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COMPARATIVE EFFICACY OF DIFFERENT TECHNIQUES TO STUDY THE EFFECT OF HYPOXIA AND SALINITY IN WHEAT

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Four different media: matrix culture, sa~d culture: hydroponic culture (N?-~ubbling) and h~~roponic culture (surface sealing) were used to study the effect of hYPOXiaand salinity on two wheat vaneties (Pb.-85, salinity and waterlogging tolerant; 7-Cerros. salinity and waterlogging sensitive), In matrix and sand culture, hypoxia was induced by water standing continuously up to the surface of pot, whereas in case of hydroponic culture, hypoxia was induced by: (a) continuous bubbling of N_2 gas into the solution (b) surface sealing through effective covering of solution with foam sheet by sealing it from all sides using packing tape. Sprouted seeds of wheat varieties were transplanted to fibre glass pots in the case of matrix and sand culture. while in hydroponic culture 10-day old seedlings ofthe varieties, raised in silica gravel, were transplanted to foam plugged holes in polystyrene sheet suspended over 25 litres of half strength Hoagland nutrient solution contained in plastic tanks. In matrix and sand culture, salinity stress was imposed at two leaf stage, whereas in hydroponic culture salt was added three days after transplanting of seedlings. Total salinity (*100 mol m^{-3} NaCI + 10 mol m^{-3} $CaCl_2$) was developed in tour equal increments in all the media. After 8 weeks of growth period harvesting was carried out. Results revealed that all the techniques used to create hypoxic environment were positively correlated with each other, Although there was a positive correlation among techniques, growth data (shoot and root fresh weight) showed more pronounced adverse effects of hypoxia only in the case of matrix culture indicating that matrix culture is the appropriate medium for such studies,

INTRODUCTION

Water-logging and salinity are the major constraints in crop production throughout the world, Waterlogging of the soil may be the result of over-irrigation, seepage from irrigation channels. mismanagement of irrigational waters, movement of water in underground aquifers or by flood control and irrigation supply dams, Pakistan is predominantly situated in the arid/semi-arid region, where evapotranspiration far exceeds the precipitation. The secondary salinization in Pakistan is primarily associated with poor management of the irrigation system, For example, low irrigation application of water has contributed a lot to salt accumulation, as one cusec water is generally applied for 135 hectares in Pakistan compared with 40 hectares or less in western parts of the United States and other countries with similar climatic conditions, As a result, irrigation water cannot wash down the salts below the root zone, which then accumulate in the surface soil. Dual stress of waterlogging and salinity for winter crops is common in salt-affected areas under rice-wheat cropping system and is a major field problem for low lying areas and sodic soils as well. Therefore, screening against the dual stress is imperative for successful cropping in such areas pending the reclamation solution of these soils. Different techniques are available to induce hypoxic stress, but which technique i~ more appropriate is yet to be established, Each technique has its own merits and demerits. In the past a number of techniques had been tried for rapid screening against salinity (Kingsbury and Epstein, I986;Qureshi et al.,1990; Aslam et al., 1993), Similarly, waterlogging conditions have been simulated through nitrogen bubbling in hydroponic culture so that dissolved oxygen could be removed (Drew. et al., 1980b), stagnating the nutrient medium by surface sealing in hydroponic culture so that no gas exchange may take place through the medium (Justin and Armstrong, 1987), or using matrix culture, and sand culture where hypoxic treatments remain saturated. All these techniques to develop hypoxic conditions can be questioned on different accounts. For example, in the case of N_2-bubbling, the gases formed during anaerobic respiration such as methane and ethylene are bubbled out from the medium (Armstrong and Gaynard,1976). Moreover. there is no rhizosphere which allows reabsorption of hormones and Fe etc. In surface sealing gas may diffuse during the maintenance of EC and pH. Thus, oxygen gradients do exist in this case which complicate results. In matrix and sand cultures, it is difficult to control oxygen and salinity. Furthermore, in sand culture, the aerobic treatments are also a bit saturated, because it does not discharge completely during recharging of the solution, Therefore. it seems appropriate to test these methods and develop a rapid and reliable technique for screening a large number of advanced lines/varieties developed by breeders against waterlogging, salinity and waterlogging x 'salinity interactions. This study was planned to select the most appropriate technique for screening of wheat cultivars against the dual stress, individually and combined together, that may correlate well with the results obtained under field conditions.

