Abstract—Plant growth is affected by the osmotic stress as well as toxicity of salt in leaves. In order to study of salt stress effects on stomatal conductance and growth rate and relationship between them as wells osmotic and Na⁺-specific effects on these traits, four bread wheat genotypes differing in salt tolerance were selected. Salinity was applied when the leaf 4 was fully expanded. Sodium (Na⁺) concentrations in flag leaf blade at 3 salinity levels (0, 100 and 200 mM NaCl) were measured. Salt-tolerant genotypes showed higher stomatal conductance and growth rate compared to salt-sensitive ones. After 10 and 20 days exposure to salt, stomatal conductance and relative growth rate were reduced, but the reduction was greater in sensitive genotypes. Growth rate was reduced severely in the first period (1-10 days) of salt commencements and it was due to osmotic effect of salt not Na⁺ toxicity. In the second period (11-20 days) after salt treatment growth reduced only when salt accumulated to toxic concentrations in the leaves. A positive relationship between stomatal conductance and relative growth rate showed that stomatal conductance can be a reliable indicator of growth rate, and finally can be considered as a sensitive indicator of the osmotic stress. It seems 20 days after salinity, the major effect of salt, especially at low to moderate salinity levels on growth properties was due to the osmotic effect of salt, not to Na⁺-specific effects within the plant.

Keywords—Osmotic stress, Relative growth rate, Stomatal conductance, Wheat.

I. INTRODUCTION

Salinity affects plant growth by the osmotic stress of the salt around the roots as well as toxicity effects caused by excessive accumulation of salt in leaves [1]. Different plant species have developed different mechanisms to cope with these inhibitory osmotic and ionic effects of salt on plant growth [2]. Plant responses to salinity occur in two phases through time: Osmotic phase is a rapid response to the increase in external osmotic pressure and occur due to the osmotic effect of the salt outside the roots. This phase immediately reduces shoot growth. Ion-specific phase is a slow response and starts when salt accumulates to toxic concentrations in the old leaves. The osmotic stress has a greater effect on growth rates than the ionic stress. Ionic stress impacts on growth much later, and with less effect than the osmotic stress, especially at low to moderate salinity levels [3].

Growth response to salinity over time has also two phases, with the first phase of growth reduction being due to osmotic effect of salt and the second due to toxic effects of salt that has accumulated over time in the leaves [4]. It has been showed that RGR reduces greater in the first periods after salt treatment and there is a partial difference in RGR between control and salt treatments during the long periods[5], [6].

The effect of salinity on photosynthesis and growth is complex. Photosynthesis is limited by both stomatal and non-stomatal factors of salt-stressed plants. Stomatal conductance is more sensitive to salinity than the non-stomatal components of photosynthesis. Stomatal conductance is a sensitive indicator of the osmotic stress because stomatal closure is often a rapid initial response to salt stress and it is reduced immediately with the onset of salinity, indicating that it responds to the osmotic stress generated by the salt outside the roots [1]. In addition to the effects of osmotic stress, apparently other factors could also be implicated in regulating stomatal function under salt stress [7]. Non-stomatal factors occurred during the long times as the salt built up in the leaves [8], and accumulation of the toxic ions in leaf cells seems to be a main cause for the non-stomatal limitation of photosynthesis [9], [10]. Stomatal factors limiting CO₂ assimilation were observed for intermediate salinity, whereas non-stomatal ones occurred at higher salinity [6]. Monitoring stomatal conductance in plants reported as one of the most sensitive indicators of stress under salinity for wheat and sorghum [8], [11]. There is a great interest in the study of relation between stomatal conductance growth rate [1], [12].

A positive relationship between stomatal conductance and relative growth rate has been found in salt-stressed durum wheat [1], [12]. Amongst wheat varieties under salt treatments genotypic variation in stomatal conductance and relative growth rate was found and salt-tolerant genotypes showed higher growth rate and stomatal conductance at salinity compared to salt sensitive genotypes [12], [1], [12].

The aim of this study was to determine the osmotic and Na⁺-specific effects of salinity on growth responses and verify a relationship between stomatal conductance and relative growth rate as a predictor of salt stress tolerance.

II. MATERIALS AND METHODS

Plant Growth Conditions

Four bread wheat genotypes including two salt-tolerant genotypes, Bam and Roshan, and two salt-sensitive genotype,
Shiraz and Qods, were used in this study. A pot experiment was conducted in the glasshouse with daily glasshouse temperature ranged from between 25°C during the day and 15°C during the night using a factorial experiment based on a randomized complete block design with three replications. Seeds were selected that were uniform in the size and weight, surface-sterilized with hypochlorite 1% and planted into pots (25 cm in diameter) containing a mixture of perlite, cocopit and vermiculite (3:3:1 by volume).

