

**TECHNOLOGICAL BREAKTHROUGHS IN  
SCREENING/BREEDING WHEAT VARIETIES FOR SALT  
TOLERANCE**

Paper presented at National Conference on “Salinity  
management in agriculture”, CSSRI Karnal, India,  
December 2 - 5 1998

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## **Technological breakthroughs in screening/breeding wheat varieties for salt tolerance**

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### **ABSTRACT**

Salinity is a major and increasing problem in irrigated areas world-wide and, although much effort has been put into the development of salt-tolerant wheat there has been little impact in farmers' fields. It is argued that to a large extent this is due to a lack of consideration of waterlogging in programmes designed to breed for salinity tolerance. This paper describes recently-developed screening techniques for use in the development of salt-tolerant, and salt- and waterlogging-tolerant, wheat genotypes and recent advances in breeding for tolerance, but is not intended to provide a comprehensive review of breeding and screening techniques.

Developments in screening under controlled field conditions using sprinkler and drip irrigation systems are described and their benefits and drawbacks noted. The potential for the development of tolerant wheats through hybridisation with wild relatives which show enhanced tolerance, including the development of *Tritopyrum*, is discussed, and progress in this field noted. Rapid progress has been made in improving yields in through the use of farmer-participatory breeding and selection methods, and an outline scheme for such a programme is presented. A screening technique for testing for combined salinity and waterlogging tolerance is proposed. It is concluded that, although progress has been slow, the availability of the new techniques will lead to much more rapid progress in the future.

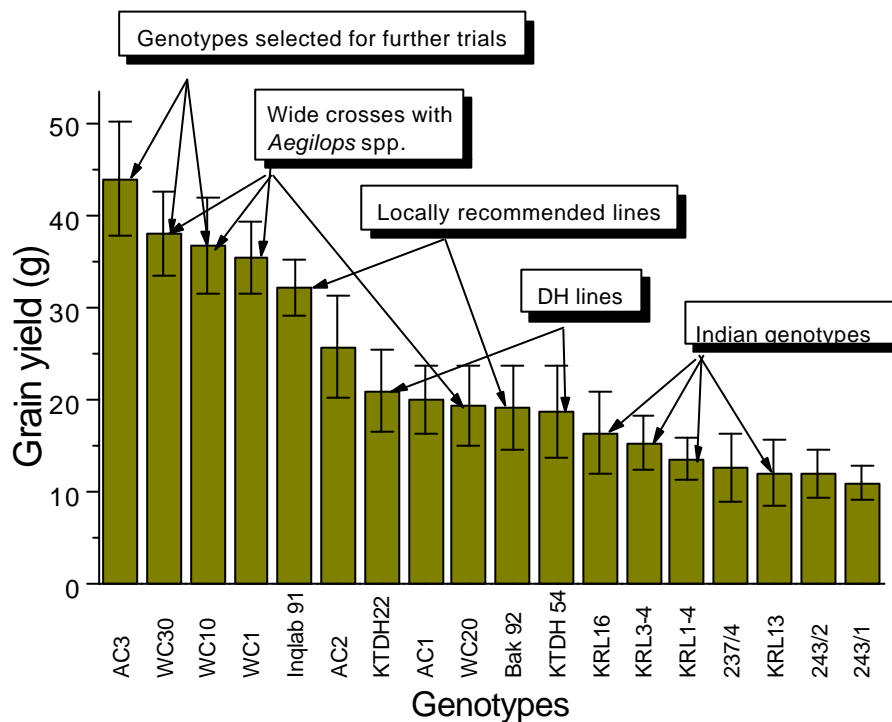
## INTRODUCTION

In both India and Pakistan, population growth will greatly increase the demand for food grain by the middle of the next century, and it will be essential for marginal lands and water to be used to increase production. In Pakistan, over 70% of the groundwater pumped is of poor quality (Ahmed and Chaudhry 1988), and 6 M ha are salt affected, mainly in the canal-command area, of which much is waterlogged. In India about 7 M ha are salt- or sodicity-affected (CSSRI Undated). Waterlogging in NW India is expected to increase 5 fold over the next 30 years (Kulkarni *et al* 1989), threatening the livelihoods of 1 million farm families and having a significant negative impact on the food production of India as a whole. In the Indian Punjab, efficient drainage systems have lowered or stabilised the watertable, which was rising by 0.25 to 0.5 m per year up to 1960, in recent years, but drainage is expensive and the area waterlogged is still very large and is expected to increase again in the future (Singh 1989). The Pakistan Government has spent large sums on reclamation, mainly on drainage, but the area outside the canal commands is untouched, and the measures have not worked on dense saline-sodic soils or where irrigation water is saline-sodic.

Yield losses of wheat in moderately saline areas of Pakistan average 65% (Quayyum and Malik 1988), and it can be assumed that the figure is similar in India. Output could be increased by increasing productivity on areas unaffected by salinity and waterlogging, but this would require additional inputs that farmers could not afford. The “Saline Agriculture” technique (Qureshi 1993) uses saline lands to provide an income for the farmer without the need for expensive drainage and reclamation work. Much of the land which cannot be reclaimed can be used profitably by growing economically important salt-tolerant trees and shrubs, *e.g.* *Atriplex* and *Eucalyptus*, as successfully demonstrated in on-farm trials in Pakistan. On land affected by moderate salinity, salt- and waterlogging-tolerant cultivars of wheat and rice can increase the income of farm families. Similar work at CSSRI has shown the potential of wheat in agroforestry intercropping systems for the reclamation of sodic soils (*e.g.* Gurbachan Singh 1994).

Many efforts have been made to improve salt-tolerance in cereals, but successful results from the laboratory have not yet been translated into the field. Richards (1995) listed only 2 grain crops for which salt-tolerant cultivars had been bred, although he ignored many Indian and Pakistani varieties

in several crops (see e.g. Mishra 1994 for India, Qureshi 1996 for Pakistan). Flowers and Yeo (1995) cited 3 salt-tolerant saltgrasses (*Distichlis palmeri*) registered in the USA in 1988, 2 lucernes (*Medicago sativa*), a meadow cord grass (*Spartina patens*) and a maize (*Zea mays*) variety, although they also mentioned other varieties that had been released in India, and concluded that the very large effort put in to the improvement of crop salt tolerance had not been translated into new varieties being grown by farmers. The Pakistan selection LU26S has shown improved yields (Qureshi *et al* 1990), but is now susceptible to rust, although further selections from it have been made and seed made available to farmers. Landraces have been identified, selected for salt-tolerance, that also have good tolerance of waterlogging, and these are being developed further (RH Qureshi, Personal Communication).

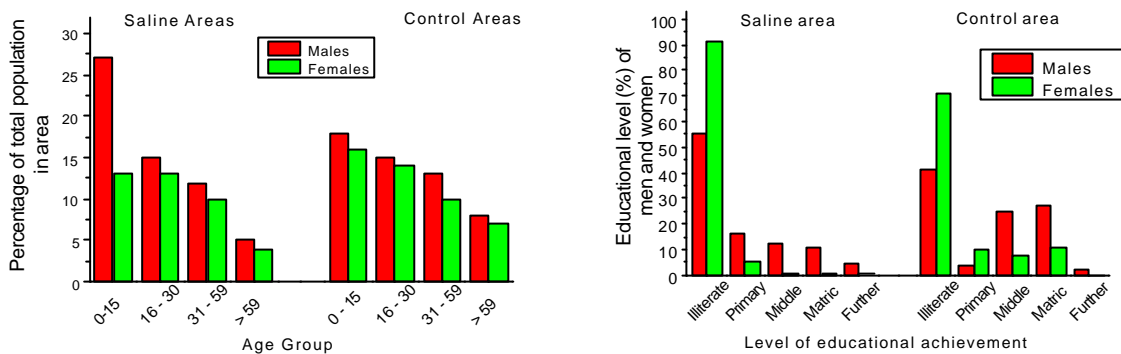


**Figure 1.** Yield performance of wheat selections, single-row trials, NWFP Pakistan 1996/97

In India, CSSRI has released the wheat KRL1-4 for saline areas. This cross of Kharchia with WL711, a variety that was also widely grown in Pakistan and Afghanistan, is popular with farmers in UP state, although not widely grown in saline areas of Haryana or Punjab (KN Singh, personal communication). However, in saline areas of Pakistan it did not do well (see Fig. 1), possibly as a result of the denser soils and greater problems of waterlogging. Conversely, genotypes selected for

Pakistan conditions have not done well in salinity trials in India. We believe that one reason for the slow progress in developing successful varieties for Pakistan in particular is the lack of *simultaneous* screening for waterlogging and salinity.

**Socio-economic factors.** Levels of human development in S. Asia are far lower than in many other areas of the developing world. In low-income families in rural India 21% of girls suffered severe



**Figure 2:** (a) Population distribution by age and gender; (b) level of educational achievement, by gender, in saline and non-saline areas of the Pakistan Punjab. Source: Ijaz and Davidson, 1997

malnutrition compared with only 3% of boys (Ul Huq 1997). In saline areas it is worse still: Ijaz and Davidson (1997) compared salt-affected and control areas in the Pakistan Punjab. The number of females in the under-15 age group in villages in saline areas was far less than expected (Fig. 2a), although the difference disappeared in older age groups, presumably as males take wives from outside, and was not shown in the control areas. It is likely that the high numbers of juvenile females “missing” are due to acute poverty in the salt-affected community. They also found that 91% of women in saline areas were illiterate, compared with 71% in non-saline areas, and that only 15% of males in the saline area were educated beyond matriculation, compared with 30% in the control area (Fig. 2b). The overall level of human development, especially among women, was far lower in saline than non-saline areas.

