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Full Length Research Paper

Physiological response of common bean (*Phaseolus vulgaris* L.) seedlings to salinity stress

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The effect of salinity stress on five cultivars of common bean: Bassbeer, Beladi, Giza 3, HRS 516 and RO21 were evaluated on a sand/peat medium with different salinity levels (0, 50 and 100 mM NaCl) applied 3 weeks after germination for duration of 10 days. Salinity had adverse effects not only on the biomass yield and relative growth rate (RGR_t), but also on other morphological parameters such as plant height, number of leaves, root length and shoot/root weight ratio. Photosynthesis, transpiration rate and stomatal conductance were adversely affected in all cultivars. Leaf osmotic potential and leaf turgor varied significantly among cultivars and salt levels. The interaction between cultivars and salt levels for photosynthesis, leaf osmotic potential and leaf turgor was highly significant at day 10 of salt treatment. The Na uptake among the cultivars varied in the order: HRS 516 <RO21 <Giza 3 <Bassbeer <Beladi. This suggests that HRS 516 was most tolerant cultivar because it was the only cultivar with the highest survival rate and no symptoms of salt stress. RO21 was the most susceptible to salinity as it showed severe symptoms of salt stress and very low survival rate.

Key words: Salinity stress, Na^+ ions, physiology, photosynthesis, water relations, common bean (*Phaseolus vulgaris* L.).

INTRODUCTION

Salinity is one of the major factors affecting agricultural productivity worldwide. In the arid and semiarid areas, it could be caused by (1) poor irrigation water which contains considerable amounts of salts, (2) accumulation of salts in the top layer of the soil due to over-irrigation, (3) proximity to the sea, and (4) the capillarity rise of salts from underground water into the root zone due to excessive evaporation. Also, low rainfall, high evaporation rate and poor water management could cause salinity related problems in these areas.

In the fertile Crescent of Jordan, Palestine, Lebanon, Syria and Iraq, and along the Nile Valley (including Egypt and Sudan), common bean is a major vegetable crop. About 20 to 30% of the bean-production areas in the Middle East are affected by soil salinity (Bayuelo-Jiménes et al., 2002b). Under such a situation, yield is expected to be low as the common bean is extremely sensitive to salinity and suffers yield losses at soil salinity levels less than 2 dSm^{-1} (Läuchli, 1984). However, common bean and other legumes are regarded as appropriate crops for the enhancement of bioproductivity and the reclamation of marginal lands, because they not only yield nutritious fodder, protein rich seeds and fruits, but also are known to enrich the soil nitrogen in symbiotic association with rhizobium (Alexander, 1984). They therefore, contribute a lot to the improvement of soil fertility in the tropical and subtropical zones where most of the soils are already salinized (Bayuelo-Jiménes et al., 2002a).

Salinity reduces the ability of plants to utilize water and causes a reduction in growth rate, as well as changes in plant metabolic processes (Munns, 1993 and 2002). Plants growing under saline conditions are stressed basically in three ways; (1) reduced water potential in the root zone causing water deficit, (2) phytotoxicity of ions such as Na^+ and Cl^-, and (3) nutrient imbalance by depression in uptake and/or shoot transport (Munns and Termaat, 1986; Lauchli, 1986; Marschner, 1995). This is attributed to the fact that Na^+ competes with K^+ for binding sites es-

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sential for cellular function (Tester and Davenport, 2003). This role makes K^+ an important element as more than 50 enzymes are activated by K^+, and Na^+ cannot substitute in this role (Bhandal and Malik, 1988). On one hand, the latter implication of these two macronutrients in salinity is thought be to one of the factors responsible for reduction in the biomass and yield components. One other hand, however, the reduction in growth is generally the Consequences of several physiological responses including modication of ion balance, water status, mineral nutrition, stomatal behavior, photosynthetic efficiency and carbon allocation, and utilization (Flowers and Teo, 1981; Greenway and Munns, 1980; Munns and Termaat, 1986). In salt-sensitive plant, shoot, and to lesser extent, root growth is permanently reduced within hours of salt tress and this does not appear to depend on Na^+ concentrations in the growing tissues, but rather is a response to the osmolatity of the external solution (Munns et al., 2000a; Munns, 2002). Although reduction in biomass, photosynthetic capacity changes in leaf water potential and leaf turgor have been reported to have a cumulative effect attributed to salinity stress (Wignarajah, 1990; Monneveux and Belhassen, 1996; Tourneux and Peltier, 1995), it is also clear that several soil and other environmental factors do influence plant growth under salinity conditions. Several studies such as genetic variability of cultivated *Phaseolus* bean cultivars exposed to salinity at germination stage (Moreno-Limon et al., 2000; Bayuelo-Jiménes, 2002a), seedling stage (Bayuelo-Jiménes, 2002a) and early vegetative growth (Bayuelo-Jiménes et al., 2002b) have been conducted. Characters like yield, survival, vigor, leaf damage and plant height, have been the most commonly used criteria for identifiing salinity tolerance (Mass and Hoffman, 1977; Shannon, 1984). Other indices of tolerance have also been proposed that are based on specific physiological characteristics. For instance, accumulation of specific ions in shoots or leaves, or production of a specific metabolite (Noble and Shannon, 1988).

