Influence of Strigolactone (GR24) as a Seed Treatment on Growth, Gas Exchange and Chlorophyll Fluorescence of Wheat under Saline Conditions

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Abstract

Strigolactones are generally considered as the signaling molecules. A pot experiment was conducted to explore the role of strigolactone (GR24) as seed treatment on wheat under saline conditions. Seeds of two wheat cultivars (S-24 and PARI-73) were sown in sand. Two salt treatments [control (non-saline) and 150 mM (NaCl)] nourished with full strength Hoagland’s nutrient solution were applied as root growing medium while four strigolactone (GR24) levels (water (0), 0.001, 0.01 and 0.1 mg L⁻¹) were applied as seed treatment for 16 h. Imposition of salt stress significantly reduced growth attributes while plants developed from seeds raised with GR24 showed variable response on growth under salt stress. Pre-sowing seed treatment with GR24 showed non-significant effect on shoot fresh and dry weight and root length. Net CO₂ assimilation rate of both wheat genotypes increased due to GR24 application. In chlorophyll fluorescence plants showed increasing response towards application of GR24 with respect to co-efficient of photochemical quenching (qP) and non-photochemical quenching (NPQ) under non-saline. Root-medium salinity significantly enhanced the concentrations of Na⁺ ions and decreased K⁺ ions in shoot and roots. Pre-seed treatment with GR24 did not modulate concentration of shoot and roots Na⁺ and K⁺ ions. Overall, the wheat cultivar S-24 showed better performance for growth parameters and non-significant behavior towards the photosynthetic rate. Both the cultivars showed uniform response in chlorophyll fluorescence. © 2017 Friends Science Publishers

Keywords: Strigolactone; Salinity; Wheat; Photosynthesis; Growth

Introduction

Environmental factors may stimulate plant growth negatively or positively. Plants response to external factors (nutrient deficiency, light, crowding and insects) through biochemical communication system. A systematic response is found in plants in response to these external factors (Stes et al., 2015). Hormones, development, as well as environmental factors regulate shoot branching through the complex interactions in which these factors interact with each other but there is currently no ideal model to explain this phenomenon (Crawford et al., 2010). Shoot architecture is a highly plastic process suggesting that plants response to signals coming from either endogenous hormones or external environment and adjust their branching characteristics to these environmental changes (Leyser, 2009; Janssen et al., 2014). Shoot branching network involves three steps: one is long distance systemic signaling, second is local gene activity and lastly perception of external signals (Wolters and Jurgens, 2009).

Strigolactones (SLs) are considered as a new class of hormones. Natural SLs isolated from root exudates and first natural SL was named as strigol. But its structural details have published after 20 years of its discovery (Besserer et al., 2006; Zwanenburg et al., 2009). Till now, 15 stimulants have been isolated from root exudates. They occur in very minute amount and production per plant may be 25–30 pg and isolation is very difficult (Koltai and Kapulnik, 2011). SLs are produced both in monocots and dicots and are generally synthesized both in roots and stems and transported to the plant apex, thereby inhibiting shoot branching (Kebrom et al., 2013; Al-Babili and Bouwmeester, 2015). Strigolactones are produced in two forms (as endogenous hormones and root secretions). Strigolactones are involved in various roles like promoting symbiotic interactions (arbuscular mycorrhizal fungi) (Evangelisti et al., 2014); enhancing cell elongation within the root but inhibiting root hairs and asymmetric root growth (Ruyter-Spira et al., 2011); affecting developmental processes (Yoneyama et al., 2013); involving in light harvesting mechanism (Mayzlish-Gati et al., 2010) and leaf senescence (Woo et al., 2001); performing cross-talk with other major hormones like auxin, ABA and ethylene (Kohlen et al., 2011); controlling leaf shape, lamina joint
inclusion (Li et al., 2014) and tiller angle (Sang, 2014); delaying leaf senescence and reducing secondary growth and controlling PCD (Programmed cell death), photomorphogenesis and modulating biotic/abiotic stress responses (Xiong et al., 2014; Ha et al., 2014; Ueda and Kusaba, 2015). Strigolactones are widely distributed and GR24 is its synthetic analogue (Gomez-Roldan et al., 2008; Tsuchiya and McCourt, 2009). Most roles are reported by application of GR24 on Arabidopsis, petunia, pea and rice (Brewer et al., 2013) and new roles are emerging continuously (Torres-Vera et al., 2014). GR24 has been initially developed for its two most important characteristics; one is its high germination activity and second is its increased stability compared to natural SLs. Only few studies are reported in literature about the hormonal function of SLs (Fukui et al., 2013).

