Theor Appl Genet (1995) 90: 1106–1111

A. Garcia · D. Senadhira · T. J. Flowers · A. R. Yeo

The effects of selection for sodium transport and of selection for agronomic characteristics upon salt resistance in rice (*Oryza sativa* L.)

Received: 1 August 1994 / Accepted: 8 December 1994

Abstract A multiple cross was constructed with the aim of combining component traits for the complex salinity resistance character. The aim was to combine donors for physiological traits with the agronomically desirable semi-dwarf/intermediate plant type and with the overall salinity resistance of the traditional tall land races. We report a study of selection strategies in the resulting breeding population. The effects of early selection for agronomic traits and early selection for low sodium transport were compared with a control population in which minimal selection was practised. Conventional selection for agronomic characters at early generations selected against low sodium-transporting (and thus potentially salt-tolerant) genotypes. In contrast, mild early selection for low sodium transport enriched the population in potentially salt-resistant genotypes but did not select against agronomic (semi-dwarf/intermediate) genotypes. It is concluded that selection for agronomic traits should be made after selection for salt resistance and, ideally, should be delayed until the population has reached near-homozygosity.

Key words Breeding · Rice · *Oryza sativa* · Salinity · Selection

Introduction

Salinity resistance in rice is now recognised as a complex character, involving the interaction of a number of component traits, which are physiological in nature and which may be inherited independently (Yeo and Flowers 1986; Yeo et al. 1990; Gregorio and Senadhira 1993; Chaubey and Senadhira 1994). Low transport of salt to the shoot is necessary, but not sufficient, to provide salt resistance in rice. Even with low rates of salt transport, time and transpiration still lead to damaging levels of salt in the leaves causing loss of productivity (Munns and Termaat 1986; Yeo and Flowers 1989). Several physiological characters which contribute to salt uptake, or which modify or mitigate the consequences of salt accumulation, have been identified and evaluated within a range of rice genotypes (Yeo and Flowers 1986; Yeo et al. 1990). Those physiological characteristics contributing to the resistance of salinity include: (1) reduced salt transport to the shoot which may be a consequence of low transpirational-bypass flow or of high water-use efficiency, (2) plant vigour which acts to dilute, through growth, the salt within the tissue, (3) compartmentation of salt away from young expanding or photosynthesising leaves (leaf to leaf distribution), (4) tolerance of salt within the tissue (tissue tolerance) which reflects differences in the distribution of salt between apoplast and protoplast or cytoplasm and vacuole (see Yeo and Flowers 1986; Yeo et al. 1990).

No present-day cultivar of rice possesses a favourable combination of these traits. The genotypes that are regarded as most resistant to salinity (such as Pokkali and Nona Bokra and similar tall land races) score highly in only vigour and low shoot sodium concentration, whilst performing poorly in compartmentation at the leaf and tissue levels (Yeo et al. 1990). Although the tall land races have been used as donors of salt resistance with some degree of success, the vigour of their plant type, although being a virtue with respect to salt resistance, is not a desirable character from an agronomic point of view. An alternative strategy is offered by the observation that there are potential donors for other component traits of salt resistance which these traditional donors lack and, furthermore, that donors for all the physiological traits can be found amongst cultivars and elite breeding lines which already have agronomic acceptability (Yeo et al. 1990). There is thus the potential for combining the component traits for salt resistance within a single genotype through a pyramiding approach to breeding (Yeo and Flowers 1986).

Communicated by G. S. Khush

A. Garcia (✉) · T. J. Flowers · A. R. Yeo
School of Biological Sciences, The University of Sussex, Brighton BN1 9QG, UK

D. Senadhira
International Rice Research Institute, P. O. Box 933, Manila, The Philippines

Such an approach immediately raises the question of how the breeding populations resulting from crosses should be handled. Some of the component traits are characterised by physiological measurements that cannot be made in early generations, either because they are not possible on individual plants or because environmental effects are expected to obscure their expression in early heterozygous generations. There is also the possibility that selection for agronomic characters and salt resistance will be mutually antagonistic. If a pyramiding approach is to be successful it is necessary not only to select suitable donors for each physiological trait but to handle the resultant breeding populations in a way that allows potentially rare recombinants to be preserved and identified.

