Scaling Up: the Essence of Effective Agricultural Research

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Abstract
Successful scaling up from laboratory research to application in the field depends on its practitioners being aware of the constraints and other interactions that arise as scaling up proceeds. Although exploration of promising ideas are often of intrinsic scientific interest, such ideas fail the test of utility if they do not get adopted by agronomists or plant breeders and if practices and cultivars based on such ideas do not get adopted by farmers. This notion of scaling up is explored here using salinity tolerance of crops as a case study, with examples drawn from gene expression, tissue culture, controlled environment studies of plants grown in hydroponics and in pots, and the behaviour of plants at the field scale. The most effective research in this arena has resulted from a culture of collegiate dialogue between scientists working at different scales.

Keywords
salinity, prebreeding, wheat, biological organisation, abiotic stress

Introduction
Plant scientists organise their thoughts by dividing the broad sweep of their subject matter into conceptual layers: community, whole plant, organ, tissue, cell, organelle, membrane, molecule (metabolite, protein) gene. These layers are essentially structural, but implied in them are a wide range of time scales of processes and interactions that are predominantly peculiar to each structural scale (Osmond and Chow 1988). We usually think of these layers as a nested hierarchy of systems and sub-systems, but it is also informative to view them as a loop in which one can travel in either direction (Fig.1).

Fig.1. Levels of organisation in crop plants, represented as a loop in which clockwise flow represents reduction, the search for mechanistic understanding at finer and finer scales, and anti-clockwise flow represents functional integration, the roles of various structures and processes in transmitting genes to the next generation. In the sense that selection of individual genotypes at the crop level (or, in natural systems, the community) determines the genetic makeup of the next generation, the “loop” is a helix when viewed across generations.
Clockwise flow denotes the familiar search for mechanistic understanding at finer and finer scales (reduction). Anticlockwise flow denotes the search for functional significance (integration). It is notable that clockwise flow essentially stops at gene. It is *anticlockwise* flow that captures the essence of biology, for unless the loop is closed none of the structures and processes that comprise it would have evolved. This point is an underlying sentiment of *Functional Plant Biology*.

In natural systems, selection pressures among organisms in communities, spiced by occasional mutations, determines what genes are transmitted to the next generation, and at what frequency. In agricultural systems, selection takes place in breeders’ plots and in farmers’ fields. Only cultivars that do well remain current. The rest are, at best, relegated to banks for genetic resources. The prediction of higher order behaviour from lower level information (“*reductionism from below*” (Weiner 1996)) has been predominantly unsuccessful.

At least ostensibly, much research in the plant sciences deals with improving the performance of the major crop plants. In trying to improve the performance of crops, the final arbiter is the farmer. New agronomic techniques or new cultivars will only be adopted if farmers find them effective. Many laboratory scientists who think that they have a great idea for improving crops find themselves eventually deeply disappointed. This is often because they try to cut across the loop in Fig.1 without taking their idea anticlockwise around it first.

There are some exceptions, which relate not to improving the performance of crops intrinsically, but to knocking out possible impediments. Bt (a gene whose product kills caterpillars) and herbicide resistance of crops are clear examples of successful genetic modification in which the path between gene and plant shown in Fig.2 works well. The persistence of the success however, depends on appropriate agronomic management to ensure that the pests and weeds do not become resistant to these measures.

There have also been successes in improving the nutritional quality of seeds by following the direct path in Fig. 2. Examples include: direct selection of natural variation (*Canola*, produced from oilseed rape by selecting lines low in the undesirable erucic acid and glucosinolates); knock-out mutation breeding (*Linola*, produced by blocking the conversion of the double-unsaturated linoleic acid into the triple-unsaturated linolenic acid (Green 1986)); and genetic transformation (*Golden Rice*, engineered to contain provitamin A).