MATERIALS AND METHODS

Hydroponic Culture (N_2-bubbling): Seedlings of the two wheat varieties Pb,-85 (tolerant to waterlogging and salinity) and Siete Cerros (7-Cerros) (sensitive to both stresses), were germinated in silica gravel (4 mm dia). For first three days. a solution having 2 mol m^{-3} $Ca(NO_3)_2$ and I mol m^{-3} $MgSO_4$ was applied and thereafter canal water was applied till the emergence of two leaves. After 10 days, the seedlings were transplanted to foam plugged holes in polystyrene sheets suspended over 25 litres of half strength Hoagland nutrient solution (Hoagland and Arnon, 1950) contained in plastic tanks (58 x 40 x 15 cm) supported on iron stand 80 cm above ground. All salinity and anaerobiosis treatments Le. non-saline aerobic (control) hypoxic, saline (100 mol m" NaCI + 10 mol m" $CaCI_2$), and saline-hypoxic treatments were completely randomized while varieties were split under each of these treatments. Twenty plants (two per hill) of each variety were grown in each split. Average of two plants in one hole was treated as one replicate. Three days after transplanting, salts (NaCI + $CaCI_2$ in the ratio of 10:I) were added to salinize the medium in increments of 25 mol m" NaCI and 2.5 mol m" $CaCI_2$ respectively for 24 hr to achieve the final salinity level. Hypoxia was induced in hypoxic treatments by discharging the solution with continuous supply of nitrogen gas (Nj-bubbling), while in aerobic treatments, the solution was air bubbled continuously with air compressor. The substrate solutions were changed weekly. In the case of hypoxic treatments it was changed after bubbling nitrogen. EC and pH of solutions were checked and maintained daily. To check the hypoxic conditions the oxygen concentration was also measured by means of oxygen meter (Table I). Fresh weight of shoot and root and tillering capacity were recorded at the time of harvest after 8 weeks of growth. At this stage, the youngest fully expanded leaves were preserved in Eppendorf tubes for extracting sap to determine Na', K+ and Cl" concentrations. Shoot dry weight was recorded after drying in an oven at 70 C.

Hydroponic Culture (surface sealing): The procedure followed was the same as given in hydroponic culture (N_2-bubbling) except that the hypoxic conditions were created by effectively covering the treatment solution in the large plastic containers with foam sheet and sealing from all sides with a packing tape. The seedlings were held in the holes made in the foam sheet. The substrate solutions were changed when required after nitrogen bubbling for replacing oxygen. The oxygen concentration was measured regularly during the experimental period. The observations and record of growth parameters were made in the same way as in case of hydroponic culture (Nj-bubbling).

Matrix Culture: Sprouted seeds of Pb.-85 and Siete Cerros (7-Cerros) were transplanted in 16 fibre glass pots with 10 L capacity having two holes, one near the top to remove the excess' solution and one near the bottom to completely remove the solution for replacement. Each pot was filled with a mixture of gravel (4 mm dial and vermiculite in the ratio of J: I by vol~ne, while I cm thick layer ofvermiculit~ was spread at the surface and solution of 2 mol m" Ca(NO')2 and I mol m" $MgSO_4$ was sprinkled to incubate the sprouts for optimum growth. The same solution was sprinkled daily to moisten the vermiculite till all the plants hacf produced their-first leaf. Half strength Hoagland nutrient solution was applied to provide complete nutrition to the plants. Seedlings were thinned to five plants pori, while two pots per treatment were used for each variety.

Two levels of salinity viz. nonsaline and saline (l00 mol m" NaCI + 10 mol m" $CaCI_2$) and two levels of anaerobiosis Le. aerobic and hypoxic were developed. Salinity stress was imposed at the two leaf stage. The stress was imposed gradually i.e. in 25 mol m" NaCI-+ 2.5 mol m" $CaCI_2$ daily increments till the final salinity level was achieved. Hypoxia was induced the next day after salinity was completed. To develop hypoxic conditions, the lower hole of the pot was plugged and respective solutions were kept standing in the pots. The substrate solutions were changed at weekly interval. In the case of aerobic treatments, respective solutions (both saline and nonsaline Hoagland solutions) were recharged daily by adding the solutions carefully and draining the excess solution through the holes at the lower side of the pots. Oxygen concentration was measured and recorded (Table I). Plants were harvested after eight weeks of growth. Shoot fresh weight, root fresh weight and tillering capacity were recorded. The youngest fully expanded leaves were preserved in Eppendorf tubes at freezing temperature for extracting the leaf sap to determine Na, K and Cl" concentrations. Shoot dry weight was recorded after drying in an oven at 70°C",

Sand Culture: The procedure was similar to th~~e used in, the case of matrix culture except that silica sand waSU-seaas--= the medium of growth instead of gravel vermiculite mixture. The development of salinity and hypoxic conditions. the method of harvesting, measurement of growth parameters and determination of ionic concentrations were all followed as in the case of matrix culture.

