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The Use of Hydroponics in Abiotic Stress Tolerance Research

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1. Introduction

Hydroponics, the 'water culture' of plants, has been used in both research and commercial contexts since the 18th century. Although now used successfully on a large scale by commercial growers of fast-growing horticultural crops such as lettuce, strawberries, tomatoes, and carnations, hydroponics was initially developed as a part of early research into plant nutrition. The idea of hydroponics, its development and improvement, stimulated the interest of plant biologists, and their research provided useful outcomes and scientific knowledge about mechanisms of nutritional toxicity/deficiency and plant development in general. Scientists discovered that plants required only a small number of inorganic elements, in addition to water, oxygen and sunlight, to grow. It was later realised that plants grew better hydroponically if the solutions were aerated. The use of hydroponics enabled plant scientists to identify which elements were essential to plants, in what ionic forms, and what the optimal concentrations of these elements were. It allowed them to easily observe the effects of elemental deficiencies and toxicities and to study other aspects of plant development under more controlled conditions. As scientists' understanding of the requirements for growing plants in hydroponics increased, the system was adopted and refined by commercial producers who found it allowed them to control environmental variables and deliver higher yields of product more reliably. Hydroponics systems are now operated in temperature- and light-controlled glasshouses which allow crop production through all seasons.

Hydroponics remains a fundamental tool for plant research. In this chapter we describe the use of hydroponics in the context of scientific research into plant responses and tolerance to abiotic stresses, drawing on our experiences at the Australian Centre for Plant Functional Genomics (ACPGF) and the University of Adelaide, as well as summarising reports in the published literature. We have also described some of the problems and uncertainties encountered in our hydroponics-based experiments. We hope that the chapter will be of benefit to scientists and other individuals with an interest in this topic.

2. Hydroponics systems for research

Plants growing in hydroponics require oxygen to be delivered to the roots, in addition to the water and nutrients supplied in growth solutions. Without constant aeration, a hydroponics system will become anaerobic and inhibit the growth of most plants. Only a small number of species (including rice) are adapted to grow in submerged environments with minimal oxygen supply. Such plants contain root structures known as aerenchyma, which are large air-filled spaces within the root that accumulate and store oxygen. The majority of plant species, however, require a continuous supply of oxygen into the growth solution for absorption by root cells. Hydroponics can be divided into two basic types depending on the method of aeration employed: (1) Flood-drain systems and (2) Continuous aeration. Both systems are routinely used in our research and in the following sections we will describe each system in more detail. While the nutrient film technique has revolutionised commercial hydroponics, it is not considered to be useful for abiotic stress research and will not be discussed in this chapter.

2.1 Flood-drain system

This type of hydroponics maintains regular mixing of growth solution by repeatedly pumping the solution into the growing vessel and allowing it to drain. Continuous movement of solution during the pumping/drainage cycles facilitates delivery of oxygen to the roots. We have not observed symptoms of depressed plant growth using this system of aeration. A large empty container (usually on the bottom) to store drained growth solution and a pump are required for the system to operate. The volume of growth solution required and the length of the drainage/pumping cycle will depend upon the size and design of the setup. The system illustrated is constructed with a lower storage/pumping tank containing 80 L of growth solution and two containers (40 L each) on the top for growing plants, and employs a 20 minute pump / 20 minute drain cycle (Fig. 1). This hydroponics system was initially designed by Mr. R. Hosking and further refined by Mr. A. Kovalchuk from ACPFG.

Flood-drain systems also require a supporting material in the growing vessel to hold the plant and to maintain a moist environment around the roots. We use polycarbonate plastic fragments, which we obtain from a local plastics manufacturer (Plastics Granulated Services, Adelaide, Australia). A selection of suitable types of plastic fragments used is illustrated in Fig. 2. The most important characteristics of the plastic fragments are: (1) chemical inertness and (2) surface wetness. The first characteristic ensures that there are no changes in the composition of available nutrients provided by the growth solution, and the second characteristic facilitates the continued supply of moisture and nutrients during draining. The plastic fragments should be washed in tap-water several times and finally rinsed with reverse osmosis water before first use to remove traces of manufacturing chemicals. With further regular washing, the fragments can be re-used many times in hydroponics systems.

We employ two methods for growing plants in the plastic fragments: either in separate tubes for individual plants (Fig. 3A), or tubs for larger numbers of plants (Fig. 3B). Growing a single plant in each tube enables the experimenter to remove individual plants for ongoing analyses (eg. imaging), and also keeps the roots separated for eventual harvest and analysis.



Fig. 1. A flood-drain 80 L supported hydroponics system in use at the Australian Centre for Plant Functional Genomics (ACPFPG).



Fig. 2. A selection of plastic fragments suitable for use as a supporting substrate in flood-drain hydroponics.

Both the tubes and tubs have a fine mesh fitted to the bottom to hold the plastic fragments in place and allow for the entry/exit of growth solution, which is pumped from below into the outer container (Fig. 3). The outer container has an overflow tube for the return of excess growth solution back to the lower storage container during the pumping period.

