

Variation and inheritance of sodium transport in rice

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Abstract

Sodium transport in rice is characterised by large variability between individual plants, and large environmental interaction. As a result of these two factors, plant sodium content is a continuous variable which is not distributed normally. This applies both to the quantity of sodium in the plant and to the concentration of sodium on a unit fresh or dry weight basis. This variability is in part because the transpirational by-pass flow, dependent upon root anatomy and development, contributes to sodium uptake. Variability in sodium content within designated cultivars is heritable and line selections diverge during recurrent selection, suggesting that selection is working on residual heterozygosity rather than on a family of homozygous lines. Varieties differ in average sodium uptake into the plant but the direct correlation of this with survival is weak. This is because other independent characters are important (and these have not been combined by natural selection nor by chance) and because overall performance is confounded by the spurious advantage of the tall (non-dwarf) plant type. This advantage is spurious because much of it is due to plant size rather than to any genetic information for salt tolerance. The benefit deriving from plant size will not be heritable in crosses with genotypes of the improved (dwarf), high-yielding plant type because the dwarfing genes are dominant. Sodium transport is heritable in crosses, and the results presented show that both low sodium transport and low sodium to potassium ratio can be selected independently of plant type. This allows the selection of dwarf plants (which are agronomically desirable) with low sodium transport (which will improve salt tolerance).

Introduction

Many traits contribute to the resistance of salinity in rice. They all act by reducing or mitigating the principal problem; which is that the influx of sodium chloride with the transpiration stream is excessive, and leads to internal concentrations of salt which are toxic (Yeo and Flowers, 1986). Concentrations of salt in the shoot can be reduced by lowering sodium transport to the shoot, by plant vigour (which provides for dilution of salt by growth) and by high water use efficiency (which reduces influx with the transpiration stream per unit growth). Compartmentation of salt within the plant (from leaf to leaf) and within the tissue (between protoplast and apoplast and between cytoplasm and vacuole) can greatly alter the impact of a given salt load in the

shoot (Flowers et al., 1991). Salt damage occurs at low *external* salinities which do not, per se, reduce growth; damage results from the *internal* accumulation of salt (Yeo and Flowers, 1986; Yeo et al., 1991). The immediate aim in breeding for increased salt resistance in rice is for a reduction in the salt concentration in the shoot.

Vigour and water-use efficiency are associated with the tall, unimproved plant type whilst modern varieties invariably include dwarfing genes (Flowers et al., 1988; Yeo et al., 1990). The dwarf plant type has a higher harvest index, better light interception because of its erect leaves, and much reduced lodging. Vigour is essential in plants that are going to be productive in saline environments (Richards, 1983), so some compromise may be necessary over the degree of dwarfing in varieties intended for saline soils. A

clear goal is to develop a plant of, at most, moderate stature with low sodium transport.

Sodium transport in rice is characterised by large individual variability which is not distributed normally (Flowers and Yeo, 1981; Yeo et al., 1988); the range in sodium concentration in the leaves of a population of even a designated cultivar grown with 50 mM NaCl is generally two orders of magnitude or more. The distribution is usually skewed and is often multimodal (Flowers and Yeo, 1981). Despite this, variability in sodium content within designated cultivars (which are expected to be almost entirely homozygous) is heritable and line selections diverge during recurrent selection (Yeo et al., 1988). Jones and Wilkins (1984) also reported an increase in the frequency of salt-resistant individuals during recurrent selection within rice cultivars. The limited data available implies that sodium transport is heritable, even though environmental effects are large.

The studies reported here compare the inheritance of sodium transport in intravarietal selections with that in doubled haploid lines derived from anther culture to assess the relative contribution of genetic and environmental effects. The distribution of sodium transport in the progeny of a four-way cross between parents differing in sodium transport and other physiological characters is then described. The data presented show that both sodium transport and sodium:potassium accumulation ratio (which are here indistinguishable) can be selected for independently of plant type (tall or dwarf).