RESULTS

Growth: Shoot fresh weight (SFW) of both the varieties decreased in all the treatments compared with control (Table 2). The average reduction of two varieties was 15.37%,57.97% and 70.68% in hypoxia. saline and saline-hypoxic treatments respectively. The data showed that the effects of hypoxia and salinity differed in intensity in both the varieties. Hypoxia alone decreased the SFW of Pb.-S5 significantly in matrix culture. hydroponic culture (N_2-bubbling), whereas there was no significant increase in hydroponic culture (surface sealing). There was a significant reduction in SFW of 7-Cerros with all the techniques. Hypoxia combined with salinity significantly reduced SFW in both the varieties. Salinity alone (100 mol m,1 NaCI + 10 mol m" $CaCI_2$) significantly reduced the SFW of both the varieties with all the techniques. When salinity was combined with hypoxia. it further decreased the SFW in both the varieties but this reduction was significant only in matrix culture. Comparison of salinity and hypoxia alone showed. that salinity caused significantly greater reduction compared to hypoxia in both the varieties for all the techniques.

A comparison among the varieties revealed that Pb.-85 performed better both under saline and hypoxic stresses. Under saline treatment, Pb.-85 significantly produced higher SFW compared with 7-Cerros in matrix and hydroponic culture (surface sealing), while there was nonsignificant increase in rest of the techniques. Under hypoxic stress, Pb.-85 significantly produced more SFW with all the techniques. Under the dual stress, again Pb.-85 produced significantly more SFW compared with 7-Crrros in matrix culture, while in other techniques nonsignificant increase was observed. The degree of correlation among the techniques for developing hypoxia/salinity in terms of effect on fresh weight production of wheat was determined to decide about the appropriateness of a particular technique for routine use as a screening procedure. The correlation was calculated by making pairs of alike variables of different techniques. Table 6 exhibits correlation coefficient among different techniques which was highly significant in all the techniques compared.

The data presented in Table 3 exhibited that the overall shoot dry weight (SOW) of the two wheat varieties decreased in all the treatments compared with control.. The decrease was almost of similar magnitude as was observed in SFW. It was observed that the effects of hypoxia and salinity differed in their intensity and were more pronounced in the matrix culture. Hypoxia caused significant reduction in SOW of Pb.-8S with matrix culture technique only, while nonsignificant reduction was observed with rest of the techniques. In 7-Cerros nonsignificant reduction in SOW was observed with all the techniques. Salinity alone significantly decreased SOW of both the varieties with all the techniques. In the saline-hypoxic treatment, SOW of Pb.-85 further reduced but significant reduction was only observed in matrix culture and hydroponic culture (surface sealing). while it was nonsignificant with other two techniques. In the case of 7-Cerros the difference between the saline and saline-hypoxic treatment in terms of reduction in SOW was only significant in matrix culture. Table 6 indicates correlation coefficient among different techniques which was highly significant.. The highest correlation 0.841 was observed between matrix culture and hydroponic culture (Ne-bubbling).

The data presented in Table 4 exhibited that the average root fresh weight (RFW) of four cultures (two varieties) was reduced by 17.41%, 56.93% and 67.47% under hypoxic, saline and saline-hypoxic treatments respectively. Hypoxia significantly reduced the RFW of Pb.-85 and 7-Cerros in matrix culture and hydroponic culture (Ne-bubbling), while it was statistically at par with other techniques. however, nonsignificant increase in RFW in Pb.-85 was observed in hydroponic culture (surface sealing). Salinity significantly reduced the RFW of both the varieties with all the techniques. Under the combined stress of hypoxia and salinity. the reduction compared with salinity alone was significant only in matrix culture in both the varieties. Table 6 reflects correlation coefficient among different techniques which is highly significant in all the cases. The highest correlation 0.767 was observed between matrix culture and hydroponic culture (Ne-bubbling).

Table 5 exhibited that hypoxia. slightly promoted the production of tillers in the two wheat varieties with all the techniques. On overall basis hypoxia promoted tillering, while salinity and salinity x hypoxia interaction reduced tiller production compared with control by 49.37% and 56.10% respectively. A comparison among varieties showed that the performance of Pb.-85 in tiller production was better than 7-Cerros under all the stresses. Table 6 shows correlation coefficient with regards to tiller production among various techniques. The highest correlation 0.761 was found between hydroponic culture (Nj-bubbling and hydroponic culture (surface sealing).

Ionic Concentration: The data summarized in Table 7 showed the effects of salinity, hypoxia and salinity x hypoxia interaction on Na+ concentration in the youngest fully expanded leaf of two wheat varieties. The comparison of treatment means revealed that the highest accumulation was observed in case of combined stress followed by salinity> hypoxia> control.. The overall accumulation of Na under salinity was 3.3 x compared with hypoxic treatment.. Under dual stress, the value was 1.7 x compared with saline treatment and 5.7 x compared to hypoxic treatment.. The results indicated that the effect of aerobic and hypoxic salinity for accumulation of Na' was additive. The varietal comparison showed that 7-Crrros accumulated significantly more concentration of Na+ compared with Pb.-8S in saline and saline hypoxic treatments with all the techniques. whereas accumulation of Na+ was slightly more in 7-Cerros within hypoxia and control treatments.