Breeding crops for improved salt-tolerance, and other remedial measures, could help reduce the social and economic disadvantages of the population, particularly women, of saline areas, and improve status, living standards, health and literacy. High value crops disappear from cultivation in saline and waterlogged areas (Joshi *et al* 1996), leading to the displacement of labour from agriculture

and wider income disparities, with labour movement restricted due to the lower productivity in moderately degraded areas and the abandonment of production altogether in severely affected areas. The introduction *and promotion* of salt-tolerant wheat will help to slow down or arrest, and in some cases to reverse, this process.

There is controversy over the relative importance of biological and engineering approaches to this problem. Some authors (e.g. Rhoades 1997) feel that the main emphasis should be upon irrigation and drainage solutions, although he agrees that there is a place for the development of tolerant crop varieties. Richards (1983 and later papers) advocated breeding for high yield rather than salinity tolerance, as due to the heterogeneity of saline fields most yield comes from those parts with low salinity. However, many fields in irrigated areas of India or Pakistan would be regarded using his criteria as of bad or extreme salinity ( $> 50\%$  with an  $EC_e \geq 5 \text{ dS m}^{-1}$ ), and so expected to show greater responses to breeding for yield under salinity, as was concluded by Kelman and Qualset (1991). The Richards (1983) argument also neglected the importance of salinity/waterlogging interactions as determinants of poor plant growth. I therefore believe that there is a place for breeding for salinity and waterlogging tolerance for wheat and other crops for high-input, salt and waterlogging-affected irrigated areas of India and Pakistan, in addition and complementary to other methods of amelioration which must also be used.

## **REQUIREMENTS FOR BREEDING FOR SALINITY TOLERANCE**

To breed for salinity tolerance, there must be inter-specific, inter-varietal (intra-specific) or intra-varietal variation, within the gene pool. It must be possible to identify the character or characters involved in the segregating generations, and the character must be heritable. There are still large gaps in our knowledge of the genetics of salt-tolerance, although it is now well-established that it is not a simple trait. As salinity tolerance varies through the life cycle of the plant, it is necessary to define closely the conditions for testing, and there are interactions with other stresses such as temperature, fertility, pests and diseases and, in particular, waterlogging. All these make breeding for salt tolerance a difficult and lengthy process, necessarily involving a number of stages, as enumerated by Shannon and Noble (1990):

1. Quantify information on the salt-tolerance of the crop, the variability within it and related species, any sensitivities to particular ions, and any environmental interactions.
2. Identify the growth stage when productivity is most limited by salinity, and assess whether this can be overcome by agronomic or other means, e.g. seed rate, altering irrigation frequencies, etc.
3. Assess whether other locally-adapted genotypes are more tolerant at the crucial growth stage.
4. Develop a procedure to screen at this stage, related as closely as possible to the prevailing field conditions.
5. Carry out field experiments to relate the screening to the field situation.
6. Evaluate a wide range of germplasm to assess the genetic variation and see if selection is possible from within the genotype, or if wide crossing or hybridisation might be better.

### **Screening criteria**

Many potential criteria or traits have been proposed for screening, often unrelated to each other and giving different estimates of salt tolerance, and the tolerance assessed at one growth stage may be unrelated to that at another. In many cases, the test criterion is also an unrealistic predictor of field performance, e.g. germination in saline solution on filter paper. In the field many seeds that could germinate under these conditions would be unable to break through a soil crust, and seedling vigour might be a better criterion. A number of workers (e.g. Shannon and Noble 1990; Flowers and Yeo 1995) have suggested that screening for salt-tolerance be carried out using physiological markers, or that physiological traits should be used as selection criteria, either singly or in combination (pyramiding), rather than selection being simply upon yield or yield components.

The sensitivity of some crops (e.g. rice, Flowers *et al* 1977) to salinity has been attributed to the inability to keep  $\text{Na}^+$  and  $\text{Cl}^-$  out of the transpiration stream. Plants limiting the uptake of toxic ions, or maintaining normal nutrient ion contents, could show greater tolerance; uptake mechanisms that discriminate between similar ions such as  $\text{Na}^+$  and  $\text{K}^+$  could be useful selection traits; and breeding for efficient nutrient uptake or low ion accumulation could be a simple way to improve salt tolerance. Selection within varieties of lines with low  $\text{Na}^+$  transport has been accomplished in rice (Yeo *et al* 1988), while variation for  $\text{Na}^+$  uptake and yield in saline conditions has been found within Blue Si-

ver wheat (Rashid, 1986; Rashid *et al* 1998; Table 1), and low Na<sup>+</sup> lines have been selected (Abdus Salam 1992). However, correlations between salt tolerance on a yield basis and ion content

**Table 1.** Classification of plants of wheat cv Blue Silver into tolerance classes when grown at 100 mol m<sup>-3</sup> NaCl.

Tolerance Class	Plants	% of population	Grain Yield (g plant <sup>-1</sup> )
Sensitive (S)	23	36	< 0.50
Moderately sensitive (MS)	15	23	0.51 - 1.00
Moderate (M)	9	14	1.01 - 1.50
Moderately tolerant (MT)	8	13	1.51 - 2.00
Tolerant (T)	9	14	> 2.01

Source: Rashid, 1986

are not always apparent, and Isla *et al* (1997) concluded that ion content should not be used to screen for salt tolerance in barley. Similarly, although Rashid *et al* (1998), found correlations between ion contents and seedling growth on an individual plant basis, on pooled cultivar data the relationship was much weaker (Table 2), due to large variations in Na<sup>+</sup> and Cl<sup>-</sup> transport in supposedly homozygous cultivars.

**Table 2.** Correlation coefficients (r) between shoot fresh weights and relative fresh weights, and inorganic ion content and osmotic potential

1. Cultivar means at each salinity level						
Salinity (mol m <sup>-3</sup> )	df	Na <sup>+</sup>	K <sup>+</sup>	K <sup>+</sup> /Na <sup>+</sup>	Cl <sup>-</sup>	OP
Fresh weights						
0	7	0.019 <sup>ns</sup>	0.370 <sup>ns</sup>	-0.065 <sup>ns</sup>	-0.529 <sup>ns</sup>	-0.269 <sup>ns</sup>
100	7	-0.653 <sup>*</sup>	-0.289 <sup>ns</sup>	0.447 <sup>ns</sup>	-0.494 <sup>ns</sup>	0.312 <sup>ns</sup>
200	7	-0.151 <sup>ns</sup>	0.601 <sup>ns</sup>	0.184 <sup>ns</sup>	-0.602 <sup>*</sup>	-0.207 <sup>ns</sup>
Relative weights						
100	7	-0.401 <sup>ns</sup>	-0.534 <sup>ns</sup>	-0.093 <sup>ns</sup>	-0.835 <sup>**</sup>	0.252 <sup>ns</sup>
200	7	-0.006 <sup>ns</sup>	0.719 <sup>*</sup>	0.044 <sup>ns</sup>	-0.763 <sup>*</sup>	-0.769 <sup>*</sup>
2. Individual plant values						
Fresh weights						
0	46	0.034 <sup>ns</sup>	0.150 <sup>ns</sup>	-0.095 <sup>ns</sup>	0.411 <sup>ns</sup>	0.146 <sup>ns</sup>
100	47	0.365 <sup>**</sup>	0.057 <sup>ns</sup>	0.304 <sup>*</sup>	0.335 <sup>*</sup>	0.247 <sup>ns</sup>
200	47	0.518 <sup>***</sup>	0.217 <sup>ns</sup>	0.520 <sup>***</sup>	0.612 <sup>***</sup>	0.336 <sup>*</sup>

In this and subsequent tables, \* denotes a significant difference at the 5% level, \*\* at 1%, and \*\*\* at 0.1%. <sup>ns</sup> denotes no significant differences

Adapted from Rashid *et al* 1999

Salt tolerance in the Triticeae is associated with enhanced ability to discriminate between Na<sup>+</sup> and K<sup>+</sup> in the soil solution and to preferentially accumulate K<sup>+</sup> and exclude Na<sup>+</sup> (Gorham *et al* 1985; Omielan *et al* 1991). Durum wheat, which lacks the D genome of bread wheat, tends to accumulate more Na and less K than bread wheat under salinity stress. The trait for K<sup>+</sup>/Na<sup>+</sup> discrimination affects transport of K<sup>+</sup> and Na<sup>+</sup> to the shoots, with little effect on root ion concentration or anion concentration in the leaves, and the main site of action is thought to be at xylem loading in the roots (Gorham *et al* 1990). It acts at all salt concentrations, but is most apparent below 100 mol m<sup>-3</sup> NaCl. At higher concentrations other mechanisms controlling ion accumulation appear to be more important. This trait has been used in attempts to confer salinity enhanced salt tolerance on durum wheat, and will be further discussed later.