Alternatively, relative growth rate (RGR) has also been used as a relative basis on which to compare growth rates of plants. However, the results from such induced salt stress studies at a period in plant growth stage may be misleading when comparing different genotypes, species or salinity treatments because the initial size of the plant can influence the size or growth rate at harvest (Hunt, 1990; Bayuelo-Jiménes et al., 2003).

Here, we are interested in screening some cultivated bean cultivars as most of them being introductions, hybrids or IBLs, have superior horticultural characteristics and are well adapted to local environments. Kingsbury and Epstein (1984) documented the importance of evaluation and exploitation of genetic variability among cultivated species of common beans alongside their wild relatives to identify tolerant genotypes that sustain reasonable yield on affected soils. Unfortunately, retrogression of alien traits from wild relatives are vulnerable to undesirable characters such as reduced viability and sterility of hybrids, as well as segregation distortion and reduced recombination in segregating generations (Rick, 1962, 1983; Foolad, 1996; Kornegay et al., 1992; Singh and Molina, 1991; Viera et al., 1989). Therefore, screening of cultivated beans rather than exploitation and breeding of salt tolerant cultivars from wild germplasm could be a short-term approach to selecting relatively salt tolerant cultivars for salt-affected soils. The comparison of growth of locally adapted cultivars based on biomass production and survival rate inferred from visual appearance of a crop, and analysis of ionic (Na^+ and Cl^-) concentrations in plant organs are good indicators to be used as effective physiological markers for salinity stress.

The objective of this study was to evaluate physiological and morphological responses of five locally adapted Sudanese common bean varieties to salinity stress. To determine the predictive screening parameters that can be applied at early developmental stage of bean plant, we have focused mainly on biomass, visual appearance and survival rate, and Na^+ uptake as practical physiological markers.

MATERIALS AND METHODS

Plant materials

Five high yielding and early maturing common bean cultivars, released by The Agricultural Research Corporation (ARC) of Sudan, namely, Bassbeer, Beladi, Giza 3, HRS 516 and RO21 were evaluated for salt stress. The characteristics of cultivars used in this study are described in Table 1.

Plant growth condition and salinity treatments

Seeds of the above bean cultivars were sown into a growth medium (sand and peat-moss at a ratio of 3:1) in a growth chamber set at the following conditions: photoperiod (16 h light and 8 h dark), temperature (24 and 20ºC) and light intensity (80,000 lux-metal halide source). Plastic pots of 25 x 20 cm size were filled with the growth medium and supplemented with Hoagland's solution adjustted to pH 5.5. The composition of the nutrient solution was made of KNO_3 (16.83 mg L^{-1}), $Ca(NO_3)_2.4H_2O$ (59 mg L^{-1}), $NH_4H_2PO_4$ (115 mg L^{-1}), $MgSO_4.7H_2O$ (123 mg L^{-1}), FeEDTA(5 mg L^{-1}), $MnCl_2.4H_2O$ (1.8 mg L^{-1}), H_3BO_3 (0.38 mg L^{-1}), $ZnSO_4.7H_2O$ (0.22 mg L^{-1}), $CuSO_4.5H_2O$ (0.08 mg L^{-1}), and $(NH_4)_6Mo_7O_{24}.H_2O$ (0.02 mg L^{-1}) .

The soil used in this experiment was 98% sand (Ikeura, 1998) with the following characteristics (Eneji, 2001): 0.03% total C, 16.7 mg kg^{-1} available P, exchangeable cations (cmol kg^{-1}): 0.4 for Ca and K, 0.2 for Mg and Na, and pH (H_20) of 6.6. Seeds of the 5 cultivars were surface sterilized with 5% (w/v) commercial bleach sodium hypochlorite (NaOCl), and germinated in a vermiculite media. After 4-7 days, 3 uniform seedlings with fully developed trifoliate leaves were transplanted into each of the plastic pots and arranged into three (3) levels of salt stress: control (0 mM NaCl), 50 mM NaCl and 100 mM NaCl. Treatments were replicated 3 times and arranged into a randomized complete block design.

Plant morphology and biomass

Plant morphological parameters such as height, number of leaves, root length, shoot and root fresh weight (FW) were obtained at 5 days intervals: day 0, day 5 and day 10 of salt treatment. The fresh

Table 1. *Agronomic characteristics of cultivars used in this study.

Cultivar	Growth Pattern	*Yield (Kg Ha^{-1})	Seed size (100 g SS)	Seed color
Bassbeer	Indeterminate	1080	35.84	white
Beladi	Indeterminate	882	30.07	,,
Giza 3	Indeterminate	1221	29.93	,,
RO21	Indeterminate	1516	27.00	,,
HRS 516	Determinate	1228	37.84	,,

*Source: Agricultural Research Corporation (ARC, Sudan) Annual Report, 1997.

shoot and root biomass were oven-dried for 24 h at 80-90°C to obtain dry weights. Relative growth rate (RGR) or the mean relative growth rate was determined as the rate of increase in total dry weight per unit of plant weight according to Hunt (1982) thus:

$RGR = (\ln W_2 - \ln W_1/t_2 - t_1)$,

RGR in $g.g^{-1}\ day^{-1}$, where, W total plant weight (g), t the time (days), and the subscripts 1 and 2 are initial and final harvest of biomass yield.