We have investigated a four-way cross (IR59462) aimed at combining two physiological traits, of low shoot sodium content and high tolerance to sodium within the tissue, with the resistance of the traditional donors, Nona Bokra and Pokkali. It has previously been demonstrated that low shoot sodium content can be selected independently of the tall plant type, which is a necessary pre-requisite for the combination of salt resistance with agronomic acceptability (Yeo 1992). The study reported here focused on sodium transport because this can be measured in individuals whereas the measurement of tissue tolerance requires replication and so cannot be determined until recombinant inbred lines have been developed. The aim of the present study was to compare the effect of three different selection regimes on the overall salt resistance of the breeding population. In the control population only the minimum selection compatible with practicality was applied. A second (bulk) population was advanced by conventional breeding methods and criteria, including early selection for agronomic traits under saline field conditions. A third (selected) population was produced by a mild (30% acceptance) selection from the control population for low sodium transport made at the F_3 and advanced subsequently by single-seed descent without further selection. The fundamental problem to be resolved was whether to select first for agronomic characters and then for salt resistance, or vice versa: Early selection for agronomic characters may reject salt-resistant lines at a time before salt-resistance characters have become fixed and so may be poorly expressed, or masked by environmental effects and field heterogeneity. Conversely, initial selection for salt-resistance traits, such as low sodium transport, may exclude agronomically desirable plants.

Materials and methods

Experimental cross

A four-way cross (IR59462) was constructed at the International Rice Research Institute (IRRI) from land races and breeding lines (Nona Bokra/Pokkali//IR4630-22-2-5-1-3/IR10167-129-3-4) of *Oryza sativa* L. This cross was made with the objective of combining salt-tolerance mechanisms into a semi-dwarf high-yielding plant type. Nona Bokra and Pokkali are traditional tall types that have good overall performance in saline soils but poor performance in terms of leaf compartmentation and tissue tolerance. The two breeding lines

Table 1 Selection procedure employed to produce the F_5 bulk population from the four-way cross IR59462

Item	F_2	F_3	F_4
Number of plants	2400	2400	1000
Season	dry	wet	dry
Soil	Salinized EC = 8–9 dSm^{-1}	Salinized EC = 8–9 dS m^{-1}	Normal
Selection	Semi-dwarf and intermediates with good fertility bulked	Semi-dwarf and intermediates with good ferility bulked	High-yielding genotypes bulked
Proportion selected	25%	25%	20%

(henceforth abbreviated in the text to IR4630 and IR10167) were used as potential donors of leaf compartmentation and tissue tolerance, as well as being of the semi-dwarf plant type (Yeo et al. 1990). The plant type of Nona Bokra and Pokkali is not favoured agronomically and although IR4630 is a semi-dwarf it has a low yielding ability due to low and erratic tillering, poor panicle exertion and high spikelet sterility, even under normal soil conditions. IR10167 possesses all the traits of a high-yielding plant type. Breeding populations derived from this cross were used, together with the parental genotypes of the cross, in the experiments described.

Selection in breeding populations

Three populations were derived from the F_2 generation of IR59462 by a range of selection methods. The *bulk* population was advanced to the F_5 at IRRI using conventional breeding criteria, including early selection for agronomic traits, as summarised in Table 1. Plants (2400) were grown in the field where they were spaced 200–300 mm apart, one plant per hill, and management included intensive protection against insect pests, addition of fertilisers (N:P:K=60:40:40 kg ha^{-1}), and maintenance of weed-free soil. The F_5 generation represents approximately 1.25% of the original F_2 genotypes (cumulative selection of 0.25, 0.25 and 0.20: Table 1). The remaining F_2 (599 seed, approximately 20% of the total F_2) were planted in a glasshouse at the University of Sussex. Of these, 371 plants (62%) produced seed within 6 months. Those that failed to do so, being both tall and daylength-sensitive and so of no possible agricultural merit, were rejected. The *unselected* population was produced by advancing all of the F_3 lines by single-seed descent to the F_5. A single plant of each F_3 line was screened non-destructively for the sodium content of the third leaf and ranked; the 100 lines with the lowest sodium content were taken as the *selected* population and advanced to the F_5 by single-seed descent without further selection (see Yeo 1992).

Growth of plants

Two separate experiments were run sequentially. The distribution of dry weight and sodium transport in the F_5 bulk population was compared with that of the four parents. A comparison was subsequently made between the three (bulk, unselected and selected) populations at the F_5.