Other physiological mechanisms, such as ion accumulation (Flowers *et al* 1977), osmotic adjustment (Morgan 1977, Shannon *et al* 1987), and production of organic solutes (Greenway and Munns 1980) have not yet shown the potential to be used as selection tools for salt tolerance in wheat. Other traits, while not contributing in themselves to tolerance, have been proposed for screening. For example, the induction and quenching kinetics of chlorophyll-a have been assessed. Krishnaraj *et al* (1993) compared 3-week old seedlings of Kharchia and the salt-sensitive variety Fielder at a range of salinities, and found that the maximum rate of fluorescence induction and quenching decreased much more in Fielder than in Kharchia as salinity increased, and could be used in screening programmes to assess the salinity tolerance of wheat genotypes. Chowdhury *et al* (1995) suggested that the electrical potential difference between the external solution and the vacuole of the outer root cells could be used to select for salt tolerance in rice and possibly other species, although no further examples of this technique could be found.

### **Characterisation and assessment of salinity tolerance**

To compare salinity tolerance, a number of models for the response of crop plants to salinity have been defined. Survival and absolute yield under saline conditions have been used in some cases, but have a number of drawbacks when comparisons need to be made. The use of relative yield com-

pared to a control allows for comparisons between crops and cultivars where the yield is expressed in different units, or where the component of economic yield differs between them. The tolerance can be described by plotting the yield or relative yield as a continuous function of soil salinity. Maas and Hoffman (1977) proposed a linear response model, containing three independent parameters, the non-saline control yield ( $Y_{\max}$ ), a salinity threshold ( $EC_t$ ), below which yield ( $Y$ ) was unaffected, and a slope ( $s$ ) representing the decrease in yield with increase in salinity above this level.  $EC_0$  represents the salinity at which there is no yield. It has been used to produce tables of salinity response functions for a number of crops (e.g. Maas and Hoffman 1977; Maas 1990).

Van Genuchten (1983) described a computer program (SALT) to fit the unknown coefficients of this and other models to experimental data, using a non-linear least-squares fit which can handle data sets with few experimental measurements, although the routines can also be handled by commercial statistical packages such as Genstat and SAS. This gives a reasonable fit for commercial yields of a number of crops plotted against the electrical conductivity of the soil saturated extract ( $EC_e$ ). At salinities above the threshold for any particular crop, the relative yield ( $Y_r$ ) could be plotted with the equation:

$$Y_r = 100 - s(EC_e - EC_t) \quad (1)$$

However, the threshold is sensitive to environmental interactions, and depends on accurate salinity measurements, as well as the number of data points taken above and below the expected threshold, and a degree of error exists in evaluating the slope at concentrations near the threshold. At high salinity, the slope tends to decrease, resulting in added uncertainty in this area. Although the model has been widely used, the concept of a salinity threshold is a simplification of the crop response, which is more often of a curvilinear form. To describe this, Van Genuchten (1983) introduced the following expression into the SALT program:

$$Y = Y_{\max} / (1 + (EC_e / EC_{50})^p) \quad (2)$$

where  $EC_{50}$  is the salinity that reduces yield by 50%, and  $p$  is an empirical constant affecting the form of the sigmoid curve, with the curve becoming steeper as the value of  $p$  increases. The model can also be formulated in relative terms, where  $Y_r = Y/Y_{\max}$ . (An example of the response curve with this model is shown later in Figure 5.) The  $EC_{50}$  has been shown to be a suitable parameter to evaluate salt tolerance (Royo *et al* 1991), as it integrates both the slope and the threshold from the

earlier model, although its use in field experimentation may be difficult where differences between genotypes are small.

### **Controlled environment screening**

Due to the problems of field screening, to be discussed later, much of the development of salt-tolerant varieties has been carried out in controlled environments. Many techniques have been used: all have their advocates and opponents, advantages and drawbacks. They include screening in hydroponic or solution culture, used by many workers (e.g. Gorham *et al* 1985 and later papers) and which allow precise control of salinity and nutrient levels, although growing plants in solution very different to the saline field situation. Usually, the parameters studied are either survival as a whole, short- or medium-term growth, or the ion contents of different plant parts. The screens can be divided into two groups: those where the seedlings are suspended over aerated solutions containing both nutrients and salt (e.g. Kingsbury and Epstein 1984, Gorham *et al* 1986, Azhar and McNeilly 1988), and those where seeds are planted in an artificial medium and irrigated with the nutrient and salt solution. Examples of the latter include sand (Sayed 1985, Munns 1985, Grieve *et al* 1993), gravel (Rawson *et al* 1988), perlite (Nevo *et al* 1993) or vermiculite (Jana *et al* 1983). Pecetti and Gorham (1997) concluded that different growing conditions could lead to different conclusions: e.g. sand culture could be more useful for selection on the basis of seedling growth, while water culture would be better for looking at Na<sup>+</sup> accumulation.

A recent development is the use of floodbenches (Akhtar *et al* 1994), where plants are grown in tanks in a substrate such as vermiculite, and the solution, containing nutrients plus NaCl at the various stress levels is pumped up at intervals and then allowed to drain. This alleviates the need for aeration, and provides a more “realistic” growing medium than pure solution culture.

### **Field screening**

#### **Natural conditions**

Field experiments in saline soils are made difficult by high spatial and temporal variability which lead to very high environmental components to the variation and make it difficult to detect differences between genotypes. Several statistical approaches have been used to address similar problems, e.g. by blocking, in particular with the use of small blocks, or by adjusting according to the values of

neighbouring plots (Pearce and Moore 1976; Bartlett 1978). These have reduced error variation and increased detection of varietal differences, and can be adapted to cope with the high heterogeneity of saline fields. Other methods such as the use of geostatistical techniques are also gaining in popularity. Methods currently being validated include the use of long rows along salinity gradients, with within-row differences being mapped and assessed using an electro-magnetic salinity sensor. These are giving good results for salinity tolerance, but have yet to be assessed for sodicity and waterlogging.

The following protocol has recently been developed by staff at the Servicio de Investigacion Agrolimentaria (SIA) in Zaragoza, Spain, for the assessment of genotypes under natural field conditions (R Aragiés, Personal Communication).

1. Locate a plot with visual symptoms of salinity and, preferably, a regular gradient in crop yield decrements.
2. Construct a detailed salinity map of the plot. The only way to do this is through  $EC_a$  (apparent soil electrical conductivity) readings of the electromagnetic sensor on a regular (ideally 2 m x 2 m) grid. Delineate the  $EC_a$  isolines, preferably using geostatistical techniques (eg using computer programs such as Surfer or ESAP).
3. Sow the crop or cultivars along the direction of the maximum salinity gradient, in bands of 4 or 5 rows, depending upon between-row spacing.
4. Use conventional cultural and agronomic practices.
5. Take regular  $EC_a$  readings (every 15 days and/or after each irrigation) on the previously-used grid to ascertain the temporal variation of soil salinity.
6. Carry out **at least** 3  $EC_a - EC_e$  calibrations ( $EC_e = a + b \cdot EC_a$ ) during the growing season. Ensure the number of data points each time is greater than 10. Measure the  $EC_e$  of the soil in the crop rootzone. From the average calibration, convert the  $EC_a$  maps to  $EC_e$  maps.
7. Harvest grain yield (or other target crop variable) in selected small areas by hand, preferably where the crop appears to be uniform (area around 0.5 - 1.0 m<sup>2</sup>). In an experiment in Spain, an area of 4 m<sup>2</sup> was harvested by machine with poor results. Harvest or

sample over the maximum number of possible points (ideally 20 per genotype or other treatment) from areas of low to high yields.

8. Plot the yield -  $EC_e$  observations. These  $EC_e$  values are the time-weighted average values obtained from the  $EC_e$  maps delineated in point 6 above. Decide the salt response model that best fits these observations. If necessary, carefully delete statistical outlying points. Estimate the salinity tolerance parameters (eg the threshold  $EC_e$  and the slope of the line, or the  $EC_{50}$  and values of  $p$  and  $Y_{max}$ ).

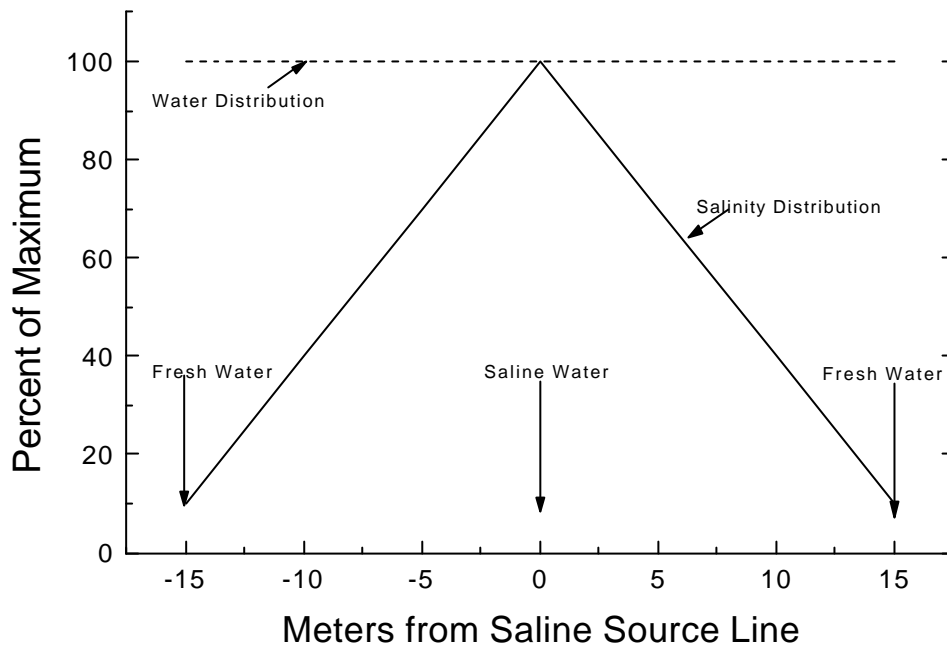
### Controlled conditions

Attempts have been made to overcome the drawbacks of screening in controlled environments and in natural conditions by developing techniques for screening in controlled field conditions.