Data given in Table 8 indicated that hypoxia and salinity altered the concentration of K+. A comparison of treatment means showed that maximum accumulation of K' was observed in saline treatment followed by control. hypoxic and saline-hypoxic treatments. The varietal comparison showed that Pb.-85 had greater accumulation of Kt under all the stresses. Under hypoxia alone. Pb.-8S accumulated significantly more concentration of K' with all the techniques except in sand culture where nonsignificant increase was observed. In saline treatment, Pb.-85 accumulated significantly more K' compared with 7-Cerros in all the techniques. The performance of Pb.-85 towards accumulation of K+ compared to 7-Cerros was found better under combined stress of salinity and hypoxia.

Table '9 showed that hypoxia, salinity and the dual stress increased Cr concentration. The maximum Cr concentration was observed in saline hypoxic> saline> hypoxic> control treatments. The accumulation of Cr was almost of the same magnitude as was observed in accumulation of Na' in the youngest fully expanded leaf. Under hypoxia alone 7-Cerros had more .but nonsignificant concentration of Cr than Pb.-85. Under both aerobic andhypoxic - salinity. 7-Cerros had significantly higher concentration of Cr compared with Pb.-85 with all the techniques.

DISCUSSION

Role of the Culture Medium in Determining the Growth Response of Wheat: The comparison among different techniques employed for the development of hypoxia/salinity exhibited that maximum shoot fresh weight was produced by matrix culture followed by hydroponic culture (surface sealing) > hydroponic culture (Ne-bubbling) > sand culture. which reflects that the medium used also played an important role in such studies.

In general, when hypoxia was induced, the reduction in growth of wheat was not so severe. In the case of hydroponic culture (N_2-bubbling), gases formed during anaerobic respirations are bubbled out by the system (Armstrong and Gaynard, 1976). Moreover, there is no rhizosphere which allowed reabsorption of toxics, hormones, However, in the case of hydroponic culture (surface sealing), apart from the problem of rhizosphere, gases may diffuse during the maintenance of EC and pH of the solution. In sand culture, recharging and changing of the solution took a long time during draining which complicated the results. Furthermore, the effect of hypoxia was more pronounced in matrix culture compared to other techniques. In view of the performance in matrix culture and problems of hydroponic culture and sand culture. the matrix culture technique was considered to be more appropriate for screening of wheat genotypes. The effect of hypoxia alone and along with salinity was more severe in case of 7-Cerros compared with Pb.-85,which produced more SFW with all the techniques reflecting better tolerance ofPb.-85 to oxygen deficiency than 7-Cerros.

The salinity also reduced the shoot growth. The reduction by salinity alone was more severe than with hypoxia alone. Hypoxia in the presence of salinity caused more reduction in shoot fresh weight compared with control, but the difference of combined stress with salinity alone was only significant in matrix culture technique which again reflected a more severe effect in matrix culture only; such differences were nonsignificant with rest of the three techniques. In saline treatment. the performance of Pb.-85 was better than 7-Cerros with all the techniques. Under the combined stress, again Pb.-85 significantly produced more shoot fresh weight in matrix culture only, whereas nonsignificant increase was observed with rest of the techniques. This reflected that the differences in growth due to salinity varied with the medium used for growth; matrix culture made the differences more prominent in this case.

The reduction in shoot dry weight with different techniques was almost of similar magnitude as was observed in the case of SFW. The ratio between SFW and SOW was almost 8%, 7%. 9% and 9% with the matrix culture, sand culture, hydroponic culture (N_2-bubbling) and hydroponic culture (surface sealing) respectively, which indicated the difference in tissue water contents. The ratio in SFW and SOW was wide in case of hydroponic system which indicated more succulence of plants grown in this system which would mean less severe effect on plant growth. This may be an important consideration in the application of results obtained in hydroponic system directly in the field. Hypoxia caused reduction in SOW with all the techniques but significant differences were observed, in both the varieties with matrix culture technique only. Under dual stress, the significant differences compared with salinity were only observed in the matrix culture technique under both the varieties.

The root fresh weight did not follow the pattern of shoot weight. However, the effect of hypoxia was more pronounced in the matrix culture, while the detrimental effect of dual stress was significant with the matrix culture technique only. Tillering was promoted by the induction of hypoxia in all the techniques except sand culture. It might be due to more number of adventitious roots formation under hypoxic conditions leading to an increase in the number of tillers as well (Nawaz, 1993). However. hypoxia combined with salinity reduced the tillering production. Pb.- 85 produced more tillers in all the treatments with all the techniques compared with 7-Cerros. These trends show that shoot and root weights are better indicators than the tillering capacity to study the interactive effect of hypoxia and salinity on wheat genotypes and suitability of a technique.