Materials and methods

General

Seeds of cultivars, breeding lines and land races of *Oryza sativa* L. were obtained initially from the International Rice Research Institute, Los Baños, Philippines. Plants which were grown for seed were raised in a glasshouse which was heated to a minimum of 20°C by night and 25°C by day. Maximum daytime temperatures were limited to $28 \pm 3^\circ\text{C}$ by automatic venting and supplementary lighting provided a minimum of $400 \mu\text{mol m}^{-2} \text{s}^{-1}$ (photosynthetically active

radiation) for 12 h per day. Sodium transport experiments were conducted in a controlled environment chamber with a 12 h photoperiod of $400\text{--}500 \mu\text{mol m}^{-2} \text{s}^{-1}$ (photosynthetically active radiation) at 27°C and 1.5 kPa saturation vapour pressure deficit. The dark period was of 25°C and 0.6 kPa saturation vapour pressure deficit. In both glasshouse and controlled environment conditions the mean air velocity was approximately 0.5 m s^{-1} .

Selection based on sodium transport

Seeds were germinated on nylon mesh floating on nutrient solution (Yoshida et al., 1986) and transplanted at seven days into boxes of the same nutrient solution. Plants were salinised with a low concentration of sodium chloride (25–50 mM) at 12–14 days. About 6 days later, the third leaf was excised for analysis and the plants returned to non-salinised nutrient solution and subsequently grown to maturity in non-salinised conditions (see Yeo et al., 1988). The leaves were analysed for sodium (and potassium as well in some cases). In some experiments the data are given as a sodium concentration on a dry weight basis, in others the data is in the form of a sodium content (μmol per leaf). Individuals were ranked according to the sodium content of (or concentration in) the sampled leaf, and selected on this basis. The range in overall sodium transport between individuals is very much greater than differences in the distribution of sodium between leaves within the same plant, and the correlation between leaf-3 sodium and whole shoot sodium is very high.

Intravarietal selection

From an original population of *Oryza sativa* cv. IR36, groups of plants with the lowest and highest sodium transport were selected and grown for seed. At subsequent generations, selection was made for low sodium transport within the low-sodium population and for high sodium transport within the high sodium population. The frequency distribution of sodium transport in the population diverged over five generations of recurrent selection (Yeo et al., 1988) after which there was no further change. The material used

here had passed through six generations of recurrent selection, then random samples of the resulting populations were bulked once without selection. Two hundred plants of each population were grown in nutrient solution, salinised, and the shoots harvested for analysis.

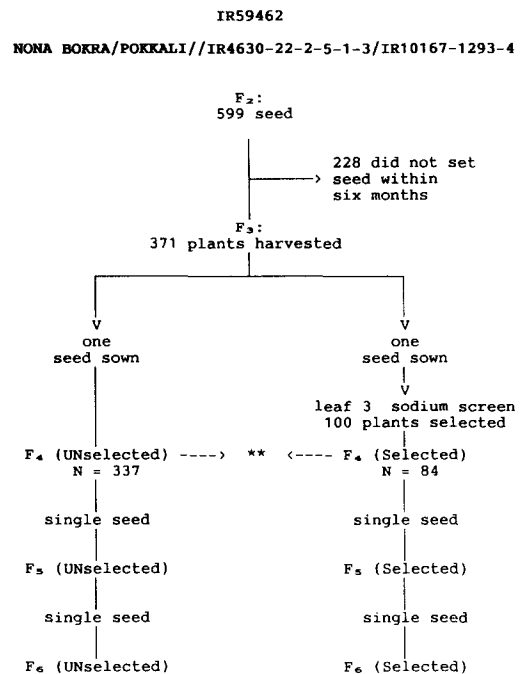
Doubled haploid lines derived from anther culture

Seed of the parental population of *Oryza sativa* cv. IR43 and anther culture derived lines were kindly supplied by Dr F Zapata (International Rice Research Institute, Los Baños, Philippines). One hundred plants of the parental population and of each of a number of anther culture derived lines were grown in nutrient solution, salinised, and the shoots harvested for analysis.