**Triple-Line-Source Sprinkler.** The Triple Line Sprinkler (TLS), was developed and validated in Zaragoza, Spain (Aragüés *et al* 1992; Royo and Aragüés 1993). It consists of 3 parallel sprinkler lines, with lateral spacing equal to the wetted radius of the sprinkler. The outer laterals apply fresh and the central one saline water with a 1:1 ratio by weight of NaCl and hydrated  $CaCl_2$ , providing a continuous linear salinity gradient on each side of the centre line and uniform water application (Fig. 3). Salinity gradients are not significantly different from one side to the other when the laterals are parallel to the prevailing wind and when used on soils with good infiltration. Soil salinity, assessed using an EM38 electromagnetic sensor at a given distance from the laterals was even: it increased over the growing season, and was higher in the top than the lower part of the soil profile, due to limited soil water infiltration. However, there was some leaf injury due to sprinkling with the saline water, mainly in the older, lower portion of the crop canopy.

The TLS was appropriate for salinity crop studies on soils allowing high leaching fractions when the system operated under low wind conditions. However, later work (Aragüés *et al* 1994; Benes *et al* 1996a; 1996b) raised concerns over leaf absorption of  $Na^+$  and  $Cl^-$  ions in this system, despite the use of pre- and post-washing to mitigate it. In particular, Gorham *et al* (1994) noted that varieties of barley possess different mechanisms of foliar and root uptake of salts, and when grown in hydroponics showed different rankings in leaf ion concentrations depending upon whether absorption took place via the roots or through the leaves. Benes *et al* (1996a) suggested that genotypes that were

salt tolerant due to their root systems efficiently restricting  $\text{Na}^+$  and  $\text{Cl}^-$  transport to the shoots might

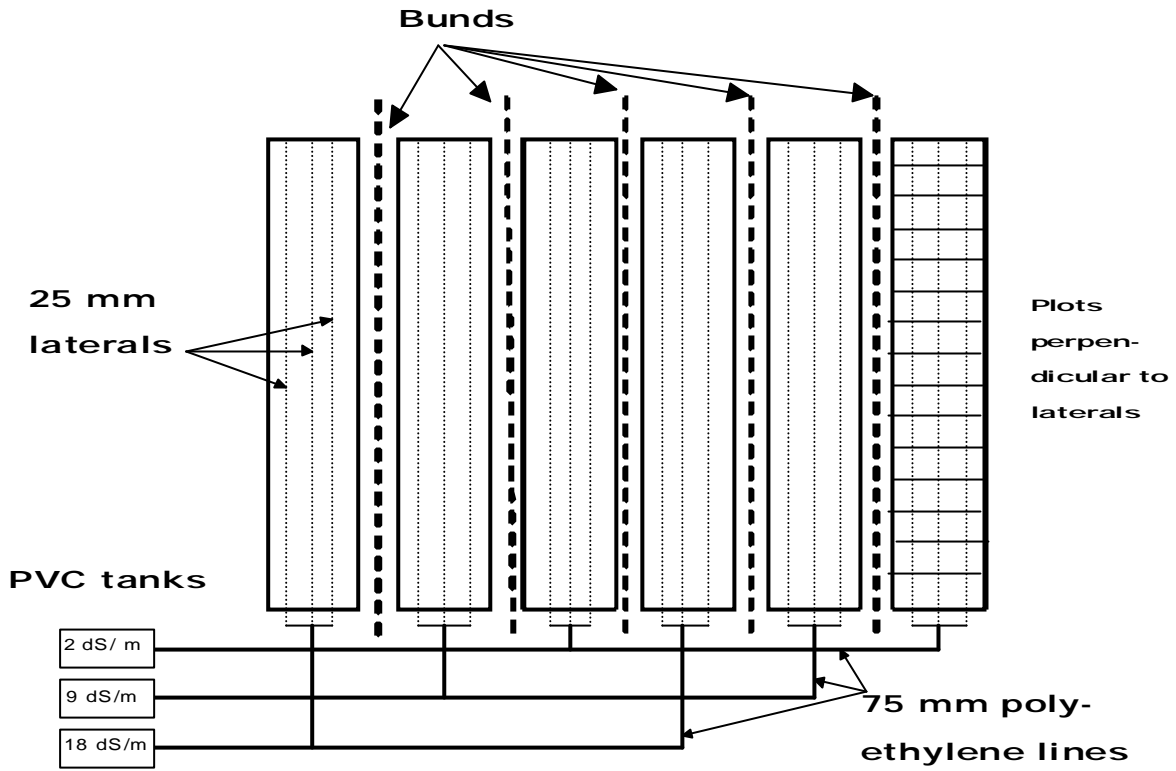


**Figure 3:** Schematic representation of the distribution of saline and fresh water in the Triple Line Sprinkler system (TLS) in Zaragoza. Source: Aragués et al (1992)

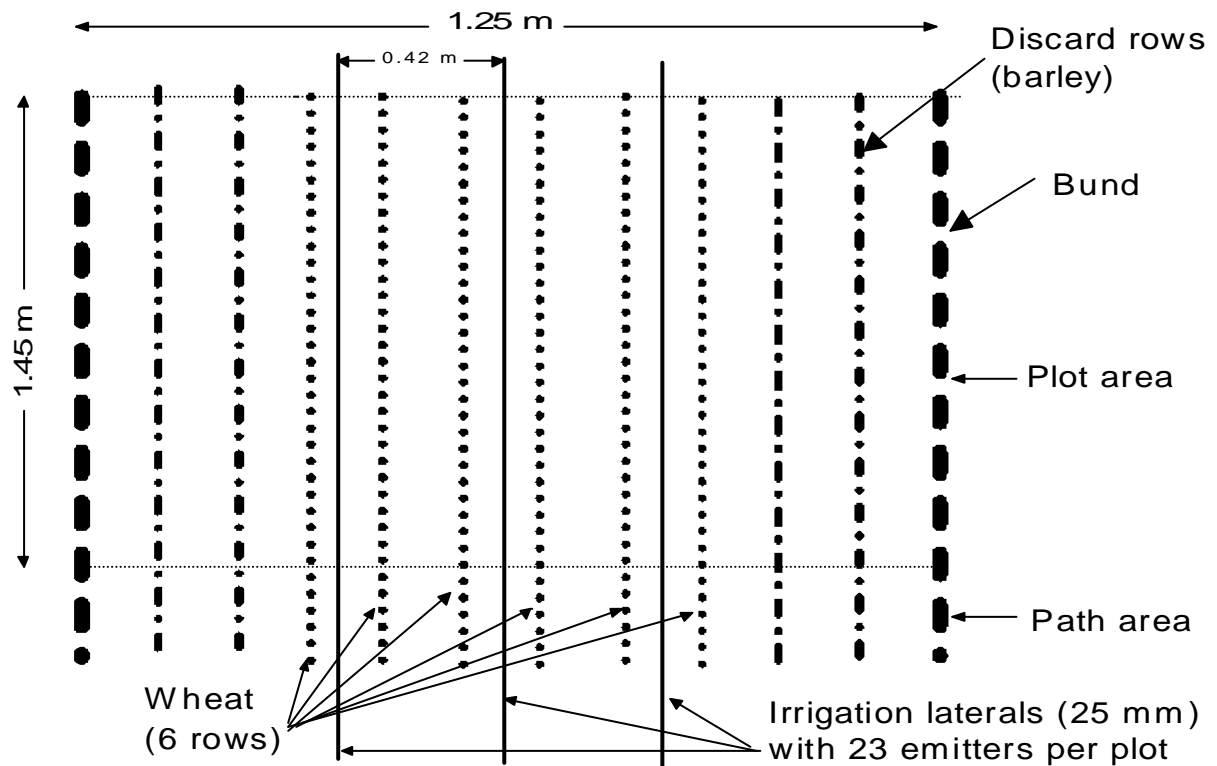
not show equal tolerance in sprinkler systems where the main absorption was via the leaves.

**Drip Irrigation System.** As a result, a controlled drip irrigation system has subsequently been developed. This consists of three 3000 L PVC tanks, a pumping system, three 75 mm-diameter polyethylene lines (one for each saline treatment) and 25-mm diameter laterals with emitters located 0.2 m apart (Fig. 4a). Plots of 1.45 x 1.25, containing six rows of plants and three irrigation laterals located 0.42 m apart, were established.