Plant water relations (leaf osmotic potential and leaf turgor potential)

Leaf osmotic potential (Ψ_s) was measured using an osmometer (OM 801, VOGEL GMBH, Marburger Strasse, Germany) and leaf turgor potential (Ψ_n) was determined using a pressure chamber according to techniques followed by Scholander et al. (1965). Samples for osmotic potential, collected at day 0, 5 and 10, were wrapped in an aluminum foil and immediately frozen in liquid nitrogen -179°C and stored at -20°C (Rekika et al., 1998) until measurement. Frozen leaf samples were thawed and squeezed with a forcep in a 1 ml ependorf tube, centrifuged, and the resulting sap pipetted into a 500 µml eppendorf tube for measurement.

Relative water content (RWC) was calculated according to Weatherly (1950). Whole leaf or leaf disks (1.5 cm) was weighed immediately after collection or punching (fresh weight, FW) and placed in a Petri dish containing wet filter paper and kept at 4°C in the dark. After 24 h, the turgid weight (TW) was obtained. For the dry weight (DW), leaf disks were oven-dried for 24 h at 80-90°C and weighed.

Photosynthesis, stomatal conductance, and transpiration rate

Leaf gas exchange, including stomatal conductance (g_s, mol H_2O $m^{-2}s^{-1}$), transpiration rate (E, mmol H_2O m^{-2} s^{-1}) and net photosynthetic assimilation rates-PAR (A, µmol CO_2 m^{-2} s^{-1}) was determined after 6 h of daylight on the fully expanded leaf using LI-COR 6400 instrument (LI-COR Inc, Lincoln, NE, USA). All measurements were made at photosynthetic photon flux (500-600 nm) of 600 µmol m^{-2} s^{-1}. Radiation within the cuvette was supplemented with a halogen lamp (100 W). Three fully expanded leaves were sampled per cultivar and salt treatment.

Ion analysis

Ion analysis of sodium was carried out using the hot water method. Three grams of finely grounded shoot were dissolved (eluted) in 30 ml distilled water and agitated in a rotary shaker set at 90ºC and 150 rpm for 2 h. While boiling the samples were removed one by one and hand-shaken to ensure a homogenous mixture, then centrifuged in a refrigerated-centrifuge (HITACHI Himac CR 20B2, Hitachi Corporation, Japan) at 3x 10^3 rpm and 25°C for 5 min. The samples were filtered using a cloth (miracloth, CALBIOCHEM, Biosciences, Inc. La Jola, CA., USA). The resulting filtrates were stored at a temperature range of 0 - 4°C until measurement. Sodium ions were determined by an atomic absorption/flame emission spectrophotometer AA6700 (Shimadzu Corporation, Kyoto, Japan).

Statistical analysis

Data were statistically analyzed by the analysis of variance using the Statveiw software. LSD test was used to separate the treatment means which differed significantly.

RESULTS

Plant morphology and biomass measurements

The summary of the analysis of variance for the parameters studied during the experiment showed that salinity stress had adverse effect on the biomass yield, water relations, ion uptake, and photosynthesis of the five cultivars. Differences in shoot dry weight (SDW) were highly significant among the salt levels and cultivars. HRS 516 excelled the other cultivars in TDW while RO21 had the least (data not shown here). No interaction was observed between cultivars and salt levels for SDW.

Root dry weight (RDW) and root length (Figure 2) were not significantly different among cultivars but there were significant differences among salt treatments at day 5 and 10. There was an increase in root dry weight (RDW) at 50 mM NaCl which then dropped considerably at 100 mM NaCl as shown in Figure 1. The root lengths of all cultivars were reduced salinity by although cultivar RO21 had slightly longer roots (Figure 2). This suggests that both RDW and root length were adversely reduced as salinity increased.

On the other hand, root/shoot ratio was significantly different among cultivars. In the non-saline (control) treatment and 100 mM NaCl salinity, HRS 516 had the best shoot/root ratio. At 50 mM NaCl salinity, the ratios for HRS 516 and Giza 3 were comparable except at 100 mM salinity, RO21 had the lowest ratio.

Total relative growth rate (RGR_t)