Breeding population

A four-way cross (IR59462) was constructed at IRRI by Dr D Senadhira from land races and breeding lines which differed in their performance in physiological traits, including a range of average sodium transport (for detailed information see Table 4 in Yeo et al., 1990). Part of the F_2 was handled as shown in Figure 1. Each seed was planted and grown to maturity. Growth was in restricted conditions to conserve space, as is normal practice. Only those plants which would be totally unacceptable in agronomic terms were rejected; these were all both tall and daylength-sensitive and had failed to set seed within six months. Seed was collected from 371 plants and this is referred to as the 'unselected' population, it was comprised of tall and dwarf types in approximately equal numbers. A single seed from each plant was grown to maturity. One seed from each plant was screened for the sodium content of the third leaf (as described above for intravarietal selection) and ranked; the lowest one hundred plants were taken as the 'selected' population and grown to maturity.

Both populations are being advanced in non-saline conditions by single seed descent without selection (other than rejection of plants that fail to set seed). The comparisons reported here were made after one further generation. At this time the breeding population will retain appreci-



** indicates the stage at which the present comparisons were made.

Fig. 1. Flow diagram showing selection within the four way cross IR59462.

able levels of heterozygosity; lines are considered 'near-homozygous' at about F_6 . The lower-term aim is to compare these methods with other conventional plant breeding strategies, including early selection for agronomic traits and initiation of line selections from early generations.

Ion analysis

Excised leaves or whole shoots were dried and extracted in acetic acid (100 mM) for 2 h at 90°C and sodium (and potassium where this was also measured) were determined in the extract by atomic absorption spectrophotometry (Pye Unicam SP9 800).

Statistical analysis

In most cases, the ion concentrations are not distributed normally. Comparisons were made using the Kruskal-Wallis generalisation of the Mann-Witney-Wilcoxon test using the statistical analysis software MINITAB (Minitab Inc).

Results

Variation in intravarietal selections and in anther culture derived lines

The frequency distributions of sodium transport in populations selected within *Oryza sativa* cv. IR36 through six recurrent selections for low and high sodium transport, followed by one cycle of multiplication without selection, differ at $p = 0.001$ (Fig. 2). However, both selected populations still exhibit large variability. Figure 3 shows frequency distributions of sodium transport for a parental population (in this case of *Oryza sativa* cv IR43) and two doubled haploid lines derived from it via anther culture. The anther culture

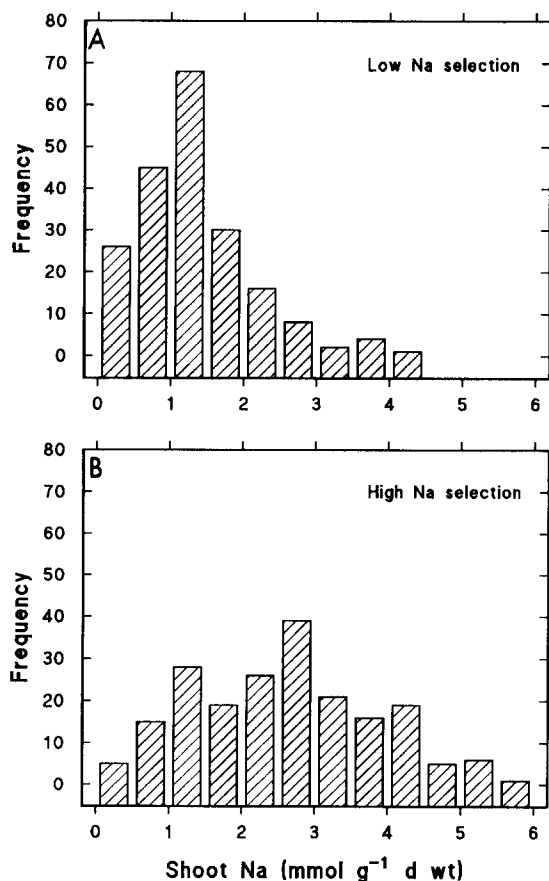


Fig. 2. Frequency distribution of sodium concentration in the shoot of intravarietal selections within cv. IR36. Lines were selected recurrently for low (A) and high (B) sodium transport for six generations and then bulked once without selection. The populations differ at $p < 0.001$.