![Diagram](attachment:Fig2.png)

**Fig.2.** A modification of Fig.1 to illustrate scaling up (anticlockwise flow) from fine levels of organisation to whole plants and crops. The direct path from gene to plant works well for genes that are not involved in vital processes, such as Bt, herbicide resistance, and nutritional quality of seeds. Adaptation to stress requires anticlockwise flow of ideas through several levels of organisation to attain success at the level of the crop. Many ideas that do not explicitly consider the problems of scaling up fail, as depicted by the arrows leaving the loop – see text for examples.
The history of improving the performance of crops, whether in yield, productivity, or environmental viability, has shown that almost all of the direct success has come from cleverly focussed empirical breeding and new agronomic techniques. But we are now faced with a slow down in the rate of genetic progress by empirical means (Fischer and Edmeades 2010), and we have the challenge before us of making use of our knowledge of the workings of plants to modify and to incorporate specific traits into breeders’ lines (see, for example Passioura (2007b)). Such traits can relate to any of the levels of organisation in Fig.1

Before looking at how best to do that, it is worthwhile looking for, and learning from, common patterns of success and failure in the past. Just as genes can head for oblivion during selection among genotypes, ideas can head for oblivion (Fig.2) as we try to scale up by moving anticlockwise around the loop in the hope of improving productivity.

Salinity tolerance, the central theme of this special issue of *Functional Plant Biology*, affords a wide range of examples of attempts to translate research at lower levels of organisation to performance of whole plants in controlled environments, and, most difficult of all, to crops in the field. Several such examples are discussed below, against a backdrop of the most important physiological features and behaviours of salt-affected wheat and barley, briefly summarised as follows.

The sequence of events following exposure of a plant to salt is first, that leaf expansion slows immediately. It may even stop completely before recovering somewhat to a value substantially less than that of the controls. This is primarily an osmotic effect, and is essentially a result of water stress (Passioura and Munns 2000). Within a day or two, however, the osmotic effect is overtaken by a signal from the roots which maintains slow leaf expansion (Munns et al. 2000). Meanwhile, transporters limit the amount of sodium entering the roots, pump much of what gets in back out of the roots into the medium, and remove sodium from the transpiration stream as it flows to the shoot (Munns and Tester 2008; Zhu 2002). After some time, the remaining sodium that reaches the fully expanded leaf blades slowly accumulates until the leaves starts to senesce, then die. This process is delayed by the extent to which sodium can be sequestered in the vacuoles. Eventually, however, the loss of photosynthetic area greatly reduces the growth of the plant, which in severe cases will die. These processes, together with other important features affecting salinity tolerance are summarised, with additional references, in Table 1.
Table 1 Some features or behaviours at various scales pertinent to growth response of salt-affected wheat and barley

<table>
<thead>
<tr>
<th>Organisational level</th>
<th>Feature, process</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fully expanded leaf</td>
<td>Na steadily accumulates in leaves until they can hold no more and lose photosynthetic activity</td>
<td>James et al. (2002)</td>
</tr>
<tr>
<td>Leaf expansion zone</td>
<td>Slow leaf growth</td>
<td>Greenway and Munns (1980)</td>
</tr>
<tr>
<td></td>
<td>Negligible sodium or chloride</td>
<td>Munns et al. (1982)</td>
</tr>
<tr>
<td></td>
<td>Substantial supply of photosynthetic</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Likely elongation control by signals from roots</td>
<td>Ternaat et al. (1985); Pérez-Alfocea et al. (this volume)</td>
</tr>
<tr>
<td>Cells</td>
<td>Na sequestered in vacuole (cheap osmoticum to help maintain turgor)</td>
<td>Flowers et al. (1977); Blumwald, et al. (2000)</td>
</tr>
<tr>
<td></td>
<td>Compatible solutes in cytoplasm</td>
<td>Wyn Jones and Storey (1978)</td>
</tr>
<tr>
<td>Enzymes</td>
<td>No evidence of differences in properties of major enzymes between glycophytes and halophytes</td>
<td>Greenway and Osmond (1972)</td>
</tr>
<tr>
<td>Transporters</td>
<td>Cell specific expression of transporter genes that control traffic of sodium (Na exclusion by roots, sequestration of Na in leaf sheath parenchyma)</td>
<td>Moller et al. (2009); Tester and Davenport (2003)</td>
</tr>
<tr>
<td>Genes involved</td>
<td>HKT1;4 (Nax1), HKT1;5 (Nax2, Kna1), SOS1, NHX1, HvNax3</td>
<td>Huang et al. (2006); Byrt et al. (2007); Martinez-Atienza et al. (2007); Shavrukov et al. (2010)</td>
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</table>