The relative growth rates (RGR_t) of the five cultivars were

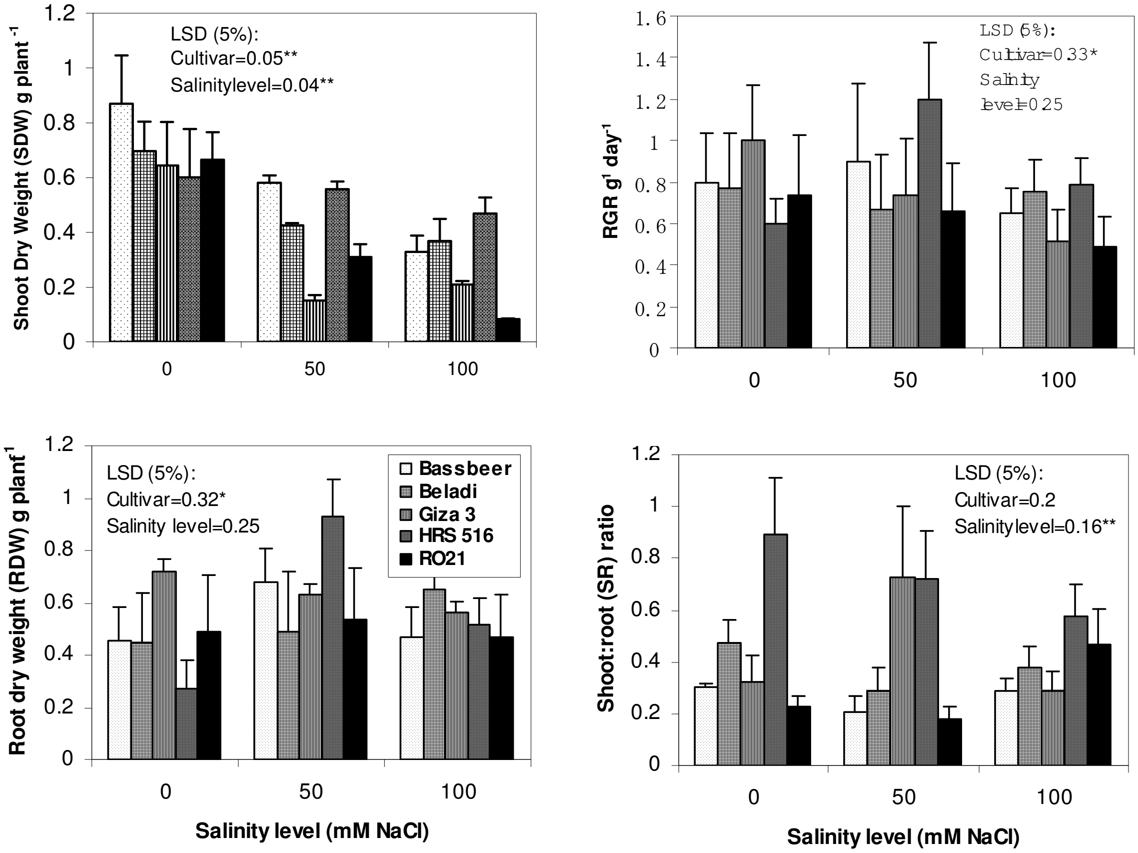

Figure 1. Effect of salinity on shoot dry weight (SDW), root dry weight (RDW), relative growth rate (RGR) and SR ratio on five cultivars of *Phaseolus vulgaris* L. and * and ** indicate significance at P≤0.05 and 0.01.

almost similar under non-saline conditions but increased under the saline condition of 50 mM NaCl and then declined considerably thereafter. HRS 516 had much or slightly higher RGR_t at 50 and 100 mM NaCl salinity. Plant height differed significantly among cultivars but not among salinity levels as shown in Fig. 2. However, the number of leaves was as significantly affected by salinity levels as by cultivars (Figure 2).

Plant water relations (leaf osmotic potential and leaf turgor potential)

Figure 3 shows the changes in leaf turgor and leaf osmotic and turgor potential of the bean cultivars under various salinity levels. Although values for leaf turgor potential varied significantly among cultivars under non-saline conditions (control), there was a marked decrease at 50 and 100 mM NaCl for all cultivars. The osmotic potential of leaves under non-saline conditions was relatively stable for all cultivars ranging from -1.05 to -1.6 MPa, but it decreased as salinity intensified with values ranging from -1.66 to -3.68 MPa (50 mM NaCl) and -2.8 to -5.8 MPa (100 mM NaCl). Differences in the leaf osmotic potential among cultivars, salt levels and interaction were highly significant. Leaf osmotic potential was found to be directly proportional to salt stress. Interestingly, the leaf osmotic potential of cultivar HRS 516 was not significantly affected by salinity stress [-1.102 MPa (control), -1.476 MPa (50 mM NaCl) and -1.345 MPa (100 mM NaCl)]. At 100 mM NaCl, RO21 exhibited the lowest leaf osmotic potential which was half as much as other cultivars. Generally, the leaf osmotic potential decreased as salt level increased.

Photosynthesis, stomatal conductance and transpiretion rate

The effect of the three levels of salt stress on photosynthesis was examined after 6 h of daylight (Figure 4). Photosynthesis was significantly impaired as salinity increased in all cultivars. Also the interaction of salt x cultivar on photosynthesis was highly significant. Although cultivar HRS 516 exhibited the lowest photosynthetic activity among other cultivars, it had the highest value at the final days of salinity treatment (Figure 4). The stomatal conductance of the cultivars declined with the advance in growth stage (age) and further as salinity increases (Figure 4). The stomatal conductance of all the cultivars ex-