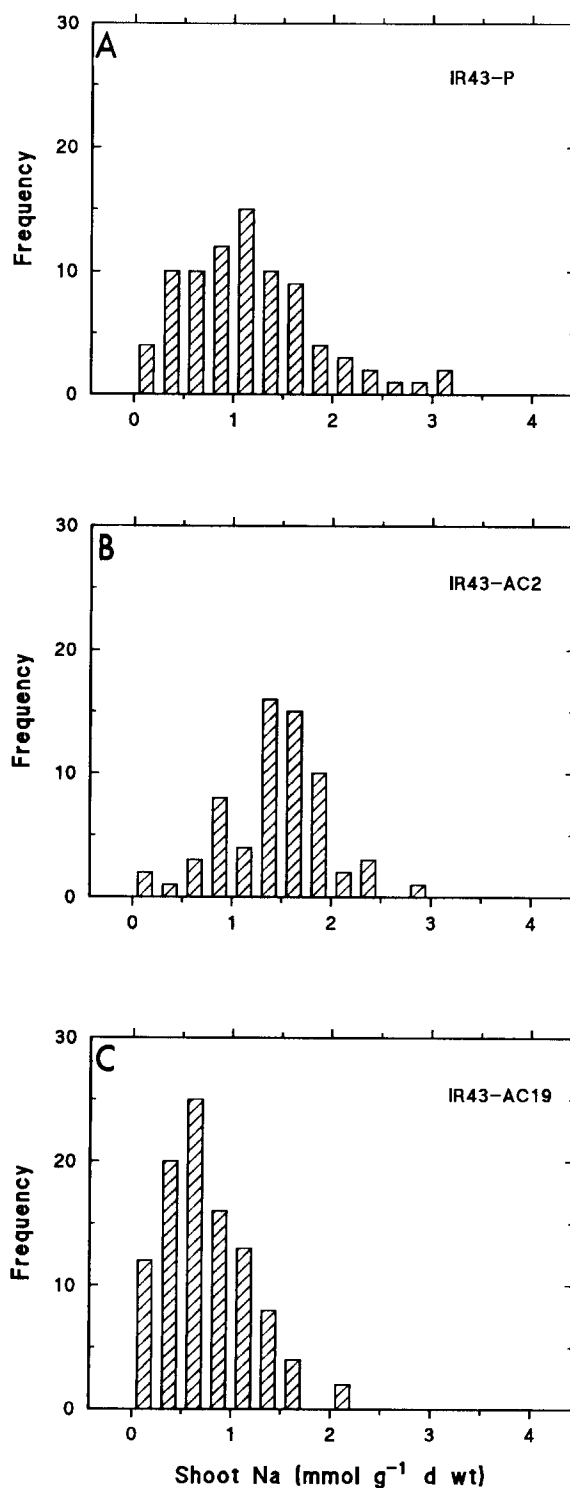


Fig. 3. Frequency distribution of shoot sodium concentration in cv IR43 (IR43-P, parental line (A)) and in two anther culture lines derived from it (IR43-AC2 (B) and 19 (C)). The lines and parent all differ at $p < 0.001$.

lines differ from the parental line and from each other at $p = 0.001$, but both still show very large variability.

Selection in breeding populations

Sodium transport

Since there is very little retranslocation of sodium in rice, the sodium content directly reflects the quantity of sodium transported. The sodium transport by the selected and unselected populations (see Fig. 1) differed significantly at F_4 . The range of sodium transport in the unselected population was very extended with a suggestion of being bimodal. By contrast, the selected population was heavily skewed towards low transport and the median approximately half of that in the unselected population (Fig. 4).