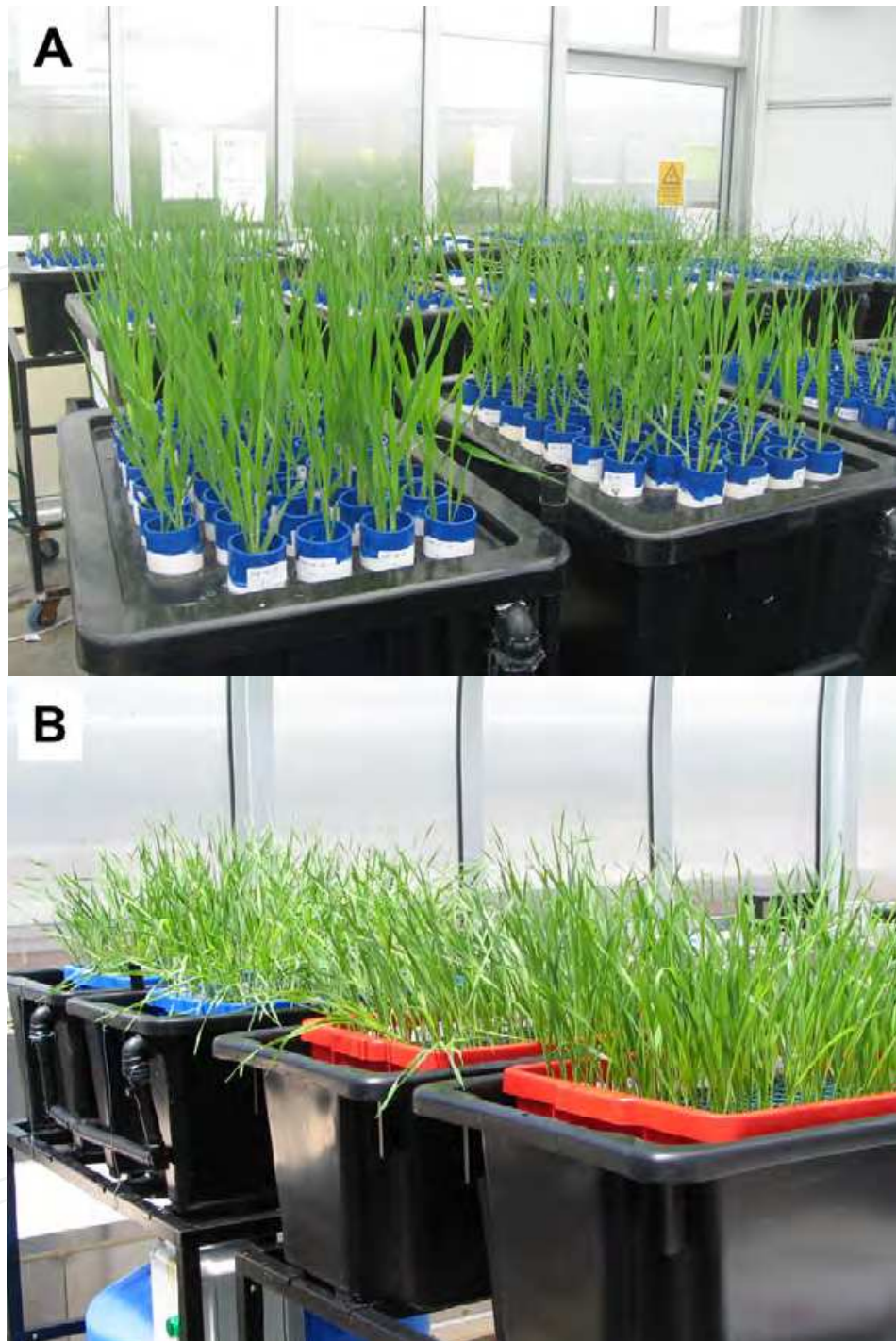


Fig. 3. Close view of barley plants in individual tubes (A) and in tubs/buckets (B) in flood-drain supported hydroponics.

The optimum age and size of plantlets for transfer to hydroponics is species-dependent. We pre-germinate wheat and barley seeds for 4 to 5 days in petri dishes or trays lined with damp paper and covered with plastic. Seedlings are transplanted when the roots are between 1 and 2 cm in length. Although the young plants recover from transplanting after 2 to 3 days, we usually allow 7 to 10 days before applying an experimental treatment. It is also possible to germinate seeds directly in the substrate, and this may work well for certain species.

2.1.1 Alternative substrates for supported hydroponics

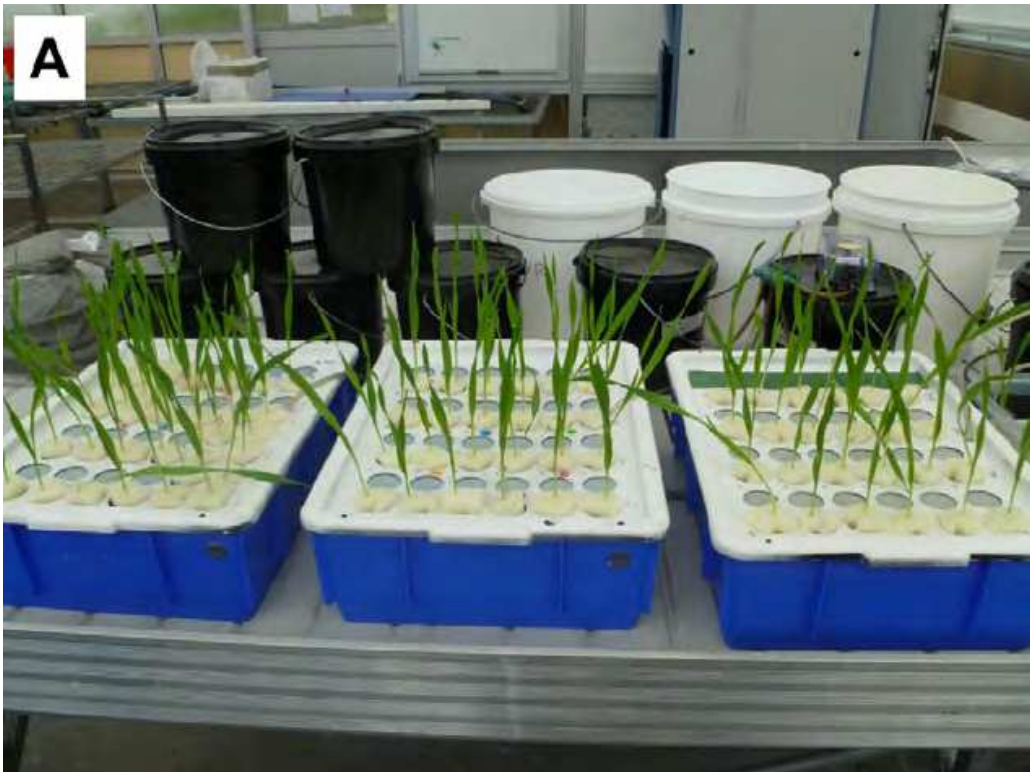
While we prefer to use plastic fragments as a solid supporting substrate for growing plants in flood-drain hydroponics, there are a range of other suitable supporting materials. The simplest to use are quartz gravel or river sand, and both are widely used in such systems (Boyer et al., 2008; Dreccer et al., 2004; Greenway, 1962; Munns & James, 2003; Rawson et al., 1988a, 1988b). However, small quartz particles can easily block and damage the pumping system. Other suitable substrates may include artificially manufactured expanded clay balls, vermiculite (Forster et al., 1994; Gorham et al., 1990, 1991) or perlite, and rockwool (Gorham, 1990; Gorham et al., 1991). Some substrates are also suitable for small, 'passive' hydroponics systems, where plants are grown in small pots containing the substrate and sitting in a tray, with growth solution supplied via capillary action from below. Expanded clay balls are manufactured in many countries with different brand, for example, Hydroton in Australia, Keramzit in Russia and Blahton in Germany. An example of this type of hydroponics using expanded clay balls is illustrated in Fig. 4. Caution is given that such a system can only be used for relatively short growth periods (less than 4 weeks) and that there is less control over the supply of nutrients to the plants, due to the potential for light-stimulated algal growth and high rates of evaporation from the tray. Unlike plastic fragments, these alternative substrates are also not chemically inert and may leach mineral ions into the growth solution or alter nutrient availability. Such substrates are less suitable for studies of mineral element deficiencies and toxicities.

2.2 Continuous aeration

The engineering requirements of hydroponics with continuous aeration are much less complex than for flood-drain systems. However, this system depends on constant aeration. A selection of aerated hydroponics setups used in our Centre is presented in Fig. 5. Good aeration in small volumes (12 L or less) is achieved using commercially available aquarium pumps, plastic tubing and aeration stones. In continuous aeration systems, the roots of germinated seedlings are directly submerged in aerated growth solution and the shoots supported to grow above the solution. One way to do this is with soft pieces of foam wrapped around the seedlings and held in holes drilled into a lid covering the hydroponics container, Fig. 5A and 5B (Drihem & Pilbeam, 2002; Gorham et al., 1987; Shah et al., 1987). Other alternatives include the use 'rafts' of polystyrene on the surface of the growth solution (Bağci et al., 2007; Ma et al., 2007), also with holes for holding the growing seedlings, the use of rockwool or agar plugs in open-ended Eppendorf tubes held in suitably sized holes drilled into the container lid, and placement of growing seedlings directly in open-ended plastic tubes held in the container lid. The size of hydroponics containers and the type of support can be varied to suit the needs of the researcher. For example, a miniature hydroponics system was designed using a 200 ml pipette tip box, where seedlings are held directly in open-ended 500 µl Eppendorf tubes (Fig. 5C). A further alternative uses 4 L lunch-box style containers purchased with plastic rack inserts (Décor, Australia). A layer of plastic mesh is sewn onto the insert, and the level of growth solution is adjusted so as to just reach the surface of the mesh (Fig. 5D). Wheat or barley seedlings can be grown directly on plastic mesh, Fig. 5D (Watson et al., 2001), acrylic grids (Kingsbury et al., 1984) or anodised aluminium mesh (Shah et al., 1987) without further support.