Salinity tolerance: successes and failures in scaling up from research in laboratories and controlled environments to behaviour of crops in the field

Gene expression
There has been much interest in using functional genomics and related technology to discover genes that could help plants cope better with salt, but little of practical importance has emerged, primarily because of the multigenic nature of salinity tolerance (Flowers 2004). Much of this research has, in the past, involved suddenly exposing plants to such high concentrations of salt (> 100 mM) that the cells of the roots plasmolyse (Munns et al. 2002), i.e. the membranes surrounding the cell contents are torn off the cells’ walls as the contents are shrunk by the surrounding salt sucking out the water; this induces major trauma that rarely if ever occurs in nature. Exploring gene expression in such circumstances is irrelevant to how plants behave in the field. Further, constitutive promoters have typically been used in transgenics, whereas cell-specific expression (Tester and Davenport 2003) and stress-inducible promoters (Kasuga et al. 1999; Flowers 2004) are more appropriate. Unfamiliarity with cell biology may lead to major misleading artefacts.
One can overcome the trauma of osmotic shock by increasing the concentration of salt gradually, in several small steps over a few days rather than in one large step, but even this may uncover little useful genetic variation in salt tolerance. The problem is that it can take weeks for such variation to become evident. This is because the main defence of plants against salt is to exclude almost all of it from the water that they take up, and it is variation in how much salt gets through and then builds up to damaging concentrations in the leaves that strongly influences a plant’s salt tolerance (Munns 2005). Laboratory experiments on salinity that are uninformed by processes occurring in whole plants over several weeks have little hope of being useful in improving productivity.

Tissue culture
Similarly, a few decades ago there was much interest in trying to select salt tolerance at the cellular level in cell culture. Cells that survived the challenge of salinity could be used to regenerate plantlets. But conditions in tissue culture do not duplicate the conditions within plants, and cells can be much more tolerant of salinity than whole plants (Flowers et al. 1985). The regenerated plantlets turned out to be no more salt tolerant than normal, unselected, plants (Dracup 1993).

Analysis of leaves
Schachtman and Munns (1992) observed that it is the slow build up of salt in the expanded leaves over days to weeks that eventually damages those leaves, leading to major loss of photosynthetic activity. From this knowledge, Munns et al. (2002) developed an assay for salt tolerance of whole plants based on measuring the salt concentration in emerged leaves of ten-day old plants grown in gravel culture – essentially supported hydroponics – with constant salt concentrations in the medium and realistic conditions of evaporation. The rationale was that the measurements, essentially of rates of uptake of Na\(^+\) into emerged leaves, would be good predictors of the timing of the onset of the later damage.

This assay proved to be so effective that it led to the discovery of two novel transporter genes in durum wheat, Nax\(_1\) and Nax\(_2\) (Table 1). Durum wheat cultivars have been notoriously more sensitive to salinity than bread wheat, because genes for protective transporters in bread wheat reside on its D genome, which is lacking in durums. The discovery of a salt tolerant durum genotype and its crossing with salt-sensitive durum cultivars generated populations segregating for salt tolerance (Munns et al. 2003). Using the leaf assay on these populations grown in gravel culture revealed the presence of two major genes for salt tolerance called Nax\(_1\) and Nax\(_2\) (Lindsay et al. 2004). Both Nax\(_1\) and Nax\(_2\) markedly reduced the amount of salt arriving in the xylem to the leaves. Nax\(_1\) removed Na\(^+\) from the xylem in roots and the base of leaves, Nax\(_2\) was confined to the roots (James et al. 2006). Nax\(_1\) was subsequently cloned by fine mapping as the Na\(^+\) transporter \(HKT1;4\), and Nax\(_2\) as HKT1;5 (Byrt et al. 2007)). This success was based on a simple and effective assay whose invention was inspired by knowledge of the slow development of salt damage in the mature leaves.