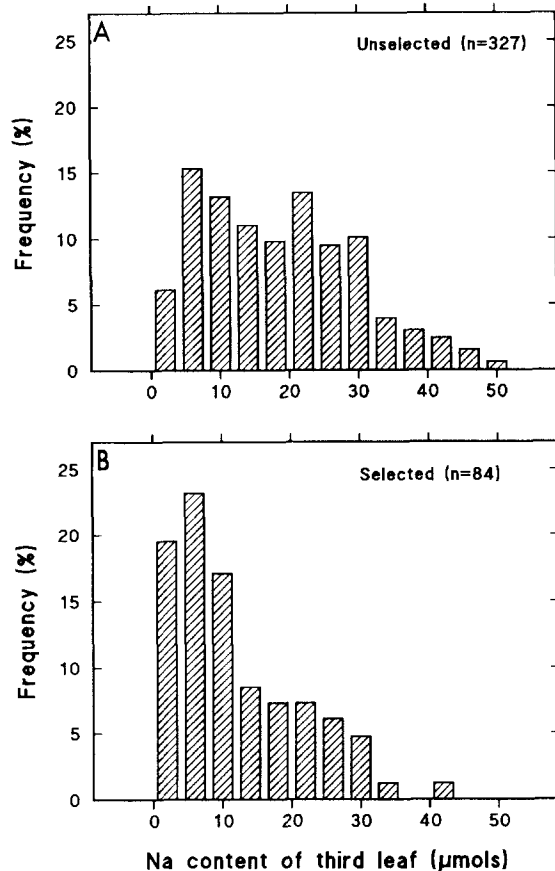


Fig. 4. Frequency distribution of the sodium content of the third leaf in unselected (A) and selected (B) populations from the four way cross IR59462. The populations differ at $p < 0.001$.

Sodium/potassium selectivity

The sodium:potassium ratio in the leaves followed a similar pattern, except that the bimodality of the distribution in the unselected population was more clearly pronounced (with peaks below 1.0 and about 2.0; Fig. 5). The second peak was practically absent in the selected population (Fig. 5).

Plant type

There was a wide range in plant size because dwarfism was present in two of the four parents, while the other two were non-dwarfed land races. To assess the impact of selection for low sodium transport the individuals in the populations were visually classified as tall or dwarf. The unselected population had a small majority of tall types, and tall and dwarf types were almost

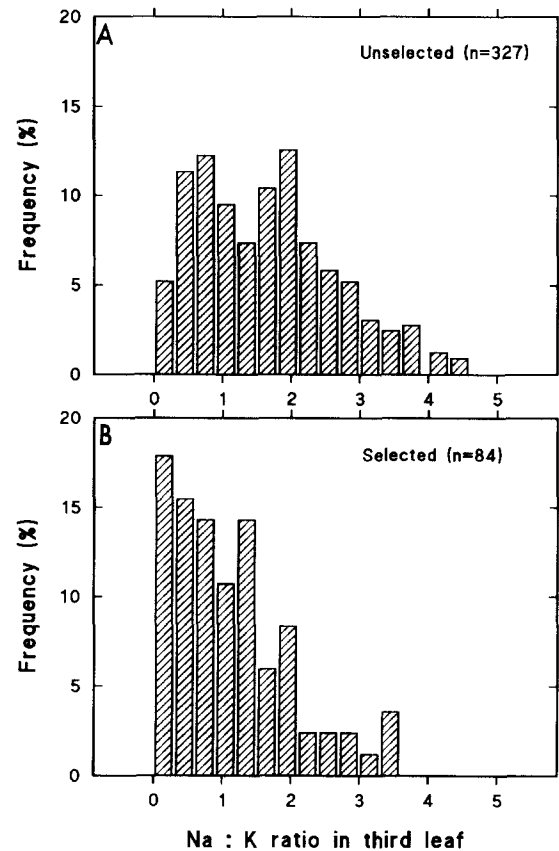


Fig. 5. Frequency distribution of sodium:potassium ratio in the third leaf of the unselected (A) and selected (B) populations from the four way cross IR59462. The populations differ at $p < 0.001$.

Table 1. The frequency of 'tall' and 'dwarf' plant types in the unselected and selected populations from IR59462. Plants with abnormal (stunted) growth were excluded

| Population | Tall | | Dwarf | |
|------------|------|------|-------|------|
| | n | % | n | % |
| Unselected | 175 | 53.5 | 134 | 41.0 |
| Selected | 40 | 47.6 | 42 | 50.0 |

equal in the selected population (Table 1). Sodium transport and sodium:potassium ratio were not dependent upon plant type; the frequency distribution of Na:K ratio in the different types in the unselected population is shown in Figure 6. The distributions do not differ significantly, although the bimodality is more pronounced in the tall than in the dwarf sub-population. The frequency distributions of sodium content alone are not shown, but the general appearance was similar.