The ionic concentration of Na", K+ and Cr of all the plants grown in different treatments using different techniques were also determined. In general, plants grown in sand culture had lesser Na' and K+ concentration than with hydroponic system and matrix culture, especially in the salinity treatments. while Na" concentration in Pb.-85 was significantly higher in matrix culture than all others. Cr concentration was generally higher in sand culture and lower in hydroponic culture, again indicating the cultural specific efTects. It was observed that compared to 7-Cerros, Pb.-85 had lesser concentration of Na' and Cr and more of K' in all the treatments with all the techniques. This trend corresponds well with the earlier studies of Greenway (1965) in barley. Yeo and Flowers (1982) in rice, Marcar and Termaat (1990) and Schachtman and Munns (1992) in wheat and in eucalyptus. They showed that less Na and high K was related to better tolerance of the species/varieties. The increase in salinity increased the accumulation of Na' in the leaves almost with all the techniques. It was also observed that K· concentration decreased both with hypoxia and hypoxic-salinity, in both the varieties with all the techniques. while its concentration was increased with the salinity alone in Pb.-85. This could be due to genetic difference in the two wheat varieties and this is in line with most of the earlier reports (Nawaz, 1993; Akhtar, 1995). In generaL the effect of hypoxia at seedling stage of wheat was not as pronounced as of salinity. Therefore, the selection of growth medium capable of highlighting the differences in varietal response was crucial for routine use for rapid screening. It has been observed that correlations in terms of growth parameters i.e. shoot fresh weight, shoot dry weight, root fresh weight and tillering capacity with all the techniques was highly significant (Table 6), indicating that probably anyone of the above techniques for screening of wheat against hypoxia could be used successfully.

It has been discussed earlier that detrimental effects of hypoxia alone and hypoxic salinity were significant in both varieties only with the matrix culture technique. which reflected that this technique could elaborate the differences more successfully in wheat growth due to hypoxia and was thus more appropriate for screening of wheat genotypes. The superiority of matrix culture over other techniques was due to the presence of vermiculite that adsorbed nutrients from the solution and root exudates as well, so that the conditions

Table 1. Levels of oxygen (mg L-1) in aerobic/hypoxic treatments of different techniques during the growth period of wheat

Treatments	Matrix culture	Sand culture	Hydroponic culture (N z-bubbling)	Hydroponic culture (surface sealing)
Control	4.08*	2.88	6.09	6.97
Hypoxic	2.23	2.15	1,92	2.10
Saline	3.88	3.05	7.15	7.19
Saline-hypoxic	2.15	2.08	1,97	2.15

* Average of four readings.

Table 2. Effect of hypoxia and salinity developed with different techniques on shoot fresh weight (g plant") of wheat

Treatments	Varieties	Matrix culture	Sand culture	Hydroponic culture (N2.bubbling)	Hydroponic culture (surface sealine;)	Mean absolute yield	Relative yield (% of control)
Control	Pb.-85	51.87ati'	35.37 a	43.20 a	42.20 a	43.160	100.00
"	7-Cerros	51.50 a	33.42 a	40.35 ab	40.11 a	41.34	100.00
Hypoxic	Pb.-85	43.75 b (84.33)	32.56 a (92.03)	38.71 b (89.61)	4261 a(100.99)	39.41	9130
"	7-Cerros	36.94 c (71.73)	28.40 b (84.96)	30.52 c (75.65)	32.58 b (8122)	32.11	77.67
.Saline	Pb.-85	34.52 c (66.54)	1394 c (39.41)	14.63 d (33.87)	18.09 c (42.86)	20.29	47.02
"	7-Cerros	28.73 d (55.79)	12.11 cd (36.23)	9.99 de (24.75)	10.08 d (25.12)	15.23	36.83
.Saline-hypoxie	I'b.-85	20.79 e (40.07)	12.60 cd (35.62)	13.60 de (31.46)	13.82 cd (32.74)	15.20	35.21
"	7-Cerros	11.93 f(23.17)	9.21 d (27.54)	8.69 e (2154)	8,47 d (21.12)	9.58	23.16
Mean		35.00.	22.20	24.94	25.99		

• NaCl 1001110lm ' + eaCh 10 mol m-5, (t average of 10 values,: average ot 40 values,: average of80 values.
Values in parentheses indicate percent of control; means with different letters differ significantly according to DMRT (I' = 0.05).

