GENOTYPE DIFFERENCES IN RESISTANCE TO MOISTURE STRESS IN BARLEY.

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SUMMARY

The experiments in this thesis were conducted to investigate the response of barley genotypes to moisture stress. The stresses were applied to seedlings by flooding the rooting medium with polyethylene glycol or by withholding water and to older plants by withholding water at specific stages of development. The study aimed to determine if specific plant characteristics, particularly ability to accumulate proline, could be used as a tool to select cultivars resistant to stress.

The first two experiments stressed twenty genotypes at the three-leaf stage by flooding the rooting medium with polyethylene glycol for 72 or 144 hours. In neither experiment did Xylem Water Potential, Ψ(xylem), fall or free proline increase as rapidly as predicted from other studies. Genotypes differed in Ψ(xylem) and free proline at the conclusion of stress in the first experiment but only for Ψ(xylem) in the second. Variability associated with the measurement of proline was very high. The proline accumulated by the various genotypes in these experiments did not agree with the results reported by Singh *et al.* (1973d) but this may have been due to differences in the exclusion of the osmoticum over the extended stress period. Ability to accumulate proline was not related to leaf survival or to any measure of recovery.

Proline accumulated to high levels in the two experiments where stress was imposed on seedlings by withholding water. These experiments suggested that genotypes differed in their ability to accumulate proline and that the variability associated with proline measurement was due to differences in the time at which accumulation commenced. Proline differences were related to performance during stress but not to the recovery on the relief of stress. Proline levels did not fall over the five day recovery period, however, so it could not have served as a source of carbon or nitrogen over this period. Genotypes also differed

in water status at the conclusion of stress in these experiments. The differences were a real effect and not an interaction with method of measurement as, in one experiment, Ψ(xylem) and Relative Water Content (RWC) were related ($r = 0.79^{**}$ for $n = 10$). The differences in water status were related to leaf area, stomatal density and post-stress recovery.

Stress was applied to older plants to coincide with specific developmental periods (jointing, pre-anthesis or post-anthesis) or for specific periods of development. Stress was imposed by withholding water until soil water potential had fallen to -15 bars but in some treatments stress was only for a single cycle while in others it was cyclic. Genotypes generally differed in Ψ(xylem) but the differences were not related to those in the seedling experiments. They also varied for free proline content but these differences were also not related to the seedling experiments. Measurement of genotype reaction to stress at the later stages was complicated by problems of escape, avoidance and poor yield performance of the unstressed plants of the late maturing genotypes. Important yield component responses were the late tillers which formed on the relief of stress before anthesis, tiller mortality in post-anthesis stress and percentage of fertile florets which was important in harvest index and grain yield responses. No particular component, however, was significantly more important than the others in determining genotype response to stress at any stress stage. Neither was Ψ(xylem) nor free proline important in determining genotype response to stress after removing the influence of anthesis data.

Genotypes varied in the efficiency with which they used water to produce dry matter. These differences were maintained across experiments. They also varied in the efficiency with which they used water to produce grain. This character was not as stable across water treatments or experiments but genotype ranking tended to remain relatively

stable. No measured character was related to Water Use Efficiency for either dry weight or grain yield but these characters could be important in determining production in dry environments. Water Use Efficiency for grain yield, however, could only be used in the selection of parental material or in the later stages of the evaluation of potential cultivars as it would be difficult to measure in large populations.

While ability to accumulate proline was apparently related to production during a period of stress in the seedling experiments, it did not describe significant amounts of the variability in resistance to moisture stress in the experiments with older plants. This may have been due to the difficulty in controlling escape and avoidance mechanisms in these experiments. It is suggested that the most successful means of determining the usefulness of this character in selecting cultivars resistant to moisture stress would be to develop near-isogenic lines and then compare their performance when stressed.

STATEMENT

This thesis contains no material which has been accepted for the award of any other degree or diploma in any University and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference is made in the text.

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1. INTRODUCTION

Moisture deficit stress is an important yield limiting factor in Australian cereal growing where the environment is characterised by erratic rainfall which can be limiting for crop growth at any time during the growing season (Sparrow, 1977). A cereal cultivar bred for this environment should be able to capitalise on favourable seasons and also produce relatively high yields of good quality grain when moisture is limiting.

Cereal breeding programmes generally select for adaptability to this region by testing lines over years and sites in the hope that the sampled environments will cover the range to be expected. The choice of this breeding method is dictated by poor definition of the physiological and morphological characters required by the ideal cultivar. In particular, no general hypothesis exists that defines the characteristics required by genotypes resistant to moisture stress or the way that such resistant genotypes would react when moisture is sufficient. Characters which have been proposed include early maturity, rooting depth, stomatal size and control, heat tolerance and, more recently, physiological characteristics such as chloropyll stability and ability to accumulate proline.

This study aimed to investigate the response of a group of barley genotypes to moisture deficit stress at various growth stages and to try to relate some of the plant characters to the responses obtained. It was particularly stimulated by the work of Singh *et al.* (1972) which suggested an association between ability to accumulate proline and performance across environments including some that were moisture deficient.

2. LITERATURE REVIEW

2.1 Introduction

Water is required for all plant growth. A shortage of water is reflected in many plant processes (Levitt, 1972; Hsiao, 1973) and inevitably in reduced growth, or death if the shortage is sufficiently severe. Most agricultural regions experience periods of drought (Laude, 1971) and drought resistance has been included in the aims of breeding programmes for many crops including rice (IRRI, 1975), wheat (Hurd, 1971) and barley.

Selection for drought resistance is often hampered by the unreliability of the occurrence of drought periods. Laboratory tests for the character would be useful, at least in reducing the population size to be tested under field conditions (Hurd, 1971). The identification of plant characters which might be used in such tests has been limited by poor definition of water status (Sullivan, 1971) or the failure to understand the complex nature of drought resistance (Levitt, 1972).

The first part of this review considers the definition of water stress and the measurement of water status. The influence of water stress on many aspects of plant growth and metabolism is then discussed. The final section deals with resistance to water stress, its definition, measurement and associated plant characteristics.

2.2 Denifition of Water Stress

Levitt (1972) attempted to define biological stress in terms analagous to physical stress and strain. Thus biological stress was defined as "any environmental factor capable of inducing a potentially injurious strain in living organisms". Since stress due to a deficiency of water is more common than that due to excess, water deficit stress is

commonly referred to as water (or moisture) stress. It is not necessary that physical definitions should apply to the biological system but Taylor (1968) proposed a similar definition without consideration of the stress and strain theory of physics - "whenever the conditions of water are unfavourable to optimum plant growth, the plant is said to be under water stress". Both definitions imply that water stress is measured in terms of water status and is recognised in terms of plant reaction to that water status.

2.3 Measurement of Water Status

Water status can be measured in both the soil and the plant. Measurement of plant water status is more valuable in the study of plant reaction to water stress (Slatyer, 1967) and the failure to do so has limited progress in this field (Sullivan, 1971).

Water Potential

Slatyer and Taylor (1960) outlined the case for using water potential as a measure of water status in plants and this has become the most widely accepted measure (Boyer, 1969; Hsiao, 1973). The concept of water potential was developed in response to the need for a unified terminology describing the physical state of water in the soil-plant-atmosphere system (Slatyer and Taylor, 1960; Taylor and Slatyer, 1961). The chemical potential of water in the multi-component system containing inert solids, reactive solids, solutes, gases and water is the partial Gibbs free energy of the water in the system (Taylor, 1968). It is best understood as the capacity of the water to do work, i.e. to move from a higher to a lower potential energy (Taylor and Slatyer, 1961; Kramer, 1969). Water potential (Ψ_w) is defined as the difference in the chemical potential of water in a system and pure free water at atmospheric pressure and the same temperature

(Taylor and Slatyer, 1961). Since differences in temperature have a complicated effect on Ψ_w, they should be avoided during evaluation (Taylor, 1968). Water potential is often quoted in pressure units (usually bars) which are obtained by dividing energy units by the partial molal volume of water (Slatyer, 1967).

Total water potential can be expressed in terms of its component potentials (Taylor and Slatyer, 1961; Boyer, 1969).

$$\Psi_w = \Psi_g + \Psi_s + \Psi_m + \Psi_p$$

Ψ_g is gravitational potential,

Ψ_s is solute (osmotic) potential,

Ψ_m is matric potential,

Ψ_p is turgor potential.

The gravitational potential is important in water having considerable vertical extent, such as water in trees, but is usually negligible for cereals (Boyer, 1969). Both osmotic and matric forces reduce potential and are therefore negative. Distinguishing between osmotic potential due to dissolved solutes and matric potential due to adsorption and surface tension is often difficult, particularly at low water potentials (Wilson, 1967), but the contribution of matric potential is frequently considered to be negligible (Barrs, 1968b). Turgor pressure is normally a positive hydrostatic pressure in the cell. As water becomes limiting and total water potential falls, turgor potential also falls rapidly. Wilting is a visible sign of low turgor potential in the plant (Slatyer, 1969).

Many methods have been used to measure Ψ_w (Barrs, 1968b; Sullivan, 1971) but the thermocouple psychrometer has steadily gained popularity in recent years and is accepted by many as the standard for Ψ_w measurements (Sullivan, 1971). The thermocouple psychrometer requires strict control of temperature (Barrs, 1968b) and for this reason is not

readily adapted to field studies. Dewpoint hygrometers (Baughn and Tanner, 1976) have overcome this problem to some extent, but the pressure chamber has often been used for field studies because it can handle a relatively large number of samples and is inexpensive.

First used by Dixon (1914) and Haines (1935), the pressure chamber was widely accepted only after the work of Scholander *et al.* (1964; 1965; 1966). It has been used for many species (Boyer, 1967; De Roo, 1969). Since the effect of pressure is thermodynamically equivalent to that of solutes and other components of the total water potential, the pressure chamber can be used to give an approximation to water potential. A leaf (or petiole) is cut from the plant and sealed in the chamber so that only the cut end emerges. Compressed air or nitrogen is let into the chamber and the pressure at which the meniscus returns to the cut end is equal and opposite to the tension in the leaf before it was excised (Barrs, 1968b). This is usually referred to as xylem water potential. The pressure is related to water potential by:

$$\Psi_w = \Psi(\text{xylem}) + \Psi_s(\text{xylem}), \quad \text{(Boyer, 1969)},$$

where Ψ_w is the water potential, Ψ(xylem) is the negative component of water potential in the cell sap which is measured as a positive pressure in the chamber and Ψ_s(xylem) is the osmotic potential of solutes in the xylem sap.

Ψ_s(xylem) is usually low and can be ignored, although it can be important in saline conditions (Scholander *et al.*, 1966; Boyer, 1967). Since pressure changes in proportion to the Kelvin temperature, measurements are relatively insensitive to temperature so the pressure chamber is suited to field use (Boyer, 1969).

The pressure chamber technique has been compared with thermocouple psychrometry for a number of species (Boyer, 1967; De Roo, 1969; Barrs *et al.*, 1970; Frank and Harris, 1972) and with the dewpoint

hygrometer in five species (Baughn and Tanner, 1976). Agreement was generally satisfactory but the relationship does vary with species and physiological age. Where absolute measurements are important the pressure chamber should be calibrated with the psychrometer but this may not be necessary when comparison of values within an experiment is all that is required (Boyer, 1969).

Water Content

Water potential has not been measured in all studies of plant water deficit and many have recorded water content directly. The most acceptable method of measuring water content is as a function of water content at full turgidity (Slatyer, 1967). Relative Water Content (RWC) has been defined as:

$$RWC = \frac{(\text{fresh weight} - \text{dry weight}) \times 100}{(\text{fully turgid weight} - \text{dry weight})}$$ (Weatherley, 1965)

Relative Water Content, formerly called Relative Turgidity (Weatherley, 1950) is measured in a similar way to the Water Deficit (WD) of Stocker (1928) except RWC = 100 - WD.

The technique of measuring RWC has been refined many times (Barrs and Weatherley, 1962; Catsky, 1965; Barrs, 1968b; Hewlett and Kramer, 1963). Fully turgid weight is obtained by floating tissue, usually leaf discs, on distilled water. Dry weight loss due to respiration is minimised by using a short flotation time, usually 4 hours (Barrs and Weatherley, 1962), with a compensating light intensity (Barrs, 1968b). Since the turgid water content of the leaf discs is affected by the temperature and humidity at which they are floated (Werner, 1954), they should be treated at constant temperature and in closed petri dishes.