Salinity causes adverse effects on crops (Shahbaz et al., 2012; Perveen et al., 2015). Abiotic stresses greatly reduce the crop productivity and salinity is also important among all abiotic stresses (Mehta et al., 2010; Shahbaz et al., 2013). Approximately salinity affects 1% agriculture land (Tuteja, 2007). Salt greatly suppresses the photosynthesis process in plants and this suppression depends on different factors. These factors are photosynthetic pigments, stomatal performance and generation of essential metabolites and antioxidants (Aziz et al., 2013; Nusrat et al., 2014). Salt stress not only suppresses photosynthetic activity but also photosynthetic machinery of plants is inhibited. Salt stress also affects the cell organelles like chloroplast and it is the site for most of photosynthetic processes (PSI and PSII) and generation of reactive oxygen species (ROS) (Nusrat et al., 2014). Plants avoid salt stress by various means like shoot damage but better root growth has been reported (may be due to exclusion of sodium) (Perveen et al., 2013). Under salt stress, stomatal aperture decreased and resulted in low CO$_2$ availability to plants (Kausar and Shahbaz, 2013). Plants develop multigenic responses/mechanisms for salt tolerance; these will then regulate many physiological/biochemical processes (Shabala et al., 2010; Rafique et al., 2015; Shabbir et al., 2015). Salinity disturbs uptake/distribution of essential nutrients and balanced absorption (Srineng et al., 2015). Excess Na$^+$ in root cells competes with K$^+$ for uptake causing ionic imbalance (Bavei et al., 2011). Uptake of K$^+$ is reduced due to having similar physiochemical properties of Na$^+$ under high salinity (Hossain et al., 2011). Salt stress induces disturbance in various metabolic processes such as photosynthesis, stomatal regulation and protein synthesis (Nusrat et al., 2014).

Wheat is grown all over the world and covered larger cultivated land, nourishes most of the world’s population. Same situation in Pakistan (largest food grain crop/large proportion of cultivated land). There is no information available about the influence of pre-sowing seed treatment of strigolactone (GR24) on wheat under saline conditions.

The present study performed to explore whether or not strigolactone (GR24) could enhance the antagonistic impacts of salt stress (NaCl) on wheat.

Materials and Methods

A pot experiment was conducted to explore the effect of pre-seed treatment with strigolactone (GR24) on wheat under saline conditions. Seeds of two wheat cultivars [salt tolerant cultivar S-24 (Shahbaz et al., 2008) and salt sensitive cultivar PARI-73 (Kamboh et al., 2002)] were obtained from Ayub Agricultural Research Institute, Faisalabad, Pakistan. Seeds of both cultivars (S-24 & PARI-73) were surface sterilized in sodium hypochlorite and soaked for 16 h in four levels of GR24 solution (Distilled Water, 0.001, 0.01 and 0.1 mg L$^{-1}$) at room temperature. After 16 h pre-sowing treatment, seeds were placed on blotting paper to remove additional moisture. Strigolactone (GR24) (M. wt. 298.2) was obtained from Department of Organic Chemistry, Radbound University Nijmegen Heyendaalseweg 135, HG 03-0186525 AJ NIJMEGEN, The Netherlands. Ten seeds per pot were shown in plastic pots containing thoroughly washed river sand. Thinning was performed to six plants per pot after thirty days of sowing. Salinity (150 mM NaCl) treatment was applied to fifty day-old plants. There were two salt (NaCl) levels [control (0 mM) and 150 mM (NaCl)]. Hoagland’s nutrient solution (full strength) was applied @ 2 liters/pot every week. Salt treatment (in full strength Hoagland’s nutrient medium) was applied in an aliquot of 50 mM solution/pot every day so that desired level was attained. Data for morphological and physiological attributes were recorded of 4 weeks of salinity treatment. Two plants were uprooted carefully, washed with distilled water and recorded shoot and root fresh weights and length. The plants were oven-dried to constant weight and dry weights were recorded. In addition, following physiological attributes were recorded.

