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A NEW RAPID AND SIMPLE METHOD OF SCREENING WHEAT PLANTS AT EARLY STAGE OF GROWTH FOR SALINITY TOLERANCE

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Abstract

As efficient, reproducible, economical and simple mass screening technique for the selection of salt tolerant wheat genotypes has been developed. This method is able to identify genetic variation in salinity tolerance in breeding material or in a large number of genotypes of wheat. The germinated seeds of wheat were transplanted in vermiculite filled Japanese paper pots. As the first leaf blade became fully expanded, the Japanese paper pots along with seedlings were transferred to NaCl solution. The NaCl was increased stepwise in two third seedlings, up to the desired salinity levels which were 200 or 100 mol m^{-3} NaCl and one third Japanese paper pots remained only in nutrient solution as control. Two salt tolerant and two salt sensitive cultivars were included in the study.

The plants were grown for 15 days in full strength nutrient solution or at desired NaCl level. The shoot weights were used as estimate of plant salinity tolerance. Shoot fresh weight (SFW), shoot dry weight (SDW), Na^+ and K^+ contents were measured for the assessment of salt tolerance. The validity of this technique was tested by growing the same cultivars at the same salinity levels in hydroponics or in soil filled pots. Salt tolerance of the cultivars was compared in three screening methods. Good reproducibility of the results among three screening methods authenticated the validity of the Japanese Paper Pot technique for the assessment of salinity tolerance at the early stage of plant growth in cereals.

Introduction

As a first step for growing crops in saline soils, the availability of a technique which could effectively and rapidly assess salt tolerance is important. Salt tolerance can be measured by a number of criteria. Munns & James (2003) reviewed a wide range of screening techniques for salinity tolerance. These procedures are laborious, expensive and time consuming. For practical purposes, a good mass screening technique should be efficient, reliable, reproducible and simple. Any or all of these screening methods could conceivably be used as selection criteria, but none of these methods yet has proven wholly reliable or been widely accepted. For screening plant material, a variety of cultural techniques like culture solution salinized by salt (Kingsbury & Epstein, 1984), artificially salinized soil in pots (Sayed, 1985), growing plants in normal soil and irrigation with saline water (Farah & Anter, 1978) have been used. Also an attempt was made to screen genotypes in naturally saline soils (Aslam *et al.*, 1993). However, screening a large number of genotypes for salinity tolerance in the field is difficult, due to spatial heterogeneity of soil chemical and physical properties, and to seasonal fluctuation in rainfall, humidity and temperature (Srivastava & Jana, 1984). On the other hand solution culture does not truly represent field conditions. In general, it has been found that the early stage of plant growth is the most sensitive phase of plant development and most of the research work on salt tolerance in different crop species reported previously (Flower & Yeo, 1981, Kingsbury *et al*., 1984, Bogemans *et al*., 1990, Yeo *et al*., 1990) has been based upon plant assessment made at this stage.

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The lack of reliable large scale screening technique is still a great problem in genetic improvement of salt tolerance of crop plants. Therefore, it has also been endeavored to study the possibility of finding a relatively quick screening method for screening populations of breeding material or a large number of genotypes. Keeping this in view, in these studies an attempt was made to develop a simple technique using Japanese paper pots to screen a large number of plants at early stage of growth for salinity tolerance and to relate the results with those obtained with the other experiments in solution culture or soil culture.

Materials and Methods

These studies were undertaken to develop a suitable method for screening of wheat plants at early stage of growth for salinity tolerance. Three methods, two already known (hydroculture and soil filled pots) and one new (Japanese paper pots) were compared. Plant material consisted of two salt tolerant bread wheat *(Triticum aestivum* L.) cultivars, Chinese Spring and Lu 26 and two salt sensitive spring wheat cultivars, Sonora 64 and Glennson. The salt tolerance ability of these cultivars has been reported in many studies (Qureshi *et al*., 1980, Kingsbury & Epstein, 1984; Rawson *et al*., 1988, Ashraf & McNeilly, 1988) and in our previous studies (data not presented). A split plot design with three replications was followed. The salt treatments were randomized as main plots and cultivars as sub plots. The plants were tested at three salinity levels i.e., 0 (control), 100 and 200 mol m^{-3} NaCl. The plants were grown in a controlled environment cabinet operating at 18±1^{o}C with a 16 h photoperiod provided by fluorescent lighting giving 400 µmol photons m^{-2} s^{-1} and 70% R.H. In these studies shoot weights were used as measure of salt tolerance of genotypes. Results were analysed using Excel software package to calculate correlation and to assess significant differences among salinity levels, genotypes and replications by analysis of variance.