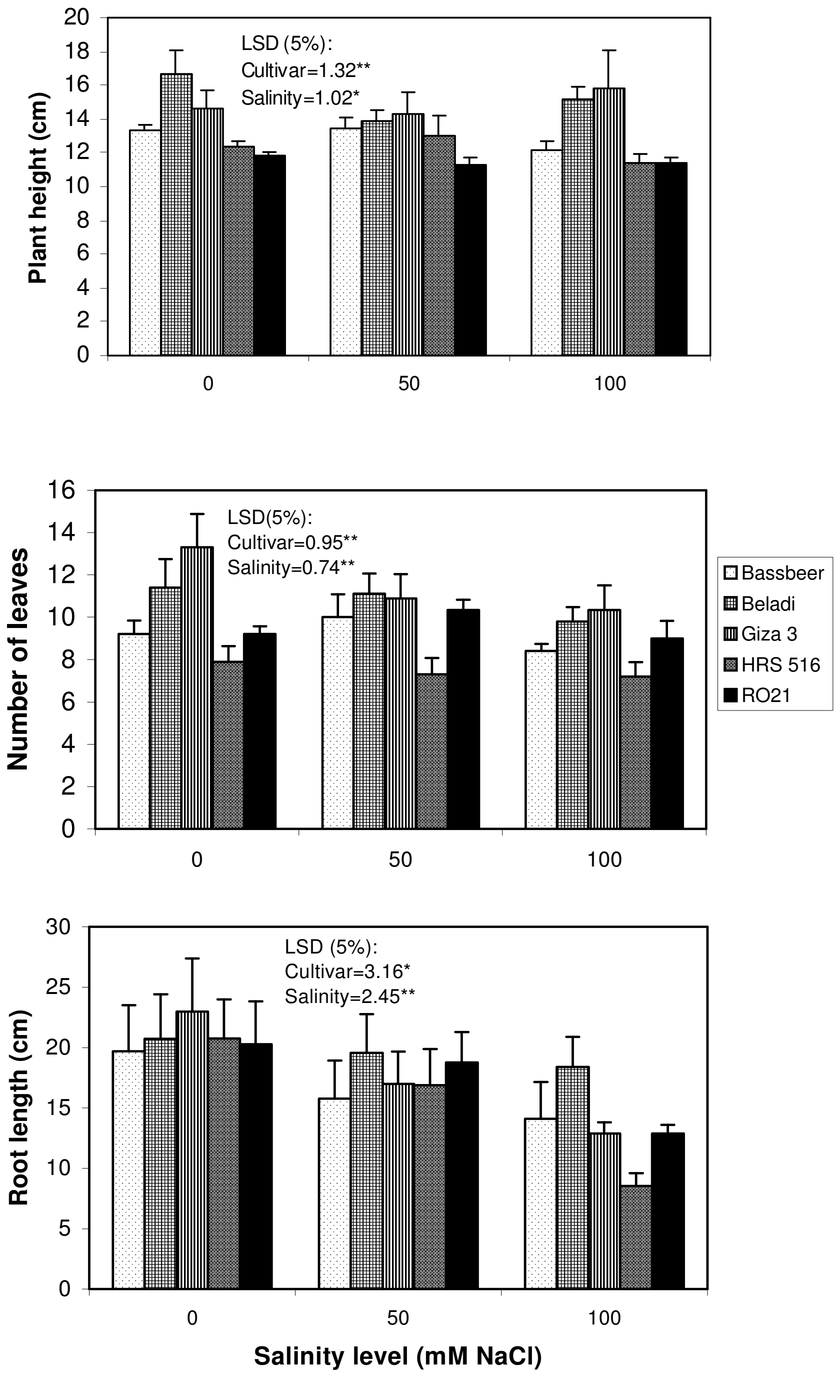

Figure 2. Effect of salinity stress on plant height, number of leaves and root length of five cultivars of *Phaseolus vulgaris* L. * and ** indicate significance at P≤0.05 and 0.01.

cept RO21 was lowest at 100 mM NaCl. The Transpiration rates of the five cultivars, under non-saline condition were not very much different throughout the experiment but they declined significantly as salinity intensified.

Ionic analysis

The ionic analysis performed for sodium concentration in shoot tissues (Figure 5) showed that HRS 516 accumulated the least amount of Na, suggesting that it is the most tolerant of salinity. Conversely, RO21 had the high-