Fig. 4. The use of expanded clay balls (A) as a substrate for a small, 'passive' hydroponics system, showing broccoli (B) and saltbush, *Atriplex* ssp. (C) after a light salt stress (50 mM NaCl). (Figure provided by Ms J. Bovill, ACPFG and students at the University of Adelaide).



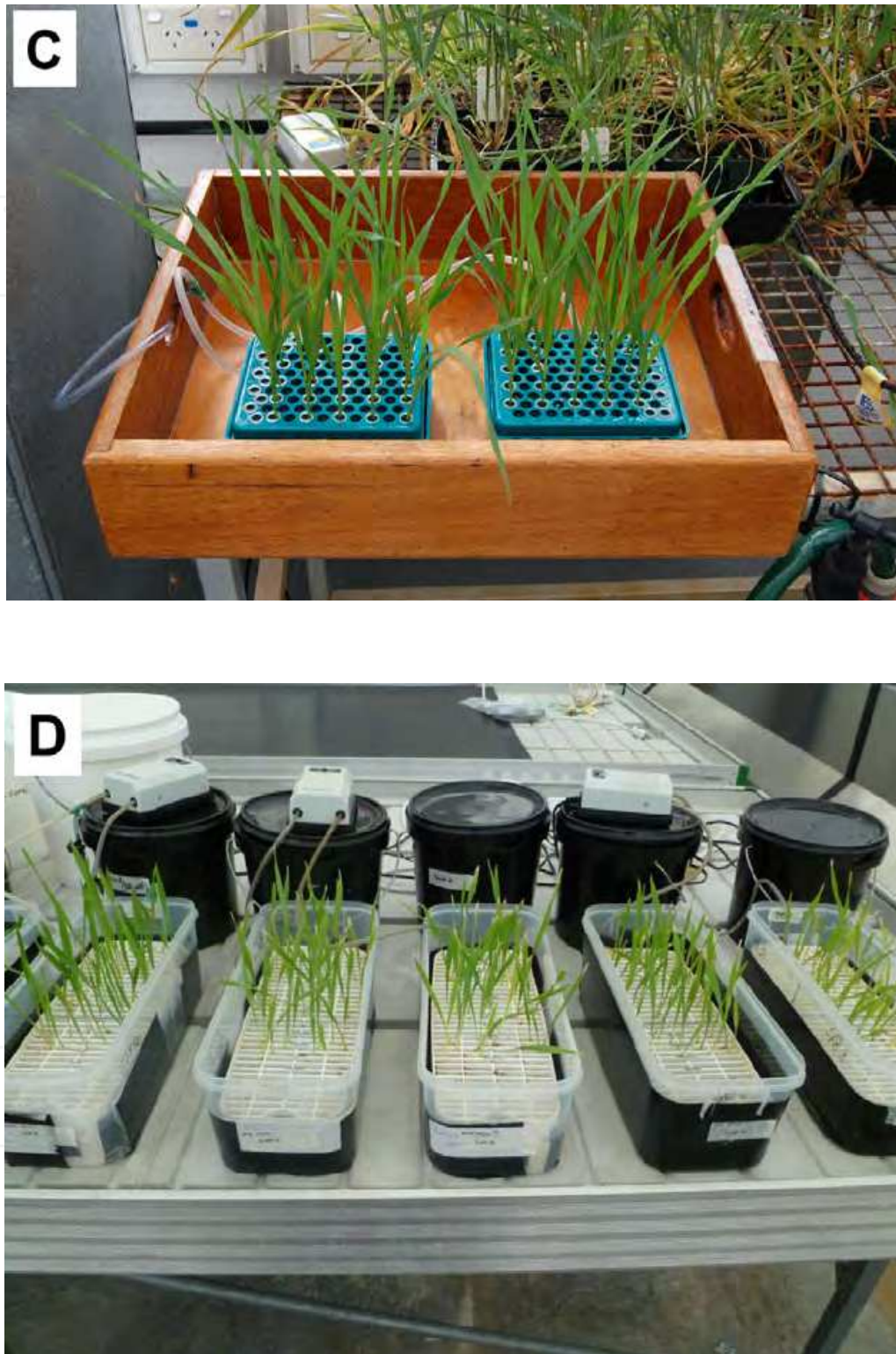


Fig. 5. Examples of continuous aeration hydroponics setups used by researchers at ACPFG. (A) 12 L boxes with foam supporting growing seedlings, or (B) with 10 ml open-ended plastic tubes. (C) Miniature hydroponics in 200 mL pipette tip boxes (image provided by Mr J. Harris, ACPFG). (D) 4 L lunch boxes with plastic inserts and mesh.

3. Composition of nutrient solutions

All nutrient solutions used for hydroponics culture of plants are essentially derived from the original protocol developed by Hoagland and Arnon (1938). A typical growth solution consists of the following essential macro-elements: nitrogen (N), potassium (K), phosphorus (P), calcium (Ca), magnesium (Mg) and sulphur (S); and micro-elements: a soluble form of iron (Fe), boron (B), copper (Cu), manganese (Mn), nickel (Ni), zinc (Zn), molybdenum (Mo) and chlorine (Cl), and, for leguminous species requiring N fixation, cobalt (Co). Sometimes, silicon (Si) and selenium (Se), while not essential elements, are considered beneficial to plant growth and are also included (Epstein, 1994, 1999; Lyons et al., 2009). The standard growth solution used by researchers at ACPFG has previously been published (Genc et al., 2007; Shavrukov et al., 2006). However, we have recently further improved the composition of the growth solution for use with wheat and barley based on tissue nutrient analysis (Table 1). We also found that other species, such as rice, have a high requirement for ammonium nitrate (5 mM).

Elements	Salts used	Final concentration	
		Published protocol (Genc et al., 2007; Shavrukov et. al., 2006) (mM)	Improved protocol (mM)
N	NH ₄ NO ₃	0.2	0.2
K, N	KNO ₃	5.0	5.0
Ca, N	Ca(NO ₃) ₂	2.0	2.0
Mg, S	MgSO ₄	2.0	2.0
P, K	KH ₂ PO ₄	0.1	0.1
Si*	Na ₂ SiO ₃	0.5	0.5
Fe	NaFe(III)EDTA	(µM) 50.0	(µM) 100.0
B	H ₃ BO ₃	50.0	12.5
Mn	MnCl ₂	5.0	2.0
Zn	ZnSO ₄	10.0	3.0
Cu	CuSO ₄	0.5	0.5
Mo	Na ₂ MoO ₃	0.1	0.1
Ni	NiSO ₄	0.0	0.1
Cl**	KCl	0.0	25.0

* Silicon is not an essential element and may be omitted from the growth solution, depending on the experiment and plant species grown.
** MnCl₂ was reduced to 2 µM in the improved protocol to optimize Mn nutrition. However, as MnCl₂ is the only source of Cl, additional chloride was supplied as KCl to avoid Cl deficiency.

Table 1. Composition of growth solutions used in plant nutrition studies by researchers at ACPFG and the University of Adelaide, including an earlier published protocol and an improved protocol for culture of wheat and barley.