Seeds were imbibed in aerated water for 24 h after which they were germinated on grids floating on nutrient solution (Yoshida et al. 1976, modified by reducing the phosphate concentration to half and replacing sodium salts with potassium salts). Seedlings were transplanted at 8–9 days into black plastic containers (280 × 160 × 90 mm), up to 50 seedlings per container, and grown in the same nutrient solution. Seedlings were grown in a controlled environmental chamber with a 12-h photoperiod of 350–450 mol m^{-2} s^{-1} (PAR) at 27°C and with a 1.5 kPa saturation vapour pressure deficit. The dark period was of 25°C with a 0.6 kPa saturation vapour pressure deficit. The mean air velocity was approximately 0.5 m s^{-1}. Plants were salinized with 50 mol m^{-3} NaCl at 12 or 14 days and subsequently sampled at 24 or 29 days, for parental comparison and F_5 comparison, respectively.

Plant analysis

Plants were divided into roots and shoots, dried at 50°C for 24 h and their respective dry weights determined. Whole shoots were extracted in 100 mol m^{-3} acetic acid for 2 h at 90°C and sodium and potassium determined on the extract by atomic absorption spectrophotometry (Unicam SP919, linked to Unicam 919 atomic absorption software). Plant heights of the F_5 were measured and plants were categorised arbitrarily as tall or semi-dwarf/intermediate on the basis of having respectively an overall height of greater or less than 450 mm at the time of sampling.

Statistical analysis

To facilitate comparisons, the data for the four parents were combined, each parent being represented equally (100 plants per parent) in the resultant 'pooled parents' data. The data were then analysed using the statistical analysis software Minitab (Minitab Inc). The shoot ion concentrations and dry weights were not distributed normally and comparisons were made using the Kruskal-Wallis generalisation of the Mann-Witney-Wilcoxon test.

Results

Comparison of parental lines with the F_5 bulk population

The F_5 bulk population was semi-dwarf, as would be expected after repeated selection for agronomic traits. Figure 1 shows the frequency distributions of shoot dry weights for the pooled parents and for the F_5 bulk population: only the lowest weight categories of the parents are represented in the F_5 bulk.

Shoot sodium transport of the bulk population was significantly (P=0.001) higher than that of the pooled parents, median values for sodium shoot concentrations were 1.39 and 0.99 mmol g^{-1} d wt for the bulk population and the pooled parents respectively, in a direct comparison of these two populations. The sodium content of the F_5 bulk population indicated that this population was almost totally depleted in the lowest sodium concentration (i.e. potentially most salt-resistant) categories.

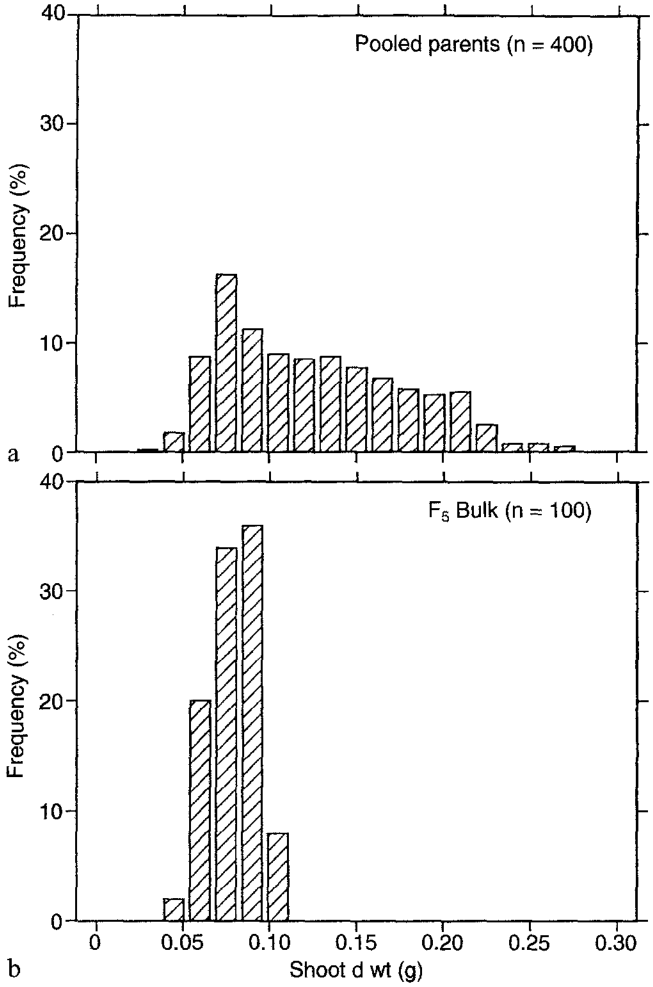

Fig. 1 Frequency distributions of shoot dry weight (g) for the pooled parents (Nona Bokra, Pokkali, IR4630, IR10167) (**a**) and for the F_5 bulk population derived from the four-way cross (IR59462) (**b**), with n in parenthesis. The four parents are represented equally in the pooled parents. The two populations differed at P<0.001