The relationship between RWC and Ψ_w may change with plant age, part of plant, season, species or previous stress (Slatyer, 1969b; Jarvis and Jarvis, 1963; Knipling, 1967; Jones and Turner, 1978) and this must be considered where RWC is used to estimate Ψ_w. While the components of water potential are important in governing physiological response to water deficit, the relationship between RWC and Ψ_w (termed the moisture release curve) may be a useful indicator of physiological change. Jones and Turner (1978), for example used this relationship as part proof of osmotic adjustment as a result of prior stress.

2.4 Effect of Water Deficits on Physiological Processes

Growth and development of plants is the result of many interconnected processes and it is difficult to isolate the influence of lowered water status on the individual processes. Growth, for example, may be restricted by the direct influence of water deficit on cell elongation or division or indirectly through the disruption of carbohydrate or nitrogen metabolism. Metabolic processes, on the other hand, may be inhibited by restricted growth.

Plant Growth

One of the first processes to be affected by mild stress is cell enlargement (Hsiao, 1973). Leaf elongation in maize, soybean, sunflowers, cotton and *Vicia* sp. was reduced at leaf water potentials below about -2 bars and all growth ceased when water potential fell below -4 to -12 bars (Boyer, 1968; 1970a; 1970b; Shin and Lemon, 1968; Lawlor, 1969; Acevedo *et al.*, 1971). Elongation appears to be dependent on turgor pressure (Vaadia *et al.*, 1961; Cleland, 1971). Mild stress, while delaying elongation, appears to have little permanent effect. Re-watering of very mildly stressed maize resulted in virtually instant leaf elongation (Hsiao *et al.*, 1970; Acevedo *et al.*, 1971) with no net

loss of size compared to the well watered control. Green *et al.* (1971) showed that for *Nitella* a critical turgor must be exceeded before irreversible cell enlargement can occur. Evidence for a critical value has also been found with soybean, wheat and rye (Green and Cummins, 1974).

Cell wall synthesis is also slowed by mild stress (Ordin, 1958; 1960) but this is probably due to feedback inhibition triggered by reduced elongation (Hsiao *et al.*, 1970).

Cell division is affected by mild stress (Gardner and Nieman, 1964) but is has been widely held that it is not as sensitive as elongation (Vaadia *et al.*, 1961; Slavik, 1966; Gates, 1968; Slatyer, 1969; Cleland, 1971). This view is based on the evidence that cell number is frequently of the same order in stressed plants and controls (Maximov, 1929; Petinov, 1965) and may explain the rapid growth following stress relief (Gates, 1955a; 1955b; Petrie and Arthur, 1943, Hsiao *et al.*, 1970). Gardner and Nieman (1964), however, showed that the DNA increase in cotyledonary leaves of radish was reduced by 60% as Ψ_w fell from 0 to -2 bars. Since cell number is usually closely related to DNA content (Nieman and Poulson, 1962), cell division would appear to be at least as sensitive to water stress as is elongation, but unlike elongation, division continued at a slow rate until severe conditions existed. Clough and Milthorpe (1975) demonstrated that cells of developing tobacco always divided at the same size so elongation and division are probably closely related.

These effects of mild stress on cell elongation and division may not be important for growth while there is an opportunity for recovery at night (Hsiao *et al.*, 1970) when turgor potential may increase (Slatyer, 1969). If stress is sufficiently severe, however, or extends for a long period the reduction in leaf area resulting from

reduced elongation could be important in restricting assimilation by the plant (Fischer and Hagan, 1965).

The properties of *Nitella* cells changed during a prolonged stress period and this allowed the resumption of growth at low turgor potentials (Green *et al.*, 1971). Once turgor pressure has fallen to zero the most likely mechanism that can restore growth is osmoregulation, a decrease in cell Ψ_s to the extent that Ψ_p becomes positive.

Osmoregulation, or osmotic adjustment, is now well recognised as a process which allows plants to adjust to salinity (Bernstein, 1961; Stewart and Lee, 1974). It has not been accepted as an important phenomenon in water stressed plants (Hsiao, 1973) although recent work has suggested a role for osmotic adjustment in maintaining the turgor of crop plants subjected to water stress (Begg and Turner, 1976; Hsiao *et al.*, 1976). Jones and Turner (1978) provided evidence for osmotic adjustment in sorghum but suggested that there may be species differences.

Carbohydrate Metabolism

Photosynthesis is reduced by water stress (Ashton, 1956; Brix, 1962; El Sharkawy and Hesketh, 1964). Many studies suggest that stomatal closure, by restricting carbon dioxide diffusion, is the dominating influence on photosynthesis (Brix, 1962; Barrs, 1968a; Boyer, 1971a; Jones, 1973; Hsiao and Acevedo, 1974). Non-stomatal effects were important at mild stress levels in studies with tobacco (Redshaw and Meidner, 1972) and sunflower (Keck and Boyer, 1974) but were not important in cotton (Troughton, 1969) or soybean (Boyer, 1970b) until severe stress levels were reached. There is some evidence for a critical water potential above which photosynthesis is not affected (Brix, 1962; Hsiao, 1973).

Many studies have also shown a critical threshold value of leaf Ψ_w or RWC above which leaf resistance, and hence stomatal opening, remained constant (Sanchez-Diaz and Kramer, 1971; Turner, 1974; Parameswaran, 1975). The actual value varied with the species (Sanchez-Diaz and Kramer, 1971; Turner, 1974), genotype (Henzell *et al.*, 1976) or environment (Jordan and Ritchie, 1971; Brown *et al.*, 1976). Values of Ψ_w for stomatal closure have varied from -7 bars for tomato (Duniway, 1971) to -20 bars for sorghum (Turner, 1974). Turner also found that the turgor potentials at stomatal closure in tobacco, maize and sorghum were similar, while the leaf water potentials were quite different.

An after-effect of stress on stomata has been reported (Stalfelt, 1955; Milthorpe and Spencer, 1957; Glover, 1959), the stomata failing to open when the stress was relieved. Fischer *et al.* (1970), working with tobacco, found most of the after-effect disappeared after one day but there was a residual effect until the fifth day. It was greater in tobacco than in *Vicia faba*. The after-effect of stress on stomata is probably the reason for the reported after-effect on photosynthesis (Schneider and Childers, 1941; Upchurch *et al.*, 1955; Ashton, 1956).

Stomatal closure results in increased leaf temperature and this has been implicated in some plant responses to water stress (Henckel, 1961; Crafts, 1968). The actual rise in temperature will depend on the environmental conditions, radiation load and heat transfer coefficient, but for most situations the temperature rise has been calculated or measured to be only a few degrees (Gale and Hagen, 1966). This would be unlikely to limit many plant processes (Hsiao, 1973).

Non-stomatal effects on photosynthesis may be due to greater resistance to CO_2 movement from the intercellular space to the chloroplasts or to altered activity of the chloroplasts (Hsiao and Acevedo, 1974). In sunflowers, there is reduced activity for electron transport in the chloroplasts of stressed tissue (Boyer and Bowen, 1970; Keck and Boyer, 1974), while the Hill reaction appears quite resistant to stress (Sullivan and Eastin, 1974).

Respiration is also slowed by severe stress (Hsiao, 1973; Slatyer, 1973), but the effects of mild stress are not clear. Some authors have reported an initial increase in respiration rate when stress is imposed gradually (Stocker, 1960) followed by a reduction (Heath and Meidner, 1961; Brix, 1962). When stress is imposed quickly, the initial increase may not be observed (Slatyer, 1967).

With increasing water stress, changes in the balance between photosynthesis and respiration are complex and may be affected by other plant processes. It is possible, for example, that growth reductions could lead to an inhibition of photosynthesis through reduced sink strength, slower translocation and accumulation of assimilates at the source (Wardlaw, 1967; 1969) resulting in feedback inhibition of photosynthesis (Burt, 1964). This chain of events is not universal, however, as Johnson and Moss (1976) did not find reduced translocation as a result of stress and Wardlaw (1969) found no evidence of feedback inhibition of photosynthesis. Thus the situation is complex and probably varies with many factors such as non-stress growth rates, sink strength, species and environment (Johnson and Moss, 1976).

An increase in the sugar content of the leaves at the expense of the starch content when stress is severe has been reported (Iljin, 1957; Stewart, 1971), although this has not always been observed (Wadleigh and Ayer, 1945; Woodhams and Kozlowski, 1954). Such an

increase in sugar content has been attributed to increased amylase activity (Spoehr and Malner, 1939) or decreased invertase activity (Marranville and Paulsen, 1970). Hsiao (1973) disputed the former explanation as the sugars resulting from amylase activity would be glucose and fructose, not the sucrose normally found. Hiller and Greenway (1968) found that the increased sugar content probably came from increased synthesis and not from starch hydrolysis. It has been suggested that this increase in sugar content is a drought tolerance mechanism, acting to protect proteins from loss of the water of hydration (Maximov, 1929; Parker, 1972).

Nitrogen and Nucleic Acid Metabolism

Most studies have shown a decrease in the protein content of stressed tissue (Shah and Loomis, 1965; Stutte and Todd, 1969), although Chen *et al.* (1964) found an initial increase in stressed citrus seedlings followed by a decline and then another increase. Singh *et al.* (1973c) found that the protein content of barley seedlings stressed with polyethylene glycol of -20 bars osmotic potential increased at a declining rate for the first 20 hours, remained constant for the next 20 hours and then decreased. Reduced protein content may be due to either increased hydrolysis or reduced synthesis. Hydrolysis does increase with stress (Petrie and Wood, 1938) but usually only at severe stress levels (Lahiri and Singh, 1969). Protein synthesis may be reduced at mild stress levels (Hsiao and Acevedo, 1974) and stressed, or previously stressed, tissue has a reduced capacity to incorporate labelled amino acids into proteins (Barnett and Naylor, 1966; Ben Zioni *et al.*, 1967).

Ribonuclease activity increases in severely stressed tissue (Kessler, 1961) and this probably explains the reduced levels of RNA (Gates and Bonner, 1959). This decrease in RNA is accompanied by reduced protein synthesis (Chen *et al.*, 1968) and injury and senescence (Wyen *et al.*, 1969).

Reduced protein synthesis is probably associated with an increase in the proportion of inactive ribosomes at the expense of active polysomes (Bewley, 1973). This has been attributed to the increased activity of ribonuclease (Genkel *et al.*, 1967; Sturani *et al.*, 1968; Bewley, 1973) but this explanation is not supported by recent evidence which has shown a lack of correlation between the increase in ribonuclease activity and the decrease in numbers of polysomes (Hsiao, 1973; Dhindsa and Bewley, 1976). Polysome loss may rather be due to polysomes failing to re-initiate after the ribosomes have run off the RNA (Dhindsa and Bewley, 1976).

Earlier reports of altered base ratios in the nucleic acids (Kessler and Frank-Tishel, 1962; West, 1962) have not been supported (Hsiao, 1973) although a change in the type of protein in stressed *Avena* coleoptile cells was reported by Dhindsa and Cleland (1975).

There is no general pattern in the reaction of enzymes to water stress (Todd, 1972). Certain enzymes such as nitrate reductase (Huffaker *et al.*, 1970) are very sensitive to the effects of mild stress, while others, such as ribonuclease and amylase are resistant and may increase in activity. The sensitive enzymes may be those with a very short life that are quickly influenced by a general reduction in protein synthesis (Hsiao, 1973).

Proline Accumulation

Along with the reduced protein synthesis induced by water deficit, the amino acid content of the plant also changes (Kemble and MacPherson, 1954; Petinov and Berko, 1965; Savitskaya, 1965; Singh *et al.*, 1973c). The most dramatic change in the water stressed tissue of many species is an accumulation of proline (Barnett and Naylor, 1966; Routley, 1966; Stewart *et al.*, 1966; Singh *et al.*, 1972). Free proline content increased hundredfold in stressed tissue of barley and even larger increases were reported in radish (Chu, 1974).