3.1 Maintenance of pH

Most plant species will grow optimally in nutrient solutions of acid to neutral pH (range 5.5 – 7.5) (Dreccer et al., 2004; Drihem & Pilbeam, 2002; Dubcovsky et al., 1996; Kronzucker et al., 2006; Munn & James, 2003), although there is some species variability in optimum pH required for growth. The nutrient solutions used in our research do not require pH adjustment to stay within the optimum range if replaced regularly and if silicon is omitted. If Si is included, careful attention must be paid to achieving and maintaining an appropriate solution pH (see Section 3.2 below).

Depending on the experiment, strict maintenance of pH may be required. Occasionally, pH values outside of the optimum range are needed for study of certain abiotic stresses. For example, aluminium toxicity studies are conducted at low pH (4.5 or less) to ensure the presence of soluble, phytotoxic Al^{3+} (eg. Collins et al., 2008; Famoso et al., 2010; Pereira et al., 2010). Maintenance of pH can be achieved by several means. Nutrient solutions can be buffered with low concentrations of a suitable zwitter-ionic buffer that is not phytotoxic, eg. 2 mM MES (2-[N-morpholino]ethane-sulphonic acid)-KOH (Genc et al., 2007). Alternatively, solution pH may be monitored and adjusted frequently. This task can be done either manually or automatically (eg. Jarvis & Hatch, 1985). For automated pH adjustment, an automatic pH controller is attached to each hydroponics unit (eg. Cole-Parmer 5997-20 pH controller, SML Resources International, USA). As solution pH moves above or below the set pH value, acid or base is automatically dispensed into the solution to return the pH to the set value (Deane-Drummond, 1982; Wheeler et al., 1990). However, pH automation is costly, particularly for multiple hydroponics units.

3.2 Silicon in nutrient solutions

Despite the beneficial effects of Si on plant growth (Epstein, 1994, 1999), there is no consensus on whether or not it should be included in nutrient solutions. In his studies with silicon, Epstein (1994) concluded that “omission of Si from solution cultures may lead to distorted results in studies on inorganic plant nutrition, growth and development, and responses to environmental stress”. The author advocated the addition of Si in solution culture to represent its abundance in soil solution (0.1 - 0.6 mM), but he did not acknowledge that there are many environmental sources of silicon, including mineral salts and water and even particulate SiO_2 in the atmosphere, which may provide enough Si for plant growth and development.

In our experience, the application of Si to hydroponically-grown wheat and barley did not result in significant differences in plant growth in non-stressed conditions. However, silicon may impact on plant responses to abiotic stresses. For example, we have observed that rice shows different responses to boron toxicity when grown with or without added silicon. One of the genes underlying boron toxicity tolerance in barley, *HvNIP2;1*, encodes a transporter belonging to the aquaporin family which facilitates transport of both B and Si (Chiba et al., 2009; Schnurbusch et al., 2010). This gene is also present in rice (Ma et al., 2006) and may explain the observed interactions between Si nutrition and B toxicity. Furthermore, the addition of silicon has been found to increase levels of observed tolerance to salinity, drought, high and low temperature and metal toxicities (reviewed in Ma & Yamaji, 2006).

Silicon is produced commercially as a crystalline powder, sodium silicate pentahydrate (eg., Chem-Supply, Australia), or as a liquid in the form of sodium silicate solution (water glass; eg., Sigma, USA). Both forms of silicate are suitable for hydroponics. However, careful attention must be paid to achieving and maintaining an appropriate pH for plant growth (acid to neutral range) when Si is used (Dubcovsky et al., 1996). The addition of either form of silicon increases the pH of the growth solution to above 7.0, and may cause the precipitation of other nutrients out of solution.

3.3 Form of nitrogen and pH maintenance of nutrient solutions

It is well known that nitrogen is one of the most important elements necessary for plant growth. A detailed review of this topic is outside the scope of this chapter. However, we would like to mention a practical issue relating to the use of nitrogen in hydroponics and consequences for nutrient solution pH. Two major forms of nitrogen are used in hydroponics: ammonium (NH_4^+) and nitrate (NO_3^-), and usually both forms are included in nutrient solutions. When both molecules are present in growth solution, NH_4^+ cations are often preferentially absorbed by plant roots over NO_3^- anions (eg. Gazzarrini et al., 1999). The different depletion rates of NH_4^+ and NO_3^- will result in an altered growth solution pH. For wheat and barley, we found that nutrient solutions containing equimolar (eg. 5 mM) concentrations of ammonium nitrate and potassium nitrate rapidly acidify as NH_4^+ is preferentially taken up by the growing plants and protons (H^+) are released to maintain charge balance. Such high concentrations of ammonium can also be deleterious to plant growth. Britto & Kronzucker (2002) reported that seedlings growth of barley was reduced considerably at 10 mM NH_4^+ compared to 1 mM NH_4^+ . Similarly, we observed that the growth of bread wheat seedlings was significantly reduced (37%) at 5 mM NH_4^+ compared to 1 mM NH_4^+ when grown in nutrient solution containing 5 mM KNO_3 / 2 mM $\text{Ca}(\text{NO}_3)_2$. (Genc et al., unpublished; Fig. 6). An optimum ammonium concentration of 0.2 mM NH_4^+ (with 5 mM KNO_3) was identified for hydroponics solutions for wheat and barley (Genc et al., unpublished). Interestingly, similar ratios of ammonium (20 – 200 μM) to nitrate (1 – 5 mM) have been measured in soil solutions of fertilised agricultural soils (Owen & Jones, 2001). Similar experiments would need to be conducted to optimise ammonium to nitrate N ratios for different species and different compositions of nutrient solution. For example, we use higher concentrations of ammonium N for the culture of rice in hydroponics. When the ratio of ammonium to nitrate N is optimised, solution pH remains relatively stable if replaced regularly to avoid depletion of either form of N.

4. Application of abiotic stresses in hydroponics systems

Research into plant tolerance of abiotic stresses, including salinity, drought, toxic concentrations of boron, aluminium or other elements and elemental deficiencies, is of fundamental importance for sustainable and secure agriculture into the future. In this context, hydroponics is a major scientific modelling tool, facilitating precise control over the treatment and consistent observations of treatment effects. Importantly, hydroponics enables observations to be made of intra- and inter-specific genetic variation in plant responses, in terms of levels of tolerance shown and the specific tolerance mechanisms that are employed.



Fig. 6. Seedlings of bread wheat (cv Krichauff) after growth in hydroponics for four weeks in the presence of 1 mM and 5 mM of NH_4NO_3 . Growth solutions were supplied with 5 mM KNO_3 and 2 mM $\text{Ca}(\text{NO}_3)_2$.

4.1 Salinity

Salinity is a major abiotic stress across agricultural regions worldwide, with a significant impact on cereal production (Colmer et al., 2005; Flowers & Yeo, 1995; Rozema & Flowers, 2008; Steppuhn et al., 2005a, 2005b). Salinity occurs either as a result of deforestation and rising saline water tables (dryland salinity) or irrigation with saline water (irrigation salinity) (Rengasamy, 2006). While soil salinity is complex, NaCl is considered the primary contributing salt as it is abundant in many soils and has a very high solubility (Rengasamy, 2002).