Gas Exchange Characteristics

Gas exchange characteristics were recorded using Infra-red gas analyzer LCA-4 ACD (Analytical Development, Hoddesdon, UK) at vegetative growth stage from flag leaf. Gas exchange attributes like net CO$_2$ assimilation rate (A), transpiration rate (E), sub-stomatal conductance (Ci), stomatal conductance (gs), water use efficiency (A/E) and (Ci/Ca) from 11:00 am to 1:00 pm. States of convenient infrared gas analyzer amid information estimations were: encompassing weight (P) 98.8 kPa; gas stream rate (U) 251 $\mu$mol s$^{-1}$; concentration of ambient carbon dioxide was 350 $\mu$mol mol$^{-1}$.

Chlorophyll Fluorescence

The polyphasic rise of fluorescence transients was measured by means of an OS5P Modulator Fluorometer (ADC
BioScientific Ltd, Great Amwell Herts, UK) (Strasser et al., 1995). All leaf samples were kept in dark for 30 minutes before measurements of activity of photosystem-II.

**Mineral Nutrients (Na⁺ and K⁺) Determination**

Mineral ions (Na⁺ and K⁺) in shoot and root were determined by following Allen et al. (1985). Hundred mg dried ground shoots and root material was digested in 2 ml of H₂SO₄ in a digestion flask. After digestion, the mixture was diluted with distilled water up to 50 mL, filtered and filtrate was used for Na⁺ and K⁺ ions determination with the help of a flame photometer (Sherwood, 410).

**Statistical Analysis**

The three-way analysis of variance (ANOVA) was applied by using the package of COSTAT software (Cohort Software Berkeley, California) to analyze the significance of data. The experiment was laid down in a completely randomized design (CRD) with four replications.

**Results**

Imposition of saline conditions (150 mM NaCl) significantly reduced the shoot and root fresh and dry weights of both wheat cultivars i.e. S-24 and PARI-73. Seed treatment with various levels of GR24 significantly reduced the root fresh and dry weights, while it did not alter the shoot fresh and dry weights of both wheat cultivars. Cultivar S-24 showed higher growth as compared to PARI-73 (Table 1; Fig. 1).

Shoot and root lengths of both wheat cultivars significantly reduced under saline conditions. Application of GR24 as pre-seed treatment further reduced the root length in both the cultivars while behavior was variable with respect to shoot length under saline and non-saline conditions (Table 1; Fig. 1). Cultivar S-24 was superior as compared to those of PARI-73 for both shoot and root lengths under both saline and non-saline conditions.

Salt medium markedly reduced the total leaf area per plant of both the wheat cultivars i.e. S-24 and PARI-73. Pre-seed treatment with GR24 further reduced total leaf area per plant under saline condition while total leaf area per plant did not affect under non-saline conditions (Table 1; Fig. 2). Cultivar S-24 showed better performance as compared to PARI-73 with respect to total leaf area per plant under both saline regimes.

Chlorophyll fluorescence attributes like photochemical quenching (qₑ), electron transport ratio (ETR) and efficiency of photosystem-II (Fv/Fm) did not alter either by salt stress or pre-seed treatment with GR24 (Table 2; Fig. 2). Seed treatment with GR24 slightly enhanced the co-efficient of non-photochemical quenching (qₑ) and non-photochemical quenching (NPQ) in both wheat cultivars, while salt stress tremendously reduced both these attributes of both the wheat cultivars (Table 2; Fig. 2).

**Fig. 1:** Growth attributes of salt stressed and non-stressed wheat plants raised from seed treated with GR24

Net CO₂ assimilation rate (A) of both wheat cultivars (S-24 and PARI-73) reduced significantly under saline conditions. Applications of various GR24 levels showed an increase in A in both wheat cultivars under salt stress (Table 2; Fig. 3). Both cultivars showed uniform response. GR24 application did not alter transpiration rate (E), sub-stomatal conductance (Ci), water use efficiency (A/E) and Ci/Ca. In S-24, seed treatment with GR24 slightly reduced stomatal conductance (gₛ). Transpiration rate (E) was not modulated by imposition of salinity. Seed treatment with GR24 slightly increased stomatal conductance (gₛ) only @ 0.1 mg/L level under saline and non-saline stress in both wheat cultivars. Among both wheat cultivars, E was high in PARI-73 as compared to S-24 while wheat cultivars did not differ significantly with respect other gas exchange attributes (Table 2; Fig. 3).

