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Full Length Research Paper

A study of wheat genotypes screening for yield in variably saline fields

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In four wheat trials at saline sites in coastal Bangladesh soil salinity changed continuously as the season progressed. It changed both laterally and by depth even at a sub-meter scale. The water table also moved deeper. Despite the dynamic nature of salinity and its spatial variability, the 112 individual plots in each trial maintained their general ranking for soil salinity, all changing in concert. Plot grain yield was best correlated with salinity measured as an average of 0-90 cm deep soil cores extracted within a month of sowing. Yield declined linearly and sharply at approximately 14 g/m^2 (146 kg/ha) per dS/m. Standard randomisation techniques failed to distribute genotypes evenly across salinity levels in the trials. Consequently, those genotypes with most replicates fortuitously in lower salinity plots had the highest average yield. In standard screening trials lacking salinity tracking in all plots, those genotypes would have been incorrectly labelled most salt tolerant. We suggest that highly variable saline sites to be used for species and genotype screening should be mapped for salinity prior to sowing, and plots marked out to cover the range of salinity likely to produce some yield (0-20 dS/m in wheat). Selected plots should then be labelled for their relative salinity and divided into four salinity categories, high to low. Each genotype and check variety should be randomly allocated to all four salt categories at sowing. At grain harvest this design provides a four-point curve of yield versus salinity for each genotype. Assessing each genotype curve against the site curve, generated from amalgamated data of all genotypes, provides a genotype ranking of salinity tolerance and first-order tolerance benchmarking.

Key words: Salinity, Bangladesh coastal zone, wheat yield, salinity tolerance screening, spatial and temporal changes in field salinity.

INTRODUCTION

“Breeding for improved salinity tolerance (ST) is the only feasible way of improving yield….in saline soils” (Genc et al., 2007). ST is generally considered a complex amalgam of traits for salt exclusion by roots, tissue tolerance of salts, osmotic regulation associated with open stomata, and water use efficiency. To these mechanisms might be added others that would be required in particular environments where the growth-limiting salts might differ and differ both spatially and temporally (reviewed by Munns 1993). But critically, in the crop context, ST must be associated with high yield.

Decades ago, Richards (1983) concluded that because

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salinity is so variable in the field and that the majority of yield comes from patches where salinity is least, the simple approach is to select for high yield for these patches, i.e. in the absence of salt. In favour of his approach is the possibility that, in some saline environments, plants that avoid salinity rather than tolerate it might achieve higher yield. This might be through roots exploiting less saline parts of the soil profile, or by changed phenology (flowering time) associated with more rapid inherent growth rate (Rawson et al., 1988). In some saline locations farm management can also practice avoidance by planting appropriate duration crops early in the season before salinity rises into the root zone and harvesting before yield is reduced (Saifuzzaman et al., 2011). So best yields in particular saline soils could be achieved without ST. Munns and Richards (1998) suggested that genotypes with high transpiration efficiency and deep roots could perform better in some saline soils than genotypes selected solely on ST traits.

Salinity during the dry season is a major constraint to crop yields in southern Bangladesh, particularly in the coastal zones (Dalgliesh and Poulton 2011).There, land totalling around 0.88 million hectare is saline so is not cropped during the dry-season (MPO 1986). Furthermore, salinity is slowly intensifying and spreading inland (SRDI 2010). In a program to provide farmers with cereals that might produce profitable yields in these saline soils, the Wheat Research Centre (WRC) of the Bangladesh Agricultural Research Institute (BARI) has been conducting breeding and screening trials at multiple locations with and without salt. WRC's breeding task is particularly difficult because they have concomitantly been selecting for high temperature and minimal water applications, both significant constraints to growth and yield in the southern coastal zones of the country. While varieties tolerating higher temperatures have been released as a result of nursery trials, only one considered to have some ST has emerged from the program (Barma et al., 2011). However, they have not been selecting via ST traits, but simply for highest yield in saline soils. Using the conventional trial design of four replicate blocks with genotypes randomised within blocks they found that genotype rankings were not consistent between seasons or saline sites. From the data it was not clear whether this was due to unexplained variation in genotype responses to local conditions, other than salt, or whether the analysis of data might in some way be lacking. There was the possibility that varying patterns of salinity between sites and years might be exploited differently by genotypes to produce yield.

This paper attempts to assess why progress in selecting for ST in the field has been poor and concludes with suggestions as to how procedures and data analysis in field trials might be modified to accelerate the screening process. Primary aims were first to describe at a meter scale salinity distribution vertically and horizontally throughout the dry season, both in the soil and the water table, at four disparate field sites in Bangladesh, second to assess correlations between the changing salinity patterns and yield, and third to propose simple designs for screening trials that would provide nominal curves of yield response to salinity by each genotype in the trials, thus their likely best match to differentially saline locations.

MATERIALS AND METHODS

The four saline sites chosen for trials were Benerpota, Satkhira (latitude 22.749° longitude 89.106°) in far western Bangladesh on the High Ganges River Floodplain, prone to flooding from the local tidal river system and with a clayey alluvium soil; Hazirhat, Noakhali (22.756 and 91.124°) in eastern Bangladesh on the Young Meghna Estuarine Floodplain, a calcareous alluvium with no significant local rivers: Amtali, Barguna (22.084 and 90.228°) on the Ganges Tidal Floodplain with grey floodplain soils: and Kuakata, Patuakhali (21.833 and 90.113°) with a calcareous alluvium but at the southerly extreme of central mainland Bangladesh in the Bay of Bengal. All are between 2 and 7 metres above sea level. From the coordinates provided above in brackets, site locations can be seen from countrywide to field scale on Google Earth satellite maps. These maps are free to browse on the Internet. The trial sites Benerpota, Hazirhat, Amtali and Kuakata were chosen because they are geographically diverse and represent those regions of southern Bangladesh that have large tracts of agricultural land that remain fallow during the dry season, primarily because of salinization.