Hydroponics is highly suitable for the study of salinity tolerance. However, there are several issues to be considered. The addition of NaCl should not be made in a single application because it will cause osmotic shock and may kill the plants. Unless osmotic stress tolerance is of interest to the researcher, salt should be added in increments until the final desired concentration is reached, to allow plants to adapt to osmotic stress. Depending on the plant species and aim of the experiment, low, moderate or severe salt stress may be applied. Typically, we add salt twice daily (morning and evening), in 25 mM or 50 mM NaCl

increments (Shavrukov et al., 2006, 2009, 2010a, 2010b), in agreement with other salinity research groups (Boyer et al., 2008; Dreccer et al., 2004; Forster et al., 1990, 1994; Gorham, 1990; Munns & James, 2003; Rawson et al., 1988a, 1988b; Shah et al., 1987; Watson et al., 2001). We have found that suitable salt stress levels are typically 100-150 mM NaCl for bread wheat (Dreccer et al., 2004; Gorham et al., 1987; Munns & James, 2003; Shah et al., 1987), 150-200 mM NaCl for barley (Forster et al., 1990, 1994; Gorham et al., 1990; Rawson et al., 1988a, 1988b; Shavrukov et al., 2010a), and 250-300 mM NaCl for tolerant cereals such as wild emmer wheat, *Triticum dicoccoides* (Shavrukov et al., 2010b), and for saltbush, *Atriplex* ssp. and other halophytes (Flowers et al., 1977). While there are reports of salinity experiments in hydroponics using NaCl concentrations of 300 mM NaCl (Huang et al., 2006), this represents a salinity level of half the strength of sea-water and most plants would be severely stressed in such a treatment.

The addition of NaCl requires supplementation with additional Ca^{2+} . Symptoms of calcium-deficiency are observed when plants are grown in hydroponics under salt treatment and not provided with extra Ca^{2+} (Cramer, 2002; Ehret et al., 1990), and leaves from these plants are calcium-deficient (eg. Francois et al., 1991). Increasing NaCl in hydroponics solutions reduces the activity of Ca^{2+} in solution (Cramer & Läuchli, 1986), and supplementary Ca^{2+} should be added to compensate. The amount of supplementary calcium (as a ratio of $\text{Na}^+ : \text{Ca}^{2+}$) required varies depending on the concentration of added NaCl and the overall composition and pH of the growth solution, and can be determined using speciation prediction programs such as Geochem-EZ: <http://www.plantmineralnutrition.net/Geochem/geochem%20home.htm> (Shaff et al., 2010); or Visual MINTEQ: <http://www2.lwr.kth.se/English/OurSoftware/vminteq/index.html> (Gustaffson, 2008). Recent empirical studies in wheat using the growth solution provided in Table 1, found that the most appropriate $\text{Na}^+ : \text{Ca}^{2+}$ ratio in solution, resulting in tissue Ca^{2+} concentrations of salt-affected plants similar to those of control plants, was 15 : 1 at 100 mM NaCl (Genc et al., 2010). This study also showed that excessive use of supplemental Ca^{2+} could induce additional osmotic stress and nutritional deficiencies such as magnesium, and thus should be avoided.

In salinity research, hydroponics can be used to study sodium accumulation during short-term (7 - 10 days) and long-term (up to maturity) studies. We generally grow wheat and barley in hydroponics in the presence or absence of NaCl for four weeks to determine overall salinity tolerance measured as relative growth (growth in NaCl treatment relative to non-saline conditions). Plants can be easily removed from hydroponics and separated into roots, shoots and leaves (if required) for destructive analysis. Non-destructive measurements can also be made: selected individuals can be removed from hydroponics, their fresh weights obtained, then transplanted to pots of non-saline soil for recovery and cultivation to maturity if seeds are required. We achieve high grain yields from wheat and barley and their wild relatives when transplanted from saline hydroponics to soil-filled pots, provided this is done when the plants are less than four weeks old.

We have also successfully grown wheat and barley plants in flood-drain hydroponics systems to maturity. With regular replacement, hydroponics growth solutions provide the growing plants with sufficient nutrients and there is no inter-plant competition for nutrients as may occur in soil. However, following tillering, the growing plants compete for light and space around the above-ground plant parts. Provided these factors are optimized, plants can

be grown to maturity as shown for bread wheat in Fig. 7. This hydroponics study demonstrated both symptoms of growth depression in plants and an increased rate of plant development under salt stress (Fig. 7).

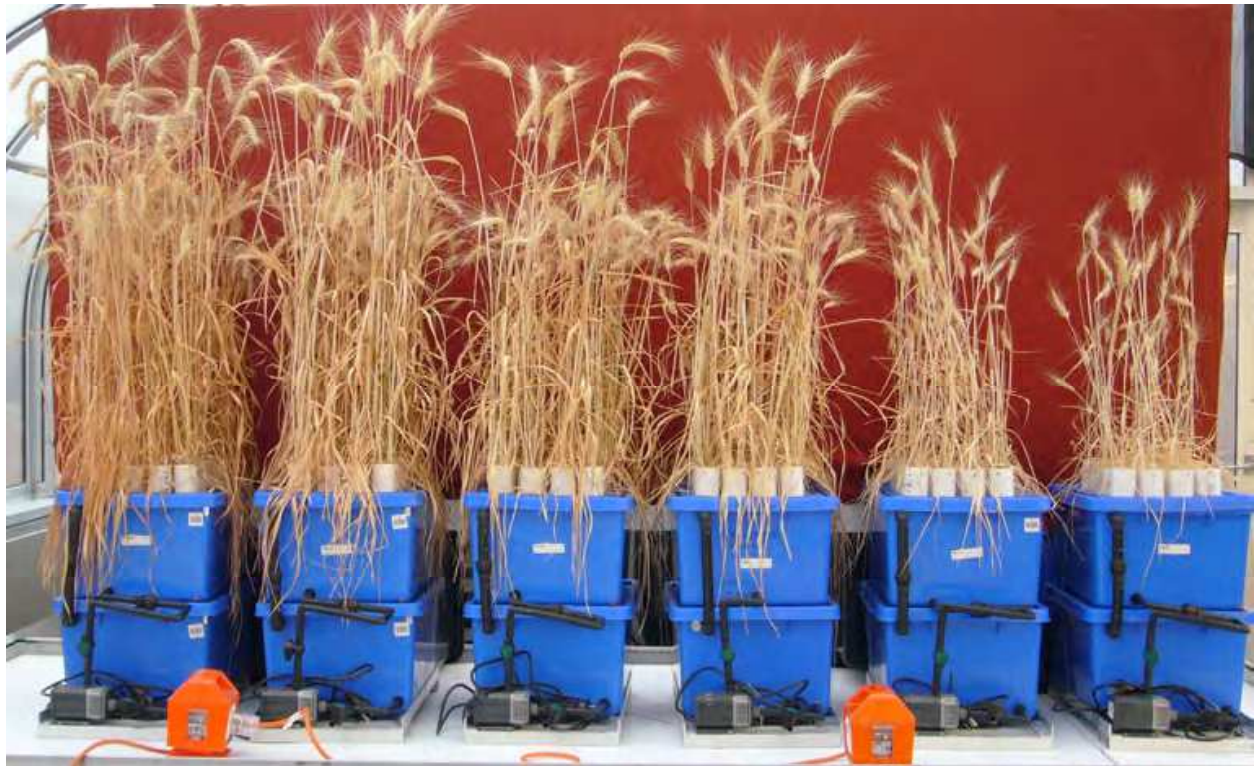


Fig. 7. Flood-drain supported hydroponics in 20 L containers and tubes, showing the appearance of bread wheat plants grown at different concentrations of NaCl to maturity (from left to right: 0, 50, 75, 100, 150 and 200 mM NaCl).