The pots were irrigated with tap water and plants were grown during autumn. One week after sowing, pots were irrigated with a quarter strength Hoagland’s solution and one week later, solution was made up to half strength modified Hoagland’s solution.

At approximately 20 days following sowing, in order to the vernalization requirement of genotypes, the pots were moved outside and exposed to temperatures as low as -10 °C. Vernalized seedlings were placed back in the greenhouse after 4 weeks exposure to low temperatures and plants were thinned to five per pot. Plants were kept under the natural light of day with supplementary light that was kept 14 h photoperiod with irradiance at plant level of 1000-1100 µmol m⁻² s⁻¹ (PAR). At the leaf 4 stage plants were subjected to two levels of salt treatment 100 and 200 mM NaCl and the remaining pots contained plants in half strength modified Hoagland’s solution with an EC of 1.5-2 dS.m⁻¹ as a control treatment. 25 mM NaCl was added twice a day (at 7:00 am and 5:00 pm) over 2 and 3 day to a final concentration of 100 and 200 mM, respectively, and supplementary CaCl₂ was also added to give a final concentration of 8 mM for 100 mM NaCl and 12 mM for 200 mM NaCl. Salt treatments were maintained for 70 days.

Due to electrical conductivity (EC) changes in each pot, once a week after salt initiation conductivity was recorded in pots drainage water by using a digital conductivity meter (Inolab Level 1, wtw. Weilheim, Germany) to estimate evaporation and the water consumption of the plants. Conductivity was conserved in a favorable amount by adding water or concentrated salt to pots, as follows: 100 mM NaCl, ~8-10 dS.m⁻¹; 200 mM NaCl, ~16-18 dS.m⁻¹.

**Measurements**

For sodium (Na⁺) analysis, At 20 days after treatments when the flag leaf fully was expanded, three plants in each block for each genotype and treatment were completely harvested from the pots, oven dried at 70 °C for 48 h and ground after being weighed. Ion measurements were taken from the chloride acid (2 N) extract of the samples that had been burned at 580 °C for 4 h, using a flame photometer. 15 plants were harvested, dried after 48 h at 70 °C to measure yield and biomass in each block for each genotype and treatment.

**Relative Growth Rate**

Relative growth rate (RGR) was calculated from the increase in the dry weight of shoot at initial and final sampling. Shoot harvests were taken of nine replicate plants for each genotype and each treatment at three sampling times 0, 10 and 20 days after final salt concentrations (100 and 200 mM NaCl). Plant shoots were cut below the crown and dried at 70 °C for 48 h. Relative growth rate was calculated for each period as Eq. (1).

\[
RGR = \frac{\ln W_2 - \ln W_1}{t_2 - t_1}
\]

Where \( W \) is the shoot dry weight and \( t \) is time in days at the start and finish of each period.

**Stomatal Conductance**

Stomatal conductance was measured using an IRGA (Infra Red Gas Analyzer, LCA-4, Analytical Development Corporation, UK) on the abaxial surface of the mid portion of leaf 4 between 10:00 and 14:00 hours at two sampling times 10 and 20 days after salt commencement.

**Statistical Analysis**

Statistical analysis was done using SAS version 9.1 (SAS Institute Inc., Cary, NC, USA) and comparisons between means were made following least significant differences (LSD) between means test at a significance level of \( P = 0.05 \). The correlation coefficients between all pairs of traits were determined using SPSS version 15.

**III. RESULTS AND DISCUSSION**

**Na⁺ Concentration**

Genotypic differences in ion accumulation rate were found among different genotypes. Na⁺ concentration in the flag leaf blade of genotypes was significantly increased in response to salinity and Bam as a tolerant genotype exhibited good control of Na⁺ accumulation in the blade of flag leaf compared to other genotypes on a Na⁺ concentration basis (see Fig. 1(a)), Although this control was not evident at 100 Mm NaCl and Na⁺ concentration was almost the same for all genotypes. However, salt sensitive genotypes differed 2-fold in leaf Na⁺ accumulation from tolerant genotypes. It has been proved that tolerant genotypes maintain ion balance and keep intracellular sodium rate in stress conditions that lead to salt tolerance [14]. It seems that Na⁺ xylem loading in the roots and the rate of Na⁺ transfer from the root to shoot causes differences in Na⁺ concentrations in the flag leaf blade, indicating genotypic differences in the rate of xylem loading in the roots is caused to occur genotypic differences in Na⁺ transport to leaves [15].