Proline accumulates in every part of stressed plants of many species in response to a water stress (Barnett and Naylor, 1966; Thompson *et al.*, 1966; Singh *et al.*, 1973c) and it disappears when the stress is relieved (Singh *et al.*, 1973c). It does not generally accumulate in excised roots or in isolated apical meristems (Stewart *et al.*, 1966; Singh *et al.*, 1973b), an indication that proline is translocated throughout the plant. It was thought that proline accumulated only in green leaves in the light but it also accumulates in etiolated leaves fed sugar (Stewart *et al.*, 1966) and in osmotically stressed Jerusalem artichoke tubers (Wright *et al.*, 1977). Accumulation may depend on a suitable supply of proline precursor and respiratory substrate as the tubers of Jerusalem artichoke are rich in arginine which is the source of carbon for proline formation (Wrench *et al.*, 1977) and in fructosans to supply energy.

The mechanism of proline accumulation is still the subject of study. It apparently commences to accumulate at negative potentials greater than those which result in reduced leaf elongation (Chu, 1974) but the Ψ_w at which accumulation of proline commenced varied from about -7 to -9 bars in barley (Chu *et al.*, 1976; Hanson *et al.*, 1977) to lower than -20 bar in sorghum and sunflower (Waldron *et al.*, 1974). It probably accumulates in response to changes is osmotic rather than turgor potential (Chu, 1974). It accumulates both as a result of protein hydrolysis and of enhanced proline synthesis (Stewart, 1972), although the latter is most important (Kemble and MacPherson, 1954; Boggess *et al.*, 1976a). Proline synthesis during stress requires oxygen (Thompson *et al.*, 1966). Noguchi *et al.* (1966) suggested that light was essential for proline formation in tobacco leaves, but Singh *et al.* (1973b) found that detached barley leaves did not require light to form proline when supplied with precursors for proline formation. The relation between the presence of green tissue and the capacity to accumulate proline may be due to

compositional differences in the tissue (Wright *et al.*, 1977) and neither chlorophyll nor functional chloroplasts were essential when precursors were supplied (Singh *et al.*, 1973b).

The biosynthetic pathway for proline formation has been studied in micro-organisms (Vogel and Davis, 1952) and higher plants (Noguchi *et al.*, 1966). In micro-organisms, proline is reversibly formed from glutamic acid via glutamic semialdehyde (Figure 1a). Glutamic semialdehyde may also be an intermediate in the formation of ornithine (Morris *et al.*, 1969). The ring closure from glutamic acid to Δ'pyrroline-5-carboxylic acid (P5C) occurs spontaneously and this compound is the most likely intermediate between glutamic acid and proline in plant tissue (Figure 1b).

The route to proline accumulation may be species dependent, however, as in barley it is predominantly via glutamate and not ornithine or arginine (Boggess *et al.*, 1976a; Stewart and Boggess, 1977) while the latter two may be important precursors in bean or Jerusalem artichokes (Stewart and Boggess, 1977; Wrench *et al.*, 1977).

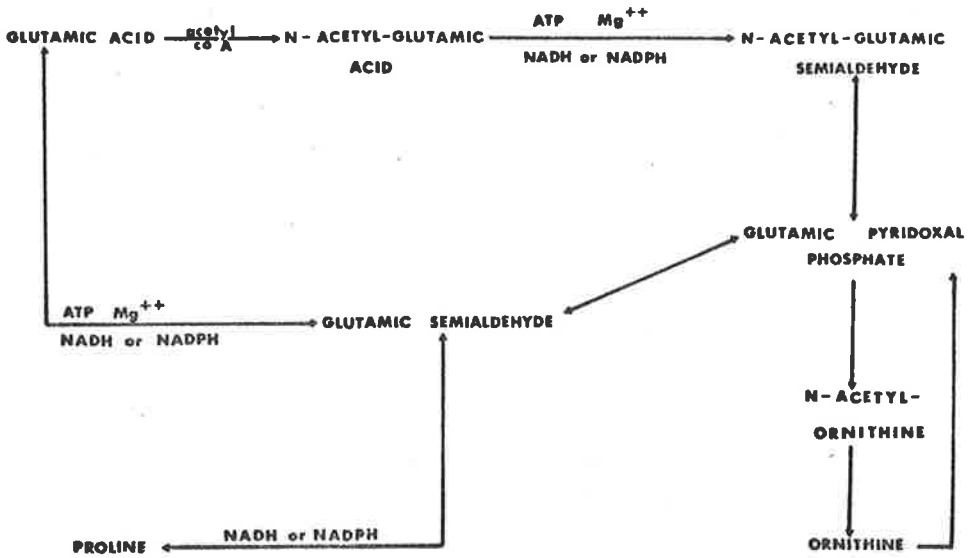
Accumulation of proline in barley apparently occurs as a result of increased synthesis and also reduced oxidation. Huber (1974) suggested that abscisic acid and salinity induced proline accumulation in *Pennisetum* resulted from increased P5C reductase activity during stress. This is unlikely, however, as there is sufficient P5C reductase activity in unstressed tissue to account for the accumulation of much higher levels of proline (Boggess *et al.*, 1976a) and the stimulation of proline synthesis probably occurs via increased P5C formation. This increased synthesis is accompanied by a loss of feed-back inhibition in stressed tissue (Boggess *et al.*, 1976b).

Figure 1.

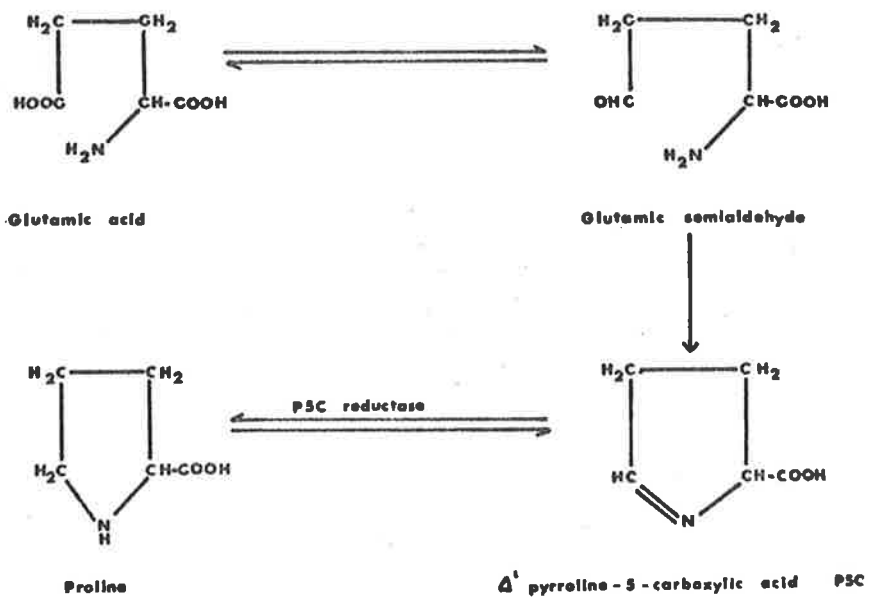
a) Biosynthetic pathway to proline and ornithine from glutamic acid in microorganisms.

b) Probable pathway for proline formation from glutamic acid in barley plants.

a)


GLUTAMIC ACID
acetyl co A
N - ACETYL-GLUTAMIC ACID
ATP Mg++
NADH or NADPH
N- ACETYL-GLUTAMIC SEMIALDEHYDE
GLUTAMIC PYRIDOXAL PHOSPHATE
ATP Mg++
NADH or NADPH
GLUTAMIC SEMIALDEHYDE
N-ACETYL-ORNITHINE
PROLINE
NADH or NADPH
ORNITHINE

b)


Glutamic acid
Glutamic semialdehyde
P5C reductase
Proline
Δ1 pyrroline - 5 - carboxylic acid P5C

Proline oxidation (proline to glutamate) is reduced to negligible rates as a result of water stress (Stewart *et al.*, 1977). This inhibition apparently does not involve the activity of P5C dehydrogenase but rather is associated with an oxidative step located on the mitochondria (Boggess *et al.*, 1978). It is possible, therefore, that proline synthesis, which occurs in the cytoplasm, may be spatially separated from proline oxidation, which takes place in the mitochondrion.

Plant Hormones

Water stress reduces the supply to the shoot of cytokinins originating in the roots (Itai and Vaadia, 1965; 1971; Itai *et al.*, 1968). There are some reports where this has not occurred (Mizrahi *et al.*, 1970), probably because stress was not severe (Hsiao, 1973). Itai and Vaadia (1965) suggested that some metabolic effects and premature senescence of stressed plants may be due to such a reduced supply of cytokinins to the leaves. Kinetin treatments of stressed tissue partially restored protein synthesis (Shah and Loomis, 1965; Ben-Zioni *et al.*, 1967).

Abscisic acid (ABA) accumulates dramatically in the leaves of wilting plants (Livne and Vaadia, 1972). Wright (1969) noted an increase in wilted, excised wheat leaves after two hours and a forty fold increase after four hours (Wright and Hiron, 1969). Accumulation was also demonstrated in the leaves of stressed, intact plants (Milborrow and Noddle, 1970; Mizrahi *et al.*, 1970; Most, 1971; Zeevaart, 1971). The accumulation of ABA in sugar cane occurred prior to wilting (Most, 1971) and in tomato and wheat resulted from *de novo* synthesis and not merely release from a bound form (Milborrow and Noddle, 1970).

ABA accumulation, or possibly the ABA-cytokinin balance has been suggested as an important stomatal control mechanism (Mizrahi *et al.*, 1970; Tal and Imber, 1971; Livne and Vaadia, 1972). Exogenously applied

ABA inhibits stomatal opening (Cummins *et al.*, 1971; Kriedemann *et al.*, 1972). Similarly, exogenous cytokinin applications caused stomatal opening (Luke and Freeman, 1968; Pallis and Box. 1970; Cooper *et al.*, 1972; Kirkham *et al.*, 1974). Cooper *et al.* (1972) reported statistical interactions between cytokinins and ABA at concentrations of 10^{-5} to 10^{-6}M. on stomatal opening as measured by transpiration rate in barley. They suggested that this response may be confined to grasses as Tucker and Mansfield (1971) found no influence of kinetin on stomata in *Commelina communis*. Hsiao (1973) questioned the role of hormones in stomatal closure, particularly cytokinins, as stomata of many species and younger leaves do not respond (Luke and Freeman, 1968; Livne and Vaadia, 1972). While hormones may not be directly responsible for stomatal closure in response to low water status, they would reinforce the response and may be involved in the changes which occur following closure.

It is also possible that ABA or cytokinin are associated with the after-effect of stress on stomatal aperture. Allaway and Mansfield (1970) considered the after-effect was due to the accumulation of an inhibitor or the deficiency of a substance which promotes stomatal opening. The suggestion that the two hormones act in this respect (Livne and Vaadia, 1972) has yet to be tested.

Applications of ABA to leaf tissue have resulted in the accumulation of proline in barley (Aspinall *et al.*, 1973) and *Pennisetum* (Huber, 1974). Both compounds accumulate in the plant during a period of water stress, but a direct relationship between them has not been established.

2.5 Effect of Water Deficit on Cereal Grain Yield

In the cereal plant, water deficit acting through the several effects on metabolism so far described has the general effect of reducing plant growth and hence biomass. The general reduction in growth may reduce grain yield but water deficit may also reduce grain yield directly through specific effects on one or more of the components of yield.

Grain yield can be considered as the product of multiplicative components. These have been separated in various ways, but they are basically a combination of:

1. Number of plants per unit area.
2. Total number of tillers per plant.
3. Proportion of the total tillers that survive to set grain.
4. Total spikelets per fertile tiller.
5. Proportion of total spikelets that set grain.
6. Weight per grain.

In cereals, like wheat, that have more than one floret per spikelet, the additional component is the total number of florets per spikelet.

Water stress at any growth stage affects that part of the plant growing most rapidly at the time and thus the associated component of yield (Aspinall *et al.*, 1964). Stress during tiller formation, for example, has its greatest effect on the number of tillers, but this can influence the later formed components, even after stress relief, through correlated responses (Grafius, 1969).

The number of plants per unit area is sensitive to stress but has been relatively neglected as an area of work because crops are not normally sown until there is sufficient water to allow germination.

Tillering is suppressed during stress, but following stress relief there may be a large temporary increase in tiller emergence (Aspinall *et al.*, 1964). The increase in tillering may not occur in the field to the same extent as in pots but this aspect requires further study, as it has been noticed in the field in some situations (Sparrow, 1977). Little information exists on the influence of stress on tiller mortality.

