Conclusions

The project has successfully established a transformation system for pearl millet. Although transformation efficiencies are currently low, work will continue towards improving the technology. A year's funding to achieve this objective and to analyze the putative transformants generated during the EU project has been secured through the DFG Germany/South African bilateral funding scheme (UH and CSIR).

Considerable progress has also been made towards the isolation of a downy mildew resistance gene. The *Dm2* gene has been located to a 4 cM interval. The publication earlier this year of the rice genomic sequence presented us with a tool to exploit our molecular markers to delineate the orthologous region in rice, which can then be used as a source for further markers or even candidate genes. It has also been shown that recombination in the vicinity of *Dm2* is high, with a genetic to physical ratio of 5 kb/cM. We are therefore optimistic that we will be able to isolate the first downy mildew resistance gene over the next year. The research is being continued at JIC through a John Innes Foundation Studentship until September 2003.

The collaborative project has also led to the testing of new genotypes in Ghana. One such line, P1449-2, was infection-free during two years of testing in the downy mildew nursery in Ghana. The use of this line in the breeding program for the improvement of local varieties is currently being explored. The nutritional composition and functional properties of the new pearl millet lines will be determined for their industrial potential by small- and medium-scale food entrepreneurs in Ghana.

Agronomy

Screening Pearl Millet Germplasm for Tolerance to Soil Salinity

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Introduction

Saline soils account for up to 580 million ha worldwide and are widespread in arid and semi-arid regions (Rengasamy 2002). Pearl millet (*Pennisetum glaucum)* is often grown in saline soils and is known to be relatively better in tolerance to salinity than other crops, particularly maize (*Zea mays)* or legumes (Ashraf and McNeilly 1987, Dua 1989). However, a well-focused search can lead to the identification of genotypes with superior tolerance. Since pearl millet is usually grown rainfed with minimum input, it is all the more important to genetically improve the adaptation of this crop to soil salinity. The improved salinity tolerant lines together with cultural management options provide greater scope for improving the crop productivity in these saline soils.

Most crop species are sensitive to salt stress during all stages of plant development, including seed germination, vegetative growth and reproductive growth. Variation in whole-plant reaction to salinity provides the most efficient initial screening for salinity tolerance (Shannon 1984, Ashraf and McNeilly 1987, Ashraf and McNeilly 1992). Therefore, the objective of this study was to screen a wide range of improved hybrid parents and germplasm lines of pearl millet for relative ability to produce more biomass under salinity during pre-anthesis stage.

Materials and methods

One hundred entries of pearl millet comprising popular varieties, hybrids and progenies were grown in a greenhouse at 20-28°C in a randomized complete block design (RCBD) with three replications. There were two salinity treatments: (1) Control: irrigated with deionized water; and (2) Saline: irrigated with 250 mM NaCl solution (EC 23.4 dS cm^{-1}), once at the time of sowing and later irrigated with deionized water. Plastic pots (12.5 cm diameter) were sealed at the bottom and filled with 1.2 kg of Alfisol mixed with diammonium phosphate at 0.25 g pot^{-1}. Sixteen seeds of each entry were sown on 29 March 2003 in four equally spaced hills in each pot and irrigated with deionized water or saline solution to

field capacity previously estimated for the soil. To avoid waterlogging during subsequent irrigations, the water needed was determined by regular weighing of representative pots. A maximum of four plants pot^{-1} were retained after thinning at 10 days after sowing (DAS) in the control. However, thinning was necessary in few saline pots, as most of them did not have the required four plants pot^{-1}. One plant per pot was sampled at 18, 25, 32 and 39 DAS. While sampling, plants were always reserved for later sampling dates; for example, if there were two plants pot^{-1} they were reserved for the third and fourth sampling. Each sampled plant was separated in root (extractable) and shoot, oven-dried at 60°C for 3 days and the dry mass then recorded. The total plant biomass for each sample was subjected to ANOVA as a two factor RCBD and the genotypic means were obtained. All the four individual sample genotypic means of total biomass produced under saline condition and the four calculated ratios of total biomass under saline condition as that of the control were used for clustering the entries into different classes using Numerical Taxonomy and Multivariate Analysis System (NTSYSPC), version 2.1 from Exeter Software, New York, USA. A similarity/ dissimilarity matrix was obtained based on Euclidean distances and thus the entries were grouped on the basis of UPGMA (unweighted pair-group method of arithmetic average).