Plants in potted soil
The roots of plants grown in hydroponic systems, whether suspended in solution or anchored in frequently-flushed coarse sand or gravel, experience uniform conditions. Salinised soil, however, is typically heterogeneous in space and in time. One can set up pots containing uniformly distributed sodium chloride, but as soon as the roots start taking up appreciable amounts of water, the distribution of the salt starts to vary. When roots take up water, almost all the salt in that water is excluded from entering the roots and thus remains in the soil where
it concentrates in the diminishing soil solution. Because roots are never uniformly distributed in a pot, the concentration of salt in the soil solution increases most where the uptake of water is greatest, usually towards the top of the pot, unless the pot is supplied with fresh water.

If a pot is not watered, the concentration of salt in the soil solution keeps increasing until it becomes so great that the roots can no longer extract the water. The plant experiences increasing osmotic stress, which may override any specific effects of sodium chloride (Rengasamy 2010, this issue). If the pot is rewatered the roots may experience the opposite: fresh water added to the top of a pot will create a zone with no or little salinity, and a cline will develop with the soil solution becoming increasingly concentrated with depth. This scenario may underlie the observation by (Tavakkoli et al. 2010) that barley is much less affected by the seemingly same exposure to salinity in soil than in solution culture.

Equally important heterogeneity may also occur at the much finer scale of the rhizosphere, where there is strong, albeit circumstantial, evidence that a build-up of solutes and thence osmotic pressure can occur at the surface of roots exposed to strong nutrient solution (Stirzaker and Passioura 1996).

Finally, there is the complication in soil but not in hydroponics that the exchangeable ions on the soil particles interact with those of added salts to produce a soil solution whose composition may be substantially different from what was aimed at (Tavakkoli et al. 2010).

The heterogeneous nature of soil and the distribution of salt within it open up the possibility that roots may be able to reduce their exposure to the salt by preferentially growing into less salty areas, thereby decreasing the great influence that the Nax genes have in hydroponics.

Field-grown plants – spatial heterogeneity

Moving to the field brings with it another rich collection of interactions. The recent discovery of widespread salinity in the commonly occurring sodic subsoils in the Australian cropping areas (Rengasamy 2002; Rengasamy 2010, this volume) has sparked great interest in breeding crops, especially wheat and barley, that cope better with salinity. This discovery was facilitated by observations in the mid 1990s by a farmer and a breeder who were puzzling about why durum wheat would grow as well as bread wheat in some seasons, but was dismally worse in other seasons (Tom Cootes and Tony Rathjen, personal communication). Knowing that durum wheat was much less tolerant of salinity than bread wheat, they augmented their observations by using an electromagnetic conductivity meter, which detected subsoil salinity.

The presence of subsoil salinity in the field amplifies some of the issues pertaining to pots, and brings in others. Subsoil salinity is often accompanied by sodicity and boron toxicity (Adcock et al. 2007). With sodicity can come the danger of hypoxia, for sodic soils are finely porous and can become almost saturated with water in wet conditions, leaving little space for air; hypoxia can worsen the exposure to salinity (Barrett-Lennard 2003; Wetson and Flowers, this issue).

The amount and pattern of the seasonal water supply can markedly influence the dependence of crops on water in the subsoil, as evidenced by the differential responses of durum and bread wheats mentioned above, and as explored with a simulation model by Rodriguez et al. (2006).
The discussion above has centred on vertical and temporal variation in salinity. Spatial variation across a field can also be large, especially in irrigated environments. Richards (1983) has argued that, where such variation is large and includes a substantial area not strongly affected by salt, it is better for breeders to select for high yield in benign conditions rather than for salinity tolerance, for the yields of crops in the benign areas of a highly variable field typically account for most of the total yield from such a field. This argument does not apply, though, where all parts of a field are affected by salinity (Flowers and Yeo 1995). The primary salinity that is common in sodic subsoils in Australia, often varies widely within a paddock. Farmers have been using electromagnetic conductivity meters to map this variation, which strongly influences the amount of water available to the plants, and they can alter their agronomic management accordingly (Whitbread et al. 2008) – for example, by applying less fertilizer where subsoil salinity is evident.