4.2 Drought

Various forms of hydroponics have been used to study drought (water stress) responses by plants, with somewhat limited success (reviewed in Munns et al., 2010). Drought is a particularly complex stress phenomenon that is difficult to model in any growth system. Water deficit may be imposed in hydroponics using osmotica such as mixed salts (eg. high concentrations of macronutrients in nutrient solution), NaCl, mannitol, sorbitol or polyethylene glycol. The applied water stress in hydroponics is more controlled and homogeneous than in soil-based systems. However, small molecules, such as mannitol, are easily absorbed by roots and move to the shoots (Hohl & Schopfer, 1991), and will affect plant metabolism and drought tolerance responses. NaCl or mixed salts may be suitable for short-term studies of water deficit (eg. Tavakkoli et al., 2010), but these will be taken up by the plants with time as well. High molecular weight polyethylene glycol (PEG) is less likely to be absorbed by plants, although uptake of PEG has been observed through damaged roots (Miller, 1987). PEG also increases solution viscosity and reduces the supply of oxygen to plant roots (Mexal et al., 1975). This can be overcome with careful supplemental oxygenation (Verslues et al., 1998). Reasonably consistent ranking of drought tolerance of wheat genotypes has been achieved using both a PEG treatment in hydroponics and drying of pots of soil (Molnár et al., 2004). We use an alternative method utilising hydroponics to

study terminal drought responses in wheat and barley. Growth solution is withdrawn from the flood/drain system described above, with plants growing in plastic fragments. Usually, up to five days of drying is allowed before plant tissue is sampled for analysis of gene expression changes relative to tissue sampled prior to the withdrawal of growth solution. While we observe variability between experiments using this method, reasonable comparisons can be made between genotypes within a single experiment. It should be noted that the withdrawal of growth solution in hydroponics systems is not suitable for long-term drought experiments because the process of roots drying between plastic fragments is relatively quick and cannot simulate processes of natural drought.

4.3 Elemental toxicities: Boron and aluminium

Excessive levels of soil boron (B) and aluminium (Al) both reduce plant growth and, in regions where they occur, significantly limit cereal production. Boron toxicity typically occurs in alkaline soils of marine origin, often in conjunction with soil salinity. Boron toxicity may also occur as a consequence of excessive fertiliser application. The effects of B toxicity include reduced root growth and shoot dry matter production, leaf necrosis, and reduced grain yield. Significant yield penalties in southern Australia due to B toxicity have been reported for wheat and barley (Cartwright et al., 1986; Moody et al., 1993). Aluminium toxicity occurs in acid soils where the main form of Al present is the soluble cation, Al^{3+} . Al^{3+} ions severely stunt root growth of cereals and other crop species and, consequently, greatly affect yield. Much of the published research into Al toxicity has relied on hydroponics experiments to measure the effects of Al^{3+} on root growth and exudation.

Boron toxicity in hydroponics is relatively easy to achieve, simply by adding boric acid (H_3BO_3) to basal nutrient solutions. For wheat and barley, we find that between 2 and 5 mM added H_3BO_3 is sufficient to see the development of B toxicity symptoms on leaves, effects on shoot growth and significant B accumulation in intolerant genotypes. Stock solutions of H_3BO_3 below 0.5 M are not adjusted for pH, and treatment levels (up to 5 mM B) do not affect the pH of the nutrient solution, or greatly change its osmolarity. It is to be noted that suitable B concentration ranges for assessing B toxicity tolerance in hydroponics would need to be determined empirically for different plant species and in different hydroponics systems and environmental conditions. For example, monocot and dicot species have different requirements for B (Asad et al., 2001), and species may also differ in B toxicity tolerance (eg. Stiles et al., 2010).

For assessment of wheat varieties for tolerance to B toxicity, we have developed a simplified hydroponics system and use relative root length as a proxy measure of tolerance (Schnurbusch et al., 2008). Seedlings are grown in a solution containing 2.5 μM ZnSO_4 , 15 μM H_3BO_3 and 0.5 mM $\text{Ca}(\text{NO}_3)_2$, supplemented with 10 mM H_3BO_3 , for 10 – 14 days, and root lengths are measured with a ruler. Relative root length (at 10 mM B compared to low B (15 μM)) is simple to score, and is much less expensive than analysis of shoot B by inductively coupled plasma emission spectrometry. The parameter correlates well with field reports of B toxicity tolerance, and also with the presence of B tolerance alleles on chromosome 7BL (Schnurbusch et al., 2008). We found, however, that this system is not suitable for barley.

Assessment of aluminium toxicity tolerance in hydroponics is complicated by a number of factors. The speciation of Al in solutions depends on both solution pH and total Al

concentration. At low pH, Al predominates as the trivalent cation, Al^{3+} , and this is known to be the major form of Al which is toxic to plants. However, the trivalent cation can complex with anions in solution, rendering it non-toxic. Hydroxyl monomers of Al may also form which are thought to be non-toxic (Parker et al., 1988), and Al readily precipitates out of solution at moderate to high pH. When precipitated, Al is not toxic to plant growth. The use of chemical speciation prediction programs such as Geochem-EZ (Shaff et al., 2010) are necessary to estimate the predicted activity of Al^{3+} in a given hydroponics solution. Careful attention to the maintenance of a low, stable solution pH is also important for obtaining reproducible experimental results. Modified hydroponics solutions (Famoso et al., 2010), or simple solutions, eg. CaCl_2 (Ma et al., 2002; Xue et al., 2006), are often used when assessing Al toxicity tolerance, to reduce the likelihood of Al forming complexes or precipitates.

The advantage of using hydroponics to study Al toxicity is that effects on root growth and exudation can easily be measured. Organic acid exudation by the roots is the major mechanism by which plants can tolerate Al and, in hydroponics, these can be collected and measured. Hydroponics has been the medium of choice for screening wheat (Delhaize et al., 1993; Sasaki et al., 2004), barley, rice (Famoso et al., 2010; Nguyen et al., 2001), maize (Magnavaca et al., 1987; Piñeros et al., 2005) and rye (Collins et al., 2008) for Al tolerance.

Boron and aluminium are examples of naturally occurring elemental toxicities and have historically been a focus of elemental toxicity research. However, there have been increasing occurrences of contamination of agricultural soils with arsenic (As) and with heavy metals including cadmium, zinc, nickel, selenium, mercury and lead. There is also a growing awareness of the potential consequences for human health of accumulation of these metals in the food chain. Hydroponics is ideal for investigating the basic mechanisms plants may possess for either reducing or avoiding uptake of As and heavy metals (eg. rice, Ma et al., 2008), or for hyper-accumulation of these elements (eg. *Pteris vittata*, Wang et al., 2002; *Thlaspi caerulescens*, reviewed in Milner & Kochian, 2008). Hyper-accumulation by plants and subsequent harvest for safe disposal is suggested as a means for removing heavy metals from contaminated sites (Salt et al., 1998). In recent research, it is emerging that both hyper-accumulation and avoidance in plants are largely due to transport processes, and these are best studied in hydroponics. Hydroponics also allows the experimenter to contain the metal elements in a closed system to ensure human safety both during the experiment and, with proper disposal and clean-up, following conclusion of the work.