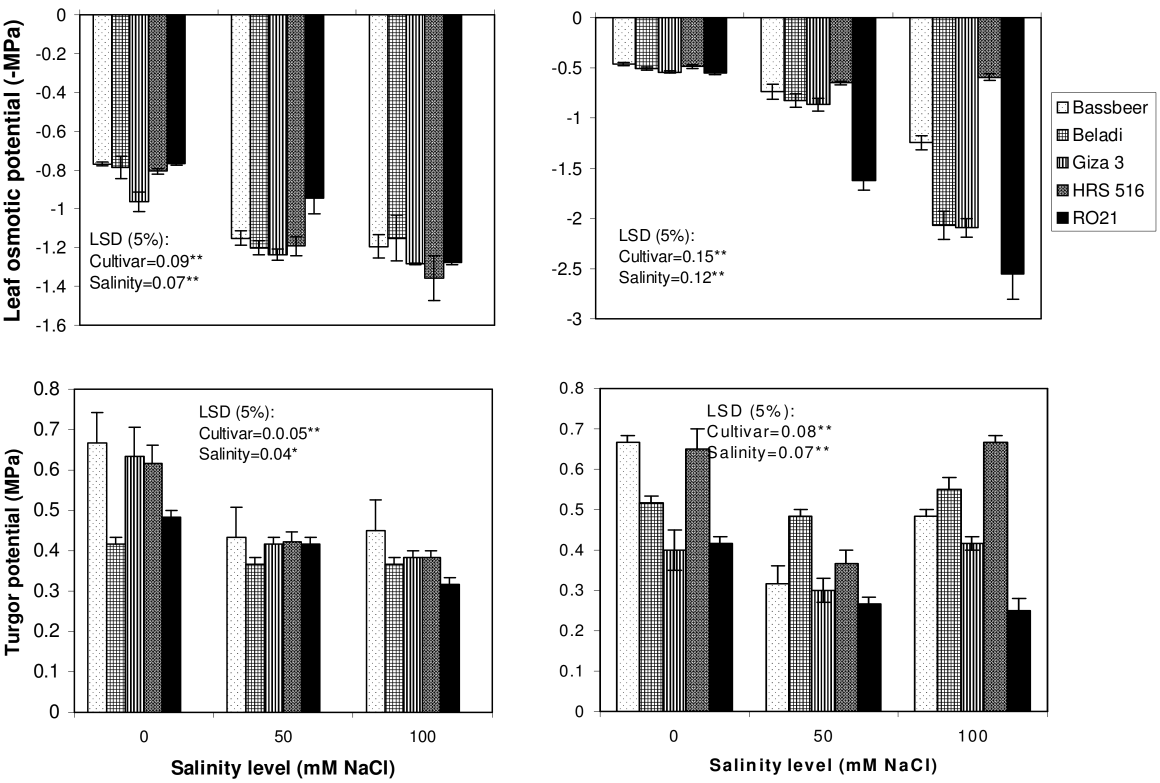

Figure 3. Effect of salinity stress on water relations; leaf turgor and leaf osmotic potential (MPa) of five cultivars of *Phaseolus vulgaris* L. at day 5 and 10 of salinity treatment as shown in (A) and (B), respectively. * and ** indicate significance at P≤0.05 and 0.01.

est Na uptake, and was thus considered the most susceptible cultivar.

DISCUSSION

Reductions in the biomass of *Phaseolus vulgaris* under saline condition were indicative of severe growth limitations. Salinity had adverse effects not only on the biomass, but also on other morphological parameters such plant height, number of leaves, root length and shoot/root ratio. In several legumes, such as faba bean (Yousef and Sprent, 1983; Zahran and Sprent, 1986), soybean (*Glycine max*) (Grattan and Maas, 1988), and bean (*Phaseolus vulgaris*) (Wignarajah, 1990), salinity was reportedly found to reduce shoot and root weights. In *Phaseolus vulgaris*, concentrations of 0.05 mol/L (50 mM NaCl) caused stunted growth due to salt-induced reduction in photosynthates (Brugnoli and Lauteri, 1991).

In this experiment, relative growth rates (RGR_t) were almost uniform at day 5 and 10 under non-saline condition but increased under saline condition (50 mM NaCl) and then declined considerably for all cultivars except cultivar HRS 516 which maintained a slightly higher RGR_t (Figure 1).

Both root dry weight (RDW) and root length of the cultivars evaluated were adversely reduced as salinity increased. This observations corroborates the findings of Cordovilla et al. (1999) that roots were more sensitive than shoots and N_2 fixation was more sensitive than was plant growth but contradicts the report by Wignarajah (1990) that salinity affected shoot growth more than root growth. However, Bayuelo-Jimenez (2002a,b) reported that salt-tolerant species (accessions) of *Phaseolus* maintained relatively high root growth even at 180 mM (nutrient solution) NaCl. The consequent increase in root to shoot growth seems to be associated with increased salinity tolerance in these species. It is possible that under salt stress the plant spends more photosynthetic energy on root production in search of water and /or reducing water loss and thus maintains a relatively high water relations (Kafkafi, 1991). Although root production was found to be continuous, plants apparently used this process as an avoidance mechanism to remove excess ions and delay the onset of ion accumulation; in tissues of less tolerant cultivars such as (RO21) root growth was not continuous. Probably, avoidance of salinity by intensive root development was dependent on species or genotypes.