Layout of trials was the same at all four sites (Photo 1). At each site, 28 wheat selections (including Triticale) were grown in randomized plots, repeated in four replicate blocks. There were 112 plots in all, each 2.5 m long by 1 m wide containing four rows of the crop planted 20 cm apart (Photo 1). Soil profile cores driven to 90 cm deep were collected from every plot every 2 to 4 weeks during crop growth. Soil cores were separated into 0-15, 15-30, 30-60 and 60-90 cm layers for salinity estimates. The soil core samples were air-dried and subsamples mixed 1:5 with distilled water then EC 1:5 ratios (dS/m) were measured with an electrical conductivity meter. EC of the added water was deducted from the meter reading. Where appropriate, EC 1:5 values were converted to ECe (dS/m) using the multipliers of Slavich and Petterson (1993), that for sand approximate 23, for sandy loam, silty loam and sandy clay loam 14, for light medium clay 8.6, and for medium clay 7.5. For all soils apart from those at Benerpota that contained more clay, the rough conversion from EC 1:5 to ECe was ×10. This approximation was supported using laboratory-analysis-based regressions of Sattar and Mutsaers (2004) for soils of the region.

Photo 1. Benerpota site on 27 February 2012, 75 DAS. In each of the 4 blocks, separated by a walkway, there were 28 plots, each of 4, 20 cm rows 2.5 m long, This layout was used at all sites. At the final harvest only the two centre rows of plots were cut. A white saline deposit can be seen on the surface of the cracking clay.

Piezometer tubes 3 m long were used to measure the depth and EC_W (dS/m) of free water in the soil profile. The tubes were inserted in each block at each site before sowing. This was to look for any correlations with EC 1:5 of the soil itself and to give an idea of where free water (the water table) might be in relation to likely rooting depth of the crop. Piezometer data for the whole season are available only from Benerpota and Amtali. Piezometer tubes are porous plastic pipes as used in shallow tube wells.

Crops were sown respectively on 14, 15, 19 and 20 December 2011 at Benerpota, Kuakata, Hazirhat and Amtali. Irrigations as deemed necessary by the local farm manager were on 5 January, 2 February, 15 March at Benerpota from the nearby lake, on 4 January and 9 February at Kuakata, on 17 January at Hazirhat, and on 9 January and 11 February 2012 in Amtali. At crop maturity, only the centre two rows of all 112 plots were harvested at each site for measurements of fresh and dry weights. These provided the final biomass and yield estimates (2.5 m × 0.4 m = 1 m² cut). Subsidiary measurements were made of culms, heads, and grains to provide data of yield components. The names and derivation of the 28 breeding selections assigned to plots are in Table 1, presented later. Shatabdi and BARI gom 25 and 26 are named Bangladesh wheat varieties, effectively high-yielding controls. They yield between 3 and 5 t/ha in non-saline conditions, yield depending on planting date and farm location in Bangladesh (Rawson et al., 2011).

RESULTS

Salt effects on seedling emergence and establishment

Seedling emergence was acceptable at Benerpota and Hazirhat where post-sowing salinity in the topsoil (0 to 15 cm) did not exceed 11 dS/m (ECe). Similarly, at Amtali, plants established well where top soil salinity was less than 12 dS/m, but they died where salinity exceeded 18 dS/m (Figure 1). At the remote southern site of Kuakata, where plant establishment was not counted, 38 of 112 plots had initial surface salinity values above 15 dS/m and many of those plots had thin canopies and yields below 0.5 t/ha. This critical range of 12 to 18 dS/m accords with Berryman and Brower (1991) in their interpretation of ECe values as affecting crop growth. They categorise 8 to 15 dS/m as moderately saline where only tolerant crops can yield satisfactorily. Very tolerant crops alone can yield at ECe values above 15 dS/m.

Table 1. Genotype reference number, name and yield ranking for saline sites at Hazirhat (H), Kuakata (K) and Benerpota (B). Rankings are based on the grain yield average of all 4 replicate plots except at Kuakata where some plots were absent. Column av is the average of all rankings for HKB.

S/No.	Variable	Yield ranking			
#	Name	H	K	B	av
1	Shatabdi	8	9	21	13
2	BARI gom 25	2	12	7	7
3	BARI gom 26	3	19	6	8
4	BAW 1142	20	20	17	19
5	BAW 1143	27	23	16	22
6	BAW 1146	5	1	9	2
7	BAW 1147	16	13	4	11
8	BAW 1148	21	16	1	14
9	BAW 1150	18	15	5	15
10	BAW 1151	7	6	3	3
11	BAW 1153	1	4	8	1
12	BAW 1154	24	11	22	20
13	BAW 1140	11	21	25	21
14	BAW 1118	6	3	19	9
15	BAW 1122	4	5	10	4
16	BAW 1130	23	24	23	24
17	BAW 1051	14	18	20	18
18	BAW 1111	10	14	15	16
19	BAW 1135	17	17	13	17
20	BAW 1138	13	7	12	6
21	BAW 1156	12	10	14	12
22	Francolin 1	26	22	27	26
23	BAW 1159	9	8	2	5
24	BAW 1160	19	27	18	23
25	BAW 1161	15	2	11	10
26	BAW 1141	26	26	26	27
27	BAW 1157	22	25	24	25
28	Triticale 1	25	28	28	28

Distribution of salinity through the soil profile and yield

The patterns for salinity distribution through time and yield are presented first for Hazirhat, located on the Young Meghna Estuarine Floodplain, and then comparisons made to the other three sites. All data are very much condensed to highlight trends.

The soil salinity profile of block 3, two weeks after sowing from one end to the other through its 28 plots is shown in Figure 2 (left). Plots varied 20-fold in average salinity by depth (solid line) from 0.5 dS/m to over 10 dS/m. Within a plot, ECe could be highest at any depth in the profile but high salinity at that depth usually meant high salinity at all depths in the plot. So salinity levels were linked vertically, indicating the dominant vertical movement of water and salt through the profile rather than in a horizontal direction. The general point here is that salinity at this time, after monsoon waters had drained away, was not necessarily most concentrated in the surface soil.