Each plot has 23 evenly-spaced emitters delivering a flow of  $12 \text{ L h}^{-1}$ , giving a uniform and complete wetting of the soil (Fig. 4b). The system utilises three salinity levels, control ( $\text{EC} = 1.5 \text{ dS m}^{-1}$ ), medium ( $10.1 \text{ dS m}^{-1}$ ) and high ( $16.7 \text{ dS m}^{-1}$ ), again made up of  $\text{NaCl}$  and hydrated  $\text{CaCl}_2$  in a 1:1 ratio by weight. The system has been in use since 1994, and has eliminated many of the problems involved with screening using the TLS, although the large number of irrigations does lead to the development of shallow root systems.



(a)



(b)

**Figure 4 (a):** Schematic representation of the drip irrigation system for testing under controlled salinity at Zaragoza, Spain; **(b)** schematic representation of the layout of a plot (not to scale)

Using these two systems, Isla *et al* (1997) found that the highest yielding barley cultivars under non-saline conditions were the highest yielding under moderate, although not under high, salinity, and concluded that Richards' (1983) generalisation that high yields in saline fields should be obtained by breeding for yield under non-saline conditions should be regarded with caution as it depended upon the severity of the stress. They also concluded that, because of the good correlations between tolerance estimated in the TLS and in the drip system, the simple and inexpensive TLS could be successfully used to screen barley for salt tolerance despite the problems of leaf ion uptake.

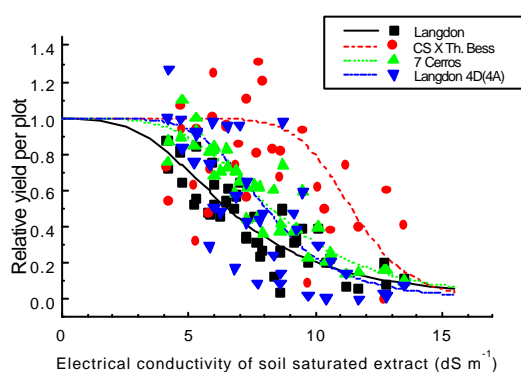
### **BREEDING for SALT TOLERANCE**

The genetic base of wheat breeding in India and Pakistan is narrow: much is simply reselected from CIMMYT germplasm. Almost all Indian salt-tolerant wheat germplasm is derived from Kharchia, and new sources of tolerance to salinity and waterlogging must be found. Little screening of landraces has been done, but these have been shown to have potential (A Rao, personal communication). There is therefore a need to develop and exploit new sources of salt-tolerant germplasm.

**The ideotype.** Donald (1968) suggested the concept of the ideotype - the ideal genotype for a particular environment, although his thinking was that it should be designed for non-stressed situations. However, Shannon (1993) suggested that its greatest potential could be in a specific stress situation. Tillering capacity is a major yield component in wheat, and the main stem yield is highly resistant to salinity at a wide range of concentrations (Maas *et al* 1993). Donald had initially proposed unicum (one tiller) wheat as a possible character for his ideotype, but this was not ideal under non-stressed conditions. However, under salinity, smaller unicum plants could be planted at high densities to maintain yields and offset the yield loss due to reduced tillering. Unicum genotypes with large ears, strong, thick straw and the ability to grow at high density could then be further developed to increase yields in saline areas.

## Wide crosses

***Thinopyrum* species** A number of wild relatives of wheat are tolerant to high salinity, for example *Lophopyron elongatum* (= *Elymus elongatus*; *Thinopyrum elongatum*) (Dvorak and Ross 1986) and *Th. bessarabicum* (Gorham *et al* 1985). *Th. bessarabicum* can withstand salinity up to 350 mol m<sup>-3</sup> NaCl, and sets seed at salinities up to 250 mol m<sup>-3</sup> NaCl (Forster *et al* 1987). It forms a 56-chromosome fertile amphiploid with hexaploid wheat, which also survived and set seed at 250 mol m<sup>-3</sup> NaCl, unlike its Chinese Spring wheat parent (Forster *et al* 1987). Salinity reduced spikelet numbers, indicating that early spikelet development may be critical in stressed plants, but there was no reduction in numbers of tillers or grains, although height and grain weight were reduced. First generation backcrosses were more tolerant than wheat, but less tolerant than the amphiploid, so full expression of the tolerance depends upon a double dose of the relevant *Thinopyrum* genes. The amphiploid has been tested under salinity in Spain and, although confirming its tolerance (Fig. 5), it also showed a great many drawbacks for direct use as a salt-tolerant crop.



**Figure 5:** Curvilinear response of grain yield per plot to soil salinity for bread and durum wheat genotypes, a wheat X *Thinopyrum bessarabicum* amphiploid, and a Langdon 4D (4A) substitution line, TLS Zaragoza 1989/90 and 1990/91

related to its ability to exclude Na<sup>+</sup> and Cl<sup>-</sup> from young leaves, inflorescences and grains (Gorham *et al* 1986), suggesting that the gene for salt tolerance on 5E<sup>b</sup> restricts the influx of these ions into developing tissues. The 5E<sup>b</sup> addition line had poor grain development at 200 mol m<sup>-3</sup>, either due to the added group 5 chromosomes adversely affecting grain morphology (Miller and Reader 1987), or because genes on other *Th. bessarabicum* chromosomes are needed for good grain development under salinity.

Forster *et al* (1988) showed that genes on homoeologous group 2 increased the susceptibility of wheat to salt, and that the 5E<sup>b</sup> chromosome from *Thinopyrum* carried a major dominant gene or genes for salt tolerance. They also showed that wheat becomes salt-susceptible after the initiation of reproductive growth, and that there were strong interactions between salinity tolerance and the genes controlling flowering time and vernalisation requirements. The tolerance of the amphiploid is

King *et al* (1996) assessed the salt tolerance of the addition lines and 5E<sup>b</sup>(5A) and 5E<sup>b</sup>(5D) substitution lines. The 5E<sup>b</sup> addition line survived better than Chinese Spring, while the substitution lines not only survived better than Chinese Spring, but also better than the addition line, showing that the gene or genes had a greater effect when substituted for a homoeologous wheat group 5 chromosome than when present as an additional chromosome. Chromosome 5E<sup>b</sup> also carries agronomically deleterious genes, and recent work aimed to introduce the relevant segment of 5E<sup>b</sup> into wheat chromosomes of group 5 through homoeologous recombination. Lines carrying 5E<sup>b</sup>/wheat group 5 recombinant chromosomes are being assessed to determine which carries the smallest chromosome fragment of 5E<sup>b</sup>, and the gene or genes conferring tolerance (King *et al* unpublished).

Alternatively, the amphiploids themselves could be developed as a new cereal (*Tritipyrum*) as was triticale. Tetraploid wheats were hybridised with *Th. bess*, and wheat-like amphiploids produced (King *et al* 1997). These varied in height and spike morphology, but in general tall parents produced tall tritipyrum. All had speltoid ears and a brittle rachis, and variation in the number of spikes and in fertility, and awns were mostly suppressed. King *et al* (1997) tested some of this material with the octaploid amphiploid Chinese Spring x *Th. bess*, and the wheat parents, at 4 salinity levels. Survival was far better in the Tritipyrum at 150 mol m<sup>-3</sup> NaCl, where 90% of the wheat died, but was much less at 200 and 250 mol m<sup>-3</sup> although little or no wheat survived at these levels. The only wheat to produce grain under salinity was Chinese Spring: the tritipyrum did, but numbers of spikes and spikelets, and seed set, were much reduced. Neodur X *Th. bessarabicum* was most tolerant at 150 mol m<sup>-3</sup>, and as the same *Th. bessarabicum* accession was used throughout this suggests that even greater tolerance could be developed by selecting specific wheat genotypes. Tritipyrum development parallels the early development of triticale, and in the long term breeding and selection predominantly within the wheat component should produce a successful new cereal.

Other work has introduced tolerance from *Lophopyron elongatum* (*Elymus elongatus*), another relative of wheat. Omielan *et al* (1991) grew this with Chinese Spring, their amphiploid, disomic substitution lines of *L. elongatum* chromosomes in Chinese Spring, and a check cultivar in the field. Under salinity, the amphiploid greatly outperformed Chinese Spring, and in the disomic substitution lines the most dramatic increase in tolerance was conferred by chromosome 3E. Farooq *et al* (1993) had similar results with *Th. scirpeum* (*E. elongatus* subsp. *scirpeus*).

***Aegilops* species.** Researchers at CIMMYT have crossed durum wheats with *Aegilops squarrosa* (= *Triticum tauschii*; *T. squarrosa*), and produced synthetic wheats with traits from disease resistance to tolerance to heat, salt, drought, and waterlogging. Through these, breeders can more efficiently transfer desirable traits into elite bread wheats. An elite set of 95 synthetics are being tested in international nurseries and by national programs for yield, disease resistance and stress tolerance, including salinity. Some outyield the best check lines, giving over 4 t ha<sup>-1</sup> (Villareal *et al* 1998). They also show promise against waterlogging, some growing almost as well in water as rice, and provide resistance to constraints such as reduced irrigation and boron toxicity, while some make better use of micronutrients. The work is being extended to synthetic durums, and lines developed with potential disease tolerance and stress resistance.