**Patterns of success and failure in scaling up**

Problems with the performance of crops are first recognised at the level of the plant or crop: disease, pests, weeds, nutrition, waterlogging, water deficits, toxicities (e.g. salinity, acidity, boron), lodging, heat damage, floral sterility, and so on. It is tempting for laboratory scientists to think of cellular or molecular solutions for solving some of these problems. There have been some outstanding successes using this approach. Incorporating genes whose products are toxic to certain pests (e.g. Bt) or that block the effects of herbicides (e.g. glyphosate resistance) has been enormously successful. Similarly, resistance to many important diseases involve one or a few genes which are likely to be amenable to genetic transformation or amenable to easy selection in breeding programs with the help of molecular markers (e.g. resistance to cereal cyst nematode (Ogbonnaya et al. 2001)).

A distinguishing feature of these genes is that they target alien organisms or molecules. They are not involved directly in the major metabolic machinery of the plant nor in the large scale processes that determine how crop plants cope productively with highly variable environments; nor do they impose major metabolic loads on the plants. Thus the shortcut between gene and plant shown in Fig. 2 can work well.

Abiotic stresses, however, impinge on plants at different scales of time and space and involve elaborate processes occurring at all levels of organisation. Salinity provides a clear example, as described above. The deep understanding we now have of how plants deal with salinity at the levels of genes, transporters, cells, tissues, organs, whole plants and crops has enabled much recent progress in developing crops that are more salt tolerant.

Operationally (sociologically?), the main ingredient of this success has been mutually illuminating dialogue between people working at different levels of organization in the plant. The important interactions are illustrated in Table 2, in which “Level N” represents any level in the loop in Fig.1, and the central practitioner is in dialogue with colleagues both above and below (Passioura 1979):

<table>
<thead>
<tr>
<th>Biological significance</th>
<th>Level N+1</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Phenomenon</strong></td>
<td>Level N</td>
</tr>
<tr>
<td>Mechanistic explanation</td>
<td>Level N-1</td>
</tr>
</tbody>
</table>
There are as many of these triads as there are levels in Fig.1 (at the “gene” end the progression is, say, into the physical chemistry of epigenetic interactions), and the most fruitful research requires ideas to be running continually, both clockwise and anticlockwise, around the loop.

To facilitate such movement is not trivial, for dialogue across levels requires the learning of other languages. Several examples are given earlier. The term “plasmolysis” does not exist in the lexicon of genomics, and familiarity with cell biology is needed to understand (and avoid) the trauma arising from severe osmotic shock. Similarly cell biologists whose experience is restricted to cell culture need familiarity with the disparate functions of various organs and tissues, the purview of plant physiology, if they are to ask questions pertinent to the behaviour of whole plants.

Learning another language is best done on a daily basis. Thus, the practitioner at the central level in the above table is best off when sharing facilities with colleagues working at the adjacent levels. But what of those colleagues? Who will teach them other languages, and thereby help them hone their ideas and generate new and better ones? It is rare for a wide spectrum of biological interests to be housed together in an interactive way, but the socio-scientific networks that abound these days can provide the wherewithal for such conversations if the interest, resolve, and recognition of the requirement are there.

In agriculture the most important conversations are those between farmers and field scientists – agronomists, breeders, and sometimes crop physiologists. Given the general observation that emerging problems, and sometimes emerging opportunities, are first seen at the level of the plant or crop, these conversations form the basis of many innovations. As von Hippel (2005) has argued, innovations are frequently made by those who are the immediate beneficiaries of them. Farmers are no exception to this – one of von Hippel’s examples is that of a farmer who created the first centre pivot irrigator by coupling his motor bike, with its throttle set, to the end of a long length of irrigation pipe with the other end fixed on a spindle.

Figure 3 illustrates the richness of these conversations between farmers and agronomists/breeders by means of the boxes above and below the main axis. Similar interactions are implied by the other arrows. An essential feature of scaling up is that the higher level brings with it a range of constraints and interactions that affect operations at that level but are generally unfamiliar to practitioners at lower levels. Plasmolysis, control of the traffic of salt around the plant, and spatial variability in salinity, are examples given earlier.
Fig.3. Schematic depiction of the types of interactions between farmers and field scientists (agronomists, plant breeders). Similar interactions across other levels are implied by the arrows to the left of the farmers and to the right of the agronomists/breeders.