By the end of the season three months later, distribution of salinity vertically was quite different (Figure 2, right; note the salinity scale is almost 3-fold that for the same plots in Figure 2, left). Concentrations were always greatest in the surface soil and variations between other depths were relatively small. In those plots where surface salinity was very high (e.g. plot 75) the tendency was for somewhat greater salinity at depth. These patterns were repeated to various degrees in other blocks at the site. Distribution of surface soil ECe across the whole Hazirhat site ranged from almost no salt to pockets that exceeded 40 dS/m. The size of the site can be gauged from Photo1. Grain yield for each plot along the length of Block 3 is included in Figure 2 (right) as a dotted line. Yield varied very significantly from plot to plot and overall

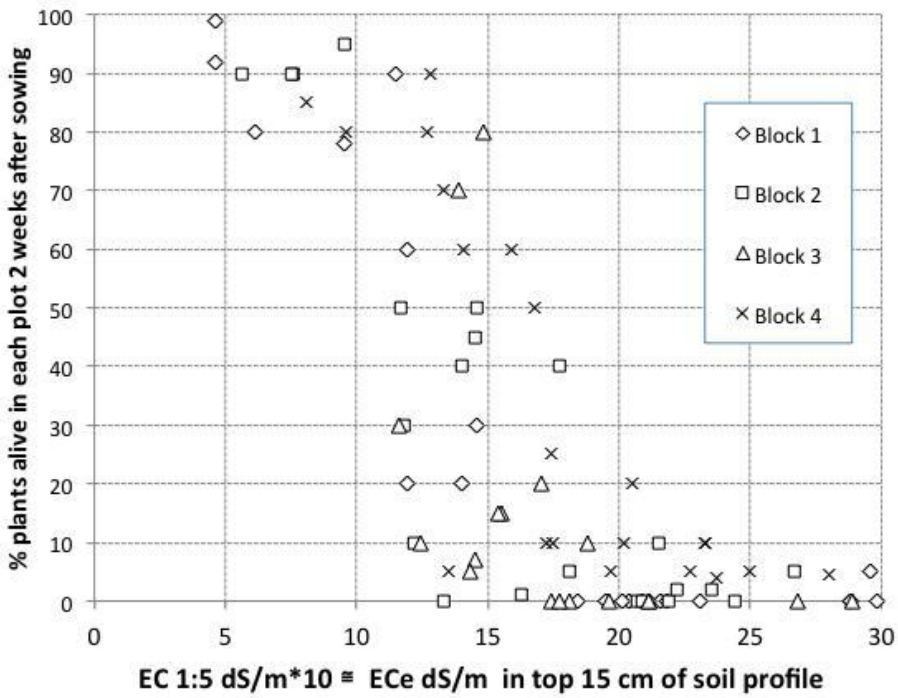

Figure 1. Percentage of seedlings establishing in Amtali plots expressed against EC1:5 (*10) as measured in the surface 15 cm of soil 2 weeks after planting. ECe dS/m approximates EC 1:5*10.

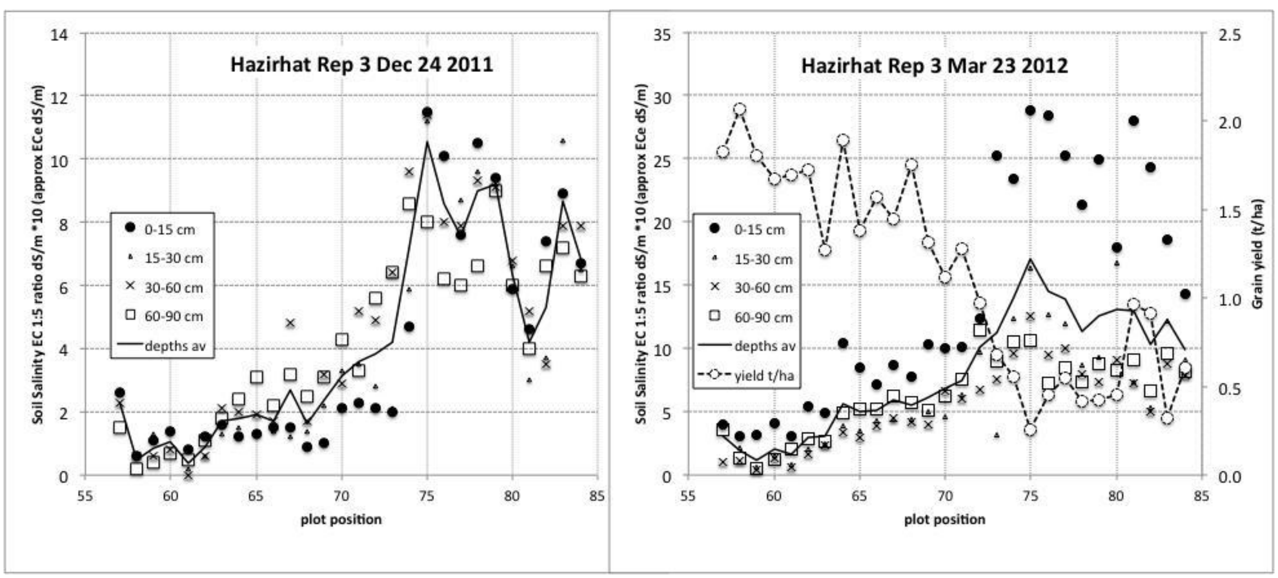

Figure 2. Distribution of salinity along the length of replicate Block 3 and down through the soil profile at Hazirhat, expressed as EC 1:5 *10 dS/m, 2 weeks after sowing (left) and after harvest (right) where the dashed line is grain yield in each plot (t/ha). EC scales are different in the graphs.

by 10 fold. The yield variation overall mirrored average plot salinity shown as a solid line, so high salinity produced low yields and vice versa.

Considering grain yield data from the 112 plots of the Hazirhat (Noakhali) site and their overall relationship with salinity, there was a close linear correlation ($r^2 = 0.75$, y =

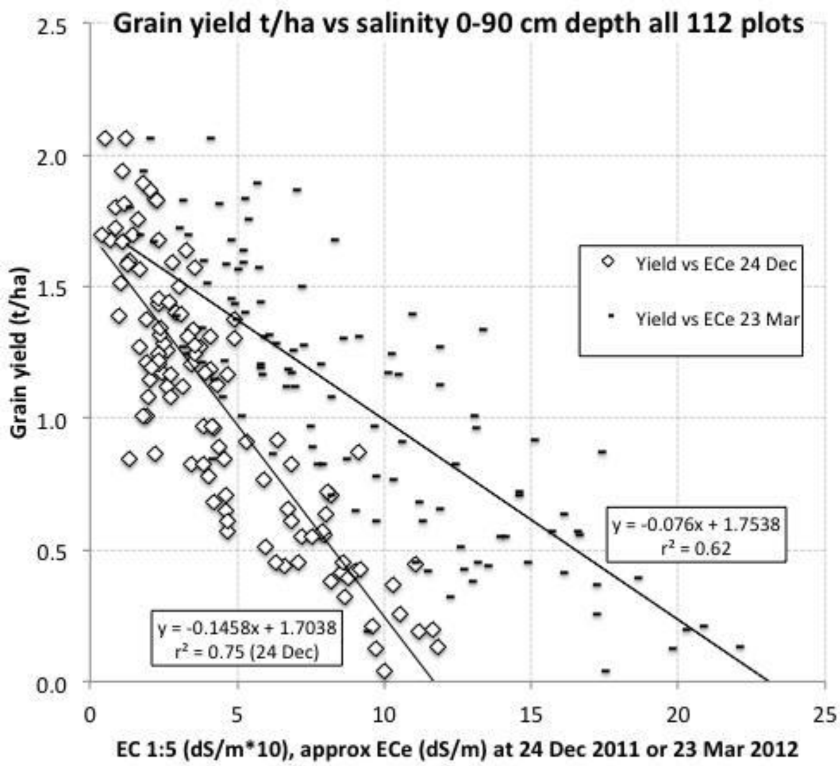

Figure 3. Plot grain yield (t/ha) versus plot salinity averaged by core depth for all 112 plots at Hazirhat. Salinity was that measured on 24 December, 2 weeks after sowing (diamonds) and for that measured at maturity (23 March, dots). Equations in boxes are for the linear regressions.