Table 3. Effect of hypoxia and salinity developed with different techniques on shoot dry weight (g plant") of wheat

Treatments	Varieties	Matrix culture	Sand culture	Hydroponic culture (N2_ bubbling)	Hydroponic culture (surface sealing)	Mean absolute yield	Relative yield (% of control)
Control	I'b.-85	6. 78 a';;:'	465 a	4.44 a	4.69 a	5.14#	100.00
"	7-Cerros	6.72 a	4.71 a	4.36 a	4.42 a	5.05	100.00
Hypoxic	I'b.-85	5.70 b (84.06)	4.39 a (94.37)	4.07 ab (90.55)	4.26 a (90.97)	4.59	8937
"	7-Cerros	4.98 c (74.19)	4.06 a (86.18)	b (80.92)	3.48 b (78.75)	4.01	78.74
.Saline	I'b.-85	3.96 d (58.38)	2.57 b (55.30)	2.08 c (46.85)	2.75 b (58.69)	2.84	55.27
"	7-Cerros	2.87 e (42.65)	193 be (4108)	125 de (28.63)	1.44 cd (32.55)	187	3673
.Saline-hvDoxie	I'b.-.85	2.62 e (38.67)	2,30 b (49.49)	169 cd (38.06)	1.87 c (39.88)	2.12	41.26
"	7-Cerros	1151'(17.10)	1.50 c (3188)	0.93 e (21.35)	089 d (2024)	1,12	21.95
Mean		4.35 ••••	3.26	2.78	2.98		

• NaCl I(){)mol m ' + CaCh I() 1110In1'";~, average ot 10 values; • average of 40 values; • average ot 80 values.
Values in parentheses indicate percent of control; means with different letters diner. significantly according to DMRT (I' = 0.05).

Table 4. Effect of hypoxia and salinity developed with different techniques on root fresh weight (g plant") of wheat

Treatments	Varieties	Matrix culture	Sand culture	Hydroponic culture (N2- bubbling)	Hydroponic culture (surface sealing)	Mean absolute yield	Relative yield (% of control)
Control	Pb.-85	12.78 ati	10.63 a	12.84 a	10.91 ab	11,79<ll'	I00.{)0
"	7-Ccrros	12.65 a	10.17 a	12.59 a	10.84 ab	11,56	100.00
Hypoxic	I'b.-85	9.72 b (76.07)	8.51 a(80.10)	10.56 b (82.21)	1183 a (108.40)	10.16	86.13
"	7-Cerros	9.52 b (75.22)	8.59 a (84.49)	9,35 b (74.23)	906 b (83.63)	9.13	78.98
.Saline	Pb.-85	6.14 c (48.02)	4.63 b (43.60)	6.65 c (51.76)	5.91 c (54.15)	5.83	49.4 7
"	7-Cerros	5.44 cd (43.02)	3.87 b (35.42)	4,41 de (35.02)	3.18 d (29.31)	4.22	36.54
.Saline-hvpoxic	I'b.-85	4.18 de (32.72)	3,38 b (31.82)	5.55 cd (43.24)	4,47 cd (40.92)	4.40	37.28
"	7·Cerros	3.26 e (25.79)	3.03 b (2977)'	3.29 e (26.13)	3.22 d (29.70)	3.20	27.68
Mean		7.96.	6.60	8.15	7.43		

• NaCI 100 mol m-3 + $CaCl_2$ 10 mol m-3,~average of 10 values, • average of 40 values, • average of 80 values.
Values in parentheses indicate percent of control; means with different letters differ significantly according to DMRT (P '=0.05).

Table 5. Effect of hypoxia and salinity developed with different techniques on the tillering capacity of wheat

Treatments	Varieties	Matrix culture	Sand culture	Hydroponic culture (N2-bubblinlt)	Hydroponic culture (surface sealin!!;)	Mean absolute yield	Relative yield (% of control)
Control	Pb.-85	9.70 a(x	7.20 a	9.80 a	9.40 a	9.030	10000
"	7-Cerros	7.30 b	6.00 ab	6.90b	7,30 b	6.88	10000
Hypoxic	Pb.-85	10.20 a(l05.15)	6,50 ab (90.28)	10,30 a (105.10)	10.80 a (114.89)	9.45	10465
"	7-Cerros	740 b(101.37)	5.70 b (9500)	720 b (104.35)	7.00 b (95.89)	6.83	99.27
.Saline	('b.-85	5.10 c (52.58)	4.20 c (58.33)	5,30 c (54.08)	6.20 b (6596)	5.2	57.59
"	7-Cerros	3.90 c (53.42)	2.80 d (46.47)	2.20 d (31.88)	2,50 c (34.25)	2.85	:l1·Q
.Saline-hypoxic	Pb.-85	4.00 c (41.24)	3.80 cd (52.78)	5.00 c (5102)	5.80 b (61.70)	4.65	SUO
"	7-Cerros	210d(2877).	2.70 d (45.00)	2.30 d (33.33)	2.2 c (30.14)	2.33	3387
Mean		6.210	4.86	6.13	6.4		

• NaCI 100 mol m-3 + CaCh 10 mol m''; (z average of 10 values; • average of 40 values; (t average of 80 values. Values in parentheses indicate percent of control; means with different letters differ significantly according to DMRT (P = 0.05).