Method 1

Screening using Japanese paper pots: Seeds of each genotype were germinated in Petri dishes on filter paper soaked with distilled water and incubated in the dark at 22^{o}C. After germination the seedlings were transferred to vermiculite-filled Japanese paper pots (Lannen Tehtaat Oy Potma Ltd, Pello, Finland) and irrigated with deionized water. As the first leaf blade became fully expanded, the seedlings along with Japanese paper pots were transferred to half strength culture solution (Hewitt, 1952) for three days and then to full strength solution. The wheat plants grown in Japanese paper pots are shown in Fig. 1. Salt was increased stepwise over five days to two third of the seedlings after which the seedlings were in 100 or 200 mol m^{-3} NaCl solution. $CaCl_2$ was also added to maintain at Na^+:Ca^{2+} ratio of 20:1. One third seedlings of each cultivar were maintained in full strength nutrient solution (control). The concentration of salt was maintained on alternate day until the end of the experiment by dipping the base of Japanese paper pots along with seedlings in freshly prepared solution of the required concentration of NaCl or full strength nutrient solution (control). Leaf 4 of the main stem (MSL4) of the plants of each genotype was sampled for Na^+ and K^+ analysis at 15 days after transfer (DAT) to the final concentration of salt or full strength nutrient solution. For this purpose the leaves were dried at 70^{o}C for 24 h and then ashed in a Muffle furnace at 500^{o}C overnight, cooled and the residue mixed with 1.5 ml of concentrated HNO_3. The samples remained in the HNO_3 for 5 minutes and then 5 ml deionized water was added to the crucibles. The concentrations of Na^+ and K^+ were determined using a flame photometer (Digi Flam, GDV, Italy). The Na^+ and K^+ contents were measured in mol m^{-3} in leaf water. The leaf

Fig.1. Wheat plants grown in Japanese paper pots.

water was calculated from the difference between fresh and dry weights of sampled leaf. On the same day plants were harvested. The fresh weights of the shoots were recorded and after drying at 80^{o}C for 48 h the dry weights were taken. The weight of already sampled MSL4 was added to obtain total fresh and dry weight of plant.

Method 2

Screening using hydroponics: Seeds of each genotype were germinated and were transferred to Japanese paper pots as mentioned in method 1. As the blade of the first leaf became fully expanded, uniform seedlings of each cultivar were transferred to 9L hydroculture containers, with their roots immersed in half strength nutrient solution (Hewitt, 1952). Nutrient solution was made with deionized water and was aerated with the help of an air pump throughout the experiment. Salt was increased stepwise over five days to two third of the seedlings after which the seedlings were in 100 or 200 mol m^{-3} NaCl solution. $CaCl_2$ was also added to maintain at Na^+:Ca^{2+} ratio of 20:1. One third seedlings of each cultivar were maintained in full strength nutrient solution (control). The concentration of salt was maintained until the end of the experiment. The seedlings were supported by 50 mm thick polystyrene sheets in which holes (45 mm diameter) were made at regular intervals to allow the shoots to emerge. The plants were held in position, in the holes, by foam rubber collars. The solution of the containers was changed after one week to maintain the salt concentration or nutrient contents. Fifteen days after transference to salt solution or full concentration of solution Na and K content of MSL4 and shoot fresh and dry weight were measured as mentioned in method 1.

Method 3

Screening using soil pots irrigated with stalinized water: Seedlings were germinated in Petri dishes. The germinated seedlings were transferred to soil filled plastic pots. Nine cm plastic pots (placed on a small Petri dish) were placed in plastic saucers. A wick was passed across the base of each pot to ensure the availability of nutrient solution according to the requirement of the plant. Both ends of the wick were immersed in the solution put in the saucer. The pots were filled with air dried clay loam soil. To ensure sufficient moisture at the time of seedling transfer, three days before transference of the seedlings the saucers were filled with deionized water. Seedlings were irrigated with deionized water till the first leaf was fully expanded. As the first leaf fully matured, two third pots were irrigated with full strength nutrient solution with a gradual addition of salt for five days to give a final concentration of 100 or 200 mol m^{-3} NaCl. $CaCl_2$ was also added to maintain at Na^+:Ca^{2+} ratio of 20:1. The remaining one third pots were with full strength nutrient solution only (control).