A successful programme to introduce tolerance from *Ae. cylindrica* to hexaploid wheat was undertaken in Pakistan (Farooq *et al* 1992a; 1992b), and six plants were obtained which produced grains at EC 15 dS m<sup>-1</sup>, and one at 20 dS m<sup>-1</sup>, although none survived to maturity at 25 dS m<sup>-1</sup>. Later (Farooq *et al* 1995a) nine more hybrids were compared with three wheats in the field. Two lines outyielded LU26S, and the overall performance of the new germplasm in saline fields was better than LU26S. This material has since been tested in the field in our own programme, and has outperformed locally-recommended genotypes and salt-tolerant cultivars (Fig. 1). Seed has also been made available to CSSRI. Other work has successfully crossed Pak81, LU26S and Chinese Spring with *Ae. ovata* (Farooq *et al* 1995b).

Over 400 *Ae. squarrosa* accessions were screened in a greenhouse using saline gravel culture and nutrient solutions (Schachtman *et al* 1991). Ten accessions selected for high and low leaf Na<sup>+</sup> were compared with Kharchia and salt-sensitive *T. turgidum* cv. Modoc. Two were highly salt tolerant in terms of relative biomass (> Kharchia) and two highly sensitive (= Modoc). Later (Schachtman *et al* 1992) they produced synthetic hexaploids from five *Ae. squarrosa* accessions varying in salt tolerance and two salt-sensitive *T. turgidum*. The relative grain yield of the hexaploids in 150 mol m<sup>-3</sup> NaCl was greater than that of the tetraploid parents, primarily due to the maintenance of grain weight under salinity.

**K<sup>+</sup>/Na<sup>+</sup> discrimination.** Gorham *et al* (1997 and references therein) summarised knowledge of the previously-mentioned enhanced K<sup>+</sup>/Na<sup>+</sup> discrimination trait. Differences in K<sup>+</sup> and Na<sup>+</sup> accumulation at < 100 mol m<sup>-3</sup> NaCl were observed in shoots of different wheat and *Aegilops* species. Hexaploid wheat showed greater discrimination in favour of K<sup>+</sup> and against Na<sup>+</sup> than tetraploid durum wheat. As *Ae. Squarrosa* also had high discrimination between K<sup>+</sup> and Na<sup>+</sup>, it was concluded that the character resided on the D genome inherited from *Ae. Squarrosa*. The trait is also present in other primitive wheats, rye, triticale and synthetic hexaploids derived from tetraploid wheat and *Ae. squarrosa*. However, it is not shown in durum wheat, cultivated barley or wild barley (*H. spontaneum*), or by perennial wheatgrasses which lack the D genome.

Experiments with disomic D genome substitution lines revealed that the trait was controlled by the long arm of chromosome 4D (Gorham *et al* 1987), and gene dosage had little effect on its expression. Dvorak and Gorham (1992) recombined the 4D chromosome with 4B, and showed that Na<sup>+</sup> exclusion and enhanced K<sup>+</sup>/Na<sup>+</sup> ratio in the shoots were controlled by a single locus, *Kna1*, on the long arm of 4D, later shown to be linked to 5 markers on the distal third of this arm (Dubcovsky *et al* 1996). The recombinant families were grown in the field (Dvorak *et al* 1994). Under salt stress *Kna1* families had higher K<sup>+</sup>/Na<sup>+</sup> ratios in the flag leaves, and higher yields of grain and biomass, than the *Kna1*<sup>-</sup> families and the parental cultivars, proving that *Kna1* was one factor responsible for the higher salt tolerance of bread relative to durum wheat (Dvorak and Gorham 1992).

The effect of *Kna1* could be modified by interaction with other genes in the D genome, and additional 4D chromatin had detrimental effects on yield, so that elimination of structural changes and unnecessary 4D material from the recombinants could increase the effectiveness of *Kna1* in a durum background. They concluded that the gene should be introgressed into cultivars for irrigated production systems, but as selection for *Kna1* in segregating populations relies on linked markers, and the only one to be mapped so far is absent in the recombinants due to the position of *Kna1*, a further marker must be identified for the work to progress.

From field work in the TLS in Zaragoza, the amphiploid Chinese Spring x *Th. bessarabicum* had the lowest yield potential (yield at low salinity), followed by the Langdon disomic 4D(4A) substitution line, then Langdon, then 7-Cerros hexaploid wheat. However, in relative terms, Langdon had

the lowest yield and the amphiploid the highest under salinity, and it was difficult to distinguish between 7-Cerros and the substitution line (Fig. 5). In the third youngest leaf at anthesis,  $\text{Na}^+$  was highest in Langdon and lowest in the amphiploid, while  $\text{K}^+$  was highest in the amphiploid and lowest in Langdon. The substitution line and 7 Cerros were intermediate, with similar contents of both ions.

In saline sand culture, there was a very high inverse correlation between  $\text{Na}^+$  and yield in a *Kna1*-containing line and a *kna1* line, which failed to produce grain. There were considerable differences within the groups, but overall *Kna1* lines had a higher mean yield. In artificial sodic soil, there was a consistent increase in  $\text{Na}^+$  accumulation with higher ESP levels in a *kna1* line, while in a *Kna1* line there were non-significant differences between  $\text{Na}^+$  at ESP 3.2 and 12.5. Although absolute grain yields were similar, relative yield was lower in the *kna1* line. It seems that, although the *Kna1* gene was not the only factor controlling ion accumulation or yield under salinity, the  $\text{K}^+/\text{Na}^+$  discrimination trait might be beneficial in breeding for sodic as well as saline conditions.

Some authors (e.g. Richards 1992) believe there is little chance of improving salt-tolerance by the introduction of alien germplasm, but these conclusions were drawn from an experiment on a drying saline soil, inappropriate to the irrigated conditions of India and Pakistan, and did not assess any hybrids in which the tolerance of wild relatives had been incorporated into existing *well-adapted* cultivars. The work described above gives us strong grounds to dispute this, and we feel that in the long term there is an excellent chance that new salt-tolerant genotypes will be developed through wide crossing to incorporate the genes for salt tolerance into elite wheat germplasm.

### **Doubled haploids**

Progress in variety production is hampered by the time taken to produce stable material, and can be accelerated by techniques such as single seed descent and doubled haploid breeding, allowing varieties to be submitted for national trials only 2 years after initial crossing. Doubled haploid breeding involves fertilizing wheat ears, in our case from crosses between Kharchia 65 and TW161,

**Table 3.** Grain yield per plot and sodium content of the leaf below the flag leaf at anthesis of wheat genotypes, drip irrigation system, Zaragoza 1994/95

	Genotype							Mean
	KTDH 9	KTDH 19	Kharchia 65	KRL 1-4	CS	CS5A (5Eb)	TW161	
Salinity (dS m <sup>-1</sup> )	Grain yield (g)							
0.0	302	946	795	602	156	359	796	565
9.0	512	506	435	510	109	274	617	423
18.0	306	277	187	213	35	119	323	223
	Sodium content (mol kg <sup>-1</sup> )							
0.0	4.0	4.0	19.2	15.9	9.3	8.5	3.1	9.2
9.0	10.8	15.5	81.6	45.9	34.8	40.6	6.6	33.7
18.0	27.4	14.1	85.1	81.8	37.6	76.6	17.2	48.5

SE of means ±	Yield		Sodium	
	salinity	salinity x genotype	salinity	salinity x genotype
	36.7**	57.6*** (48.0 <sup>a</sup> )	7.18*	11.93* (10.29 <sup>a</sup> )

<sup>a</sup> SE for within salinity comparisons

**Table 4.** Grain yield per plot (g) of wheat genotypes grown over two seasons in two fields at Gandheri, Nowshera, NWFP, Pakistan

Environment	Mean EC <sub>e</sub>	Genotypes							Mean
		KTDH19	Kharchia 65	TW 161	Mutant	Blue Silver	Bakthwar 92	WS 10	
A, 1994/95	5.54	261	431	225	115	120	237	173	223
B, 1994/95	4.03	1002	1019	681	1150	1369	1638	1499	1194
A, 1995/96	1.95	266	503	241	487	515	603	399	431
B, 1995/96	1.71	721	915	658	726	835	893	867	802

se environment means ± 47.4\*\*\*; G x E means ± 76.1(64.2)\*\*\*

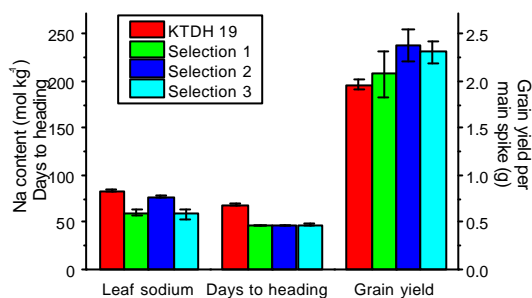
an Na<sup>+</sup>-excluding UK breeding line, with maize pollen, the maize chromosomes subsequently being eliminated during development, leaving a haploid wheat embryo. Embryos are excised 2-3 weeks after pollination and transferred to an agar medium, and the plantlets later transferred to potting soil and treated with colchicine to double the chromosomes. We have tested the resulting homogeneous and stable plants (designated KTDH19), along with the parents and other genotypes, for salinity tolerance in the Drip Irrigation System in Zaragoza, as well as in India and Pakistan. The line showed excellent tolerance in Spain (Table 3), but due to late maturity and rust-susceptibility did not yield well in India or Pakistan (Table 4), although biomass production was high.