Table 2 Shoot dry weights and plant height of the three F_5 populations (bulk, unselected and selected) derived from the four-way cross IR59462. Values are the median, first (Q1) and third (Q3) quartiles, with n in parenthesis. Differing superscript letters (^{a,b,c}) denote significant differences (P=0.01) between populations

Population	Shoot d wt (g)			Plant height (mm)		
	Median	*Q1*	*Q3*	*Median*	*Q1*	*Q3*
Bulk (n=174)	0.164^a	0.114	0.216	390^a	350	420
Unselected (n=327)	0.205^b	0.153	0.270	410^b	340	508
Selected (n=89)	0.246^c	0.192	0.314	450^c	378	550

Comparison of the three F_5 populations

The three populations differed significantly with respect to plant height and shoot dry weight. Plant height and shoot dry weight of the bulk population were significantly lower (P=0.001) than those of the unselected and selected (for low sodium transport) populations (Table 2). There was a wide range in plant size amongst the selected and unselected populations reflecting the plant types of the four parents. The bulk population was largely (95%) composed of the semi-dwarf/intermediate plant type, whilst 62% and 51% of the unselected and selected populations, respectively, were of this type. Selection for low sodium transport had the effect of increasing (P=0.01) the average plant size in the selected population with respect to the unse-

Table 3 Sodium transport to shoot (mmol g^{-1} root d wt), sodium concentration (mmol g^{-1} d wt) and Na: K ratio within shoots of the three F_5 populations (bulk, unselected and selected) derived from the four-way cross IR59462. Values are the median, first (Q1) and third (Q3) quartiles with *n* in parenthesis for A) all plants and B) semi-dwarf/intermediate plants, where tall plants (those above 450 mm at time of harvest) were excluded from the analysis. Differing superscript letters (^{a,b,c}) denote significant differences (P=0.05) between populations

Population	Na^+ transport to shoot (mmol g^{-1} root d wt)			Na^+ shoot concentration (mmol g^{-1} d wt)			Na: K in shoot		
A) All plants	*Median*	*Q1*	*Q3*	*Median*	*Q1*	*Q3*	*Median*	*Q1*	*Q3*
Bulk (n=174)	3.36^a	1.69	5.59	1.11^a	0.58	1.85	1.88^a	0.96	2.93
Unselected (n=327)	2.73^b	1.54	4.65	0.96^a	0.59	1.54	1.87^a	1.23	2.86
Selected (n=89)	1.82^c	1.06	2.97	0.59^b	0.37	0.89	1.33^b	0.87	1.95
B) Semi-dwarf/intermediates	*Median*	*Q1*	*Q3*	*Median*	*Q1*	*Q3*	*Median*	*Q1*	*Q3*
Bulk (n=166)	3.51^a	1.84	5.67	1.13^a	0.66	1.95	1.94^a	1.02	3.08
Unselected (n=203)	2.97^a	1.66	5.05	1.14^a	0.71	1.79	2.12^a	1.36	3.18
Selected (n=45)	2.07^b	1.06	3.12	0.73^b	0.45	1.05	1.41^b	1.11	2.15

lected population. This could have been in part an indirect selection in favour of vigour, and in part a reflection of the increased salt resistance of the selected population, because heights and weights are for growth in saline conditions. However, selection had not been for tall plants because the proportion of semi-dwarf/intermediates in the selected population was still 51%.

Salt uptake was expressed in terms of shoot sodium concentration (mmol g^{-1} d wt) and of sodium transport to the shoot per unit root weight (mmol g^{-1} root d wt). Frequency distributions of shoot sodium concentration (mmol g^{-1} d wt) for the three F_5 populations are shown in Fig. 2. Shoot sodium concentration of the selected population differed significantly (P=0.001) from the bulk and unselected populations. The range of shoot sodium concentration was greatest in the bulk and unselected populations whilst being heavily skewed towards low sodium for the selected population (Fig. 2). Sodium transport (mmol g^{-1} root d wt) by the bulk population was much greater than by the selected population (the median was almost double that of the selected population) and was significantly (P=0.05) greater than the unselected population (Table 3).