4.4 Nutrient deficiencies

Hydroponics has been instrumental in establishing the essentiality of most of the mineral nutrients required by plants (Jones, 1982; Reed, 1942), from the early development of nutrient solution recipes in the 1860's by the German scientists Sachs and Knop (Hershey, 1994), through to as recently as 1987 when nickel was confirmed as an essential micronutrient for higher plants (Brown et al., 1987). Hydroponics is frequently used to study the effects of mineral nutrient deficiencies on plant growth and physiology. It is particularly useful in identifying visual symptoms or critical deficiency concentrations for diagnostic purposes, characterising physiological functions of mineral nutrients, determining their uptake kinetics, studying root exudates and gene expression changes and also changes in root morphological traits in response to nutrient deficiencies. It is also commonly used to identify germplasm with enhanced nutrient use efficiency (i.e. an ability to produce greater

biomass at limited nutrient supply) for breeding programs. However, the use of hydroponics is limited to processes involving efficiency of utilisation or mobilisation within the plant rather than those operating at the root-soil interface (Graham, 1984). Like many other research groups, we routinely use hydroponics to study effects of elemental deficiencies, including phosphorus (Huang et al., 2008) and zinc (Fig. 8), on the growth and physiology of important crop species such as wheat and barley. In such studies, particular care must be taken to avoid contamination from external sources of the element of interest. For phosphorus deficiency experiments, for example, the hydroponics setup should be

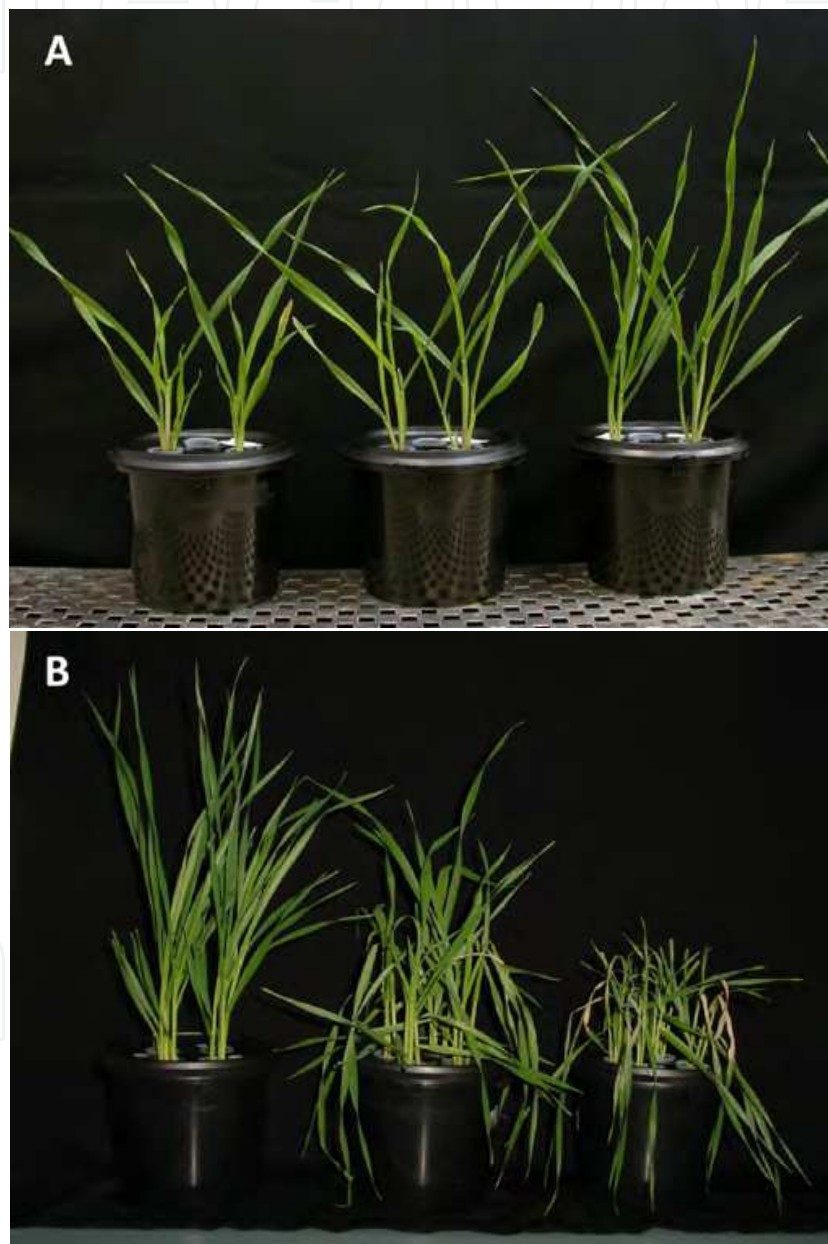


Fig. 8. An experiment designed to study Zn deficiency in barley and wheat, using aerated hydroponics in 1 L pots containing plastic fragments to support the growing plants: (A) Barley plants (cv. Pallas) grown with different concentrations of Zn (from left to right: 0.005, 0.05 and 0.5 μM Zn), and (B) Three bread wheat genotypes (from left to right: Stylet, RAC875-2 and VM506 grown with nil Zn supply).

thoroughly washed with a mild acid solution to remove all residual phosphorus, especially as phosphorus is a common ingredient in standard detergents used to wash laboratory glassware and hydroponics equipment.

5. Scaling up: From hydroponics to the field

The main advantages of hydroponics over soil-based systems can be summarised as follows; (i) there is a greater degree of control over variables and thus observations are reproducible, (ii) effects of nutrient deficiency or toxicity on plant growth can be determined more reliably, and (iii) studies on root nutrient uptake and certain root morphological traits are much easier to conduct since in soil-based systems it is often difficult to separate roots from soil particles and accurately measure nutrient concentrations or uptake by roots. This makes hydroponics ideal for studying nutrient toxicities, deficiencies and other abiotic stresses. However, it should be remembered that hydroponics is very much an artificial system, and observations may differ greatly from those made in soil-based systems.

Some reports have demonstrated strikingly similar results in hydroponics and in field trials. For example, identical Quantitative Trait Loci (QTLs) were found on the long arm of chromosome 7A in two unrelated mapping populations in bread wheat (Halberd x Cranbrook and Excalibur x Kukri) for Na⁺ accumulation in both hydroponics and in field trials (Edwards et al., 2008; Shavrukov et al., 2011). There are, however, many reported discrepancies between research findings using hydroponics and soil-based systems. Recent studies in barley, for example, suggest that responses to salinity stress (Tavakkoli et al., 2010) and drought stress (Szira et al., 2008) can vary between hydroponics and soil culture. Assessments of P efficiency in wheat also differed greatly when cultivars were grown in hydroponics compared to soil (Hayes et al., 2004). Similarly, despite the effects of B toxicity which we have observed in hydroponics largely reflecting those of glasshouse-based soil experiments as well as observations made in the field (eg. Jefferies et al., 2000 and Table 2), there are some inconsistencies. These inconsistencies are likely to be explained by

		Hydroponics		Soil	
		+ 2 mM B	+ 5 mM B	+ 10 mg B kg ⁻¹	+ 30 mg B kg ⁻¹
Shoot B (mg B kg ⁻¹)	Halberd	405	2883	900	2600
	Cranbrook	653	3667	1240	3900
Relative DW (%)	Halberd	108%	75%	58%	14%
	Cranbrook	115%	74%	50%	6%
3 rd leaf necrosis (%)	Halberd	N/A	30%	25%	30%
	Cranbrook	N/A	45%	60%	90%

N/A = not obtained

Table 2. Comparison of boron toxicity tolerance traits observed in hydroponics and soil-based experiments, for the wheat cultivars Halberd (B toxicity tolerant) and Cranbrook (intolerant). While shoot boron concentrations are comparable, relative dry weights respond differently in soil compared to hydroponics.

differences in either the physical/chemical characteristics of the growing environment, or root morphology differences created by these characteristics.