Husain and Aspinall (1970) showed that slight water stress in barley reduced the rate of initiation of floral primordia, but upon stress relief there was a period of rapid initiation. Since the number of spikelets per spike in barley is determined by the rate of primordium initiation relative to the rate of floral development (Nicholls and May, 1963), stress reduced the final number of spikelets per spike as development continued at stress levels that completely inhibited initiation.

There appears to be a particularly sensitive period in the growth of cereals just prior to anthesis when stress has a great influence on yield (Salter and Goode, 1967; Fischer, 1973). This sensitivity has been associated with male sterility caused by an inhibition of meiosis or subsequent microsporogenesis (Skazkin and Zaradskaja, 1957; Bingham, 1967). This sensitivity would be demonstrated by a lower proportion of fertile florets but this component is not often measured and the effect is usually recognised as reduced grain number per spike. Stress at anthesis can markedly reduce fertilization and grain-set in most cereals (Slatyer, 1969) but maize seems to be the most sensitive crop (Robins and Domingo, 1953).

Aspinall (1965) studied the influence of stress on grain growth in barley. Grain weight may be reduced by stress after anthesis. Stress early in the period of grain growth may limit final grain size,

even though well watered conditions are resumed before the completion of grain filling. Stress may also reduce the period for grain filling. Where stress continued over the whole period of grain filling, grain weight was reduced in studies reported by Salter and Goode (1967). Weight per grain is an important component in studies of water stress because it is the final process in yield formation and there can be no compensation for reduced grain weight (Fischer, 1973). Grain growth is also occurring at a time of maximum drought likelihood in many environments (Chinoy, 1962). Reduced grain weight as a result of water stress has been attributed to a decline in the area of photosynthetic tissues after anthesis (Fischer and Kohn, 1966) but translocation out of the leaves may also be reduced, thus lowering the supply of assimilates to the grains from this source (Wardlaw, 1967).

The possible existence of a critical period when stress has a major influence on yield has already been mentioned. This appeared to be five to fifteen days before ear emergence in wheat (Fischer, 1973). Salter and Goode (1967) surveyed the literature for many crops and found this stage to be the most critical in many of the studies. This stage also seems to be the most sensitive in barley (Skazkin and Zavadskaya, 1957; Aspinall *et al.*, 1964; Wells and Dubetz, 1966; Gardener, 1971). The existence of this as the only critical period is not universally accepted. Langer and Ampong (1970) found that stress at an earlier stage (during spikelet initiation) had a greater effect on yields of two New Zealand wheat varieties. Jensen (1971) in a field study with barley, found that the total dry matter, grain yield and straw yield were linearly related to the number of water stress days during the period between the emergence of the fourth leaf and maturity, suggesting that the effects of a daily water stress were additive, which does not support the concept of a critical period. A water stress day occurred when Ψ(xylem) fell

below a critical value but Jensen did not say whether there was any stress during the pre-anthesis period.

2.6 Definition of Drought Resistance

Levitt (1972) extended his comparison with the stress and strain terminology of physics to define a measure of stress resistance as "the stress (water status) required to produce a specific strain". The specific strain he advocated was the LD 50 - the point at which 50% of the tissues were killed (Levitt, 1964; Levitt *et al.*, 1960). He realised that resistance in the broad sense could be extended to include growth and development during stress and upon stress relief, but he preferred to emphasize survival for his physiological studies. The definition preferred in the present study, however, is based on measurement of growth and yield at a particular water status, this definition being of more use in agronomic studies (Laude, 1971).

Resistance to drought often has an even wider meaning. It may also describe the ability of a plant (or genotype) to avoid reaching a water status as low as other plants (or genotypes) under equivalent conditions. This has been recognised for many years (Kearney and Shantz, 1911) but there has been a proliferation of terminology to explain the various components of resistance. The terminology of Levitt (1972) will be followed throughout this thesis but many other terms have been used to describe equivalent components (Table 1).

Drought escape is apparent resistance when a plant (genotype) is at a less sensitive stage at the time of stress. The usual situation is where early maturing plants complete their life cycle before the onset of stress (Derera *et al.*, 1968) but escape may also be due to drought occurring at a non-critical time.

Table 1.

Alternative terms used to describe the components of water stress resistance

Author(s)					
Levitt (1972)	Maximov (1929)	Stocker (1960)	Levitt (1956)	Kearney and Shantz (1911)	May and Milthorpe (1962)
Succulents				Resistance	
Non-succulents					
Escape	Ephemerals			Escape	Escape or Avoidance
Avoidance				Evasion	Endurance with high internal water content
Water Savers	Succulents	Constitutional drought resistance			
Water Spenders					
Tolerance	True Xerophytes			Endurance	Endurance with low internal water content
Dehydration Avoiding		Protoplasmic drought resistance	Drought hardiness		
Dehydration Tolerating					

Drought avoidance describes the type of resistance where a plant (genotype) is able to maintain a higher tissue water potential than other plants (genotypes) under equivalent conditions. Water stress avoiders have been divided into the water spenders that absorb water fast enough to avoid low tissue water potentials and the water savers that prevent water loss (Levitt, 1972).

Tolerance to water stress describes the type of resistance that was first described in this section - the maintenance of growth or yield despite a low tissue water potential or the ability to recover and grow on the relief of stress. Levitt (1972) further divided drought tolerance into the dehydration avoiders and the dehydration tolerators. Dehydration avoiders prevent cell dehydration by mechanisms such as osmoregulation. Dehydration tolerators are able to withstand tissue dehydration with less disturbance to growth.

In all studies of water stress resistance, it is necessary to recognise the component being studied. The failure to do this has limited the usefulness of many studies (Sullivan, 1971). The escape or avoidance components can be estimated by measuring water status of the plants in relation to environmental water status. Tolerance is estimated by the growth or yield response to a particular water status.

The mechanisms giving water stress resistance have been extensively studied. The reaction to stress by unrelated genera have been studied to define the basic resistance mechanisms (Parker, 1968; 1972; Levitt, 1972). Other studies have emphasized the resistance by plants of the same genotype in different seasons (Parker, 1972), after hardening (Henckel, 1961) or after chemical treatment (Poljakoff-Mayber and Gale, 1972). Differences between different genotypes within a species or within closely related species is the only variability available for plant improvement (May and Milthorpe, 1962).

The variability available within the *Gramineae* will be emphasized for the remainder of this review and the resistance mechanisms available outside the grasses will only be discussed where such mechanisms are likely to be useful for cereal improvement.

2.7 Measurement of Drought Resistance

The resistance of a genotype to a stress is measured by its growth or yield response to the stress in relation to the response of other genotypes. The measurement of performance is not difficult once the character to be studied has been defined but, in water stress studies, the imposition of a defined stress is difficult.

The definition of stress under field conditions is not simple and plant breeders have often taken a more empirical and pragmatic approach. A range of genotypes is grown in a range of environments which provide differing levels of stress. The genotypes are then evaluated for their ability to perform across these environments by one of the statistical techniques that have been developed (Freeman, 1973). This type of analysis provides information on resistance to combined environmental stresses and thus to water stress only where the main environmental limitation is water.

One method for partitioning the interaction between the genotype and environment used the mean performance of many genotypes to provide a measure of the environment. Where a range of genotypes is grown over a range of sites, the regression coefficient of genotype performance on the environment mean can then be used as a measure of stress resistance, particularly where this linear response describes a large proportion of the genotype x environment interaction. This approach is illustrated by the work of Finlay and Wilkinson (1963), who grew 277 barley genotypes at eight sites over three seasons. The main difference between the sites

was considered to be rainfall. The site mean provided an indication of site worth "without the complexities of defining or analysing the interacting seasonal or edaphic factors". The regression coefficient of the yield of each genotype on the site mean was called the stability index. In this study, the yields were transformed to logarithmic values "to induce a high degree of linearity". The stability index varied considerably for different genotypes and this has been used as an indication of their drought resistance (Singh *et al.*, 1972). Biological interpretation of the stability index is difficult, however, as the logarithmic transformation places greater emphasis on performance at the low yielding sites (Knight, 1970; Lawrence, 1970). Some workers have considered the deviations from regression more important and have termed this stability (Eberhart and Russell, 1965). Sparrow (1969) overcame this confusion by suggesting that the term stability be reserved for the regression coefficient and the deviation be termed reliability. Where more than one stress is significant in the range of environments sampled, or where the levels of one environmental factor range both above and below the optimum, the deviations from regression would be large and interpretation difficult (Knight, 1970). A study of genotype x environment, whether using this regression technique or the more complex pattern analysis of Byth *et al.* (1976) may be useful for the plant breeder making a broad study or selection from a range of genotypes but it does not supply information on the mechanism of the response.

Grafius (1969) describes the use of correlation between sequential characters (the yield components) as a measure of stress. Each component is to some extent correlated to the previously formed components. The success of a genotype in an environment would depend on the influence of stress on a yield component and the compensation response of later formed components (Grafius and Thomas, 1971).

The statistical techniques are of value where the precise nature of the stresses of an environment are not known or where the biological responses and resistance mechanisms to the stresses are not well understood. These techniques will not replace a detailed knowledge of the genetic and physiological basis of genotypic differences in resistance to water stress.

Field experience of performance under dry conditions has often provided the only measures of resistance to drought. Ranking of genotypes for a character in the same order as their ranking for resistance based on field performance has been regarded as significant proof that the character is related to water stress resistance (Kaloyereas, 1958; Dobrenz *et al.*, 1969a). Apparent field resistance may be due to other factors such as disease resistance (Reitz, 1974) and the correlations may be misleading.

Survival in drought chambers which simulate atmospheric stress have been used as a criterion of water stress resistance (Aamodt and Johnson, 1936; Bayles *et al.*, 1937; Kenway and Peto, 1939). These tests have not correlated well with field performance under stress (Kenway *et al.*, 1942; Ashton, 1948, May and Milthorpe, 1962), probably because they were measuring the wrong component of resistance.

Field evaluation of growth and yield during or following stress is difficult as the stress cannot be controlled. This is particularly true where different genotypes are being compared as they are probably at different stages of development at any one time. Rain shelters have been used (Owen, 1958b), but these must involve some additional disturbance to the environment. Trenches to collect and divert rainfall have also been used (Kirby, 1970) but they are only applicable in some environments. Pot studies are of limited use (Owen, 1958a) as the stress is imposed more rapidly than would be the case in the field. This may

be an advantage in studies of the stress effect on plant development (Aspinall *et al.*, 1964; Fischer, 1973). Studies in controlled environments are also important in determining the basic plant responses to stress.

Stress may be imposed in pots by withholding water, by flooding the rooting medium with a solution of low osmotic potential or by lowering the root temperature. Osmotic solutions, such as polyethylene glycol (PEG) (Lawlor, 1969; Singh *et al.*, 1973c), mannitol (Slatyer, 1961) or sodium chloride (Nieman, 1962) have been used. The usefulness of these solutions will depend on their phytotoxicity and the extent to which they are absorbed into the plant. Polyethylene glycols of high molecular weight (PEG 4000 or PEG 6000) are probably the least toxic of these substances (Lawlor, 1969). Lowering of root temperature may be useful for imposing mild stresses if the facilities are available (Ullery, 1971).

Where growth rate following stress is the criterion of resistance, a sufficiently long period should be allowed to separate the genotypes. Laude (1971) considered the one week period commonly used was insufficient and, in studies with ryegrass, a four week recovery period was required to adequately measure the response (Corletto and Laude, 1974).

2.8 Drought Escape

Ephemerals escape drought by surviving in the most tolerant seed resting stage (Levitt, 1972). Similarly, early maturing genotypes complete their life cycle before the onset of stress or they can be planted after a stress period.

Chinoy (1962) studied the drought resistance of eight *Triticum* genotypes representing seven species. He found that escape due to early maturity was most important. Early maturity, rather than any difference in the ability of the genotypes to endure wilting accounted for most of the differences in apparent drought resistance. Derera *et al.* (1969) found that earliness accounted for 40% and 90% of the variation in drought resistance of wheat varieties in two different seasons in northern New South Wales. In both these studies the drought became increasingly severe as the season progressed.

Early maturity may improve drought resistance but reduce the yield potential in favourable seasons (Doyle and Marcellos, 1974). There would then be a balance between time of maturity and yield potential that would be different for each region depending on the drought expectancy, and each season depending on the onset of drought.