Potassium/sodium selectivity, as assessed by the ratio of Na: K within the shoot, did not differ significantly between the bulk and unselected populations (Table 3). However, the Na: K ratio within the shoot of the selected population was significantly lower than in the other two populations (Table 3). The performance of the selected population compared with the bulk population in terms of sodium uptake and K: Na selectivity was not a consequence of differing proportions of the different plant types (tall or semi-dwarf/intermediate) amongst the populations, as can be demonstrated by excluding tall plants from the analysis. Semi-dwarf/intermediate plants of the selected population performed significantly better than those of the unselected and the bulk populations in terms of lower shoot sodium concentration and higher K: Na selectivity (Table 3).

Although there were significant differences between the three populations in root, shoot, and total dry weights, these populations did not differ significantly with respect to the root: shoot ratio. Mean root: shoot ratios were 0.36, 0.37 and 0.34 for the bulk, unselected, and selected populations respectively and no correlation was found between root: shoot ratio and sodium transport within individual populations (data not shown).

Discussion

The results indicate that early selection (at the F_2 and F_3) for semi-dwarf and intermediate genotypes with good fertility under saline conditions (EC=8–9 dS m^{-1}) and later selection (at the F_4) for high-yielding genotypes under non-saline conditions resulted in an almost entirely dwarfed F_5 population which performed poorly with respect to sodium transport. Although selection for agronomic characters was clearly successful, the average shoot sodium concentration of the bulk population was higher than that of the pooled parents and sodium transport was even higher than that of the unselected (control) population. The population was drastically depleted in low salt-transporting, and therefore potentially salt-resistant, lines. Such data cast serious doubt as to the suitability of conventional selection criteria for advancing breeding populations in salinity resistance breeding programmes.

The unselected and selected (for low sodium transport) populations displayed a wide range in plant size, which reflected the different genotypes of the four parents (dwarfism was present in two out of the four parents). This indicates that selection for low sodium transport preserved a high degree of genetic variation for plant type and did not remove the agronomically desirable semi-dwarf/intermediate types.

Sodium transport is characterised by large variability due to environmental effects (Flowers and Yeo 1981; Yeo 1992). Environmental factors which affect transpiration, such as light, temperature and humidity, all affect salt uptake. Despite this, a single mild selection at the F_3 for low