Results and Discussion

The pearl millet genotypes emerged in 6 to 9 DAS in the pots irrigated with saline water whereas those in the control pots emerged within 3 to 4 DAS. However, many test entries did not emerge in the saline pots, but wherever emergence occurred, the number of seedlings were few (data not shown). Differences among the genotypes and genotype x salinity interactions existed at all stages of sampling for both absolute and relative weights (Table 1). Cluster analysis on the basis of absolute and relative biomass for four growth stages indicated about 4 major groups with a similarity coefficient of 40%. Eight entries with a skewed performance at one or two stages were excluded and grouped separately. The pots where one or two plants emerged were harvested at the fourth, or third and fourth sampling time and the sparse population in these pots permitted them to grow with relatively more vigor and less competition. Thus, though these ranked the least at the early stages (as the mean values were 0), their later performance was high. However, the rest of the genotypes can be grouped into highly sensitive, sensitive, tolerant and highly tolerant entries based on the group means of the total biomass and relative biomass in all four sampling periods (Tables 2 and 3). Almost all the entries that emerged poorly under irrigation with saline water were classified as highly sensitive. However, it is quite possible that some of the entries of this category might have the capacity to produce higher shoot dry mass at later stages if emerged successfully. Such a condition can be expected to prevail where saline water irrigations are practiced (Francois et al. 1994). Most of the highly tolerant entries such as IP 3757 are either previously documented to be tolerant or grown in Rajasthan, India where the soils are often saline (CZI 9621 bred by the Central Arid Zone Research Institute and RIB 3135-18 bred by the Rajasthan Agricultural University). From most of the populations, at least one highly tolerant progeny and one sensitive or highly sensitive progeny were identified. Some of the B-lines currently in use for hybrid development such as ICMB-00888, ICMB-91444, ICMB-93333 and ICMB-98222 also fall under the salinity tolerant category.

These experiments are being repeated to confirm the salt tolerance reaction of the 100 test entries. Also, determination of various ionic compositions of the plant tissues is being carried out to delineate the mechanisms of salt tolerance. The same material is being tested at the International Center for Biosaline Agriculture (ICBA), Dubai, UAE as part of a collaborative project on salinity tolerance.

Table 1. Analysis of variance and its significance for salinity treatments, pearl millet entries and their interactions for the total dry matter plant^{-1} of samples at different days after sowing (DAS).

	Mean sum of squares and significance level[1]			
Source of variation	14 DAS	25 DAS	32 DAS	39 DAS
Salinity levels (S)	16.03***	96.12***	249.66***	48768***
Pearl millet entries (G)	0.012***	0.097***	0.29**	1.07*
S x G	0.012***	0.087**	0.30**	115*
Residual	0.007	0.059	0.20	0.82

1. * = Significant at P = <0.05; ** = Significant at P = <0.01; *** = Significant at P = <0.001.

Table 2. Cluster group means of total biomass (g plant^{-1}), the ratio of total biomass under 250 mM saline condition as that of control on 18,25,32 and 39 days after sowing (DAS) and the comparative reaction of the tested pearl millet entries.

Pearl millet entries	18 DAS		25 DAS		32 DAS		39 DAS		
	Biomass	Ratio	Biomass	Ratio	Biomass	Ratio	Biomass	Ratio	Reaction[1]
30	0.004	0.014	0.008	0.008	0.018	0.013	0.158	0.055	Highly sensitive***
23	0.018	0.060	0.043	0.054	0.105	0.080	0.739	0.306	Sensitive***
29	0.023	0.069	0.071	0.085	0.218	0.157	1.197	0.487	Tolerant ***
10	0.036	0.102	0.242	0.283	0.485	0.342	0.974	0.373	Highly tolerant*
8	0.025	0.059	0.103	0.108	0.289	0.223	1.951	0.759	Highly tolerant* (needs confirmation)

1. Pair-wise analysis of means by multivariate analysis showed that the clusters listed with *** were different at 0.001 level of probability and * were different at the 0.05 level.