As the capacity of plants to resist water stress appears to depend on the stage of growth, a genotype may escape the effects of drought by being at a less sensitive stage at the time of stress. This type of resistance would only be important in an environment where the timing of drought was predictable but must be taken into account experimentally in studies of genotypic differences in resistance.

2.9 Drought Avoidance

The existance of "water savers" and "water spenders" was noted on p.23. One aspect of drought avoidance not often considered, however, is that restricting water use during a period of sufficient supply may delay the onset of stress when water becomes limiting. An example of this is the interaction between nitrogen and water stress (or comparison of varieties with different vegetative growth rates). High nitrogen supply promotes vegetative development and increases

water use. When water becomes limiting, the high nitrogen treatments are more affected, not only because they are using more water at the time of stress, but also because they have exploited more of the water before the dry period (Gardener, 1971).

Stomatal frequency and Size

There have been conflicting reports on the significance of stomatal frequency or size in drought avoidance. Miskin and Rasmusson (1970) found that the frequency of stomata varied from 36 to 98 per mm^2 in the world collection of barley genotypes and suggested that transpiration would be reduced where stomatal frequency was lower. Miskin *et al.* (1972) subsequently reported that in well watered barley, the genotypes with lower stomatal frequency transpired less for the same level of photosynthesis but attributed this to the dominance of mesophyll resistance (non-stomatal effects) in carbon dioxide exchange. Dobrenz *et al.* (1969b) reported that stomatal frequency was negatively correlated with the drought resistance rating of six clones of blue panicgrass (*Panicum antidotale*), the clones with the lower stomatal frequency being more resistant to seedling drought. The stomatal frequency was not associated with the water use efficiency of the clones.

Smaller stomata were found in drought resistant cultivars by Birdsall and Neatby (1944) and Asana (1962) but their studies did not adequately measure resistance.

Smaller and fewer stomata would therefore appear to be related to reduced transpiration and hence would possibly delay the onset of stress when water was limiting. The frequency and size of stomata are often negatively correlated (Miskin and Rasmusson, 1970) and it may be difficult to select for both characters. Lines of barley have been selected for high and low stomatal frequency but this had little influence on the water relations. There was a tendency for the plants

with the higher frequency to be more resistant to stress. Selection for fewer stomata had also increased stomatal size and cell expansion (Jones, 1975). Thus selection for lower stomatal frequency would need to be carefully controlled to avoid unwelcome correlated changes. There is some hope for selection, however, as Wilson (1971) was able to select for increased stomatal frequency in ryegrass without decreasing length.

Stomatal Control

Variations in stomatal control have often been associated with differences in drought resistance between species. Examples are maize compared with sorghum (El Sharkawy and Hesketh, 1964; Glover, 1959), maize with soybean (Boyer, 1970b), soybean with sorghum (Teare and Kanemasu, 1972) and maize and sorghum with tobacco (Turner, 1974). Within species variability in stomatal control has not been extensively studied. Stocker (1960), working with oats and Gautreau (1970) with peanuts both considered that early closure of stomata was an important avoidance mechanism. Conversely, two studies with sorghum (Blum, 1974a; Henzell *et al.*, 1975) found that the more drought resistant cultivars, based on previous drought tests, were the ones with less responsive stomata. Blum noted, however, that these cultivars were more resistant because they had drought tolerating mechanisms.

The stress situation will govern whether it is an advantage to the plant if the stomata close at high water potential and so conserve water but restrict carbon dioxide exchange or remain open at low potentials and thereby maintain photosynthesis. The interaction between stomatal control and other drought resisting mechanisms will also be important as illustrated in the previous paragraph.

Delayed recovery of stomata following stress relief has already been discussed. Glover (1959) showed that sorghum stomata opened earlier than those of maize when stress was relieved and sorghum recovered growth

more quickly. There is little evidence of variability of this sort within a species.

The plant hormone abscisic acid (ABA) has been implicated in stomatal control mechanisms (Livne and Vaadia, 1972; Quarrie and Jones, 1975). A "wilty" mutant of tomato only closes its stomata when ABA is applied (Tal and Imber, 1971). It has been suggested that genotypes that can produce ABA freely when stressed could be at an advantage in drought since they readily close stomata (Quarrie and Jones, 1975). A drought resistant mutant of maize had higher non-stress levels of ABA and accumulated more of the hormone during stress than did a drought susceptible variety (Larque-Saavedra and Wain, 1974). The advantage of this type of response to a water deficit will depend on the advantage of early closure of stomata, an hypothesis which has still to be proved.

Roots

Genotypes with extensive, penetrating root systems are able to extract more water from the soil and so avoid the onset of stress (Hurd, 1971; IRRI, 1976). There appears to be significant variation for this character in wheat (Hurd, 1964; 1968; 1974) and rice (IRRI, 1976) and the character has been successfully exploited in the production of two drought resistant wheat varieties (Hurd, 1974). Derera *et al.* (1969) also presented limited data which showed that genotypes with deeper root systems were more drought resistant. Variation in rooting density and distribution has been reported in barley (Hess, 1949; Lee, 1960; Hackett, 1968).

Passioura (1972) was able to improve the yield of a wheat cultivar under stress conditions by forcing it to grow on only one seminal root. Water use was restricted by this manipulation and severe stress was delayed until after more critical growth stages later in the season. He suggested that the same result could be achieved by increasing

root resistance to water flow by selecting for reduced root xylem diameter. Another alternative would be to select for fewer seminal roots (Meyer, 1976). This approach assumes that nodal roots will absorb sufficient water during water-sufficient periods to maintain adequate growth.

Levitt (1972) has suggested that genotypes able to penetrate very dry soil and so exploit deeper soil reserves would be at an advantage in drought periods. Drought resistant sugar cane varieties were able to penetrate drier soil than susceptible ones (Dastane, 1957).

Water Use Efficiency

Water Use Efficiency is the ratio of dry matter accumulated to evapotranspiration and differs from Transpiration Efficiency (Fischer, 1979) in that transpiration is used as the divisor in the latter ratio. De Wit (1958) remarked upon the predictability of Water Use Efficiency across environments. Genotypic differences in this character could be related to drought resistance under stress conditions, however, and would appear as drought avoidance.

Wright and Dobrenz (1970) found Water Use Efficiency differences in boer lovegrass (*Eragrostis curvula*) and blue panicgrass (*Panicum antidotale*) genotypes but these were negatively correlated to a seedling drought tolerance test. They found a significant correlation between seedling and mature plant Water Use Efficiency under their conditions. Passioura (1977) also reported differences for Water Use Efficiency in wheat cultivars but did not attempt to relate this to resistance. Other studies have not demonstrated genotype differences for Water Use Efficiency, however, as discussed by Fischer and Turner (1978).

Water Use Efficiency for grain yield is related directly to Water Use Efficiency as defined above by the harvest index and in his study, Passioura (1977) found that the harvest index was related to the percentage of the total water supply which was available after anthesis.

2.10 Drought Tolerance

Drought tolerance mechanisms enable plants to survive periods of low tissue water potential and to resume growth when conditions improve. Tolerance mechanisms that enable xerophytic species to withstand drought have been extensively studied but no general hypothesis on tolerance has been widely accepted (Parker, 1972; Levitt, 1972). The significance of tolerance mechanisms within species, particularly within the grasses has not been established.

There have been suggestions that mechanisms giving tolerance to one form of stress may also give tolerance to others. The associations between heat, drought and freezing tolerance in particular have been considered (Levitt, 1956; Parker, 1968; Levitt, 1972) as each stress may cause internal water deficits. The evidence for such a relationship is not strong, however (Chu, 1974) and the literature associated with resistance to other forms of stress will not be reviewed.

Sullivan and co-workers established a relationship between field drought resistance and the heat tolerance of leaf discs in grain sorghum (Sullivan *et al.*, 1968). Survival after severe drought stress in pot grown plants was also correlated with the results of the heat tolerance test (Sullivan and Eastin, 1974). The heat tolerance test is similar to the heat test used for pine needles by Kaloyereas (1958) to determine chlorophyll stability. This test was used successfully by Murty and Majumder (1962) to predict the drought resistance of rice genotypes, while Fanous (1967) failed to find any relationship in millet. Boyd and Walker (1972) used a controlled wilting of wheat leaf sections to determine chlorophyll stability and found it to be related to the ranking of cultivars for drought resistance based on field experience. A similar test was related to a crude drought resistance index for rapeseed by Richards (1978). Singh *et al.* (1973d), however, found no difference

between barley genotypes in the rate of chlorophyll loss when the seedlings were stressed osmotically.

Kessler and Frank-Tishel (1962) suggested an altered nucleotide base ratio in the RNA of stressed tissue may indicate drought resistance. Stutte and Todd (1968) investigated this proposal in wheat but found the technique to be too variable to be useful in selecting for drought resistance.

Increased capacity to bind water has been suggested as an important drought tolerance mechanism by some Russian workers (Henckel, 1964; Shchukina, 1969). Newton and Martin (1930) also found such a relationship but later workers were unable to establish its usefulness (Whitman, 1941; Carroll, 1943).

An increase in the sugar content at the expense of starch under dry conditions has been suggested as a tolerance mechanism (Levitt, 1972). This was largely based on the evidence of Iljin (1929), who found that when plants were grouped ecologically, their sugar content increased with the dryness of the habitat. The difference in drought resistance of two rice varieties was related to sugar concentration (Murty and Srinivasulu, 1968) but other studies have failed to find any relationship (Levitt, 1972).

Levitt (1972) proposed a general tolerance hypothesis based on the content of sulphhydryl groups (SH) in tissue protein. He proposed that protein aggregation and denaturation on dehydration is probably associated with the formation of disulphide bonds (SS) and loss of SH groups. A dehydration resistant plant would be able to prevent oxidation to SS bonds or prevent protein aggregation by splitting the intermolecular SS bonds which may occur. There is more evidence for a relationship between freezing resistance and SH content than for the relationship with dehydration resistance but Kaloyereas (1958) obtained a correlation

between dehydration resistance and SH content in pine. This proposition still awaits critical evaluation.

Proline Accumulation

Singh *et al.* (1972) reported a high negative correlation ($r = -0.89^{**}$) between the proline accumulated by ten barley genotypes under stress and the stability index calculated for these genotypes by Finlay and Wilkinson (1963). The use of the log yield in the latter authors' statistical method emphasizes the performance of varieties at the lower yielding, drier sites (Knight, 1970). In the study by Singh *et al.* (1972), proline was measured in the first leaf of three week old barley seedlings stressed with a PEG solution of -20 bars osmotic potential. The proline was measured 60 hours after imposing stress. This result was supported by another experiment (Singh *et al.*, 1973d) which showed that proline accumulated by five barley genotypes when stressed osmotically was related to recovery when the stress was relieved by washing the PEG from the rooting medium. The cultivars that had the higher relative growth rates for the first four days after stress relief were those that had accumulated most proline during the stress period. They also had less leaf senescence.

Conflicting data were presented by Hanson *et al.* (1977) in a study which used two of the genotypes studied by Singh (1970). They were unable to show a similar potential for proline accumulation. It was unfortunate, however, that in their study they were not able to compare the genotypes directly at a similar water potential as Proctor always reached a lower level than Excelsior. Similarly, they found that that the genotype accumulating most proline was more affected by stress but it was also more stressed. They were able to demonstrate a "trapping" of proline in necrotic tissue thus suggesting some loss of nitrogen to the plant by this means.

Blum and Ebercon (1976) worked with grain sorghum genotypes and found that the free proline content commenced to accumulate at leaf water potentials of -14 to -16 bars in plants stressed by withholding water. The genotypes differed considerably in the amount of proline accumulated at the end of the stress period. They also found that the ability to recover after relief from the stress was highly correlated with proline accumulated during the stress period, but not with the resistance of isolated tissues to desiccation.

Barnett and Naylor (1966) also found a relationship between the drought resistance of bermuda grass (*Cynodon dactylon*) clones and the ability to accumulate proline during stress. The clones which came from drier habitats were able to accumulate more proline when subjected to stress. Similar results were obtained with *Carex* sp. by Hubec *et al.* (1969) and Palfi and co-workers have suggested that drought resistant crop varieties could be selected by screening for ability to accumulate proline. They present very little critical evidence in their papers other than showing differences in ability to accumulate proline under stress (Palfi and Juhasz, 1971; Palfi *et al.*, 1973).