Saline stress (150 mM) through root growing medium significantly increased shoot and root Na⁺ ion contents in two wheat cultivars i.e. S-24 and PARI-73 (Table 3; Fig. 4). Seed-priming treatment with GR24 did not alter shoot and root Na⁺ ion in both wheat cultivars under various salt stress conditions. Imposition of salinity significantly decreased shoot and root K⁺ contents in both wheat cultivars (S-24 and PARI-73). Pre-sowing seed treatment with GR24 did not modulate the shoot and root K⁺ ions (Table 3; Fig. 4).
stress, growth inhibition is a key morphological effect under salinity. In current experiment, imposition of salt stress caused an adversarial impact on growth attributes. Under salinity stress, growth inhibition is a key morphological effect under salinity. In current experiment, imposition of salt stress caused an adversarial impact on growth attributes. Under salinity stress, growth inhibition is a key morphological effect.

Table 1: Mean squares from analyses of variance of data for growth attribute and chlorophyll fluorescence of salt stressed and non-stressed wheat plants raised from seed treated with GR24

<table>
<thead>
<tr>
<th>Source of Variance</th>
<th>df</th>
<th>Shoot fresh weight</th>
<th>Root fresh weight</th>
<th>Shoot dry weight</th>
<th>Root dry weight</th>
<th>Shoot length</th>
</tr>
</thead>
<tbody>
<tr>
<td>GR24</td>
<td>3</td>
<td>15.9ns</td>
<td>0.415*</td>
<td>0.259ns</td>
<td>0.081**</td>
<td>49.46*</td>
</tr>
<tr>
<td>Salinity (S)</td>
<td>1</td>
<td>797.7***</td>
<td>15.46***</td>
<td>11.17***</td>
<td>0.665***</td>
<td>4018.7***</td>
</tr>
<tr>
<td>Cultivars (Cvs)</td>
<td>1</td>
<td>131.6***</td>
<td>2.350***</td>
<td>2.404***</td>
<td>0.000ns</td>
<td>391.1***</td>
</tr>
<tr>
<td>GR24 × S</td>
<td>3</td>
<td>3.272ns</td>
<td>0.169ns</td>
<td>0.031ns</td>
<td>0.002ns</td>
<td>71.62</td>
</tr>
<tr>
<td>GR24 × Cvs</td>
<td>3</td>
<td>6.898ns</td>
<td>0.335ns</td>
<td>0.045ns</td>
<td>0.029ns</td>
<td>81.97**</td>
</tr>
<tr>
<td>S × Cvs</td>
<td>1</td>
<td>14.92ns</td>
<td>1.456**</td>
<td>0.230ns</td>
<td>0.089*</td>
<td>257.0***</td>
</tr>
<tr>
<td>GR24 × S × Cvs</td>
<td>3</td>
<td>5.523ns</td>
<td>0.443*</td>
<td>0.146ns</td>
<td>0.028ns</td>
<td>45.18 ns</td>
</tr>
<tr>
<td>Error</td>
<td>48</td>
<td>9.765ns</td>
<td>0.143</td>
<td>0.098</td>
<td>0.015</td>
<td>17.48</td>
</tr>
</tbody>
</table>

Source of Variance
- Root length
- Leaf area
- Fv/Fm
- ETR
- qf

Table 2: Mean squares from analyses of variance of data for chlorophyll fluorescence and gas exchange characteristics salt stressed and non-stressed wheat plants raised from seed treated with GR24