The decline in photosynthesis observed with increasing salinity could be attributed to stomatal factors. During salt stress, as well as water deficit, the concentration of CO_2 in chloroplasts decreases because of a reduction in stomatal conductance, in spite of the apparent stability of

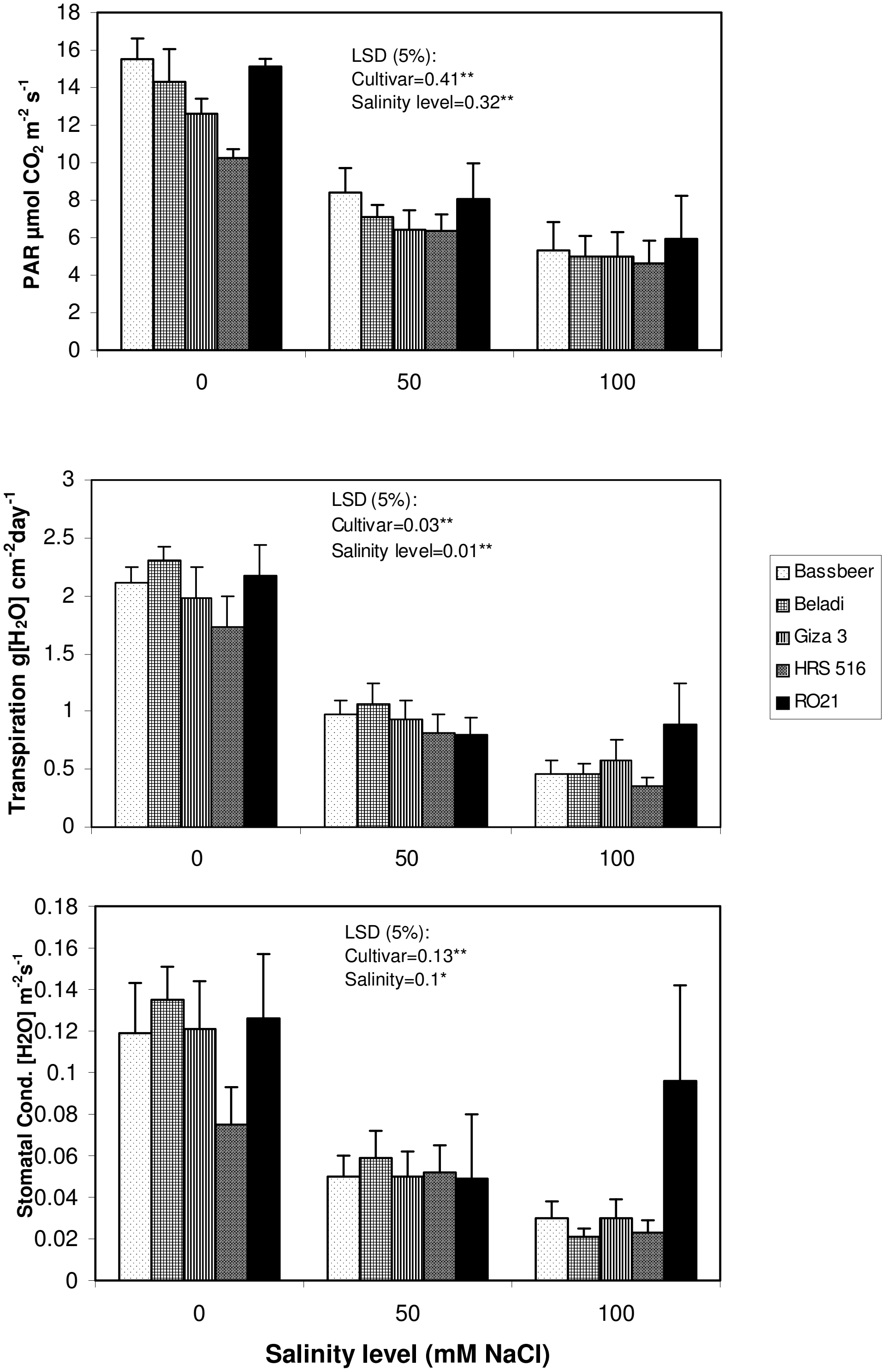

Figure 4. Effect of salinity stress on photosynthesis (photosynthetic assimilation rate, PAR), transpiration rate (E), and stomatal conductance (gs) of five cultivars of *Phasoelus vulgaris* L. * and ** indicate significance at p≤0.05 and 0.01.

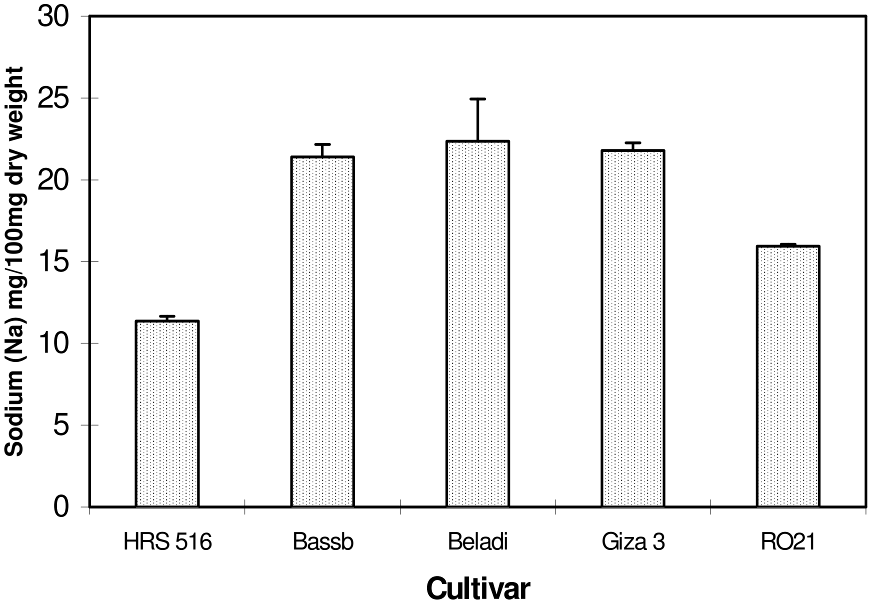

Figure 5. Sodium accumulation in the shoot of five cultivars of *Phaseolus vulgaris* L. Bars indicate standard error (SE) of means (n=3).