Waldren *et al.* (1974) disputed the suitability of proline as a selection tool based on their studies with sorghum and soybean. They claimed that proline accumulates only at high stress levels and is therefore not a sensitive indicator of stress. They implied that there were not sufficient differences between the species to be useful, but they did not examine the variability within a species.

Richards (1978) and Richards and Thurling (1979) studied the drought resistance of rapeseed and concluded that the accumulation of proline was highly related to yield under water stress conditions in five varieties. This was more apparent in *Brassica napus* than in *B. campestris*. They found a broad sense heritability of 43% when

genetic analysis for ability to accumulate proline was made in 112 related families but narrow sense heritability was only 18%. The use of proline as a selection criterion was more promising in *B. napus* than in *B. campestris*. The correlation between performance under dry field conditions and proline measured in a glasshouse seedling test was significant ($r = 0.35^{*}$).

The particular role of proline in drought resistance has not been established. It is also accumulated in a non-halophytic bacteria (Measures, 1975), marine bi-valves (Pierce and Greenberg, 1973) and plants exposed to salinity stress (Chu, 1974; Stewart and Lee, 1974). In these cases proline appears to act as an osmoregulator, where due to its low toxicity at high concentration, it prevents damage while allowing growth. There is little evidence for proline acting in this way in water stressed plants. It is possible that proline serves as a less toxic way of storing nitrogen in stressed tissue (Levitt, 1972; Slukhai and Opanasenko, 1974). Henckel (1964) claimed that the accumulation of ammonia in stressed tissue may be toxic. It has been shown that proline is non-toxic at high concentrations (Stewart and Lee, 1974) and probably exerts the least inhibitory effect on cell growth of all amino group donors (Palfi *et al.*, 1974). Singh *et al.* (1973d) did find least tissue damage in stressed barley seedlings which accumulated most proline, while Blum and Ebercon (1976) found no relationship between tissue survival and proline accumulation, nor did they find an accumulation of ammonia in stressed tissue. Barnett and Naylor (1966) suggested that proline may act as a reserve of reduced carbon and nitrogen compounds which can be used when conditions are more favourable to growth. In support of this hypothesis, proline appears to be used for this purpose in maize root tips (Barnard and Oakes, 1970) and pollen grains of rice (Ito, 1972). This appears to be the reason for better recovery of the higher proline accumulators

in the study by Blum and Ebercon (1976). While Singh *et al.* (1973d) also demonstrated increased ability to grow following stress relief in the high proline accumulators, this may have been related to better survival of leaf tissue in these genotypes. Tully *et al.* (1979) dispute these claims, however, as they did not find proline to be a major reserve of nitrogen after stress nor a major translocated nitrogen species during stress. They also found that proline was trapped in senescent tissue after stress (Hanson *et al.*, 1977). These differences have still to be resolved.

2.11 Conclusions

Water stress and stress resistance are complex characters that are not easily evaluated. A water stress must be measured in terms of water status of the plant and for this, the water potential or possibly the relationship between water potential and water content are the most suitable criteria.

It would be more precise to measure resistance to water stress by measuring tissue survival at a particular water status but this may not be a useful criterion in studies that aim to improve crop yield (Laude, 1971). In such studies it is the growth and yield response to the stress and following the relief of stress that are important.

It has been suggested that drought escape and avoidance are far more important than tolerance in crop varieties (May and Milthorpe, 1962). The significance of tolerance mechanisms in improving the drought resistance of a crop such as barley have not been adequately determined. Blum (1974a) has suggested that drought resistant sorghum genotypes combine both drought avoidance and tolerance and such combinations are likely to be important for drought resistance in other species.

Most aspects of metabolism are affected by water stress (Hsiao, 1973). It is not surpirising, then, that a number of these responses have been related to drought resistance. The work on proline accumulation, particularly that by Singh and co-workers is most interesting. It has been suggested that, at least for some species, ability to accumulate proline may indicate drought tolerance if not actually being a part of the tolerance response. Proline accumulation, either alone or in combination with other characters may then be a valuable selection tool in developing drought resistant cultivars.

3. MATERIALS AND METHODS

3.1 Choice of Genotype

Twenty-one genotypes were used for the experiments in this study (Table 2). Twelve of these, (Arivat, Asahi 2, Bankuti Korai, BR 1239, CI 3576, Excelsior, Ketch, Maraini, Princess, Prior A, Proctor and Velvon II) were chosen because they had been used in the studies of Singh *et al.* (1973d). These genotypes with the exception of Asahi 2 and Ketch, were also used in a study of genotype response to agronomic manipulation by Gardener (1971), which provided useful information on their reaction to changing environments. Clipper was added to the list as it is currently the most popular barley cultivar in Australia. These genotypes covered a very wide range of maturity types and the other genotypes were added to the list to allow comparison of different genotypes within maturity groups. Thus Mona was added to complement Bankuti Korai as a very early maturing two-row type. Cyprus Black, Greenough and Stewart were added as examples of early maturing six-row types. CPI 18197, a mid-season, two-rowed genotype was added for comparison with Clipper. Dore was chosen for early maturity but proved to be later flowering than expected. Zephyr, a two-rowed genotype was added to the list of late maturing genotypes. Hiproly, the high lysine genotype was chosen to determine whether the metabolic difference which results in high lysine grain was important to other aspects of nitrogen metabolism.

3.2 Measurement of Plant Water Status

Xylem Water Potential

A pressure chamber was used to estimate water potential. It was used in preference to a thermocouple psychrometer to ensure

Table 2.

Genotypes used in the experiments, showing origin (where known), head type and maturity rating when sown in July in South Australia

Number	Genotype	Origin	Head Type	Maturity
1.	Arivat	U.S.A.	6	Mid.
2.	Asahi 2	Japan	2	Late
3.	Bankuti Korai	Hungary	2	Very early
4.	BR 1239	Canada	6	Mid.
5.	CI 3576	Egypt	2	Early
6.	Clipper	Australia	2	Mid.
7.	CPI 18197	Algeria	2	Early to Mid.
8.	Cyprus Black	Cyprus	6	Early
9.	Dore		2	Mid.
10.	Excelsior		6	Late
11.	Greenough	U.S.A.	6 (hooded)	Early
12.	Hiproly	Ethiopia	2 (naked)	Mid.
13.	Ketch	Australia	2	Early
14.	Maraini	Italy	6	Very late
15.	Mona	Sweden	2	Very early
16.	Princess	Sweden	2	Late
17.	Prior A	Australia	2	Mid.
18.	Proctor	England	2	Late
19.	Stewart		6	Early
20.	Velvon II	Canada	6	Late
21.	Zephyr	Netherlands	2	Late

there would be no restriction on the number of samples that could be sampled daily. A small chamber was used to enable measurement of single leaves to be carried out rapidly. The leaf to be measured was excised from the plant at the junction of the lamina and sheath with a sharp blade and immediately sealed within the chamber which was sited close to the plants. Compressed Medical Air was let into the chamber quickly until the pressure was just below the expected equilibrium; the pressure was then increased slowly until a droplet of water appeared at the cut surface. The whole operation was always completed within one minute to minimise evaporation from the cut surface. In most experiments only one leaf from each plant was selected to be measured. For the glasshouse experiments the xylem water potentials were recorded between 11.30 am and 1.30 pm; this measured the potential when it was at its lowest.

Relative Water Content (RWC)

RWC was measured in only one experiment. The leaf lamina to be measured was cut from the plant at the junction of the lamina and sheath. It was then quickly cut into 2 cm sections and placed in a weighing bottle which was sealed. The bottles were then stored in cooled, insulated containers, until all samples had been collected (about 15 minutes). After the first weighing, the leaves were floated on distilled water in closed petri dishes. The dishes were maintained at 20°C under fluorescent lights at the previously determined compensating light intensity. After floating for four hours, the leaf sections were dried between sheets of tissue paper, weighed, dried at 85°C for 24 hours and weighed again. RWC was calculated from the formula,

$$\text{RWC} = \frac{\text{fresh weight - dry weight}}{\text{turgid weight - dry weight}} \times 100$$

3.3 Plant Morphological Measurements

Dry Weight

Plant or leaf dry weight was always determined by drying in a forced draught oven at 85°C for 24 hours.

Relative Growth Rate

Relative growth rate was determined from the formula:

$$RGR = \frac{\log_e \text{ dry weight (final)} - \log_e \text{ dry weight (initial)}}{\text{time}}$$

Seedling Measurements

Unless otherwise stated in the individual experiments, seedling measurements were made on three plants from each pot.

All tillers and leaves that were visible were included in the total tiller number and total leaf number. This included those tillers or leaves that had just begun to emerge. No distinction was drawn between expanding tillers or leaves and those that were fully formed.

Plant height was measured from the soil surface to the tip of the tallest leaf on the plant when it was fully extended. The tallest leaf was not always the youngest leaf.

Leaf area was measured with an electronic planimeter which had been calibrated with a metal disc. Leaf area was measured only on leaf lamina and was recorded as leaf area per pot and not the leaf area index.

Leaf survival was measured on a length basis. Leaves began to senesce at the tip and the length of the region of living tissue was measured and expressed as a percentage of the total lamina length.

Stomatal Frequency and Length

Stomatal frequency and length were measured on the abaxial surface

of seedling leaf laminae at a point 2 cm from the auricle. An imprint of the leaf surface was taken with cellulose acetate film (clear nail varnish) which was then mounted between two microscope slides for later measurement. The magnification for stomatal measurements was chosen to give 40 to 90 stomata per microscope field. Three fields were counted for each leaf replica. Stomatal length was measured at higher magnification using a calibrated ocular micrometer. Total guard cell length was measured on five stomata per replica.

Mature Plant Measurements

The height of mature plants was measured from the soil surface to the tip of the uppermost spikelet (sterile or fertile) excluding the awn.

Total tiller number, including both fertile and infertile tillers was recorded at harvest. It is possible that this measurement did not reflect the maximum number of tillers formed as tiller number was not recorded throughout the experiments. The number of fertile tillers was also recorded, a fertile tiller being defined as any tiller producing a spike, whether its florets were fertile or not.

The total number of florets (both fertile and sterile) was counted on the spikes before threshing. Spikes were then threshed using a rubbing board, the chaff removed and the number of grains counted. Floret fertility was then calculated from :

Floret fertility = (Number of grains/total florets) x 100.

The weight of grain on each spike was recorded after drying at 85^{o}C for 24 hours and this was used to calculate both grain yield and grain weight (weight per 1000 grains).

3.4 Plant Biochemical Measurements

Total Chlorophyll Content

Leaves to be analysed for chlorophyll content were removed from the plant, cut into 2 cm sections, plunged into liquid nitrogen and stored at -20°C for later chlorophyll analysis. Prior to analysis, the sample was divided into two portions, one being used to determine leaf moisture content and the other for chlorophyll determination.

Leaf sections were extracted in acetone and chlorophyll estimated from the optical density at 645 nm and 663 nm (MacKinney, 1941). The total chlorophyll content (chlorophyllα plus chlorophyllβ) was calculated from the formula:

$$\text{Chlorophyll } (\alpha + \beta) = \frac{1}{\text{dry weight}} \; (643.22\ A663 + 1623.84\ A645)$$

where A663 and A645 are the optical densities at 663 or 645 nm respectively.

Free Proline Content

Free proline was measured by the method of Faber and Aspinall (pers. comm.) which is a refinement of the technique of Singh *et al.* (1973c) based on that of Troll and Lindsley (1955).

Leaves to be measured were removed from the plant, cut into 2 cm sections, plunged into liquid nitrogen and stored at -20°C prior to analysis. The frozen tissue was divided into two portions, each portion being weighed. One portion was dried at 85°C for 24 hours and weighed again to determine moisture content. This factor was then used to calculate the dry weight of the portion used for proline analysis.