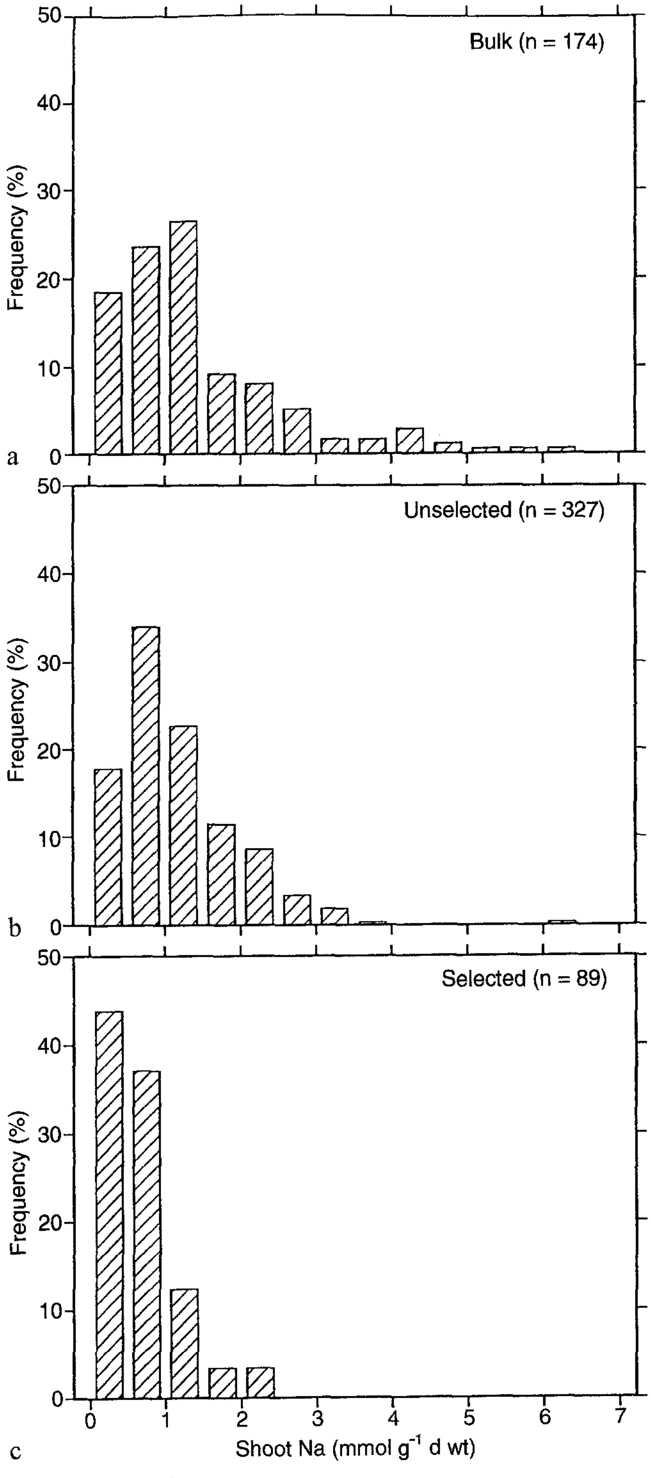

Fig. 2 Frequency distributions of shoot sodium concentration (mmol g^{-1} d wt) in the F_5 bulk (**a**), unselected (**b**) and selected populations (**c**), derived from the four-way cross IR59462. The selected population differed from the bulk and unselected populations at P=0.05

sodium transport succeeded in selecting for a heritable component of sodium transport. Low sodium transport was inherited as a characteristic of the population in the F_4 (Yeo 1992) and this was continued in the F_5. The F_5 population exceeded the performance of the unselected and bulk populations with regard to low sodium transport and high potassium: sodium selectivity.

Those accessions identified as the most resistant to salinity through mass screening trials are non-dwarf land races (Akbar 1986); however, their resistance is largely a consequence of their vigour (Yeo et al. 1990). Plant vigour will mitigate the consequences of sodium transport due to the diluting effect of growth on tissue sodium concentrations (Yeo and Flowers 1986). Amongst a large number of rice genotypes, plant vigour was the character having the strongest correlation with survival (Yeo et al. 1990). However, there is a need to develop semi-dwarf or intermediate lines (which are agronomically more desirable than the tall land races) with low sodium transport. The results confirm that low sodium transport and enhanced K: Na selectivity can be selected for independently of plant type, demonstrating the possibility of selecting for salt-resistant plants with desirable agronomic characteristics. Although salt resistance and plant type could be selected independently, our main finding was that selection for low sodium content preserved variation for desirable agronomic plant types, while early selection for agronomic characters strongly depleted the population in potentially salt-resistant genotypes.

The explanation for this may lie in the fact that salt tolerance is multigenic (Akbar et al. 1986; Hurkman 1992; Gregorio and Senadhira 1993) and is not a single character but rather the sum of several component traits (Yeo and Flowers 1986). Even a single such trait, sodium transport to the shoot, can involve the interaction of several biochemical and physiological processes. The composition and structure of cell membranes can affect passive leakage through the membrane bilayer either into the root or into the xylem. The nature and relative affinities of transport proteins and ion channels will affect ion uptake and selectivity. Root anatomy and development would be expected to affect leakage of salt with the transpirational-bypass flow (Yeo et al. 1987). Water-use efficiency will affect net sodium transport to the shoot via the relationship between transpiration and growth (Flowers et al. 1988). Any of these could be quantitative traits affected by several genes. Thus, even the single parameter of sodium transport is likely to result from the interaction and combination of several genes. Furthermore, there is evidence to suggest that both sodium content and K: Na selectivity within rice are governed by both additive and dominance gene effects, with the presence of dominant alleles that both increase and decrease salt resistance (Akbar et al. 1986; Gregorio and Senadhira 1993).

If a number of genes are likely to be involved in salt tolerance, we may surmise that recombinants possessing an optimal complement of alleles for component traits are potentially rare, and also that they may be difficult to identify in the early heterozygous generations, particularly in field situations where heterogeneity for salinity is a recognised problem (Malcolm 1983; Richards 1983). For both of these reasons it will be imperative to conserve as much genetic variability as possible for as long as possible within a breeding population in order to maximise the probability of retaining salt-tolerant genotypes. Although a population managed solely on agronomic criteria can give rise to lines with enhanced salt resistance, the present study has provided evidence that early selection for agronomic traits under saline field conditions greatly diminishes the genetic base for salt resistance; much genetic variation was lost and so the probability of retaining salt-resistant genotypes must have been greatly reduced.