<table>
<thead>
<tr>
<th>Source of Variance</th>
<th>df</th>
<th>NPO</th>
<th>qf</th>
<th>A</th>
<th>E</th>
<th>g</th>
</tr>
</thead>
<tbody>
<tr>
<td>GR24</td>
<td>3</td>
<td>0.021**</td>
<td>0.007*</td>
<td>41.36*</td>
<td>0.248ns</td>
<td>31780.5**</td>
</tr>
<tr>
<td>Salinity (S)</td>
<td>1</td>
<td>0.322***</td>
<td>0.116***</td>
<td>1227.6***</td>
<td>0.265ns</td>
<td>142689.9***</td>
</tr>
<tr>
<td>Cultivars (Cvs)</td>
<td>1</td>
<td>0.012ns</td>
<td>0.0005ns</td>
<td>46.86ns</td>
<td>2.237**</td>
<td>232.1ns</td>
</tr>
<tr>
<td>GR24 × S</td>
<td>3</td>
<td>0.0002ns</td>
<td>0.0002ns</td>
<td>26.32ns</td>
<td>0.044ns</td>
<td>31574.1***</td>
</tr>
<tr>
<td>GR24 × Cvs</td>
<td>3</td>
<td>0.002ns</td>
<td>0.0007ns</td>
<td>36.19*</td>
<td>0.850**</td>
<td>8751.3ns</td>
</tr>
<tr>
<td>S × Cvs</td>
<td>1</td>
<td>0.00005ns</td>
<td>0.000002ns</td>
<td>45.51ns</td>
<td>0.140ns</td>
<td>41005.8*</td>
</tr>
<tr>
<td>GR24 × S × Cvs</td>
<td>3</td>
<td>0.017*</td>
<td>0.006*</td>
<td>4.365ns</td>
<td>0.257ns</td>
<td>37197.8**</td>
</tr>
<tr>
<td>Error</td>
<td>48</td>
<td>0.004</td>
<td>0.002</td>
<td>11.82ns</td>
<td>0.187</td>
<td>6854.8</td>
</tr>
</tbody>
</table>

Source of Variance
- C_i
- A/E
- C_i/C_i

Table 3: Mean squares from analyses of variance of data for mineral nutrient (Na+ and K+ ions) of salt stressed and non-stressed wheat plants raised from seed treated with GR24

<table>
<thead>
<tr>
<th>Source of Variance</th>
<th>df</th>
<th>Shoot Na+</th>
<th>Root Na+</th>
<th>Shoot K+</th>
<th>Root K+</th>
</tr>
</thead>
<tbody>
<tr>
<td>GR24</td>
<td>3</td>
<td>1.882ns</td>
<td>0.309ns</td>
<td>6.313ns</td>
<td>5.266ns</td>
</tr>
<tr>
<td>Salinity (S)</td>
<td>1</td>
<td>1718.1***</td>
<td>9075***</td>
<td>492.0***</td>
<td>569.4***</td>
</tr>
<tr>
<td>Cultivars (Cvs)</td>
<td>1</td>
<td>66.83**</td>
<td>16ns</td>
<td>11.52ns</td>
<td>0.076ns</td>
</tr>
<tr>
<td>GR24 × S</td>
<td>3</td>
<td>6.244ns</td>
<td>0.815ns</td>
<td>56.29***</td>
<td>33.64***</td>
</tr>
<tr>
<td>GR24 × Cvs</td>
<td>3</td>
<td>8.180ns</td>
<td>3.424ns</td>
<td>5.819ns</td>
<td>38.91***</td>
</tr>
<tr>
<td>S × Cvs</td>
<td>1</td>
<td>12.78ns</td>
<td>5.641ns</td>
<td>104.8***</td>
<td>8.41ns</td>
</tr>
<tr>
<td>GR24 × S × Cvs</td>
<td>3</td>
<td>0.297ns</td>
<td>1.471ns</td>
<td>24.16*</td>
<td>35.42***</td>
</tr>
<tr>
<td>Error</td>
<td>48</td>
<td>6.160</td>
<td>4.477</td>
<td>5.876</td>
<td>3.522</td>
</tr>
</tbody>
</table>

* *, **, *** = significant at 0.05, 0.01 and 0.001 levels, respectively; ns = non-significant