The sample used for proline estimation was homogenised with 1500 mg Permutit resin (Decalso-F, Permutit Co. of Aust.) and 10 ml of a solution of re-distilled methanol, re-distilled chloroform and water in the proportions 12:5:3. The emulsion which formed was broken with

the addition of 8 ml water and the layers mixed thoroughly before centrifuging at 7000 rpm for 5 minutes. The upper aqueous layer was transferred to a boiling tube, 5 ml of glacial acetic acid was added followed by 5 ml ninhydrin reagent (0.125g ninhydrin, 3ml glacial acetic acid and 2ml orthophosphoric acid per sample). This was boiled for 45 min. in a boiling water bath and then cooled to room temperature. A measured aliquot of toluene, varying from 5-10ml depending on the proline content was added, the layers mixed thoroughly and allowed to stand at least 30 min. The optical density of the ninhydrin product dissolved in toluene was read at 520 nm and the proline content estimated from a standard curve constructed from L-proline standards treated in a similar way. The free proline content was then calculated in mg/g dry weight.

3.5 Experiments

Experiment 1. Osmotic stress of barley seedlings - growth cabinet.

Seeds of 20 genotypes (all from Table 2 omitting Asahi 2) were germinated for 24 hours at 20°C. Ten pre-germinated seeds were sown in each 10cm plastic pot of perlite and thinned to five seedlings three days after sowing.

The plants were grown in the growth cabinet at 20°C ± 1°C and a 16 hour photoperiod at a light intensity of 2×10^4 lux (fluorescent plus 10% incandescent). The seedlings were watered daily with 50ml nutrient solution (Table 3) for the first 14 days and 100ml per day thereafter.

There were five pots of each genotype. Three of these were randomly assigned to be stressed and two to be unstressed controls. The pots were re-randomised daily.

Table 3.

Nutrient solution used for all experiments.

Nutrient	Source	ppm in Solution
N	$Ca(NO_3).4H_2O$	1180.00
K and N	KNO_3	260.00
Mg	$MgSO_4.7H_2O$	490.00
P and K	KH_2PO_4	68.00
B	H_3BO_3	0.50
Mn	$MnCl_2.4H_2O$	0.25
Zn	$ZnSO_4.7H_2O$	0.25
Cu	$CuSO_4.5H_2O$	0.02
Mo	$Na_2MoO_4.2H_2O$	0.02
Fe EDTA	$Na_2EDTA + FeSO_4.7H_2O$	0.50

Stress was applied by replacing the nutrient solution with 250ml of a polyethylene glycol solution (molecular weight 4000) of -20 bars osmotic potential (32.4g PEG/100ml) when seedlings of all genotypes were at the three-leaf stage, 21 days after sowing.

Water potential and free proline content were measured on the first leaf of sample plants 24, 72 and 144 hours after stress imposition. A single leaf was divided longitudinally for determination of Ψ(xylem) and free proline in the 24 hours sample. The unstressed controls were measured for Ψ(xylem) and free proline content 24 and 144 hours after stress imposition, whilst the former was measured for all plants before stress was imposed.

The osmoticum was washed from the rooting medium after 144 hours of stress with six changes of distilled water and this was finally replaced with 100ml of nutrient solution. The plants were watered daily with nutrient solution for the subsequent 3 days (72 hours). Leaf survival, tiller number and plant height were then measured on five plants in each pot of two of the three stressed pots and the two unstressed pots of each genotype. The first leaf of all plants had been used for determination of Ψ(xylem) and free proline at this stage.

Experiment 2. Osmotic stress of barley seedlings - glasshouse.

The experiment aimed at quantifying free proline accumulation in twenty genotypes in response to an osmotically induced moisture stress. The genotypes were those used in Experiment 1. Five seedlings per 10cm pot were sown in perlite and watered with nutrient solution but the pots were placed in a naturally lit glasshouse.

Seedlings were stressed at the three-leaf stage, 21 days after sowing. Stress was applied osmotically as in Experiment 1. There were four pots of each genotype and these were randomised within four blocks

and all were stressed for 72 hours when the first leaf of plants were sampled for Ψ(xylem) and free proline.

Experiment 3. Seedlings stressed by withholding water - growth cabinet.

Ten germinated seeds of 20 genotypes (as for the previous two experiments) were sown in washed river sand in each 10cm plastic pot. Three days after sowing, the seedlings were thinned to five per pot. There were three pots per genotype and they were arranged in three replicates in a growth cabinet. The temperature in the cabinet was $20^{o}C \pm 1^{o}C$ and there was a 16 hour photoperiod at a light intensity of 2×10^{4} lux. (fluorescent plus 10% incandescent).

The pots were watered with 50ml nutrient solution (Table 3) daily for the first 14 days and 100ml thereafter. They were re-randomised within the replicates each day. Water was withheld on day 21 when the plants of all genotypes were at the three-leaf stage. Leaf xylem water potential and free proline were measured five days later on the first leaf of one plant in each pot and these measurements were repeated on the following day. There was no recovery period.

Experiment 4. Seedlings stressed by withholding water - glasshouse.

Ten genotypes were chosen for thus study (Asahi 2, Bankuti Korai, CI 3576, Clipper, Cyprus Black, Dore, Excelsior, Maraini, Proctor, Stewart), based on previous information of ability to accumulate proline and likely differences in ability to recover after stress.

The soil used for this experiment was a 1:1 mixture of coarse river sand and alluvial loam. Fertilizer was added during soil preparation to provide sufficient nutrients for plant growth.

Ten seeds were sown in five positions (two in each position) in 10cm plastic pots. The seedlings were thinned to five per pot after emergence. They were arranged with four in an outer circle and one in

Table 4.

Schedule of treatments for experiment 4, showing treatment number, treatment designation, water status, sampling time and characters measured. Chl = chlorophyll content; DW = dry weight; Hgt = plant height; LA = leaf area; Lvs = number of leaves; P = xylem water potential; Pr = free proline content; RWC = relative water content; S = stomatal frequency and size; Till = number of tillers; WU = water used during stress.

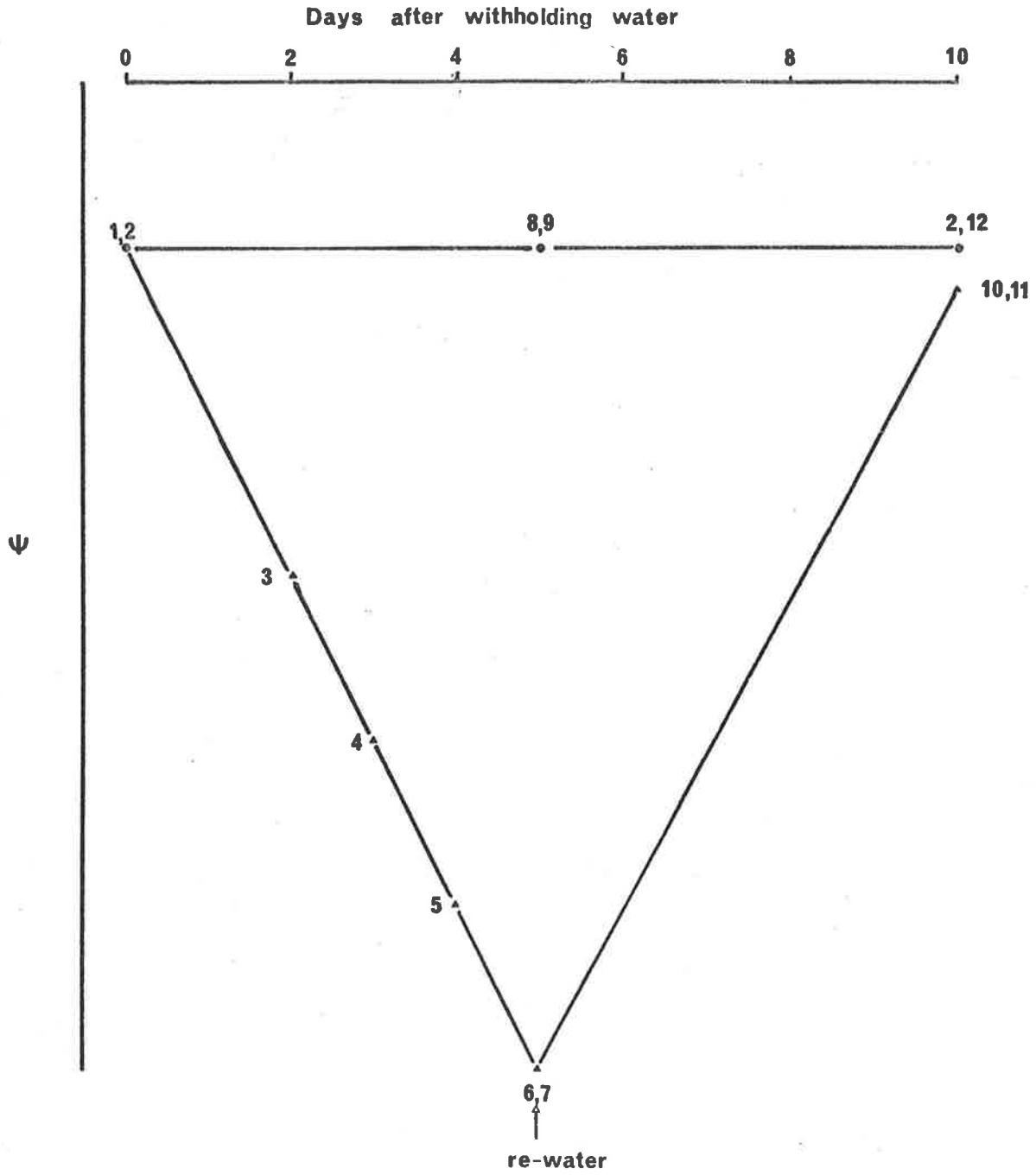
No.	Treatment Designation	Water Status	Harvest Day	Characters Measured
1.(a)	0N	Pre-stress	0	DW, LA, S.
2.(a)(b)	0N	Pre-stress	0	P, Pr.
	10N	Control	10	P, Pr, RWC
3.	2S	Stress	2	P, Pr, RWC
4.	3S	Stress	3	P, Pr. RWC
5.	4S	Stress	4	P, Pr. RWC
6.(a)	5S	Stress	5	Chl, P, Pr, RWC
7.(a)	5S	Stress	5	DW
8.(a)	5N	Control	5	Chl, P, Pr.
9.(a)	5N	Control	5	DW
10.(a)	10R	Recovered	10	P, Pr, RWC, WU.
11.(a)	10R	Recovered	10	Dw, Hgt, LA, Lvs, Hgt.
12.(a)	10N	Control	10	Dw, Hgt, LA, Lvs, Hgt.

(a) Two treatment numbers correspond to 0N, 5S, 5N 10R and 10N to provide sufficient plant material for measurement.

(b) Treatment 2 was used to provide control samples for xylem water potential and proline at day 0 and day 10.

Figure 2.

Diagram illustrating the treatment numbers assigned to the stressed (▲) and control (●) plants in Experiment 4. Treatment numbers correspond to Table 4, p. 50.


Days after withholding water
0
2
4
6
8
10
Ψ
1,2
8,9
2,12
10,11
3
4
5
6,7
re-water

a central position. The pots were filled with 80g fine gravel and 500g soil. They were free draining and were maintained at field capacity by overwatering once per day for the first four weeks. At this time all genotypes were at the three-leaf stage and the water treatments were started. Stress was imposed by withholding water, while the control pots were watered as before. Watering of the stressed pots was recommenced after five days and the experiment was terminated five days later.

The three replicates were blocked in a T pattern because there were temperature and light gradients in two directions in the glasshouse. Within each replicate, treatments 1 to 12 were assigned at random and the genotypes were randomised within treatments. The treatment numbers correspond to harvest time, water treatments and type of measurement (Table 4 and Figure 2).

The eleven genotypes from Table 2 that were not sown in this experiment in its entirety were included as part of treatment (5S) to obtain an estimate of their ability to accumulate proline under these particular stress conditions. This treatment (5S) was replicated four times.

Experiment 5. Stress at specific development stages.

Eighteen genotypes were chosen for this experiment. They included all genotypes of Table 2 with the exception of Asahi 2, Mona and Prior A. They represented a wide range of types, from early to late maturing, low to high yielding and included ten two-rowed and eight six-rowed types. It was recognised that stress would not be equivalent for genotypes with very different maturities so they were assigned to three groups of six genotypes of different maturity.

The soil for this experiment was a red-brown earth topsoil taken from the property of Mr. K. Shackley, Reeves Plains, South Australia. It was screened to pass through a 1.25 cm mesh sieve. It had very low levels of nitrogen and phosphorus (Hare, 1976). Critical soil moisture contents corresponding to soil water potentials of -0.1 bar, -0.33 bar and -15 bar were determined with a pressure plate apparatus (Table 5).