-0.146x + 1.704) when ECe averaged by depth to 90 cm, measured 2 weeks after sowing, was the gauge of salinity (Figure 3, diamonds. NB. The slope in the equation is the rate of decline in yield with ECe, or 146 kg/ha/dS/m.)The linear correlation was less close (r^2 = 0.62, y = -0.076x + 1.754) when ECe also averaged by depth to 90 cm, but measured at crop maturity, was used to gauge salinity. The correlation became poorer again when yield was related to salinity of the top 15 cm alone at crop maturity (r^2 = 0.48, data not shown). If salinity had to be measured at crop maturity, the best correlation with yield then, similar to that for the full 0-90 cm profile, was an averaged 0 to 60 cm soil core. That shorter core would be less labour to extract than the full 0 to 90 cm core.

The problem with gauging salinity effects on yield by taking soil cores at different times and to different depths is that each gives very different regression coefficients (equation slope and intercept). Accordingly, the regression of Figure 3 indicated that yield became zero when ECe measured soon after sowing was 12 dS/m while zero yields was reached at 23 dS/m when ECe was measured at maturity. Zero-yield ECe was 47 dS/m, when considering only the salinity of the 0-15 cm surface soil at maturity (y = -0.0351x + 1.65, r^2= 0.49).

Consequently, estimation of the salinity level that causes crop death or results in a yield of say 1 t/ha is very dependent on when salinity is measured and what portion of the soil profile is being considered. When yield prediction is important, the early measure at sowing becomes very useful, but when salespeople are describing salt tolerance of their seed material, the late-season values, particularly those using surface soil, are far more impressive and convincing to prospective buyers.

As described under seedling establishment, Amtali (Barguna) grey Ganges Floodplain topsoil had very high salinity levels and higher than at other locations so many plants died shortly after sowing. Plots differed widely in their salt levels, such that a 4 m move laterally could equate with surface ECe values of 30 to 12 dS/m and from no yield to over 2 t/ha (data not shown). While high and low values at the surface were reflected in the ranking of values in lower soil horizons, salt levels below 30 cm were moderate, similar to values in Figure 2 at Hazirhat. Surprisingly, for those plots where some plants survived, there was an overall correlation between grain yield (t/ha) and salinity (ECe dS/m) measured 20 days after sowing and averaged by depth to 90 cm (y = -0.114x + 1.446, r^2=0.40). This is close to the relationship

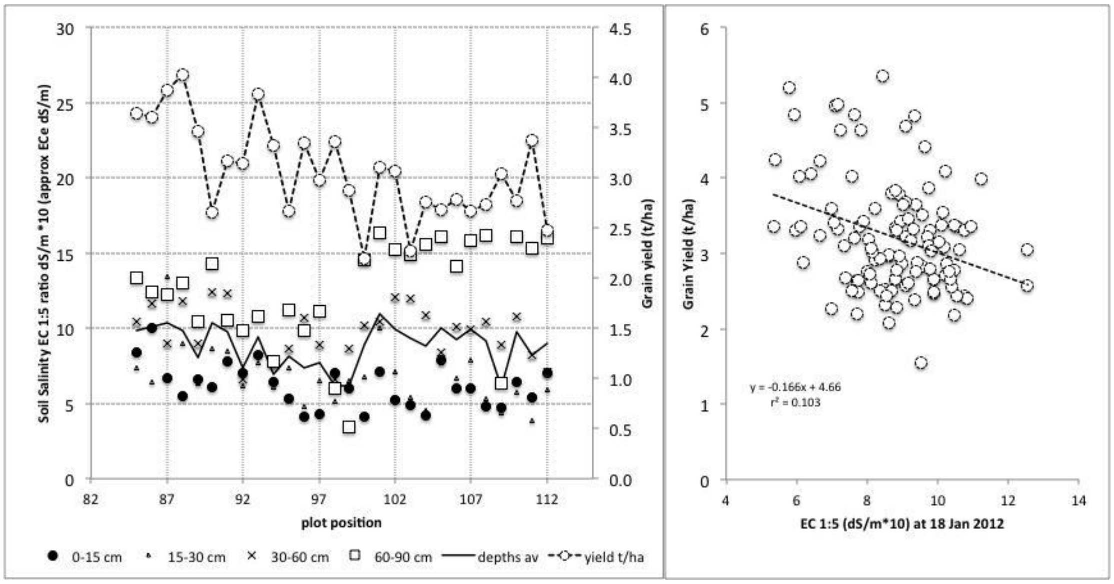

Figure 4. Benerpota salinity at different depths one month after sowing for plots of Block 4 and grain yield in those plots (left). The relationship between salinity and yield for all plots at the site is shown in the right hand figure.

for Hazirhat despite the complication of seedling deaths that had resulted in canopies with different plant densities. The relationship indicated zero yield if average soil salinity was around 13 dS/m, again as at Hazirhat. The equivalent equation between yield and ECe for the 0-15 cm soil profile only was y = -0.047x + 1.30, r^2=0.40. The relationship between yield and salinity based on soil cores taken at maturity indicated zero yield if average soil salinity was 27 dS/m (c.f. Figure 3).