Table 6. Relationship among different growth parameters under various techniques used for the development of hypoxia and salinity

Variables (growth medium)			Growth parameters	Correlation coefficients
MC	vs	SC	Shoot fresh weight	0.829**
MC	vs	HCN	"	0.846**
MC	vs	HCS	"	0.789**
SC	vs	HCN	"	0.890**
SC	vs	HCS	"	0.821 **
HCN	vs	HCS	"	0.812**
MC	vs	SC	Shoot dry weight	0.816**
MC	vs	HCN	"	0.841**
MC	vs	HCS	"	0.820**
SC	vs	HCN	"	0.777**
SC	vs	HCS	"	0.762**
HeN	vs	HCS	"	0.828**
MC	vs	SC	Root fresh weight	0.758**
MC	vs	HCN	"	0.767**
MC	vs	HCS	"	0.641 **
SC	vs	HCN	"	0.693**
SC	vs	HCS	"	0.592**
HCN	vs	HCS	"	0.681 **
MC	vs	SC	Tillering capacity	0.646**
MC	vs	HCN	"	0.756**
MC	vs	HCS	"	0.709**
SC	vs	HCN	"	0.628**
SC	vs	HCS	"	0.648**
HCN	vs	HCS	"	0.761**

MC = Matrix culture; SC = Sand culture; HCN = Hydroponic culture (N^2-bubbling); HCS = Hydroponic culture (Surface sealing); ** = Highly significant.

Table 7. Effect of hypoxia and salinity developed with different techniques on Na^+ concentration (mol m^{-3}) in the youngest fully expanded leaf of wheat varieties

Treatments	Varieties	Matrix culture	Sand culture	Hydroponic culture (N_2-bubbling)	Hydroponic culture (surface sealing)	Mean
Control	Pb.-85	8.06 e	8.71 d	7.92 d	7.99 d	8.17
"	7-Cerros	8.97 e	9.83 d	8.94 d	8.89 d	9.16
Hypoxic	Pb.-85	8.77 e	8.79 d	8.40 d	8.69 d	8.67
"	7-Cerros	9.34 e	9.74 d	9.45d	9.13 d	9.41
Saline	Pb.-85	26.28 d	20.76 c	27.75 c	28.86 c	25.91
"	7-Cerros	37.17 b	25.44 b	38.76 b	36.86 b	34.56
Saline-hypoxic	Pb.-85	34.89 c	23.82 b	40.65 b	38.40 b	34.44
"	7-Cerros	70.19 a	38.35 a	85.16 a	82.16 a	68.97
Mean		25.46*	18.18	28.39	27.62	

• NaCl 100 mol m^{-3} + $CaCl_2$ 10 mol m^{-3}; average of 10 values; • average of 40 values; ••• average of 80 values.
Values in parentheses indicate percent of control; means with different letters differ significantly according to DMRT (P = 0.05).

Table 8. Effect of hypoxia and salinity developed with different techniques on K^+ concentration (mol m^{-3}) in the youngest fully expanded leaf of wheat varieties

Treatments	Varieties	Matrix culture	Sand culture	Hydroponic culture (N_2-bubbling)	Hydroponic culture (surface sealing)	Mean
Control	Pb.-85	127.47 b	104.28 b	134.78 ab	137.95 ab	126.12
"	7-Cerros	122.77 b	98.60 b	128.36 ab	131.96 ab	120.42
Hypoxic	Pb.-85	121.95b	97.59 b	125.47 ab	130.15 ab	118.79
"	7-Cerros	104.52 c	91.06 b	101.01 c	109.19 cd	101.45
Saline	Pb.-85	137.86 a	123.69 a	143.02 a	146.15 a	137.68
"	7-Cerros	120.74 b	103.54 b	122.10 b	125.36 bc	117.94
Saline-hypoxic	Pb.-85	100.66 c	92.85b	101.18c	101.98de	99.22
"	7-Cerros	81.00 d	66.82 c	82.52 d	87.18 e	79.38
Mean		114.62 •••	97.30	117.33	121.24	

• NaCl 100 mol m^{-3} + $CaCl_2$ 10 mol m^{-3}; average of 10 values, • average of 40 values, ••• average of 80 values.
Values in parentheses indicate percent of control; means with different letters differ significantly according to DMRT (P = 0.05).