Table 3. Pearl millet entries grouped on the basis of pre-anthesis total biomass production under 250 mM saline water irrigated condition and the ratio of biomass under salinity as that of control.

Group	Entries
Tolerant	RCB-2-S1-33-1-3-2-2, ICMR 312-S1-17-2-3-1-2. MC 94 C2-S1-3-2-2-2, IP 3732,ICMV 91059-S1-17-3-3-1-2, MC 94 C2-S1-33-1-3-2, ICMR 312-S1-17-3-2-1-2, SDMV 90031-S1-60-1-1-2, ICMB 01222, MC 94 C2-S1-3-2-1-1, ICMB 98777, ICMR 356, MC 94 C2-S1-66-1-2-2, ICMP-451, CZI 98-11, ICMB 94555, ICMB 95111, AIMP 92901-SI-520-1-3-1, ICMB 95333, ICMR 312-S1-22-1-3-2-1, ICMB 02111, ICML 22, RCB-2-S1-43-3-4-2, ICMR 312-S1-22-1-3-2-1, ICMS 7704-S1-51-5-1-2. MC 94 C2-S1-36-1-3-2, 841 B, J 104 Selection, RIB 335/74 (RHB 30 Pollinator)
Highly tolerant	RCB-2-S1-24-2-3-1-2, ICMS 8511-S1-17-2-1-2. ICMB 93333, MC 94 C2-S1-3-1-1-2, HTP 94/54 (HHB 146 pollinator), ICMV 91059-S1-11-3-3-3-2, MC 94 C2-S1-89-4-2-2, ICMB 98222, RCB-2-S1-40-1-1-2-2, IP 3757, RCB-2-S1-19-2-2-1-2, ICMS 8511-S1-14-2-2-2, CZI 9621, RIB 3135-18 (RHB 121 pollinator), ICMV 91059-S1-4-2-3-2-2, ICMB 91444, SDMV 90031-S1-26-3-1-2, ICMB 00888

Acknowledgments. This research was supported by grants from the Governments of Australia, Canada, France, India, Japan, the Netherlands, Switzerland, UK and USA and the Asian Development Bank, Commission of the European Communities, Rockefeller Foundation and OPEC Fund for International Development assigned to biotechnology-assisted improvement in salinity tolerance through the GT-Biotechnology of ICRISAT. The authors gratefully acknowledge the guidance on statistics provided by Subhash Chandra, Senior Scientist (Biometrics), ICRISAT.

References

Ashraf M and McNeilly TM. 1987. Salinity effects on five cultivars/lines of pearl millet (*Pennisetum americanum* [L] Leeke). Plant and Soil 103:13-19.

Ashraf M and McNeilly TM. 1992. The potential for exploiting variation in salinity tolerance in pearl millet [*Pennisetum americanum* (L.) Leeke]. Plant Breeding 108:234-240.

Dua RP. 1989. Salinity tolerance in pearl millet. Indian Journal of Agricultural Research 23:9-14.

Francois LE, Grieve CM, Maas EV and Lesch SM. 1994. Time of salt stress affects growth and yield components of irrigated wheat. Agronomy Journal 86: 100-107.

Rengasamy P. 2002. Transient salinity and subsoil constraints to dryland farming in Australian sodic soils: an overview. Australian Journal of Experimental Agriculture 42:351-361.

Shannon MC. 1984. Breeding, selection and the genetics of salt tolerance. Pages 231-254 *in* Salinity tolerance in plants. (Staples RC and Toeniessen GH, eds.). New York, USA: John Wiley and Sons.