The Kuakata (Patuakhali) site, despite being within a few kilometres of the Bay of Bengal, was not remarkably different from the sites described for Hazirhat and Amtali. Salinity was extremely variable horizontally at the surface such that a few plots produced no yield because of high surface salts as at Amtali. Salinity values at the surface were reflected in values immediately below in the profile, though again, salinity below 15-30 cm was usually less than at the surface. Unlike at other sites, the change in salinity in any plot between early January and late March was relatively minor particularly in the soils below 30 cm, so regressions between salinity (ECe dS/m) averaged for the 0-90 cm profile and yield (t/ha) were similar regardless of when salinity was measured. Correlations were closest for salinity from the full 0-90 cm profile (y= -0.096x + 2.112, r^2=0.60) and poorest for the deepest soil (60-90 cm; r^2= 0.39) and surface soil (r^2=0.47).

Benerpota (Satkhira), with its clayey alluvium soil is adjacent to a saline lake and within 200 m of a tidal river. There were differences in salinity across the site though nowhere did the variation come close to that at other locations. There was no indication that high salinity in one part of the profile meant high salinity at all points vertically in line with that value, thus making Benerpota different from other sites. There was a tendency for highest salinity to occur in the 60-90 cm part of the profile and lowest salinity in the surface layer (Figure 4 left). Apparent almost random distribution of salt spatially was repeated in cores taken outside the irrigated areas.

As in Barma et al. (2011), yields at this site were generally very high, and in some plots seemed to correlate inversely with average 0-90 cm depth salinity (Figure 4 left). But the overall yield versus salinity correlation for the 112 plots at the site was poor (Figure 4 right) with the regression using salinity (EC 1:5*10 dS/m) measured in the month after sowing having a higher slope than at other sites (y = -0.166x +4.66, r^2=0.10).The regression with salinity measured at grain maturity was even less significant (r^2=0.02, y = -0.050x +3.89).

It is noted that despite the lack of significance in the relationship between salinity measured in the month after sowing and yield at Benerpota, the regression indicated that yield fell with increasing salinity at 166 kg/dS/m, not dramatically different from the 145 kg/dS/m registered at Hazirhat.

Soil and water table salinity trends through time: Benerpota is different

The results of any trials screening for salinity tolerance in the field will be influenced not only by where they are conducted but also by when they are conducted within the annual cycles of salinity. With the advance of the dry season, the predominant trend in salinity was the

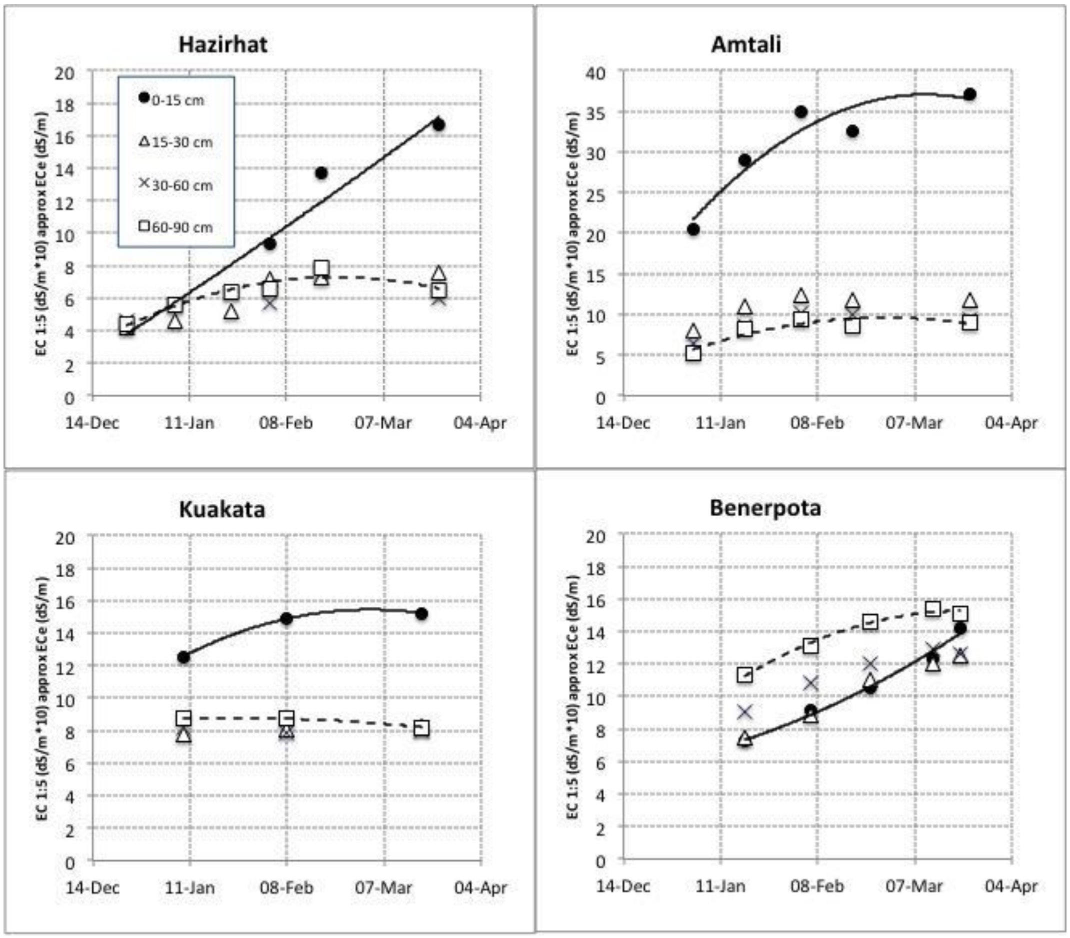

Figure 5. Soil EC 1:5*10 at different depths in the crop rooting profile through the dry season. Each point is the average of 112 plots. The vertical axis for Amtali is double that for other sites.

increasing salinization of the surface soil; salinity below 15 cm remained relatively constant (Figure 5). Benerpota had a progressive increase in salinity of all soil layers.

Water table EC_W also trended upwards, though not dramatically so, and water tables became deeper, though again the changes were of the order of centimetres rather than metres. Benerpota, while having average soil profile EC values that were within the range at Hazirhat and Kuakata (Figure 5), had very low water table salinity particularly early in the season (Figure 6, left), and that water table was very shallow and certainly accessible by roots (~40 cm deep during tillering to heading). Hazirhat also had a relatively shallow water table of less than 1 metre (Figure 6, right), but by contrast it was quite saline (10-12 dS/m). The implication here is that plants at the Benerpota site were effectively growing in a slightly saline water culture medium during early development. This shallow water table dominated any negative effects of soil salinity on growth, providing the water to generate high crop biomass and associated yield. Plants at other sites were growth restricted during early development both by high soil and water table salinity, and in the case of Amtali and Kuakata, by a water table that was too deep for direct root access.