During the experiment, on alternate days, the saucers of the pots were filled with full strength nutrient solution or full strength nutrient solution with their respective salt additions. Fifteen days after transference to salt solution or full concentration of solution Na and K content of MSL4 and shoot fresh and dry weight were measured as mentioned in method 1.

Results and Discussion

Means for SFW, SDW, Na^+ and K^+ content are shown in Table 1. SFW and SDW were significantly reduced by salinity. Overall, due to salt, SFW and SDW were more affected under hydroponics than in soil pots or in Japanese paper pots while plants growth in control conditions were similar in all the three screening methods. At the highest salinity treatment the SFW and SDW were worst affected. On the basis of shoot fresh and dry weights under saline conditions, Lu 26 was found to be the most tolerant cultivar included in the study while Sonora 64 was rated as most salt sensitive.

At 200 mol m^{-3} NaCl, the salt tolerant cultivars (Lu 26 and Chinese Spring) gave almost the same SFW when grown in pots containing soil or in the Japanese paper pots. However, when the same cultivars were grown in hydroponics they gave about three to five times less SFW at the same salinity level. For SDW, the same trend of salt tolerant cultivars was found as that of SFW at the highest salinity level. The cultivars showed salt tolerance in terms of shoot fresh and dry weights they accumulated less Na^+ in their leaves as compared to that in the salt sensitive cultivars. On an overall basis, at 200 mol m^{-3} NaCl the Na^+ content of genotypes was more than 100 mol m^{-3} NaCl. The K^+ content of genotypes was generally higher at lower salinity level than that of higher salinity level. Under saline conditions in Japanese paper pots and in soil pots, salinity tolerant genotypes accumulated more K than salinity susceptible genotypes. However, surprisingly in hydroponics salinity susceptible genotypes had less K^+ content. There is no obvious reason for the less K^+ content in hydroculture. The SFW and SDW of both salt tolerant cultivars were significantly greater than those of the salt sensitive cultivars at all salt treatments in all the three screening methods. By considering ranking of the four cultivars in respect of salt tolerance at 200 or 100 mol m^{-3} NaCl in the three methods it is apparent that although there was some deviation in the rank order among salt tolerant or salt sensitive cultivars yet tolerant and sensitive cultivars were in the same order in each of the three screening techniques. This indicates that Japanese Paper Pot method has a satisfactory reproducibility with the techniques already being used.

Table 1. Means for Na^+ and K^+ content, shoot fresh weight and shoot dry weight of the plants grown at 3 salt levels using three methods of screening.

	Japanese paper pots			Hydroponics			Soil pots		
	Salinity (mol m^{-3} NaCl)		Control	Salinity (mol m^{-3} NaCl)		Control	Salinity (mol m^{-3} NaCl)		Control
	200	100		200	100		200	100	
	Na^+ content (mol m^{-3} of leaf water)								
Chinese spring	258	165	64	251	157	78	243	172	63
Lu 26	218	123	69	269	188	47	270	162	62
Sonora 64	528	267	60	613	593	83	560	241	65
Glennson	651	348	68	754	329	76	728	283	63
Mean	414	226	65	472	317	71	450	214	63
LSD (0.05)	111	52	13	38.5	69.0	31.5	111	23.2	7.8
	K^+ content (mol m^{-3} of leaf water)								
Chinese spring	277	232	275	227	335	420	364	318	236
Lu 26	379	319	252	317	351	387	320	410	245
Sonora 64	181	211	255	490	682	341	238	355	271
Glennson	129	203	238	413	601	430	266	237	256
Mean	241	241	255	362	492	394	297	330	252
LSD (0.05)	85	80	67	30.8	80.4	131.5	85	80	67
	Shoot fresh weight (g)								
Chinese spring	10.8	14.6	22.1	227	335	420	11.2	15.3	21.7
Lu 26	12.3	15.7	25.3	317	351	387	12.2	16.5	27.4
Sonora 64	8.2	12.5	27.4	490	682	341	9.6	12.8	26.3
Glennson	4.7	11.8	25.1	413	601	430	7.5	11.3	22.9
Mean	8.9	13.6	25.0	362	492	394	10.1	14.0	24.6
LSD (0.05)	1.2	0.9	4.0	30.8	80.4	131.5	1.7	1.2	5.5
	Shoot dry weight (g)								
Chinese spring	4.5	5.2	5.5	0.638	1.678	6.3	3.4	4.9	4.7
Lu 26	3.7	5.4	5.6	0.389	0.617	5.2	3.4	5.3	5.7
Sonora 64	2.3	3.9	6.8	0.167	0.299	6.9	2.6	3.9	6.0
Glennson	1.6	2.9	5.9	0.136	0.229	5.5	2.3	3.3	4.4
Mean	3.0	4.3	5.9	0.332	0.706	6.0	2.9	4.3	5.2
LSD (0.05)	0.5	0.8	1.7	0.199	0.399	1.4	0.4	0.5	0.8

