6	86.5	87.7	88.0
KRL-99	100.0	82.1	83.4	85.5	86.2	87.5	88.9	91.3	92.1	93.2
KRL-119	99.7	85.7	86.1	86.8	88.2	89.6	90.2	91.7	93.1	93.9
Kharchia-65	100.0	91.4	92.0	93.9	94.6	95.1	96.7	97.1	97.8	98.3
AAIW6	99.3	72.3	73.1	74.3	75.5	76.4	78.1	79.4	80.2	81.1
AAIW7	99.7	74.1	75.1	76.2	78.2	80.3	81.2	82.3	82.9	83.4
AAIW8	99.7	69.5	67.6	68.6	69.9	70.7	72.6	73.7	75.7	76.9
AAIW9	99.3	75.5	66.6	67.9	69.6	70.7	71.6	72.7	73.8	74.1
C.D. (P=0.05)	NS	0.2	0.3	0.2	0.1	0.1	0.1	0.1	0.1	0.1
CV	0.4	0.1	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.1
F-test	4.6	6.0	1.8	1.1	3.0	0.7	3.0	0.6	0.5	0.5

T₀- Control; T₁- 100 mM NaCl alone; T₂- 100 mM NaCl + 50 ppm Silicon; T₃-100 mM NaCl +100ppm Silicon; T₄-100 mM NaCl+0.1µM EBL; T₅-100 mM NaCl+ 1.0µM EBL; T₆-100 mM NaCl+50 ppm Silicon+0.1µM EBL; T₇-100 mM NaCl+50 ppm Silicon +1.0µM EBL; T₈-100 mM NaCl+ 100ppm Silicon+ 0.1µM EBL; T₉-100 mM NaCl+ 100ppm Silicon+1.0µM EBL

Table 2 : Effect of foliar application of 24-epibrassinolid (0.1µM, 1.0µM) on shoot length of wheat (*Triticum aestivum* L.) varieties grown under salt stress (100mM) condition accompanied concentration of Si (50ppm, 100ppm)

Varieties	Shoot length (cm)									
	T ₀	T ₁	T ₂	T ₃	T ₄	T ₅	T ₆	T ₇	T ₈	T ₉
KRL-19	74.8	55.3	57.5	58.7	59.5	60.8	62.5	65.5	68.4	70.5
KRL-99	76.6	57.1	59.2	60.7	61.3	62.8	64.5	67.1	69.7	71.5
KRL-119	73.6	59.2	61.2	62.5	63.3	65.7	67.5	69.8	71.7	72.2
Kharchia-65	78.5	62.7	64.3	65.1	66.9	67.8	69.0	70.4	72.3	75.7
AAIW6	73.7	51.5	52.6	53.7	55.6	56.4	57.2	58.9	59.9	61.0
AAIW7	64.4	43.1	44.2	45.5	46.3	48.3	50.5	53.5	56.7	59.4
AAIW8	68.6	50.3	52.3	53.5	54.4	56.5	57.4	58.6	59.5	62.7
AAIW9	62.6	40.2	42.2	44.7	45.2	46.4	48.3	50.5	52.4	56.6
C.D. (P=0.05)	0.2	0.2	0.1	0.4	0.1	0.1	0.1	0.2	0.1	0.4
CV	0.2	0.1	0.1	0.3	0.1	0.1	0.1	0.1	0.1	0.3
F-test	52.1	1.8	4.4	0.7	0.5	1.1	0.9	1.1	1.4	1.6

T₀- Control; T₁- 100 mM NaCl alone; T₂- 100 mM NaCl + 50 ppm Silicon; T₃-100 mM NaCl +100ppm Silicon; T₄-100 mM NaCl+0.1µM EBL; T₅-100 mM NaCl+1.0µM EBL; T₆-100 mM NaCl+50 ppm Silicon+0.1µM EBL; T₇-100 mM NaCl+50 ppm Silicon +1.0µM EBL; T₈-100 mM NaCl+ 100ppm Silicon+ 0.1µM EBL; T₉-100 mM NaCl+ 100ppm Silicon+1.0µM EBL

Table 3 : Effect of foliar application of 24-epibrassinolid (0.1µM, 1.0µM) on root length of wheat (*Triticum aestivum* L.) varieties grown under salt stress (100mM) condition accompanied with two concentrations of Si (50ppm, 100ppm)