CO_2 concentration in intercellular spaces (Tourneux and Peltier, 1995). Brugnoli and Lauteri (1991) also indicated that reduced photosynthetic carbon assimilation was attributed to reduced stomatal conductance.

Since, transpiration rate followed the same trend as photosynthesis, it is clear that a reduction in photosynthesis has some effects on both stomata and transpiration as the three are integral elements of the photosynthetic apparatus of plants. It was also observed that the stomatal conductance of plants declined with age and was very low as salinity intensified. This was more apparent in plants subjected to 100 mM NaCl regardless of cultivar. During stress, carbon allocation, osmotic adjustment and accumulation of soluble sugars compete with other sinks and can affect growth (Monneveux and Belhassen, 1996).

Although values for leaf turgor varied among cultivars under non-saline conditions (control), there was a marked decrease at 50 mM NaCl for all cultivars with only slight increases as salinity increased except for cultivar RO21. Leaf osmotic potential was stable for all cultivars. Romero-Aranda et al. (2001) also reported that leaf water potential and leaf osmotic potential decreased with salinity but leaf turgor pressures were significantly higher than in control plants which suggest that bulk tissue turgor did not limit growth under the saline conditions tested. Bayuelo-Jimenez (2003), however, found that as turgor potential was maintained by or enhanced by salinity, osmotic adjustment was maintained.

Even though leaf osmotic potential increased in the other cultivars, HRS 516 maintained a relatively constant potential under saline conditions. At 100 mM NaCl, however, RO21 exhibited a higher leaf osmotic potential, twice as much as other cultivars. This could be explained from the fact that during stress, carbon allocation, osmotic adjustment and accumulation of soluble sugars compete with other sinks and can affect growth (Monneveux and Belhassen, 1996). The decrease in leaf osmotic potential may be due to the accumulation of osmolytes (cellular compatible solutes), which are direct products of photosynthesis (soluble sugars) (Monneveux and Belhassen, 1996). As a result, we concluded that the increase in leaf osmotic potential of the other cultivars was attributed to an increase in salt level. Generally, there is substantial evidence that glycophytic as well as halophytic species adjust to high salt concentrations by lowering tissue osmotic potential with an increase in inorganic ions from external solution and/or compatible solutes (Cachorro et al., 1995). Osmotic adjustment helps to maintain shoot functioning (Morgan, 1995).

Our results show that cultivar HRS 516 exhibited lower Na uptake than the others while RO21 had comparatively, the highest Na uptake. This suggests that HRS 516 is a more resistant cultivar because common bean is known to exclude Na^+ from the shoot by re-absorption of Na+ from the xylem, but takes up Cl^- in proportion to external NaCl concentrations (Jacobi and Ratner, 1974). The cultivar (RO21) with the highest Na uptake had a low survival rate with distinct visual symptoms of salinity damage. This observation tends to confirm the report which identified correlations of high shoot Na concentrations with shoot damage as a physiological marker during screening for salinity tolerance (Dasgan et al., 2002).

The low survival rates noticed for other cultivars could be explained by the fact that high concentrations of these sodium ions in the protoplasmic constituents not only effectively inhibit metabolic functions (Hartung et al., 1998), but also result to high viscosity in the cell, therefore increasing the chances of molecular interactions that cause protein denaturation and membrane fusion (Hartung et al., 1998). One interesting phenomenon about *Phaseolus* is that it tends to show signs of salinity shock at the 5th day of salt exposure and recovery in case of the salt tolerant cultivar (HRS 516).

The results here is unlikely in favor of findings of Bayuelo-Jiménez (2002a) and others that most of the cultivars of *Phaseolus vulgaris* compared to theirs wild relatives were sensitive to salinity stress because the response of HRS 516 to salinity by maintaining high dry weight and a low Na^+ concentration in shoot tissues is a unique characteristic in cultivated beans. Thus, this provides more evidence that some of the cultivated cultivars of common bean in Sudan have substantially higher degree of tolerance to salinity. This is probably due to wide crosses with wild relatives for disease resistance. These retrogressed disease resistant traits, therefore, might also be of multiple or diverse importance to other environmental stresses such as salinity.

However, to evaluate physiological and morphological responses of locally adapted common bean varieties to salinity stress, we suggest more robust methodologies, in terms of time and resources, for screening common bean for salinity tolerance. These include physiological markers such as survival rates, ion concentrations, shoot and root dry weight, shoot/root ratio and relative growth rate (RGR_t) as essential parameters for screening for salinity. However, other morphological characters like plant height, number of leaves, leaf area, and root length and density are difficult to correlate to salinity tolerance where cultivars have different growth pattern.

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