Negative correlation between Na⁺ accumulation with biomass and grain yield showed that tolerant genotypes
reduces leaf Na⁺ accumulation and maintains a high photosynthetic activity for more biomass production and also supplies assimilate from leaves to the growing ear and grain during pre-anthesis and grain filling stages [16], and finally increases grain yield. Although, Na⁺ has been also found to play a key role in osmotic adjustment that may decrease Na⁺-specific effect by osmotic adjustment [17].

**Biomass Production**

Shoot biomass of all genotypes was substantially reduced by salinity, but significant genotypic differences was found only after 10 days exposure to salt with the effect on growth rate confined largely to the first 10 days, in where sensitive genotypes showed greater reduction (42.9%) in biomass production than tolerant ones (22.2%)(data not shown). At maturity, salinity caused a significant reduction of 18.5% and 50.4% in shoot biomass at 100 and 200 mM NaCl, respectively. Shoot biomass was significantly reduced by an average 20% for the tolerant genotypes, and an average 11% for the sensitive genotypes at 100 mM NaCl, while this reduction in all genotypes was to a similar extent 50% at 200 mM NaCl (see Table 1). Results indicated that shoot biomass is a trait which showed differences more than other traits and has been used as an index for salt tolerance [18].

Negative correlation between shoot biomass and Na⁺ accumulation \( (r=-0.877) \) (see Table 2) suggested that Na⁺ concentrations in leaves can be considered as a trait for salt tolerance NaCl was reduced to a similar extent for all genotypes (about 50%), despite greater Na⁺ concentration by the sensitive genotypes, indicating that osmotic stress effects may negatively influence carbon assimilate and photosynthesis and lead to reduction in growth not Na⁺-specific effects within the plant.

**Grain Yield**

Grain yield of main stem was reduced at both salinity treatments, but significant genotypic differences were substantially found at 200 mM NaCl. It was significantly reduced by 53.3% at 200 mM NaCl, but at 100 mM NaCl, there was no significant reduction apart from Qods by 17.8%. At 200 mM NaCl, sensitive genotypes showed greater reduction (66.5%) in grain yield compared to tolerant ones (40.3%) (see Fig. 1 (b)). The significant negative correlation between Na⁺ concentration and grain yield \( (r=-0.882) \) (see Table 2) indicates that the reduction in source activity may affect the accumulation of photo-assimilates within the grains and confirmed that salt-sensitive genotypes with higher Na⁺ concentrations were affected more by salinity than tolerant genotypes. A negative correlation has already been found between Na⁺ accumulation and salt tolerance in terms of grain yield [20], [21].

Na⁺ concentration in the flag leaf of all genotypes was to a similar extent at 100 mM NaCl, despite lower grain yield in Qods by 17.8% reduction (see Fig. 1 (b)), (see Table 2), indicating that the main effect of salinity on grain yield and growth was due to osmotic effect of the salt, not Na⁺-specific effect. It has been proved that at low to moderate salinity levels, osmotic stress impacts on growth with more effect than ionic stress within the plant [16], [2].

At 200 mM NaCl, grain yield in sensitive genotypes with a greater Na⁺ concentration was significantly decreased more than tolerant genotypes (see Table 2), indicating these responses are probably due to the Na⁺-specific effect of the salt which can negatively influence reproductive growth [2].

It has been reported that salinity causes genotypic differences in growth response without involving salt toxicity [19], [12] and in high salinity levels, grain yield and biomass were reduced due to the osmotic effect of the salt not to a Na⁺-specific effect [16], [19].

**Stomatal Conductance**

Salinity negatively affected flag leaf stomatal conductance of all genotypes. This parameter was one of the photosynthetic factors that decreased immediately in all plants during the first days after salt treatment (data not shown). Stomatal
conductance substantially reduced at both experimental periods (10 d and 20 d after salt comencement) (see Table 1). After 10 days, stomatal conductance was reduced by 50.8% and 61.6% relative to control in plants exposed to 100 mM and 200 mM NaCl respectively. There was less variation among genotypes in control compared to saline conditions. Salinity reduced stomatal conductance in different genotypes and the largest reduction was observed in sensitive genotypes, Qods and Shiraz, while tolerant genotypes especially Bam showed smaller reductions. On the other hands, Roshan and Bam showed the latest stomatal closure in response to salinity, indicating that stomatal conductance is a key mechanism for salt tolerance and these genotypes might be used as a source of osmotic stress tolerance in breeding programs [1] (see Table 1).