Table 9. Effect of hypoxia and salinity developed with different techniques on Cl^- concentration (mol m^{-3}) in the youngest fully expanded leaf of wheat varieties

Treatments	Varieties	Matrix culture	Sand culture	Hydroponic culture (N_2-bubbling)	Hydroponic culture (surface sealing)	Mean
Control	Pb.-85	48.68 f	57.63 cd	48.12 d	43.66 d	49.520
"	7-Cerros	55.09 ef	64.67cd	58.85 cd	54.42 d	58.26
Hypoxic	Pb.-85	50.93 ef	55.90 d	49.25 d	44.35 d	50.11
"	7-Cerros	57.29 e	64.56 cd	59.83 cd	55.21 d	59.22
Saline	Pb.-85	70.52 d	72.99 c	68.36 c	67.80 c	69.92
"	7-Cerros	88.00 c	97.36 b	88.20 b	90.37 b	90.98
Saline-hypoxic	Pb.-85	94.69 b	96.78 b	98.78 b	97.62 b	96.97
"	7-Cerros	128.43 a	188.31 a	130.60 a	134.23 a	117.89
Mean		74.20*	78.53	75.25	73.46	

• NaCl 100 mol m^{-3} + $CaCl_2$ 10 mol m^{-3}; average of 10 values, • average of 40 values, ••• average of 80 values.
Means with different letters differ significantly according to DMRT (P = 0.05).

become closer to the field situation (Drew and Sisworo, 1979; Trought and Drew, 1980. At an early stage of hypoxia, shoot and root growth can appreciably be damaged simply as a consequence of inadequate supply of oxygen to the root. In vermiculite gravel + culture, further damage may be caused due to accumulation of some solutes, or gases at injurious concentrations as found in the soil (Drew and Lynch. 1980a). Thus, matrix culture (vermiculite + gravel) being. simpler. easier to work with and more effective in highlighting the differences in varietal response, is considered more appropriate for such studies.

REFERENCES

Akhtar, J. 1995. The response of wheat *(Triticum aestivum L.)* and wheat *Thinopyrum Amphiploids* to the interactive effects of salinity and hypoxia. Ph.D. Thesis, University of Agriculture, Faisalabad, Pakistan.

Arrnstrong, W. and TJ. Gaynard. 1976. The critical oxygen pressures for respiration in intact plants. Physiol. Plant. 37: 200-206.

Aslam, M., R.H. Qureshi, and N. Ahmad. 1993. A rapid screening technique for salt tolerance in rice *(Oryza sativa* L.). Plant Soil, 150:99-107.

Drew, M.C. and EJ. Sisworo. 1979. The development of waterlogging in young barley plants in relation to plant nutrient status and changes in soil properties. New Phytol. 82:301-314.

Drew. M.C. and J.M. Lynch. 1980a. Soil anaerobiosis, microorganisms and root function. Ann. Rev. Phytopathol. 18:37-66.

Drew. M.C.. A. Charnel, J.P. Garrecand A. Fourcy. 1980b. Cortical air spaces (aerenchyma) in roots of corn subjected to oxygen stress. Plant Physiol.. 65:506-511.

Greenway, H. 1965. Plant responses to saline substrates VII. Growth and ion uptake throughout plant development in two varieties of *Hordeum vulgare.* Aust. J. Biol, Sci. 18:763-779.

Hoagland. D.R. and D.1. Arnon. 1950. The water culture method for growing plants without soil. Calif. Agri. Exp. Stn. Circ. No.347. 39 pp.

Justin, S.H.F.W. and W. Armstrong. 1987. The anatomical characteristics of roots and plant response to soil flooding. New Phytol. 106:465-495.

Kingsbury, R.W. and E. Epstein. 1986. Salt sensitivity in wheat. I. A case for specific ion toxicity. Plant Physiol. 80:651-654.

Marcar, N.E. and A. Termaat.. 1990. Effects of root zone solutes on *Eucalyptus cama/dulensis* and *Eucalyptus bicostata* seedlings: Responses to Na+, Mg^{2+} and Cr. Plant Soil, 125:245-254.

Nawaz, S. 1993. Salinity x hypoxia interaction in wheat. Ph.D. Thesis, University of Agriculture, Faisalabad, Pakistan.

Qureshi, R. H., A. Rashid and N. Ahmad. 1990. A procedure for quick screening of wheat cultivars for salt tolerance. In Genetic Aspects of Plant Mineral Nutrition (Ed: N. El Bassam, M.Damborth & B.C. Laughman). Kluwer Academic Publishers, Netherland. 315-324.

Schachtman, D.D. and R. Munns. 1992. Sodium accumulation in leaves of *Triticum* species that differ in salt tolerance. Aust. J. Plant Physiol.. 19:331-340.

Trought, M.C.T. and M.C. Drew. 1980. The development of waterlogging damage in wheat seedlings *(Triticum aestivum* L.). I. Shoot and root growth in relation to changes in the concentrations of dissolved gases and solutes in the soil solution. Plant Soil, 54:77-94.

Yeo, A.R. and TJ. Flowers. 1982. Accumulation and localization of sodium ions within the shoot of rice *(Orl':=a sativa* L.) varieties different in salinity resistance. Physiol. Plant. 56:342-348.