**General discussion**

Although I have focussed on salinity in the above, a similar set of scaling arguments apply to other abiotic problems, such as drought, discussed in some detail in Passioura (2007a), and waterlogging, which brings with it the danger of anoxia (Barrett-Lennard 2003; Colmer and Voesenek 2009).

With the latter, anaerobic biochemistry, though of great intrinsic interest, is of secondary importance to the development of aerenchyma, continuous open corridors within roots that enable oxygen to diffuse for tens of centimetres within roots growing in waterlogged soil. Another adaptation is the ability of the roots to recover rapidly from the transient waterlogging that commonly occurs in many dryland cropping soils (Setter and Waters 2003). There is genetic variation in these developmental processes that could one day be harnessed, perhaps twenty years hence, to provide more tolerant genotypes. Meanwhile, when scaling up to farmer’s fields, it is better to seek an agronomic solution than a genetic one. Preventing waterlogging in the surface soil by growing plants on raised beds can substantially increase yields in areas where the excess water can be drained away (Bakker *et al.* 2005).

**Trait-based breeding**

Plant breeders have made, and are continuing to make, substantial progress in improving water-limited productivity. They have done so mostly by traditional empirical means, though augmented over the past few decades by more powerful statistics that enable them to distinguish promising breeding lines more clearly, and by selection based on molecular markers. The only agronomic or physiological traits they use routinely are flowering time, which is essential to fit the variety to the target environment, and height.

Meanwhile, there is very large R&D expenditure on abiotic pre-breeding around the world. Australia spends about as much on pre-breeding as on breeding of wheat and barley, and there is concern that little of this effort has resulted in new varieties (Anon 2009). A major difficulty is that there has not been a well-developed procedure for scaling-up, an issue that was explored in a recent workshop which brought together agronomists, breeders, and a range of pre-breeders from molecular biologists to crop physiologists (Passioura 2007b).

Scaling up at this advanced level is exceedingly difficult. An essential tool in this process is the setting up of field phenotyping sites to explore and expedite the introduction of novel traits into advanced breeding lines in realistic environments using breeding lines adapted to the target environments. In the context of getting a radical new cultivar into the hands of farmers it is salutary to remember that it took 8 years of shuttle breeding, with two generations per year, to produce the first commercial cultivars of the semi-dwarf wheats that were the basis (together with semi-dwarf rice) of the Green Revolution (Reitz and Salmon 1968) – this, despite the seemingly simple task of incorporating a single dwarfing gene that could be easily selected for.

**Scaling up of agricultural R&D**
How then can we best facilitate the scaling up of agricultural R&D, whether within the R&D community, or between it and farmers and their advisers? One impediment is the prevailing language, which has many terms implying one-way flows of information rather than interactions across scales. “Technology transfer” is the most potent of these, but several others, such as “extension” and “delivering outcomes” have similar connotations. All imply that R&D professionals produce solutions to agricultural problems which are then “delivered” to receptive farmers, or perhaps thrust upon, or seductively sold, to recalcitrant ones. In fact, one can argue that what agricultural research mainly produces is useful management options and cultivars for farmers to choose among when dealing with their highly variable environments as each season, or run of seasons, develops.

This language of “technology transfer” is appropriate in many circumstances, but gives no hint of the mutually stimulating dialogues that take place between many farmers and agricultural scientists and that greatly expedite scaling up. This two-way flow of information alerts the scientists to problems and operational constraints that may accompany an increase in scale, and helps train the intuition of the farmers by giving them a deeper understanding of the processes going on in their crops and pastures and in the ground under their feet – after all, it is the activity of well-informed inventive farmers that leads to many agricultural innovations.

Dialogues between agricultural scientists working at different scales are as important as wellsprings of innovation as those between farmers and scientists. The challenge is to create R&D institutions, of which farmers and their advisers are an essential part, in which the informal dialogues that stimulate sensible scaling up can be fostered across all scales from molecular genetics to farms. The notion of the value-chain is well recognised beyond the farm gate. It is of equal importance in fostering innovation before the farm gate.

Acknowledgements
I am indebted to Tim Flowers, John Kirkegaard, Rana Munns, Richard Richards, Richard Stirzaker, Anton Wasson and Michelle Watt for their penetrating comments on the manuscript and for countless highly illuminating conversations.
References