The salt-tolerant genotype Roshan had the highest stomatal conductance under both salinity levels and experimental periods. The rapid stomatal closure of Shiraz after the start of salt stress might have resulted in increased salt accumulation (see Fig 1 (a)) (see Table 1). In contrast, the salt-tolerant genotype Roshan seems to have better control over stomata (see Fig 1 (a)) (see Table 1). In Table 2, it has been suggested that the decrease in stomatal conductance might be the most important adaptive mechanisms of salinity tolerance in rice [22] [23].

However, it is suggested that the severe reductions in stomatal conductance and transpiration rate represent adaptive mechanisms to cope with excessive salt [24].

It has been observed that root signals presumably cause a large decrease in stomatal conductance of wheat genotypes under salinity [8]. The Na+ concentration in flag leaf blade of the tolerant genotypes was significantly lower than that of the in tolerant genotypes (see Table 2), and this could be attributed to the initial period of acclimation following exposure to salt stress, when a reduction in stomatal conductance and transpiration will also result in reduced salt uptake, as most of this uptake in rice is known to occur passively, through the transpiration stream [25].

**Relative Growth Rate (RGR)**

Plant growth of all genotypes was inhibited by salinity. Relative growth rates did not differ greatly between genotypes in the control apart from Bam (see Table 1). However, the RGR of all genotypes decreased significantly due to salinity. Salinity significantly affected RGR at both experimental periods (1-10 d and 11-20 d after salt) (see Table 1).

Significant difference in RGR between genotypes and treatments in both periods was found but results showed that RGR was reduced severely during the first period (1-10 d), and growth rate was higher at second period (11-20 d) (see Table 1). Salt-tolerant genotypes showed higher growth rate at

### TABLE I  EFFECT OF DIFFERENT SALINITY LEVELS ON SHoot BIOMASS, GRAIN YIELD, HARVEST INDEX AND RELATIVE GROWTH RATE OF FOUR WHEAT GENOTYPES DIFFERING IN SALT TOLERANCE EXPRESSED AS THE PERCENT OF CONTROL (70 DAYS OF SALT TREATMENT)

<table>
<thead>
<tr>
<th>Genotype</th>
<th>NaCl (mM)</th>
<th>Mean Changes Mean</th>
<th>Changes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Shoot biomass (g)</td>
<td>% C</td>
<td>Stomatal conductance (mmolm/s²)</td>
</tr>
<tr>
<td>Roshan</td>
<td>0</td>
<td>3.70 a</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>3.17 abc</td>
<td>85.9</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>1.80 e</td>
<td>48.7</td>
</tr>
<tr>
<td>Bam</td>
<td>0</td>
<td>2.9 cd</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>2.7 cd</td>
<td>92</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>1.5 e</td>
<td>52</td>
</tr>
<tr>
<td>Shiraz</td>
<td>0</td>
<td>3.53 ab</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>2.88 cd</td>
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<td></td>
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<td>1.66 e</td>
<td>47</td>
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<tr>
<td>Qods</td>
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<td>3.01 bc</td>
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</tr>
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<td></td>
<td>100</td>
<td>2.36 d</td>
<td>78.4</td>
</tr>
<tr>
<td></td>
<td>200</td>
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Mean Square

<table>
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<tr>
<th>Genotype</th>
<th>Mean Square</th>
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</thead>
<tbody>
<tr>
<td>0.523</td>
<td>25137 **</td>
</tr>
<tr>
<td>9.18 **</td>
<td>141018 **</td>
</tr>
<tr>
<td>0.056 **</td>
<td>5702 **</td>
</tr>
</tbody>
</table>

Mean Square

<table>
<thead>
<tr>
<th>Genotype/Salt</th>
<th>Mean Square</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0022**</td>
<td>27624**</td>
</tr>
<tr>
<td>0.0059**</td>
<td>96869**</td>
</tr>
<tr>
<td>0.0009**</td>
<td>22588**</td>
</tr>
</tbody>
</table>

Means within a column followed by the same letter are not significantly different (P = 0.05), according to Least Significant Difference Test (LSD).

n.s: Non-significant, **: significant at 0.01 and 0.05 probability levels, respectively.