SCREENING GENOTYPES FOR YIELD AT SALINE SITES

The field trials at the four saline sites followed a conventional design used for screening varieties for yield. There were four replicate blocks with the 28 genotypes being randomly allocated to plots within each block. Plots were large enough to provide reasonable estimates of yield and yield components. Table 1 presents the genotype rankings for yield with separate columns for each site. Amtali site is excluded because too many replicate plots were empty due to extreme salinity.

Some genotypes ranked highly for yield across the three sites (#2, 6, 10, 11, 15, 20 and 23) while 4, 5, 16, 22, 24, 26, 27 and 28 were consistently poor. In the normal course of events, and after statistical analysis, the low-ranking genotypes would be excluded from further trials in the assumption they were salt sensitive, while

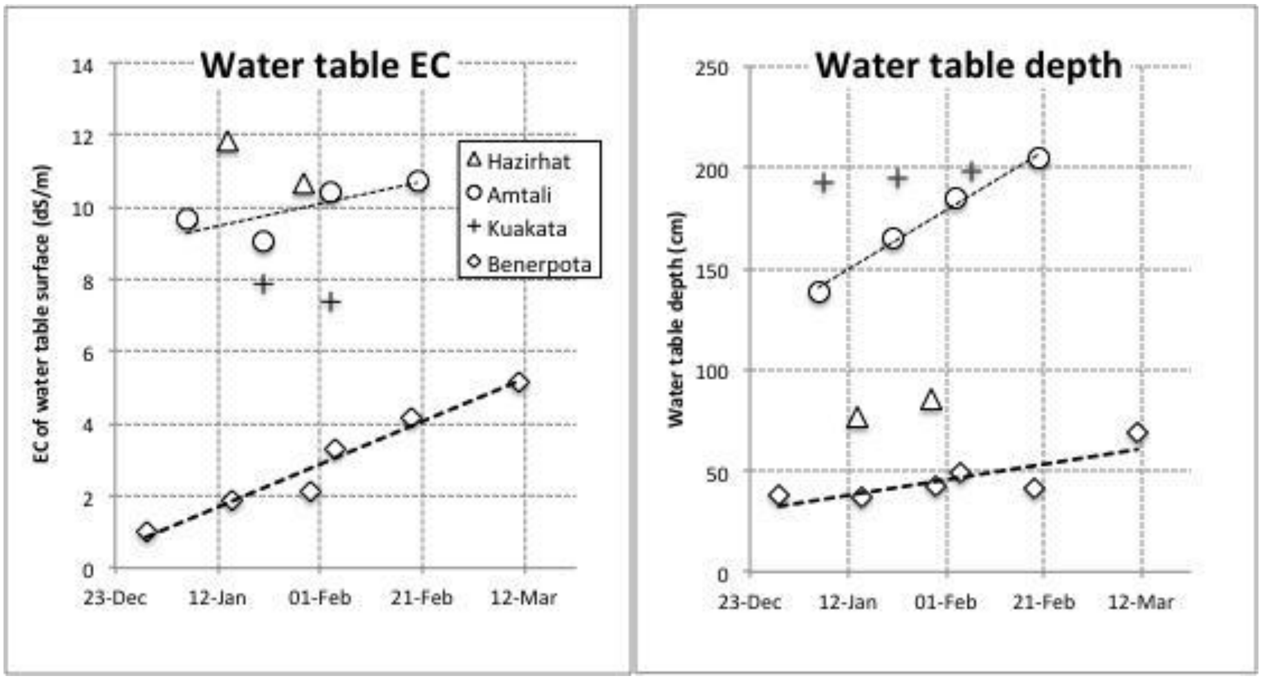

Figure 6. Water table EC (dS/m) and depth (cm) during the dry season at four salinity-screening sites. Regressions are drawn through Amtali and Benerpota data.

those with high average yield would be retained for further testing. However, normally, individual plots would not be rigorously tracked for salinity throughout the season as in this study. More likely a few well-spaced EC samples would be taken within each block to characterise its relative salinity and to identify the salinity class of the site.

Weighting yield of genotypes against the salinity of their plots

Rigorous salinity tracking in these trials showed that, by chance, genotypes 6, 11, and 15 had been allocated mainly to plots that had relatively low salinity at both Hazirhat and Kuakata (see #11 on Figure 7, identified by open circles, average yield 1.6 t/ha), so in reality had not been tested for salinity tolerance. Conversely, some of the low-ranking genotypes had inadvertently been sown in predominantly high salinity plots at both sites (see #26 on Figure 7, identified by open squares, average yield 0.5 t/ha). Even with more replication and more sites, it would take a few seasons to reliably find genotypes with the highest level of salinity tolerance using the conventional approach to field screening.

The Hazirhat data sets, when graphed by individual plots, indicate that the response of yield to salinity is curvilinear (Figure 7, dotted line, and as in Barma et al., 2011 for the previous season); averaging the genotype data removes the tails of the curves making the response seem linear.

If we can assume in the first instance that a curve such as in Figure 7 is a standard response for wheat yield to salinity at that location, then any genotypes with data above the curve particularly at high salinity will be salt tolerant and those below will be sensitive. Genotypes above the curve at low salinity will be potentially high yielding but not necessarily salt tolerant. Selecting above-curve genotypes at any category of salinity along the curve would indicate the genotypes best suited to any salinity category.

This approach works well for categorizing genotypes for degree of salt tolerance in theory, but in practice it has two problems when using the current data. First, too few genotypes span a wide range of salinity, so not all can be ranked within each salinity category. Second, unaccounted-for variation between plots, likely due to factors other than salinity, produced a few plot salinity-yield relationships that were a long way off the standard curve. Initially we wondered if roots from one plot were accessing adjacent plots so were exposed to an average salt level for the three plots. This concern was despite harvesting only the 2 central rows of 4-row plots. To test this Figure 7 was redrawn using average salinity of each three plots against yield. The curve regression r^2 value fell from 0.71 to 0.24 (data not shown) implying there was little cross-plot access by roots.