Varieties	Root length (cm)									
	T ₀	T ₁	T ₂	T ₃	T ₄	T ₅	T ₆	T ₇	T ₈	T ₉
KRL-19	10.0	6.5	7.6	8.7	4.4	4.1	5.1	4.5	5.8	5.1
KRL-99	11.1	7.1	8.3	10.3	5.7	4.9	6.1	5.4	7.1	6.1
KRL-119	12.0	8.6	9.6	11.7	7.5	6.6	7.9	7.1	8.5	7.9
Kharchia-65	12.3	10.1	11.4	12.5	9.0	8.5	9.3	8.8	9.7	9.9
AAIW6	12.8	5.8	6.7	7.6	5.1	4.9	5.7	5.2	6.1	5.6
AAIW7	11.7	5.2	6.1	7.3	4.9	4.1	5.4	4.5	6.0	4.8
AAIW8	13.2	6.1	7.5	8.6	5.5	4.7	6.0	5.0	6.4	5.8
AAIW9	10.6	4.3	5.7	6.8	3.8	2.8	4.2	3.1	4.8	3.6
C.D. (P=0.05)	0.5	0.3	0.2	0.1	0.1	0.2	0.1	0.1	0.1	0.1
CV	1.8	1.5	0.9	0.7	0.7	1.2	0.6	0.8	0.7	0.6
F-test	2.7	0.3	1.0	0.2	0.3	0.4	0.7	1.8	0.1	0.8

T₀- Control; T₁- 100 mM NaCl alone; T₂- 100 mM NaCl + 50 ppm Silicon; T₃-100 mM NaCl +100ppm Silicon; T₄-100 mM NaCl+0.1µM EBL; T₅-100 mM NaCl+1.0µM EBL; T₆-100 mM NaCl+50 ppm Silicon+0.1µM EBL; T₇-100 mM NaCl+50 ppm Silicon +1.0µM EBL; T₈-100 mM NaCl+ 100 ppm Silicon+0.1µM EBL; T₉-100 mM NaCl+ 100 ppm Silicon+1.0µM EBL

shoot length under controlled condition as well as in all the treatments (*viz.*, T₁, T₂, T₃, T₄, T₅, T₆, T₇, T₈, T₉) (Table 2). A reduction in shoot length due to NaCl treatment among tolerant as well as non-tolerant varieties was observed. Among the non tolerant varieties the decrease was more severe. Among salt tolerant varieties the maximum shoot length (75.5 cm) was recorded in tolerant variety Kharchia in treatment T₆. Whereas the minimum shoot length (42.2 cm) was recorded in non-tolerant variety AAI W9 in treatment T₂ in comparison to their control. Result showed that application of 24-EBL and silicon was able to overcome adverse effects as imposed by NaCl in tolerant and non tolerant varieties.

There was a significant difference in root length under controlled and all the stages of treatments (*viz.*, T₁, T₂, T₃, T₄, T₅, T₆, T₇, T₈, T₉) (Table 3). Among salt tolerant varieties the maximum root length (9.9 cm) was recorded in variety tolerant Kharchia in treatment T₉. Whereas the minimum root length (2.8 cm) was recorded in non-tolerant variety AAI W9 in treatment T₂ in comparison to their control. Result showed that application of 24-EBL and silicon was able to overcome adverse effects as imposed by NaCl in tolerant and non tolerant varieties.

The reduction in root and shoot development may be due to toxic effects of the higher level of NaCl concentration as well as unbalanced nutrient uptake by the seedlings. High level of salinity may have also inhibit the root and shoot elongation due to slowing down the water uptake for overall osmotic adjustments of the plant body under high salt stress condition. After applying foliar application of Br root length of plant start decreasing. The chemical purification of brassinolide (BL), the active component of BR, has allowed the assignment of this hormone to a diverse variety of physiological responses including cell elongation, reduced root elongation, leaf bending and unrolling (epinasty), pollen tube growth, thermo tolerance, and induction of ethylene biosynthesis (Mandava, 1988; Arteca, 1995; Salchert *et al.*, 1998 and Dhaubhadel *et al.*, 1999) wheat cultivars. From the above experiment it can be concluded that root growth was found to be highly sensitive parameter and the varieties (KRL19, KRL99, KRL119, Kharchia) seem to be relatively tolerant with respect to others.

Conclusion :

It could be concluded that salinity adversely affected the growth of wheat plants expressed as seed germination

(%) and morphological parameters as shoot length and root length. The interaction between salinity levels with EBL and silicon treatments might protect the plants and enhanced growth criteria and could be useful for increasing grain yield. Hence, it could be recommended to spray plants which grown in saline regions or irrigated with saline water with EBL and silicon to overcome the destructive effect of salinity. Thus, EBL and silicon application is most beneficial to plant growth under stress condition.

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