The bulk population was advanced at IRRI with the view of generating elite breeding lines as donors of salt resistance. Since such elite breeding material was intended for widespread use maximum yield potential was an over-riding concern and maximum selection importance was given to agronomic characteristics known to be associated with high yield potential. However, if lines are to be developed for use primarily in saline conditions, some of the priority given to maximum yield potential under ideal conditions might be relaxed (Yeo 1994). A combination of good salt resistance and acceptable agronomic characteristics might offer the best immediate prospects for good yields under saline conditions. Delaying selection for agronomic characters might not select the best possible agronomic genotypes, though in our study this approach did retain a combination of low salt-transporting and desirable semi-dwarf/intermediate lines.

Single-seed descent has the advantage of retaining the greatest proportion of possible genotypes within a breeding population; however, the sheer numbers and effort involved to advance a breeding population to near-homozygosity are such that this may not always be practical. We have provided evidence that a single mild selection for low sodium transport at the F_3, which reduced the size of the breeding population by two thirds, retained variation for salt transport and for plant type. It is concluded that selection for agronomic traits should be made after selection for salt resistance and, ideally, once the population has reached near-homozygosity.

Acknowledgements This work was supported by the United Kingdom Overseas Development Administration.

References

Akbar M (1986) Breeding for salinity tolerance in rice. In: IRRI (eds) Salt-affected soils of Pakistan, India, Thailand. The International Rice Research Institute, Los Baños, The Philippines, pp 39–63

Akbar M, Khush GG, HilleRisLambers D (1986) Genetics of salt tolerance in rice. In: IRRI (eds) Rice genetics. The International Rice Research Institute, Los Baños, The Philippines, pp 399–409

Chaubey CN, Senadhira D (1994) Conventional plant breeding for tolerance to problem soils. In: Yeo AR, Flowers TJ (eds) Soil mineral stresses: approaches to crop improvement. Springer-Verlag, Heidelberg, pp 11–36

Flowers TJ, Yeo AR (1981) Variability in the resistance of sodium chloride salinity within rice varieties. New Phytol 88:363–373

Flowers TJ, Salama FM, Yeo AR (1988) Water-use efficiency in rice (*Oryza sativa* L.) in relation to resistance to salinity. Plant Cell Environ 11:453–459

Gregorio GB, Senadhira D (1993) Genetic analysis of salinity tolerance in rice (*Oryza sativa* L.). Theor Appl Genet 86:333–338

Hurkman WJ (1992) Effect of salt stress on plant gene expression: a review. Plant and Soil 146:145–151

Malcolm CV (1983) Wheatbelt salinity, a review of the salt land problem on south-western Australia. Techn Bull No. 52. Western Australian Department of Agriculture, Perth

Munns R, Termaat A (1986) Whole-plant responses to salinity. Aust J Plant Physiol 13:143–160

Richards RA (1983) Should selection for yield in saline regions be made on saline or non-saline soils? Euphytica 32:431–438

Yeo AR (1992) Variation and inheritance of sodium transport in rice. Plant and Soil. 146:109–116

Yeo AR (1994) Physiological criteria in screening and breeding. In: Yeo AR, Flowers TJ (eds) Soil mineral stresses: approaches to crop improvement. Springer-Verlag, Heidelberg pp, 37–59

Yeo AR, Flowers TJ (1986) Salinity resistance in rice (*Oryza sativa* L.) and a pyramiding approach to breeding varieties for saline soils. Aust J Plant Physiol 13:161–173

Yeo AR, Flowers TJ (1989) Selection for physiological characters – examples from breeding for salt tolerance. In: Jones HG, Flowers TJ. Jones MB (eds) Plants under stress. Cambridge University Press, Cambridge pp 217–234

Yeo AR, Yeo ME, Flowers TJ (1987) The contribution of an apoplastic pathway to sodium uptake by rice roots in saline conditions. J Exp Bot 38:1141–1153

Yeo AR, Yeo ME, Flowers SA, Flowers TJ (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

Yoshida S, Forno DA, Cock JH, Gomez KA (1976) Laboratory manual for physiological studies of rice (3rd edn.). International Rice Research Institute, Los Baños, The Philippines