### TABLE II  COEFFICIENT CORRELATIONS BETWEEN TRAITS OF FOUR WHEAT GENOTYPES GROWN UNDER DIFFERENT SALINITY LEVELS

<table>
<thead>
<tr>
<th>Traits</th>
<th>Na+</th>
<th>Grain yield</th>
<th>Shoot biomass</th>
<th>Stomatal conductance</th>
<th>RGR (10-20 d)</th>
<th>RGR (1-10 d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RGR (1-10 d)</td>
<td>-0.688**</td>
<td>0.679**</td>
<td>0.628**</td>
<td>0.443**</td>
<td>0.743**</td>
<td>1</td>
</tr>
<tr>
<td>RGR (10-20 d)</td>
<td>-0.773**</td>
<td>0.635**</td>
<td>0.787**</td>
<td>0.345**</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Stomatal conductance</td>
<td>-0.732**</td>
<td>0.532**</td>
<td>0.690**</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shoot biomass</td>
<td>-0.877**</td>
<td>0.792**</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grain yield Na+</td>
<td>-0.882**</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

n.s: Non-significant, **: Significant at 0.05 probability level, *: Significant at 0.01 probability level
salinity compared to sensitive ones [1], [12]. Although, Shiraz had higher RGR in control conditions but it was suffered the largest growth reduction at salinity in both periods (Table 1). Our results were consistent with a two-phase model has been proposed for salinity effects on RGR in which growth affected only in the first periods after exposure to salt [5], [6]. In the first period after exposure to salt, salinity had more severe effect on RGR than second period. Shoot dry weight was also affected more by salinity in the first period compared to second period, with a greater reduction (42.9%) in sensitive genotypes compared to tolerant ones (22.2%)(data not shown).

A negative correlation has found between RGR and Na+ concentrations at the first period \((r= -0.773)\) and the second period \((r= -0.678)\). In spite of increased Na+ accumulation at the second period (data not shown), higher negative correlation at the first stage suggested that more reduction in first stage was due to osmotic stress effect on growth rate not Na+ toxicity. Higher correlation between shoot biomass and RGR in the first period \((r=0.787)\) versus second period \((r=0.628)\) also suggested that salinity reduced growth rate more during the first period of salt treatment, via biomass reduction being due to the osmotic effect of salt. It has been found that the effect of salinity on RGR during the first stage of salt treatment is probably associated with a fall in net assimilation rate due to lower average leaf photosynthetic rate [6].

Relation Between Stomatal Conductance and Growth Rate

The decline in stomatal conductance occurred in parallel with a significant reduction in growth rates. Higher stomatal conductance under stress was correlated to higher growth rates and shoot biomass [1], [12]. There was a positive relationship \((r=0.443)\) between stomatal conductance (10 days after salt) and the RGR of the shoot between 1-10 days in salt (see Table 2). Roshan and Bam with a higher stomatal conductance in salt tended to have a higher RGR. Further, positive correlation between stomatal conductance and grain yield \((r=0.590)\) confirms significant relationship between higher stomatal conductance and higher yield in field-grown plants [26]. The decrease in stomatal conductance might limit photosynthesis and reduce leaf growth rate, as described for other plant species [27].

Stomatal conductance showed a positive correlation with shoot biomass \((r=0.690)\) and grain yield \((r=0.532)\). On the other hand, reduced stomatal conductance caused a reduction in photosynthesis and biomass production and finally grain yield. Roshan and Bam with higher stomatal conductance had a lower reduction in biomass production and grain yield compared to sensitive genotypes, Qods and Shiraz with lower stomatal conductance in salt conditions. It has been proved that the higher stomatal conductance could be useful to select for yield potential in plants under salt-stressed conditions [28], [29]. These results showed that higher stomatal conductance in salt conditions will confer salt tolerance to current genotypes in terms of growth rate, biomass and grain yield.

IV. CONCLUSION

Lower extent of Na+ accumulation in growing photosynthetic tissues with higher stomatal conductance and growth rate in saline conditions are associated with the improved salt tolerance that can lead to lower grain yield loss. Higher stomatal conductance in salt is related to higher assimilation and there was a positive relationship between stomatal conductance and relative growth rate in salt, suggesting stomatal conductance can be used as a surrogate for growth rate. These relationships showed that stomatal conductance can be a reliable indicator of growth rate and also can be used as a reliable screen in tolerance to osmotic stress caused by salinity for wheat genotypes. As the osmotic effect of salt was more severe than Na+ specific effect at moderate to high salinity, therefore, developing osmotic stress tolerance can be used as a potential to improve salt tolerance in wheat breeding programs.

ACKNOWLEDGMENT

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