The system has been modified slightly at CIMMYT by the use of cut tillers in sucrose solution in growth chambers. Contamination was controlled by using sulphurous acid in the growth medium, and emasculation was by hot water rather than by hand, using a water bath at 43°C and a spike treatment of exactly 3 minutes. Selfed seeds were identified by their large size and well-formed endosperm, and discarded. This technique was claimed to have a success rate of 100% in 10 F<sub>3</sub> populations (Mujeeb Kazi *et al* 1998).

A DFID-funded project (R6438) has provided funding for two breeders from CSSRI to work on this technique, and as a result a number of doubled haploid genotypes, developed using Indian and Pakistani varieties, are undergoing testing in India. We expect that production of this material will begin at CSSRI within the next twelve months.

## **Mutation**

Flowers and Yeo (1995) reported that out of almost 500 crop varieties developed by mutation, the technique had had little application to breeding for any stress tolerance, probably as mutation worked best with factors controlled by single genes rather than complex quantitative characters such as salt tolerance. Salt-tolerant rice has been developed through mutation at CSSRI, but there is no evidence that salt-tolerant wheat has yet been produced by mutation breeding specifically for the trait, although a number of barley mutants have been found to be salt-tolerant (Pakniyat *et al* 1997). However, our work has shown that mutation can induce earliness in salt-tolerant but late maturing material (AR Mahar, Unpublished data). Seeds of the late maturing, salt-tolerant doubled haploid line KTDH19 were irradiated, and selection for earliness continued until the M<sub>5</sub> generation, when



**Figure 6:** Grain yield, days to heading and leaf Na<sup>+</sup> content of M<sub>5</sub> selections from irradiated KTDH 19. Source: Mahar, 1999

selection was carried out for yield under saline conditions. The work produced genotypes maturing almost 3 weeks earlier than the parental material, with no reduction in the salinity tolerance: indeed some lines had slightly increased yield or lower sodium, and one had the added benefit of awns, which were absent in the unirradiated controls (Fig. 6).

### Marker-assisted selection

Salinity tolerance is one of a number of traits that exhibit continuous variation. Such quantitative traits, under the control of a number of genes, may be referred to as polygenic and in the past have been analysed by standard biometrical methods. These remain very useful, but cannot yield information about the effects of individual genes, only the sum of their effects. However, if such polygenes are closely linked to other genes with clearly visible effects, then the latter can act as markers to trace the inheritance of the linked polygene. Developments in this field are being covered in more detail in another paper (Flowers 1998), and this paper will simply mention some of the work in which our colleagues at the John Innes Centre, Norwich, have been involved.

Wheat doubled haploid lines have been examined to identify QTLs associated with salinity responses: a population of doubled haploid lines has been tested in saline environments and mapped with over 300 markers (S.A. Quarrie, Personal communication). Productivity under saline conditions was associated largely with genes affecting flowering time and ion accumulation, and many QTL effects were around the vernalisation response gene *Vrn1* on chromosome 5A. Traits regulating productivity under stress conditions can be identified by comparative QTL analysis and measures of productivity such as biomass production or yield. The coincidence of major QTLs for 2 traits such as leaf ion content and grain yield would indicate their likely association, and the identification of alleles of markers associated with major QTLs for improved salt-tolerance would allow the techniques to be used to improve the efficiency of selecting for better salt-tolerance. As no QTLs for salt tolerance have been identified in genotypes adapted to the Indian sub-continent, this would be a

major benefit for breeders, as the high variability of soil salinity and sodicity means that heritability of tolerance under standard selection conditions is often very low.

### **Tissue culture**

The use of tissue culture to improve salt-tolerance has been studied in a number of crops, and has the advantages that large cell populations can be maintained under precisely-controlled conditions, and that techniques such as mutation, haploid production, somatic hybridisation and transformation are easy to carry out under such conditions. However, it has proved very difficult to maintain the selected character during regeneration, and little is known of how the cellular response compares with that of the whole plant. Although a few improvements in salt-tolerance from tissue culture have been demonstrated in the laboratory (Timm *et al* 1991; Barakat *et al* 1996) there are no reports of this material having been tested in the field.

### **Molecular biology**

Recent advances have broadened the possibilities for manipulating genes, both at the cell and at higher levels, and gene identification, isolation and transformation are now realities, although their use to develop salt-tolerant crops is hampered by the multigenic nature of salinity tolerance and the complicated response of the plant as a whole. Stress-induced changes in gene expression can occur: for example Ramagopal (1987) found that salt stress reduced mRNA in barley roots by between 20 and 30%, with 21 new RNA species being induced and others inhibited. Salt-induced proteins have been noted in several species including a hybrid between wheat and *Elytrigia* (Gulick and Dvorak 1987). These techniques are still in early stages of development, and will become increasingly important in the future. It is likely that it will be possible to transfer genes for salt tolerance by the direct injection of DNA or through bacterial or viral plasmid vectors. Due to the lack of current knowledge of the number and position of specific genes for salt tolerance, techniques to transfer substantial portions of the genetic material from one species to the other may be the most useful.

### **Farmer Participatory Methods**

Witcombe *et al* (1996) found that few farmers in marginal areas of developing countries had adopted improved cultivars, often because they had not been exposed to suitable alternatives to their own landraces, but they were willing to take part in trials to identify improved material, and to

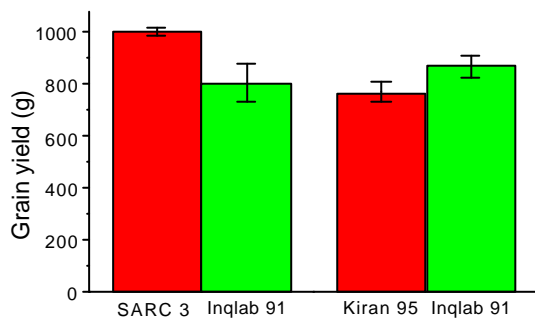
use genotypes so identified. With his colleagues (Joshi and Witcombe 1996; Sthapit *et al* 1996) he distinguished two participatory approaches in plant breeding: participatory varietal selection (PVS)

**Table 5.** Methods of varietal selection with varying degrees of farmer participation

Methods in increasing order of farmer-participation	Evaluation includes	Example institutions
1. Researcher-managed and evaluated on-station trials; farmers may visit station to identify farmer-acceptable material	Yield data; possibly farmer evaluation	Research
2. Researcher-managed on-farm trials, replicated design; farmers may be involved in evaluation	Yield data; possibly farmer evaluation	Research
3. Farmer-managed, replicated design, on-farm trials, with scientists' supervision; several entries per farmer	Yield data; farmers' perceptions	Research
4. Farmer-managed, unreplicated design, on-farm trials; one cultivar per farmer; replication across farmers	Yield data; farmers' perceptions	Research Extension, NGO
5. Trials as in 4	Farmers' perceptions only	NGO Extension Research
6. Farmer-managed trials; no formal design either within a farm or across farmers	Informal, anecdotal	NGO Extension Research

Source: Witcombe *et al* (1996)

and participatory plant breeding (PPB).



**Figure 7:** Performance of new salt-tolerant selections compared with local variety (Inqlab 91) in farmer managed trials near Mardan, NWFP Province, Pakistan.

One variety, SARC3 (a selection from LU26S), gave a yield increase of 20% over the local material.

PVS was used to identify farmer-acceptable cultivars of rice and chickpea in India (Joshi and Witcombe 1996). This involved the determining of farmers' requirements, a search for released and non-

PVS (summarised in Table 5) is a rapid and cost effective way of identifying farmer-preferred cultivars so long as a suitable choice of genotypes exists, but if this is impossible then the more resource-demanding PPB can be used. PPB (summarised in Table 6) can use previously-identified genotypes from PVS as parents. Figure 7 shows preliminary results from a PVS trial in NWFP Province, Pakistan (equivalent to stage 4 in Table 5), where farmers were given two salt-tolerant genotypes that had come through our current programme to compare with the locally-grown variety.

released cultivars that matched those needs, and the testing of the material found in farmer-managed trials. Acceptable cultivars were found among released varieties, but not among the recommended material for the area (adjoining rainfed areas of Gujarat, MP and Rajasthan). They concluded that the lack of adoption of new cultivars was because resource-poor farmers had not been recommended or exposed to the most appropriate cultivars under the existing variety recommendation and popularisation system, and that adoption rates would be improved by increased farmer participation, especially the systematic testing in zonal trials of locally-popular cultivars. A more liberal release system, and a more open system of providing seeds of new cultivars to the farmers would be needed.