For SFW, SDW and Na^+ content, there was a significant ($p<0.01$) positive correlation of hydroponics and soil filled pots with Japanese paper pots. SFW and SDW had a significantly negative correlation with Na^+ content of the leaves which indicate that better growth performance of salt tolerant cultivars was due to the exclusion of Na^+ from shoots.

The similarity of results obtained from the three methods indicated a high reproducibility of the results and their close association (significant correlation) among each other authenticated the validity of the Japanese Paper Pot technique. The ranking pattern of various cultivars in salt tolerant and salt sensitive groups indicates that the performance of the cultivars tested remained fairly constant for various screening methods.

Apart from the use of a wide range of screening tests for the evaluation of salt tolerance in early stages of growth there is no standard and widely accepted screening method. Many attempts have been made to devise a rapid and easy screening method. These tests customarily involve exposure of plants to saline media, followed by some measure of injury such as visual assessment of leaf damage (Shannon, 1978), survival (Dewey, 1962, McGuire & Dvorak, 1981) and ion uptake (Yeo *et al*., 1990). When large populations are to be screened for their reaction to saline medium, the available facilities (pots or hydroculture tanks) are usually too limited for the inclusion of all material in one study. Subsequently, the material has to be screened in batches over a long time (Shannon *et al*., 1984). When the population is broken down into separate batches, though using other genotypes as a check, the comparison of genotypes between batches may be biased by some modification in the environment in different batches. Moreover significant genotype x batch interaction may arise.

For practical purposes, a good mass screening method should be efficient, reproducible, reliable and simple and for this purpose selection of a suitable criterion for assessing the relative salt tolerance in various methods is important. In the present study, three screening methods were tested to determine the consistency in response of various cultivars grown in two salt levels and in control. The cultivars showed similar response to salt in all the methods used in the study which indicates that any of the techniques is effective for assessment of salt tolerance. The hydroponics and soil pots have already been used in many studies (Sayed, 1985; Heakal *et al*., 1990; Aslam *et al*., 1993, Munns & James, 2003). However, for the determination of salt tolerance of wheat lines the use of Japanese paper pots is reported first time. As compared to other two techniques Japanese paper pots method is easy, simple and economical. Japanese paper pot method is more comparable with screening in saline soil conditions than that of hydroponic as solid media is provided to roots. In addition a large number of plants at early stage of growth could be screened in a small area with this method e.g., 3000 plants can be screened in $1m^2$ area. In this way, the difficulties arising by dividing populations into batches can be overcome, since using Japanese paper pots in a glasshouse thousands of genotypes could be screened. By using Japanese paper pots access to individual plant is easy as compared to other methods. Another advantage of this method is that plants are screened at very early stages of growth and since it is non-destructive, the tolerant plants can be transferred later to water culture, pots or a saline field for further assessment of slat tolerance at subsequent stages of growth. In this way population size for testing at later stages of growth will be reduced.

The preference of plants at early stage of growth under saline conditions has been considered highly predictive of the response of adult plants to salinity (Norlyn, 1980; Kingsbury & Epstein, 1984; Azhar & McNeilry, 1987; Munns & James, 2003. While working with barley, wheat and seven grass species, Ashraf *et al*., (1986a, 1986b) screened seedlings of these species and derived plants that showed considerable tolerance to salinity at the adult stage. Thus, plants at early stage of growth screened for salinity tolerance by using Japanese paper pots could show considerable salinity tolerance at the later stages of growth.

From the data presented here, it is easy to conclude that screening at the early stage of plant growth in Japanese paper pots is a convenient and fairly reliable technique for determining differences with respect to salt tolerance in a large number of wheat genotypes. Field screening techniques confront the biggest problem of high degree of soil heterogeneity (Richards, 1983) and only a limited number of genotypes can be handled. However, it is suggested that after screening a large number of genotypes in Japanese paper pots, the selected lines must be evaluated in saline soils before recommending them for cultivation in saline soils.