We persisted with the idea of ranking genotypes for three salt-tolerance categories using the above and below the curve approach to indicate better and worse

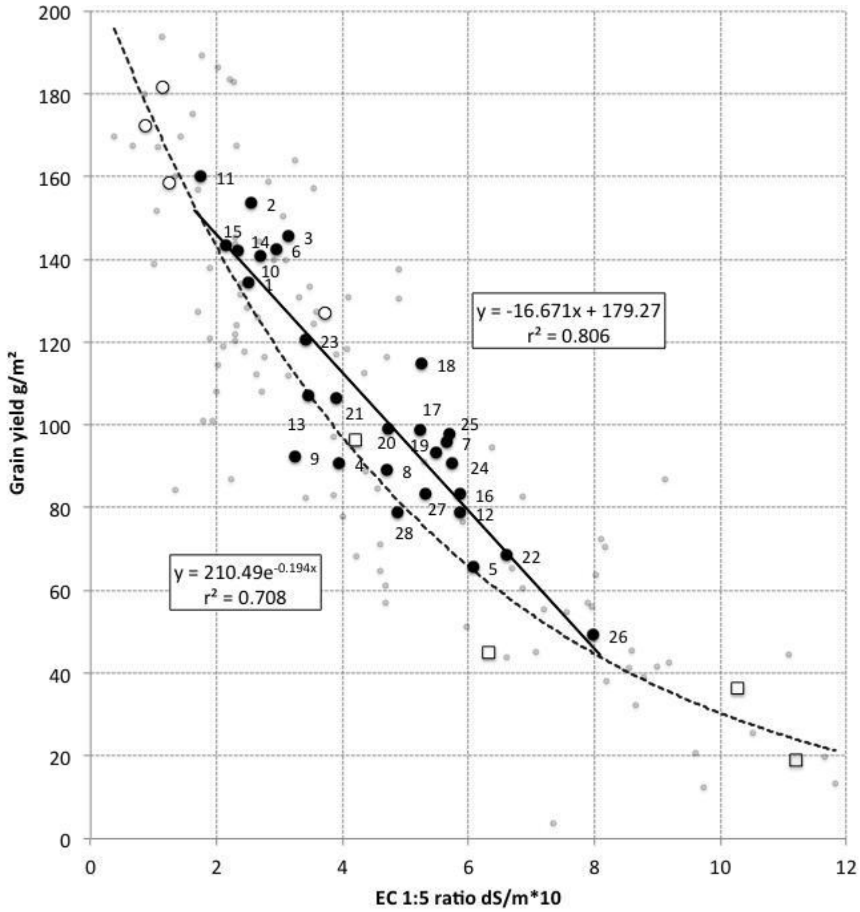

Figure 7. Grain yields of plots at Hazirhat against plot EC 1:5*10 averaged over depth (0-90 cm at 24 Dec, 2 weeks after sowing). The 112 plot data are shown as small dots and defined by an exponential curve, while the data averaged for each genotype (numbered as in Table 1), are defined by a linear regression. The 4 replicate plots of genotypes 11 (open circles) and 26 (open squares) are identified to show their positions in relation to the yield-salinity curve.

Table 2. Genotypes categorised for yield in the lower, medium and higher salt categories at each site. Salt categories are not equival ent across sites. Genotype identification numbers are as in Table 1. Categories for good and bad are based on above or below the site regression line on each figure.

	Better yielding genotypes within each salt category		
	Lower salt at site	**Medium salt at site**	**Higher salt at site**
Hazirhat Noakhali	11, 2, 3,	25, 19, 16, 18, 24	22,26
Kuakata Patuakhali	11, 6, 10, 15	15,20,25, 14, 1,	5, 13, 19
Benerpota Satkhira	2, 3, 9, 23	15, 20,25, 19, 8, 10, 11, 21	5,7
Conventional 3-trial ranking of 14 best genotypes for yield (as in Table 1)	(1st) 11, 6, 10, 15, 23, 2, 3 (best ranked lines are growing in lower average salinity, by chance)	20, 14, 25,7, 21, 1, 8 (14th)	

adaptation, but we added the data from Kuakata and Benerpota to the assessment after graphing them independently as in Figure 7 (graphs not shown). Table 2 shows the genotypes that ranked highest for yield within each of the salt categories. There is some agreement across sites.

Of particular interest is the overall conventional ranking for yield averaged from the three sites, transferred from Table 1, and shown in the bottom section of Table 2. This conventional ranking does not take into account different salinity of plots, simply recognizing the three sites as saline. The seven top-ranked genotypes are all from the

Table 3. Response of yield components to salinity (EC dS/m*10) as described by coefficients of determination of linear regressions for all 112 plots in the Hazirhat trial. Multiply the slope and intercept by 10 to give kg/ha for yield and biomass. Benerpota trial coefficients are in brackets. No linear trends were significant at that location.

	r^2	Slope(dS/m)	intercept
Yield (g/m^2)	0.74	-14.6(-16.5)	170(466)
Biomass (g/m^2)	0.77	-37.8(-43.6)	473 (1289)
Culms per m^2	0.62	-14.3 (-8 4)	232(404)
Grains per culm	0.18NS	-0.70 (0.03)	19.8(27.2)
Weight per grain (mg)	0.67	-1.52(-1.13)	42(42)
Grains per m^2	0.68	-323(-116)	4279 (10916)

lower salt category, that is, are yielding best because they are exposed to less salt. The next seven best-ranked genotypes are mainly from the medium salt category. It could be argued therefore that a standard screening trial for salinity tolerance is likely to be primarily a trial that ranks the salinity of plots and the associated genotype rankings are fortuitous. This is an extreme view but could play a part. The discussion section will present a modified design for a salinity tolerance screening trial based on what we have found.

The plasticity of yield as salinity increases

If different genotypes are to differ in their yield tolerance of salt, how will they achieve that in terms of yield components? The basic assumption is that a tolerant line might not yield quite so well in the absence of salt as a sensitive line, but would yield better at high salt. So the curve for yield tolerance might be the same shape as in Figure 7 but swivelled at some mid-point. How sensitive is each component of yield to salinity, so what relative capacity is there for each component to increase genotype salt tolerance and achieve that new curve? The examples in Table 3, showing the coefficients of linear regressions, are from Hazirhat using salinity measured two weeks after sowing. Benerpota coefficients are shown in brackets for comparison.

The slopes in Table 3 indicate the effect of each dS/m (approximately ECe) on the component measured while the intercepts are the average values at zero salinity for the site. Thus yield at Hazirhat declined by 146 kg/ha per dS/m from a high of 1700 kg while at Benerpota the decline was similar at 165 kg/ha though from a much greater high of 4660 kg. While the Benerpota data are not significant statistically, they indicate that salinity impacted slightly later in crop development, largely after numbers of ear-bearing culms had been determined.