Discussion

In current experiment, imposition of salt stress caused an adversarial impact on growth attributes. Under salinity stress, growth inhibition is a key morphological effect (Shahbaz and Ashraf, 2013; Zulfiqar and Shahbaz, 2013; Nusrat et al., 2014; Vu et al., 2015; Masood and Shahbaz, 2016) and marker, which has been studied in various crops. In this experiment GR24 was used as Strigolactone analogue to study effect of GR24 and salt stress (150 mM)
in two wheat cultivar S-24 & PARI-73. Seed treatment with GR24 slightly reduced growth attributes except shoot fresh and dry weight and root length. In the current study, reduction in shoot length might be related to suggestion that strigolactone not only involved in regulating shoot branching and root development but also to maintain shoot elongation (Ruyter-Spira et al., 2011; Germain et al., 2013) suggested that dwarfism developed in plants not due to increased branching but might be due to low resources for main stem to develop. Strigolactone stimulated internode elongation (plant height) leading to better gain yield and biomass independently from gibberellin (Boyer et al., 2009). But in contrast to our results it has also been reported that various concentrations of GR24 (10^{-6} to10^{-8} M) were supplied as exogenous application to *Nicotiana benthamiana* seedlings caused biomass accumulation. Similar findings also previously reported regarding increased germination and biomass accumulation (Daws et al., 2008). Salt-induced decrease and increase in efficiency of PS II and non-photochemical quenching (NPQ), respectively in wheat (Akram et al., 2012) and rice (Habib et al., 2013). Electron transport membrane system and Photosystem II (PSII) activity impaired in plants by the salt stress (Ashraf and Ashraf, 2012; Kanwal et al., 2013). Photosynthesis mechanism is a key physiological process disturbed by salinity and damaging effects extensively studied on various crop such as wheat and rice (Cha-um et al., 2010). The performance of photosynthetic process is affected by salinity like change in the metabolic processes or stomatal limitation to CO₂ diffusion (Chaves et al., 2009; Abbasi et al., 2014). Salt stress causing decrease in substomatal CO₂ concentration on one side but increased due to limitation in CO₂ consumption because of less
photosynthesis (Elfeel and Abohassan, 2015). In present study, net CO₂ assimilation rate and stomatal conductance were decreased in both wheat cultivars under saline conditions, nonetheless, the lessening in these properties are enhanced by seed treatment of GR24. Photosynthetic rate (A) and stomatal conductance (gₛ) expanded by seed treatment of GR24. Improvement in photosynthesis process is a comprehensive outcome of increased CO₂ fixation and assimilation and light use efficiency (Wang et al., 2010). However, in our experiment seed treatment showed very slight effect on photosynthetic efficiency. Only NPQ and qₑ showed positive response towards application of GR24 as seed treatment. The difference might be due to different environmental conditions on plant species. Essential mineral nutrients uptake decreased due to imposition of salinity among various crops such as in rice (Shahbaz and Zia, 2011), wheat (Perveen et al., 2012) and sunflower (Shahbaz et al., 2011) etc. Sodium uptake increased during salinity due to high availability of Na⁺ contents in root growing medium. For K⁺ contents, various findings have been reported in various crops i.e. salinity may not alter K⁺ uptake and may considerably decreased K⁺ contents both in roots and shoots (Shahbaz et al., 2011). Root growing medium significantly increased shoot and root sodium contents and decreased K⁺ contents in two wheat cultivar under saline regimes (Perveen et al., 2012), which are in agreement with our findings. Seed-priming treatment with GR24 did not increase/decrease shoot and root Na⁺ and K⁺ ion in two cultivars under two salt stress conditions.

Conclusion
Salt stress adversely affected not only growth of both wheat cultivars, but also severely disturbed gas exchange characteristics and chlorophyll fluorescence. Seed treatment with GR24 slightly increased net CO₂ assimilation rate (A) and slightly reduced stomatal conductance (gₛ) and had non-significant effect on transpiration rate (E), sub-stomatal CO₂ concentration, water use efficiency and C/Co ratio. Salinity significantly reduced the non-photosynthetic quenching (NPQ) and the co-efficient of non-photosynthetic quenching (qₑ) of two cultivars while GR24 application slightly enhanced NPQ and qₑ. Salinity significantly increased and decreased shoot and root Na⁺ and K⁺ ion contents, respectively in wheat cultivars. Seed-priming treatment with GR24 did not alter shoot and root Na⁺ and K⁺ ion contents in both wheat cultivars under various salt stress conditions.

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