Discussion

Source of variation

The distributions in Figure 2 are closely comparable with those determined at an earlier generation of recurrent selection (Yeo et al., 1988). This suggests that a dividing line between the heritable and non-heritable components of sodium transport had been reached; the limit of divergence during recurrent selection being taken to mark the limit of the heritable component. The differences in the populations were preserved during multiplication without selection. Change during recurrent selection in highly-developed cultivars implies that the variability is due to residual heterozygosity rather than that the cultivar is a family of homozygous lines; in which case all change should be achieved in a single generation (Yeo et al., 1988). Cultivars of inbreeding species are not so homozygous as has often been supposed (Simmonds, 1979); and heterozygosity may have accumulated for characteristics which have not been subjected to selection pressure whilst it may have been practically eliminated by repeated selection in characters of agronomic interest.

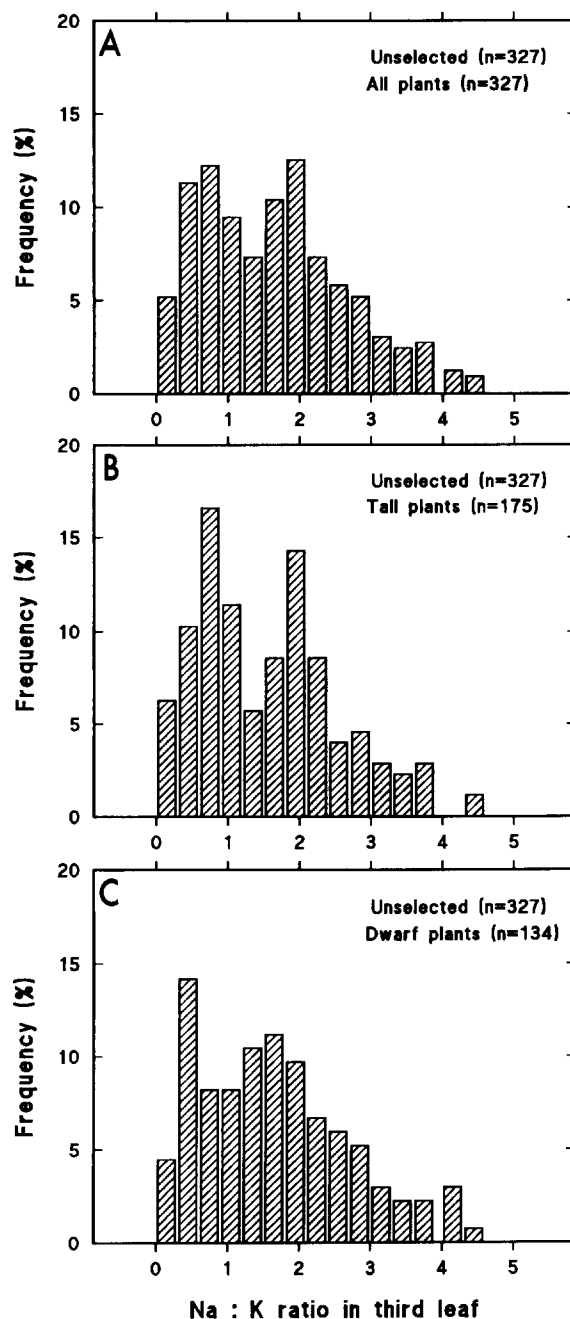


Fig. 6. Frequency distribution of the sodium:potassium ratio in the third leaf of the unselected population of IR59462 (A), classified according to plant type (tall (B) and dwarf (C)).

In the production of anther culture derived lines, anthers are cultured in vitro to generate haploid plantlets. The chromosome number of the plantlet is then doubled to produce a diploid plant, completely homozygous at all loci, called a

doubled haploid (Poehlman, 1987). The plant is then increased and evaluated in the same way as a breeding line. These homozygous lines also exhibited large variability (Fig. 3). This supports the conclusion that recurrent selection had utilised the heritable component of sodium transport and that the variability remaining was a large environmental effect.