Biomass declined progressively with increasing salinity (~400 kg/ha per dS/m, Table 3) in part because tillering was curtailed. This reduces the development of adventitious roots that are associated with tillers. Hazirhat crops declined from their potential of 250 ear-bearing shoots per m^2 to 50, a loss of 80% of yield potential with salinity (a loss of 14 culms/m^2 per dS/m). The effect of reduced tillering produced a greater than 80% effect on the number of grains/m^2 and those grains were 30-40% smaller. In essence, salinity reduced the expression of yield incrementally through crop development and no stage was protected. All are expressions of reduced growth and the co-variation of many factors. Consequently, a salt-tolerant genotype should demonstrate its tolerance even in the early stages of tillering.

DISCUSSION

The first aim of this study, to describe salinity on farms representative of saline Bangladesh, showed salinity to be a continuously moving target throughout the dry season. Salt concentrations changed both through the soil profile and laterally, even at a sub-meter scale. Changes also occurred in the water table that moved deeper as the season progressed. And changes differed between trial sites. Despite this complexity, some things were generally clear at three of the sites: First that salt concentration was strongly linked vertically through the soil profile and often weakly linked laterally (high salt at depth meant high salt at the surface). Second, that the ranking of plots for salt concentration (averaged by depth) remained similar throughout the season at a site (the most salty plots at sowing were most salty at harvest). Third, that wheat yield in plots declined almost linearly with increasing salinity. Fourth, and surprisingly, that plot measurements of soil salinity taken at the start of the season (averaged over the full 90 cm profile) predicted the yields that plants would produce 90 days after sowing. The early measurement of salinity was indeed better related to yield than most subsequent measurements. There could be modifiers to these conclusions. For example, very high surface salinity at sowing weakened or killed seedlings despite salt being minimal further down the soil profile (at Amtali site). Also, the relationships were weakened if crop roots were bathed in the soil solution for much of the season such as at Benerpota site. Nevertheless, there were enough constants to allow us to make some suggestions as to how methodologies of screening species or genotypes for salinity tolerance in the field might be improved.

The genotype screening trials, accompanied by rigorous plot salinity measurements, produced some interesting findings. Most important was that four-fold replication and randomisation of plots within the four blocks was not sufficient to distribute genotypes evenly across soil salinity levels at a site. By chance, a particular genotype could have all four replicates in low salt plots. Since yield fell rapidly with increasing salinity, that genotype would yield well overall and with little statistical variation and rank highly in the trial. It would be wrongly assessed as salt tolerant even though its salinity tolerance had not been tested in any replicate. Without consideration of the individual plot EC measurements, the genotype rankings for yield would have been taken as their rankings for salinity tolerance in each trial. Greater replication, more sites and more years would approach correct tolerance rankings, but at the cost of time and labour. There is a better and quicker way for tolerance screening in the field that is detailed now.

One clear finding that has been well documented was that salinity of the surface 0-15 cm soil at sowing strongly impacts on yield through seedling death if levels are above 12 dS/m, and that if levels approach or exceed 20 dS/m nothing survives. This means that establishment screening trials could be run at variably saline locations such as Amtali over 3 weeks after sowing, and repeated throughout the dry season. But it would be important to measure EC of the surface soil over the whole proposed site before allocating genotypes to plots, as all genotypes should be represented in all general salinity categories between 12 and 20 dS/m. This approach would benchmark genotypes for seedling survival, provide a general curve of response to salinity averaged for genotypes across the site, and provide a first-order estimate of the response of individual genotypes. It is noted that use of a ground conductivity meter such as the EM38 would make salinity mapping of the site much quicker and less arduous. But regular calibration against the coring method used in this study is still required.

The extreme spatial variation in salinity at three of our four sites at the scale of a meter should not be considered a problem in screening for salinity tolerance but rather as a bonus. In effect, such a site can be visualised as a range of salt treatments, not neatly arranged in adjacent rectangles, and rectangular blocks, but as an almost random scatter. Once the scatter has been mapped for salinity levels and the site visualised back into rectangles of plot size appropriate for the species tested (we used 2.5 × 1 m, a minimum for wheat), the marked plots can be allocated to salinity treatments and genotypes. Our observation was that within a site of approximately 20 × 20 m, plot salinity (soil cores taken shortly after sowing and averaged to 90 cm depth) ranged between almost zero in some plots to around 12 dS/m. Though average salinity increased more than two-fold with advance of the dry season, the ranking of plots for salinity changed little. So plots initially nominated as low or high salt remain as such till crop maturity.

We are suggesting that after such a site has been scanned for salinity and the plot arrangement mapped out, the plots should be listed on the basis of their salinity and the list split into four salinity classes. Each of the four classes represents a level of salinity in which every genotype will be represented randomly. This design will provide, at grain maturity, an overall curve of yield versus salinity for the site such as in Figure 7 (with its 112 data points). Additionally, it will provide curves of yield versus salinity for each genotype with every curve having a data point within each salinity class. Genotype yield curves that fall above the site curve should represent an enhanced level of tolerance for the site. Those falling below will indicate reduced tolerance of the conditions at the site.

The best design would replicate this plan at the same site, to test whether apparent responses to salinity held, but in the Bangladesh context, where three distant sites had similar characteristics of salt distribution, it may be more efficient to do replication of sites. That would highlight the impacts of local factors on genotype ranking for yield in saline soil.

The expectation from published data with isolines of wheat in the field (James et al., 2012) is that an isoline with ST will have equal yield at no salinity to that without ST. But the yield difference will increase progressively with increasing salinity as the intolerant line loses yield faster than the tolerant one. In that study, the yield difference at high salinity was approximately 300 kg due to the presence of the *Nax* sodium exclusion genes. However, the presence of other traits such as a changed root: shoot ratio, allowing longer roots to access deeper less-saline soils or a low salinity water table (Munns and Richards, 1998), might result in a curve that would rotate around a moderate level of salinity. These lines would not be salt tolerant but equally usefully for yield enhancement, salt avoiding.

Now that the trait of sodium exclusion has been firmly linked with growth and yield in saline fields using specific genes (James et al., 2012) and with a wider range of varieties (McDonald et al., 2012), it is critical to assess for presence or absence of this trait in any future screening trials. For each genotype tested in field trials, this requires parallel measurement of Na+ in leaves 3 or 4 of plants that have been grown in saline solutions. Less Na+ equates with higher yield. The field trials will indicate whether other unspecified traits can lead to improved yield at saline sites whether via ST (sodium exclusion) or salinity avoidance enhancement, by being salt avoiding.

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