Many nutrients in soil do not exist at high concentrations in soil solution, but are instead bound to negatively-charged surfaces of clay or organic matter particles, or are precipitated as mineral salts. Nutrients are only released into solution to replace those taken up by plants. The soil solution is thus strongly buffered, maintaining low but stable nutrient concentrations. By contrast, many nutrients in hydroponics solutions are necessarily supplied at much higher concentrations. This makes studies of nutrient deficiencies particularly difficult. Frequent solution replacements, or large volumes of solution, are necessary to maintain low and relatively stable concentrations of an element of interest. Alternatively, it is possible to try to mimic the buffering ability of soil and maintain stable concentrations of a particular nutrient by adding resins (eg. Asad et al., 2001) or chelating agents (eg. Chaney et al., 1989; Norwell & Welch, 1993; Rengel & Graham, 1996) to the nutrient solution. In soils, there is also a gradient of nutrient concentrations established across the rhizosphere as roots take up and deplete nutrients from their surrounds, so that the nutrient concentration at the root surface is much lower than in the bulk soil solution. It is not possible to establish a similar gradient in nutrient concentrations in well-stirred hydroponics solutions.

Soils are also heterogeneous environments, with spatial variability in water and nutrient availabilities and physical characteristics. This heterogeneity cannot be replicated in hydroponics. In soil, plants are able to respond to heterogeneity by investing greater root growth in either nutrient- or moisture-rich patches and avoiding hostile micro-environments (Jackson et al., 1990). Research into these types of plant responses can only be done in soil. Mycorrhizal fungi and other soil biota form close associations with plant roots in soil, and these are particularly important for phosphorus, and also zinc and copper uptake, by plants in these environments (Smith & Read, 2008). Nodulation of roots by *Rhizobium* spp. is also vital for nitrogen uptake by leguminous plants (Kinkema et al., 2006). Such interactions between plants and rhizosphere microorganisms, and the implications for abiotic stress tolerance can only be studied effectively in soil.

Although hydroponics allows the experimenter unrestricted access to roots and thus easy assessment of root traits under different stress conditions, it is widely acknowledged that root morphological traits of hydroponically-grown plants may be very different to those of plants grown in soil or other solid media. This may directly affect any conclusions drawn about the tolerance of plants to abiotic stresses. For example, nodal roots originate from either the stem or the mesocotyl between the base of the shoot and the base of the primary root. They are believed to be largely responsible for exploring surface soil layers, and nodal root morphology may contribute greatly to determining the drought tolerance and P uptake efficiency of plants (Ho et al., 2005). However, nodal roots will develop differently in hydroponics and soils because of seed placement differences between the two systems. Development of root hairs also differs between soil- and hydroponically-grown plants. Root hair length and density is reduced in solution culture compared to soil for both maize (Mackay & Barber, 1984) and barley (Gahoonia & Nielsen, 1997; Genc et al., 2007). Root hair length/density is directly correlated with plant uptake of phosphorus (Bates & Lynch, 2000; Gahoonia & Nielsen, 1997) and is also related to zinc uptake efficiency (Genc et al., 2007). Research also suggests that the rate of appearance and maturation of a suberised exodermal

layer in roots differs for hydroponically grown plants. The exodermis forms a barrier between the root and the external environment, controlling water and solute influx, and thus is potentially critical for tolerance to water stress (Cruz et al., 1992; Hose et al., 2001). It has been found that there is more rapid suberisation of the exodermal layer in maize roots grown in moist air (aeroponics), vermiculite or stagnant conditions compared to aerated hydroponics (Enstone & Peterson, 1998; Zimmerman & Steudle, 1998). Moreover, in barley, nodal roots are more extensively suberised than seminal roots (Lehmann et al., 2000), and thus hydroponically grown barley roots will have limited suberised exodermal layers compared to equivalent soil-grown plants. Researchers remain unclear as to the morphology of soil-grown roots and how they respond to abiotic stresses in field conditions. Hydroponics, aeroponics, pot and/or field sampling have gone some way towards examining root traits, but the future development of DNA profiling and sophisticated imaging technologies (eg. LemnaTec) will improve our understanding of the role of roots in abiotic stress tolerance. It is likely that root adaptations are a very significant component of stress adaptation.

6. Conclusion

Hydroponics is a particularly useful research tool used to study plant responses to abiotic stresses, including salinity, boron and aluminium toxicities, nutrient deficiencies and to a lesser degree, drought. Here we have described two hydroponics systems used by researchers at ACPFG and the University of Adelaide, both of which are suitable for abiotic stress tolerance research. The particular advantages of hydroponics are that treatments can be precisely controlled, and plant responses can be accurately and reproducibly determined. Genetic variation in abiotic stress tolerance, both between and within species, can be assessed with confidence. Roots of hydroponically-grown plants are easily accessible, allowing, for example, morphological traits to be examined, short-term uptake experiments to be conducted and root exudates to be collected for analysis. However, it should be remembered that hydroponics is a unique, artificial system for growing plants and is not a substitute for soil. There are many differences between soil- and hydroponically-grown plants, as well as fundamental differences in the supply of water and nutrients, which are important to consider when researching abiotic stress tolerance. We have summarised some of the limitations relating to using hydroponics as a research tool in this chapter, and caution that ultimately, validation of abiotic stress responses and characteristics must be made in soils and in field conditions.

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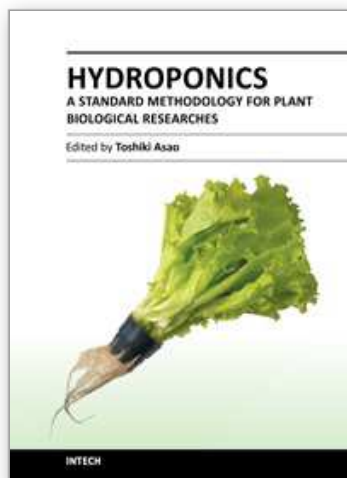
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Hydroponics - A Standard Methodology for Plant Biological Researches

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Hydroponics-A standard methodology for plant biological researches provides useful information on the requirements and techniques needs to be considered in order to grow crops successfully in hydroponics. The main focuses of this book are preparation of hydroponic nutrient solution, use of this technique for studying biological aspects and environmental controls, and production of vegetables and ornamentals hydroponically. The first chapter of this book takes a general description of nutrient solution used for hydroponics followed by an outline of in vitro hydroponic culture system for vegetables. Detailed descriptions on use of hydroponics in the context of scientific research into plants responses and tolerance to abiotic stresses and on the problems associated with the reuse of culture solution and means to overcome it are included. Some chapters provides information on the role of hydroponic technique in studying plant-microbe-environment interaction and in various aspects of plant biological research, and also understanding of root uptake of nutrients and thereof role of hydroponics in environmental clean-up of toxic and polluting agents. The last two chapters outlined the hydroponic production of cactus and fruit tree seedlings. Leading research works from around the world are brought together in this book to produce a valuable source of reference for teachers, researcher, and advanced students of biological science and crop production.

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