References

Ashraf, M. and T. McNeilly. 1988. Variability in salt tolerance of nine spring wheat cultivars. *J. Agron. Crop Sci*., 160: 14-21.

Ashraf, M., T. McNeilly and A.D. Bradshaw. 1986b. Tolerance of Sodium chloride and its genetic basis in natural population of four grass species. *New Phytol*., 103: 725-734.

Ashraf, M., T. McNeilly and A.D. Bradshaw. 1986a. The potential for evolution of salt (NaCl) tolerance in seven grass species. *New Phytol*., 103: 299-309.

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

Azhar, F.M. and T. McNeilly. 1987. Variability in salt tolerance in *Sorghum bicolor* (L) Moench under hydroponic conditions. *J. Agron. Crop Sci*., 195: 269-279.

Bogemans, J., J.M. Stassart and L. Neirinckx. 1990. Effect of NaCl stress on ion translocation in barley. *J. Plant Physiol*., 135: 753-758.

Dewey, D.R. 1962. Breeding crested wheat grass for salt tolerance. *Crop Sci.,* 2: 403-407.

Farah, M.A. and I.M. Anter. 1978. Salt tolerance of eight varieties of rice. *Agric. Res. Rev*., 56: 9-15.

Flowers, T.J. and A.R. Yeo. 1981. Variability in the resistance of Sodium chloride salinity within rice (*Oryza sativa* L.) varieties. *New Phytol*., 88: 363-373.

Heakal, M.S., A.S. Modaihsh, A.S. Mashhady and A.I. Metwally. 1990. Combined effect of leaching fraction, salinity and potassium content of water on growth and water use efficiency of wheat and barley. *Plant Soil*, 125: 177-184.

Hewitt, E.J. 1952. Composition and preparation of nutrient solution. In: *Sand and Water Culture Methods Used in the Study of Plant Nutrition*. Farnham Royal: Commonwealth Agricultural Bureaux. pp. 187-191.

Kingsbury, R.W. and E. Epstein. 1984. Selection for salt resistant in spring wheat. *Crop Sci.,* 24: 310-315.

Kingsbury, R.W., E. Epstein and R.W. Pearcy. 1984. Physiological response to salinity in selected lines of wheat. *Plant Physiol.,* 74: 417-423.

McGuire, P.E. and J. Dvorak. 1981. High salt tolerance potential in wheat grass. *Crop Sci.,* 21: 702-705.

Munns, R. and R.A. James. 2003. Screening methods for salinity tolerance: a case study with tetraploid wheat. *Plant Soil*, 253: 201-218.

Norlyn, J.D. 1980. Breeding salt tolerant crop plants. In: *Genetic Engineering of Osmo-regulation*. (Eds.): D.W. Rains, R.C. Valentine, A. Hollaender. Plenum Press, New York, pp. 239-309.

Qureshi, R.H., R. Ahmad, M. Alyas and Z. Aslam. 1980. Screening of wheat (*Triticum aestivum* L.) for salt tolerance. *Pak. J. Agric. Sci*., 17: 19-25.

Rawson, H.M., R.A. Richards and R. Munns. 1988. An examination of selection criteria for salt tolerance in wheat, barley and triticale genotypes. *Aust. J. Agric. Res*., 39: 759-772.

Richards, R.A. 1983. Should selection for yield in saline conditions be made on saline or non-saline soils. *Euphytica*, 32: 431-438.

Sayed, H.I. 1985. Diversity of salt tolerance in germplasm collection of wheat (*Triticum* spp.). *Theor. Appl. Genet*., 69: 651-657.

Shannon, M.C. 1978. Testing salt tolerance variability among tall wheatgrass lines. *Agron. J*., 70: 719-722.

Shannon, M.C., G.W. Bohn and J.D. Mccreight. 1984. Salinity tolerance among muskmelon genotypes during seed emergence and seedling growth. *Hort. Sci*., 19: 828-830.

Srivastava, J.P. and S. Jana. 1984. Screening wheat and barley germplasm for salt tolerance. In: *Salinity Tolerance in Plants: Strategies for Crop Improvement*. (Eds.): R.C. Staple, G.H. Toenniessen John Wiley and Sons, New York, pp. 273-283.

Yeo, A. R., M.E. Yeo, S.A. Flowers and T.J. Flowers. 1990. Screening of rice *(Oryza sativa* L.) genotypes for physiological characters contributing to salinity resistance, and their relationship to overall performance. *Theor. Appl. Genet*., 79: 377-384.

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