**Table 6.** Methods of plant breeding in self-pollinated crops with varying degrees of farmer participation

Technological breakthroughs in screening/breeding wheat varieties for salt tolerance	
Methods in increasing order of farmer-participation	Site specificity Example
1. All generations grown by plant breeders on station; farmers involved at pre-release stage or even after release	Wide adaptation targeted; early generations may all be in single location followed by multi-locational testing CGIAR, NARS, Developed country programmes
2. Early generation (F <sub>2</sub> or F <sub>3</sub> ) in farmers' fields; all other generations and procedures with plant breeder on station	Single location testing for F <sub>2</sub> or F <sub>3</sub> Thakur (1995)
3. Best advanced lines at F <sub>7</sub> or F <sub>8</sub> given to farmers for testing; closest method to participatory varietal selection since farmers given nearly-finished product	Easy to test best advanced lines across locations Galt (1989)
4. From F <sub>3</sub> or F <sub>4</sub> onwards farmers and plant breeders collaborate to select and identify the best material on farm (and also on-station); farmers select; plant breeders facilitate the process; release proposal prepared by plant breeder	Possible to run selection procedures on early generations in more than one location Sthapit et al (1996)
5. Breeder gives F <sub>3</sub> or F <sub>4</sub> material to farmers; all selection left to farmers; at F <sub>7</sub> or F <sub>8</sub> or later, breeders monitor diversity in farmers fields and identify best material to enter in conventional trials	Extremely easy to run selection schemes in many locations Salazar (1992)
6. Trained expert farmers make crosses and do all selection with or without assistance from breeders; breeders can place best material in conventional trials	Specific to farmers' requirements None yet; second-generation technology

Source: Witcombe *et al* 1996

The PPB was conducted in high-altitude areas of Nepal, and aimed to breed acceptable varieties with the minimum resource use, and to use farmers' knowledge in the breeding programme (Sthapit *et al* 1996). Farmer participation began at the F<sub>5</sub> stage, and was carried out over two seasons in two villages. Farmers were willing participants, and made successful selections in the segregating material. There were large differences in the farmers' preferences between the F<sub>5</sub> bulks, and the most preferred were rapidly adopted. The best variety did well in the formal trials system in Nepal, and was much better than varieties produced by conventional centralised breeding. Overall, the group concluded that, compared with conventional techniques, PPB was more likely to produce farmer-acceptable products. These techniques were initially used in marginal environments, but a programme is under way at present to extend them to high potential areas such as irrigated, salt-affected land in the Indian Punjab. An outline scheme for a participatory breeding programme is presented in Table 7.

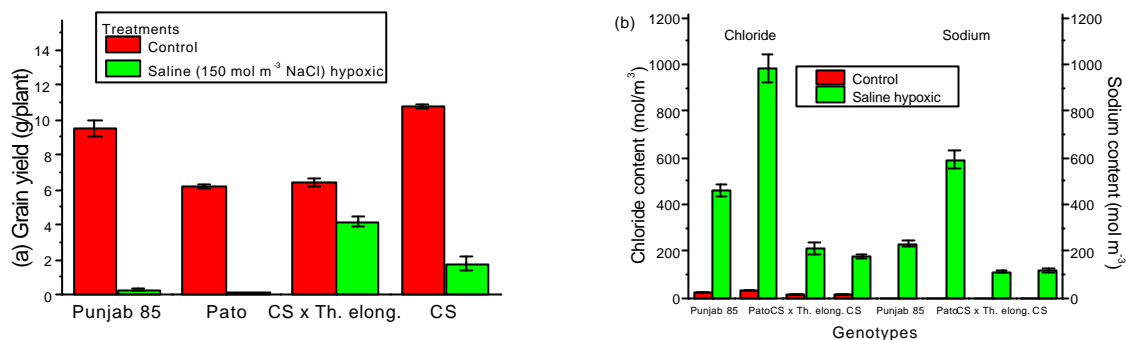
**Table 7.** A participatory plant breeding scheme in self-pollinating crops

Year	Generation	Farmers' role	Breeders' role
1	P <sub>1</sub> x P <sub>2</sub>	Can assist in choosing parental material	Make crosses
1	F <sub>1</sub>		Grow F <sub>1</sub>
2	F <sub>2</sub>	Can grow on-farm	Select from large plot with spaced plants
3	F <sub>3</sub> rows	Visit nursery for joint evaluation	Joint evaluation and evaluate in disease nursery
4	F <sub>4</sub>	Evaluate each other's material and select within their own bulk	Survey farmers' fields and evaluate in disease nursery
5	F <sub>5</sub>	Continue best bulks with single-plant selection; best material given to other farmers	Sample 100 - 400 single plants from most widely accepted bulk
6	F <sub>6</sub>	As above	Grow progeny row nursery (PRN) to select "true to type" rows
7	F <sub>7</sub>	As above	Submit entry in formal trials with seed from PRN; use farmer-saved seed for farmer trials; and multiply breeder seed from PRN seed
8	F <sub>8</sub>	As above	Use breeder seed for formal trials system
9	F <sub>9</sub>	As above	Submit release proposal based on formal trial data and farmer data

Source: Witcombe *et al* 1996

### SCREENING FOR COMBINED SALINITY AND WATERLOGGING TOLERANCE.

As noted earlier, salinity in irrigated areas is very often accompanied by waterlogging, but very little information on the combined effects of these stresses has been published. The development of crops tolerant to both salinity and hypoxia is likely to require the transfer of genes from other species, and the development of simple methods to identify desirable individuals from large populations. Studies of salinity-waterlogging interactions could throw light on the problem of breeding cereals capable of high yields on saline soils, and it is likely that there will be little improvement until cultivars are developed with tolerance to simultaneous salinity and hypoxia, which causes very large increases in salt uptake (Barret Lennard 1986). We believe that such genotypes will soon be available, for the following reasons.



**Figure 8:** Effect of saline hypoxia on (a) yield and (b) leaf sodium and chloride contents of wheat genotypes and a wheat X *Thinopyrum elongatum* amphiploid. Sodium contents in the controls are negligibly small. Source: Akhtar *et al* 1994

We now know the location of the gene responsible for  $K^+/Na^+$  discrimination in hexaploid wheat (Gorham *et al* 1987). Secondly, cereals vary in tolerance to hypoxia (or waterlogging). Aerenchyma formation, an adaptation to hypoxia in the crown roots of cereals, is associated with increased diffusion of  $O_2$  to the root tips (Trought and Drew 1980; Thomson *et al* 1992). In stagnant nutrient solution, triticale cv. Muir had twice the aerenchyma percentage of wheat (Thomson *et al* 1992), and in field experiments waterlogged for 30 days, it outyielded 6 wheat cultivars and another triticale (Barrett-Lennard and Minkey, unpublished data).

Akhtar *et al* (1994) showed strong possibilities for improving the performance of cereals in these environments by simultaneously inserting genes for salinity and waterlogging tolerance. They compared growth and ion relations in wheats and amphiploids of Chinese Spring x *Thinopyrum* species. Saline hypoxia reduced growth, water use, grain (Fig. 8a) and straw yields in wheat, but NaCl or hypoxia alone had smaller effects. Saline hypoxia also reduced  $K^+$  and increased the  $Na^+$  and Cl<sup>-</sup> (Fig. 8b) concentrations in cell sap. A Chinese Spring x *Th. scirpeum* amphiploid had no significant decrease in grain yield under saline hypoxic conditions, and the lowest percentage increase in leaf  $Na^+$  and Cl<sup>-</sup> concentrations. Wheat accumulated more  $Na^+$  and Cl<sup>-</sup> than the amphiploids in saline hypoxic conditions, and in a second experiment a Chinese Spring x *Th. elongatum* amphiploid was in relative terms the most tolerant genotype tested, although its absolute yield was low.

To develop salinity and waterlogging tolerant wheats, rapid methods for screening large segregating populations of young plants will be needed. This may be possible by selecting plants with the lowest leaf concentrations of  $Na^+$  and Cl<sup>-</sup>, although this technique has problems. At low external concentra-

tions Cl can be taken up actively, while at high external concentrations uptake is mostly passive (Greenway 1965), and some plants selected for low Cl uptake could have poorer rather than better growth. Concentrations of Na<sup>+</sup> and Cl will also be affected by the time that a leaf has been transpiring, and segregating populations of wheats crossed with wild grasses will inevitably contain individuals with widely differing rates of leaf production, so techniques would have to ensure that comparisons were made between similarly aged leaves. The screening will require the collection of very large numbers of leaf samples in a relatively short time, presenting considerable logistical problems, and requiring laboratories able to rapidly analyse very large numbers of samples so that the desirable plants can be removed from the stress before they died.

Alternatively, the rates of production and senescence of leaves on the main shoot could be compared, which would integrate the performance of the whole plant and would not be confounded by the leaf selected for analysis. Valid comparisons could be made between plants with widely differing rates of leaf production, and screening could be conducted over a longer period of time (the time for most plants in the population to produce 2-3 leaves), so avoiding the logistical problems above, and could be conducted by groups without access to analytical laboratory equipment.

The ideal program will probably combine the techniques, using leaf counts as a preliminary stage to eliminate 80-90% of the individuals in a segregating population, allowing a more detailed screening of the remaining material using ion regulation criteria.

## CONCLUSIONS

Despite slow progress to date, it is possible that we are on the verge of major improvements in stress tolerance in wheat, in particular for saline and waterlogged situations. Techniques now exist to allow meaningful screening for salt tolerance to be carried out in field conditions. Novel methods such as doubled haploid breeding have accelerated variety production, and developments in wide hybridisation and genetic engineering will soon allow the incorporation of tolerance from related but poorly-adapted genotypes into elite bread and durum wheat cultivars. The active involvement of farmers will lead to breeders developing material that is acceptable to farm families, and will further accelerate the introduction of new cultivars.

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