Two factors contribute to the extent of the variability. Sodium uptake is mediated, in part at least, by the transpirational by-pass flow (Yeo et al., 1987). The bypass-flow is leakage along a direct apoplastic contact from the external medium to the xylem in regions of the root where the endodermis has not yet differentiated or has been disrupted, such as by the development of lateral roots (Dumbroff and Pierson, 1971; Peterson et al., 1981). This is a small and very variable percentage of the transpiration stream but it becomes important as a feature in ion transport at high transpiration rates and high external concentrations (Pitman, 1982; Sander-son, 1983). Transpiration rates in rice are substantial, as befits a tropical C_3 plant which, together with most of its genus, is a native of freshwater marshes (Oka, 1988). The bypass-flow is dependent upon root morphology and developmental anatomy and so will be influenced by the way in which a particular root system has grown. In addition, any factor which affects the transpiration stream will affect sodium uptake. Most environmental variables (such as light, temperature, air movement) affect plant transpiration. For these two reasons, a large, non-heritable component of sodium transport is to be expected.

Sodium transport and sodium:potassium selectivity

The ion content can also be expressed as a sodium:potassium ratio (Figs. 5, 6). Much of this apparent sodium:potassium selectivity is due to a variable sodium uptake superimposed upon a very much more constant, and normally distributed potassium uptake (data not shown, but see Flowers and Yeo, 1981). If the sodium and potassium are entering via different pathways then this apparent selectivity may bear little relation to the well-documented case for the

Triticeae (Gorham et al., 1987). In the Triticeae, leakage pathways (such as the by-pass flow) are presumably much less significant than in rice. There was a suggestion that selectivity in rice does have an additional component; the frequency distributions for Na:K selectivity were more strongly bimodal than was sodium transport alone (Fig. 5). But it will be difficult to recognise a selectivity character in rice against the variability in sodium transport per se.

Whatever the physiological basis of the individual plant differences in sodium and potassium transport, selection for low sodium:potassium ratios (i.e. high potassium:sodium selectivity) is achieved empirically (Fig. 5).

Relationship to plant type

When a large number of accessions of rice were studied, plant vigour had the strongest correlation with overall performance as assessed by survival (Yeo et al., 1990). The most vigorous accessions are of course those which have not been dwarfed, and the accessions identified as the most salt resistant in mass screening trials (of which more than 90,000 have been made; Akbar, 1986) are non-dwarf land races. Low sodium transport is not, however, a characteristic of the tall plant type; their advantage lies with their growth rate which dilutes the salt (Yeo et al., 1990). They also have a higher water-use efficiency, which is conferred by their morphology rather than differences within the leaf (Flowers et al., 1988). The tall plant type is not favoured in modern plant breeding because of the greater yield potential of the dwarfed types. This would leave an insoluble problem if plant type and salinity resistance were not separable. Studies of growth showed that both tall and dwarf types responded similarly to salt, and it was concluded that there was no inherent disadvantage in dwarf types provided that sodium transport could be reduced to compensate for their reduced vigour (Yeo et al., 1991).

Screening to date has selected as donor parents vigorous accessions of the tall plant type, but the need is for parents of dwarf or moderate stature with low sodium transport. The selected population showed a dramatic reduction in sodium transport and in sodium:potassium ratio

(Figs. 4, 5), but was composed almost equally of dwarf and tall plant types (Table 1). These results demonstrate that variability in sodium transport can be produced in a cross, and selected for in a breeding population, independently of plant type. Although screening of existing genotypes has mostly found tolerance which is associated with the tall plant type, these results show that selection within new breeding populations can produce the desired combination of low sodium transport with dwarf plant type.

Intensity of selection

An important question in the handling of breeding populations is how strongly to select and how early. The earlier and the stronger the selection pressure the simpler the logistics. But the variation seen in recurrently selected intravarietal lines and anther culture derived lines shows that the predictive value of individual performance is heavily modulated by environmental and developmental factors. Selection of single individuals would not, in these circumstances, be expected to be an ideal procedure. A selection of one hundred plants at F_3 (just over a quarter of the population; see Fig. 1) appears at this stage to have been effective. A comparison of the populations produced by a range of selection methods will first be made at F_5 and will be reported in due course.

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