The soil was mixed in batches sufficient to fill three pots and the required nutrients (Table 6) added during mixing. Before mixing, the soil was treated with Nemagon (R) as the larvae of the cereal cyst nematode (*Heterodera avenea*) were detected in test samples.

The pots were tin-plate, non-draining and lined with a plastic bag. They were cylindrical with a surface area of 250 cm^2 and were 34 cm deep. They contained 11.56 kg over dry soil when packed to a bulk density of 1.4.

Seed was germinated for 2 days at 20^{o}C. Five seeds were sown in each pot, four in an outer circle and one in a central position. The pots were arranged in an evaporatively cooled glasshouse. Reserve seed was sown in a similar soil and transplants replaced the few obvious failures.

When seedlings had established, 500g of fine gravel was applied to the surface as a mulch to prevent excessive evaporation from the soil and to prevent erosion of the soil during watering. A circle of aluminium coated Sisalation (R), 15cm high, was used to shade the base of the plants to control the profuse tillering normally found in pot grown plants. The plants were supported during growth to prevent lodging (Plate I).

The pots were weighed, weights recorded and filtered rain water added each week. Supplementary watering was required every two to three days when the plants were growing actively and the amount of water

Plate I.

One pot used in Experiment 5 showing the lined tin-plate pot, circle of aluminium coated Sisalation and dowell to support plants.

Similar pots were used for Experiment 6.

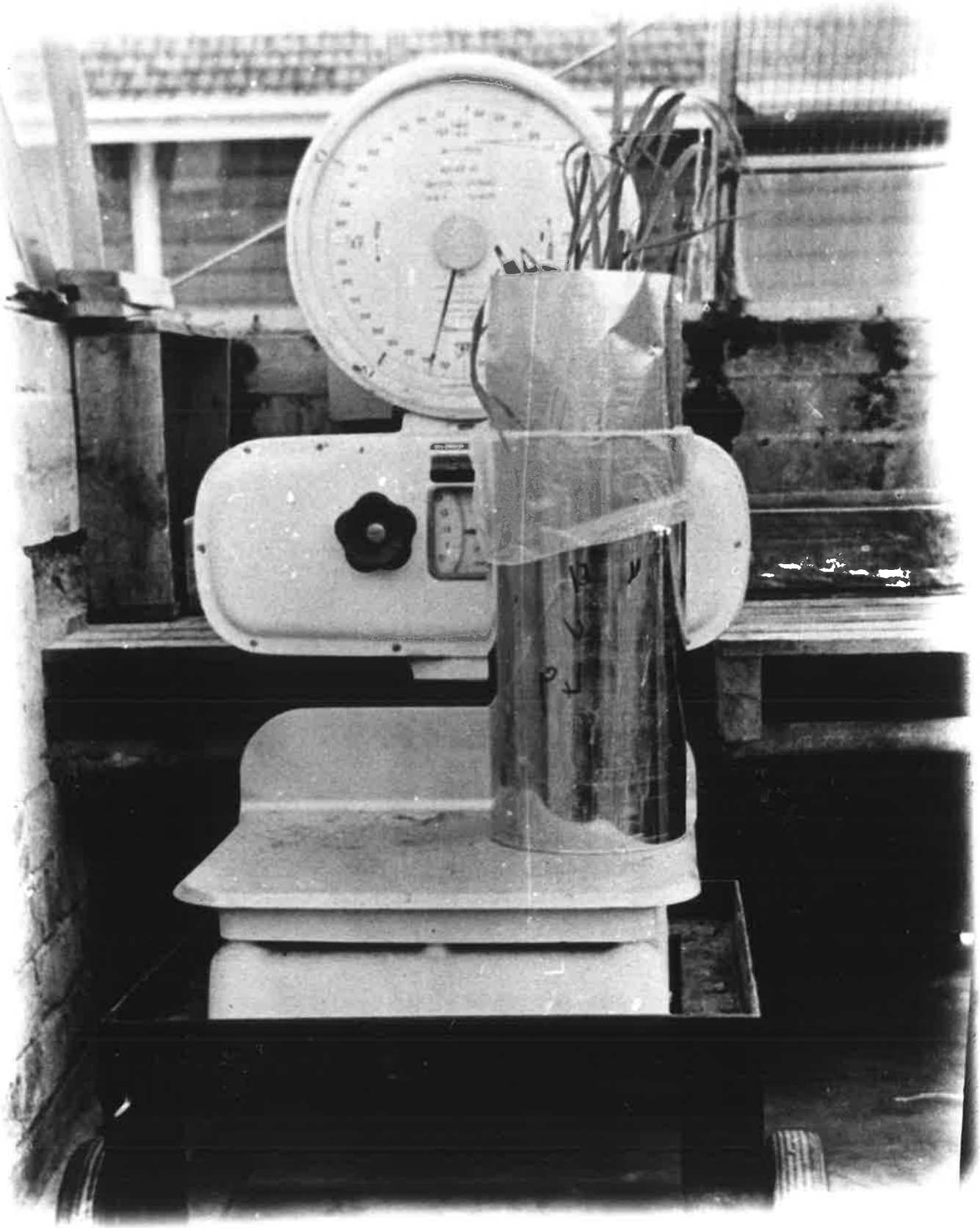


Table 5.

Water content of the red-brown earth soil used in Experiment 5 at critical soil water potentials.

Soil Water Potential bar	Water Content %
-0.10	16.0
-0.33	10.2
-15.00	4.6

Table 6.

Nutrients supplied to the red-brown earth soil used in Experiment 5.

Element	Source	Weight per pot (mg)
N	NH_4NO_3	355.00
P	$CaH_2(PO_4)_2.H_2O$	810.00
K	K_2SO_4	270.00
Mn	$MnSO_4.H_2O$	45.20
B	H_3BO_3	14.90
Zn	$ZnSO_4.7H_2O$	29.80
Cu	$CuSO_4.5H_2O$	0.75
Mo	$Na_2MoO_4.2H_2O$	37.20

required was determined by sample weighing. Water was added to maintain the soil water potential between -0.1 and -0.33 bars at all times other than during a stress cycle.

The experiment was a factorial design of four replicates, the factors being genotype and water treatment which were completely randomised within replicates. Stress was applied by withholding water until the soil water potential fell to -15 bars as determined by pot weight. At this stage the pot was re-watered and maintained at field capacity for the remainder of the growing season. The water treatments were:

Water Treatment 1: The unstressed control.

Water Treatment 2: (Vegetative Stress), stress applied during the vegetative stage with a maximum just prior to jointing.

Water Treatment 3: (Pre-anthesis stress), stress applied to reach a maximum just prior to anthesis.

Water Treatment 4: (Post-anthesis stress), stress was applied after anthesis and was relieved when the soil water potential had fallen to -15 bars but water was then withheld for the remainder of the growing season. This cyclic stress was prompted by the finding of Aspinall *et al.* (1964) that a single stress after anthesis would have little effect, particularly as the plants were able to reduce the soil water potential to -15 bars in a few days.

The stage of development for the timing of the stress was determined at the time when three of the five main stems in the pot were at the specified stage.

At the end of the stress period (or after the first stress cycle in Water Treatment 4), the youngest fully expanded leaf of two plants were sampled for measurement of xylem water potential and free proline. Two leaves were sampled at the same time from the corresponding unstressed control plants (Water Treatment 1) for the same measurements.

Plants were watered until mature and the pots no longer required significant quantities of water. This corresponded to loss of chlorophyll from the leaves. Each tiller was tagged at anthesis and the tillers measured separately for a range of plant characters when they were mature. These were tiller height, total dry weight, grain yield, tiller number, number of full and sterile florets and grain weight.

Experiment 6. Stress maintained for specific development periods.

This experiment was conducted in a glasshouse with temperature controlled at 22°C to reduce any possible interaction between temperature and stress. The stress treatments were designed to extend over specific developmental phases.

The experiment was restricted to eight genotypes as space was limiting and these were chosen to provide four genotype pairs of comparable maturity. Bankuti Korai and Mona were very early two-row types; Cyprus Black and Stewart, early six-row; Dore and Clipper, mid-season two-row; Princess and Proctor, late two-row.

The soil was sterilized river loam and coarse river sand mixed in equal proportions. Sufficient nutrients were added during mixing to support adequate growth (Table 6). Soil water content at specific water potentials were determined with a pressure plate apparatus (Table 7).

The soil was dried below 4% water content before potting. The pots were the same as those used for Experiment 5 and were filled with 13.5 kg soil packed to a bulk density of 1.5.

Table 7.

Water content of the soil used for Experiment 6 at critical soil water potentials.

Water Potential bars	Water Content %
-0.1	16
-0.33	10
-1.0	6
-15.0	4

Fourteen seeds were planted in each pot in seven positions and after establishment were thinned to seven per pot. Three of the water treatments required stress from establishment. These plants were watered lightly to ensure germination and the stress treatments were imposed immediately the seedlings emerged.

The water treatments were:

Water Treatment 1: (HHH) High water regime from sowing to harvest (control).

Water Treatment 2: (LHH) Low water regime from sowing to jointing and a high regime thereafter.

Water Treatment 3: (LLH) Low water regime from sowing to anthesis and a high regime thereafter.

Water Treatment 4: (HHL) High water regime from sowing to anthesis and low thereafter.

Water Treatment 5: (LLL) Low water regime from sowing to maturity.

Soil water content was allowed to vary between 16% and 10% in the high water regime, while for the low regime the water content was allowed to vary between 6% and 4%. The pots were weighed and watered every second day, the amount of water to be added being calculated from the pot weight after adjustment for the fresh weight of growing plants (estimated from the weight of plants grown under equivalent conditions). Supplementary water was required twice each day, when plants were growing actively, to maintain the water treatments. The amount of water given to the pots was determined from past water use. All water supplied was recorded.

The temperature in the glasshouse was maintained at $22^{\circ}C \pm 2^{\circ}C$. It was controlled via an exchange unit and air movement within the confined space was extremely high and contributed to the very high plant water consumption.

There were three replicates. The water treatments were randomised within the replicates and the genotypes randomised within the treatments. This split plot design was chosen to minimise the effects of competition that could result from the influence of stress on plant height. The genotypes and water treatments were re-randomised each time the pots were weighed.

At five separate times during the experiment, the youngest fully expanded leaf from each stressed and corresponding control treatment was sampled for measurement of xylem water potential and free proline. Anthesis date of the main stem on each plant was recorded by tagging. The mature plants were harvested individually at the conclusion of the experiment and dried at 85^{o}C for 48 hours. The characteristics recorded were total dry weight, tiller number (fertile tillers only), total florets per spike, percentage of fertile florets, grain weight and grain yield per plant.

4. RESULTS

Experiment 1. Osmotic stress of barley seedlings - growth cabinet

Twenty genotypes grown in a growth cabinet were stressed at the 3-leaf stage with a polyethylene glycol (PEG) solution of -20 bars osmotic potential. Stress was maintained for 144 hours and recovery was measured 72 hours after washing the PEG solution from the rooting medium. The experiment was designed to examine the change in xylem water potential in the 20 genotypes over the stress period and to relate this to the accumulation of free proline. Recovery was measured in terms of plant height, tiller number and leaf survival.

Plants wilted rapidly when PEG was applied but the water potential as measured by the pressure chamber did not fall as fast as was expected over the stress period (Figure 3). The average water potential after 72 hours of stress was -9 bars compared to -28.3 bars reported by Singh *et al.* (1972) when they stressed barley seedlings for a similar period. The average water potential after 144 hours of stress had fallen to -12.1 bars.

Genotype means for xylem water potential were significantly different but the differences were not significant within times (Table 8).

Water potential was also measured in the unstressed replicates. The genotypes were not significantly different but there were differences between the times (Table 9 and Figure 3). Nutrient solution was watered into the unstressed pots just prior to the measurement at 144 hours and this was probably the reason for the high Ψ(xylem) in these plants at that time.

Free proline was at a low level in all unstressed plants (Figure 4) and the genotypes were not significantly different. The

Figure 3.

Xylem water potential (bars) in the first leaf of stressed (▲) and unstressed (●) plants of 20 barley genotypes 24, 72 and 144 hours after stress imposition.

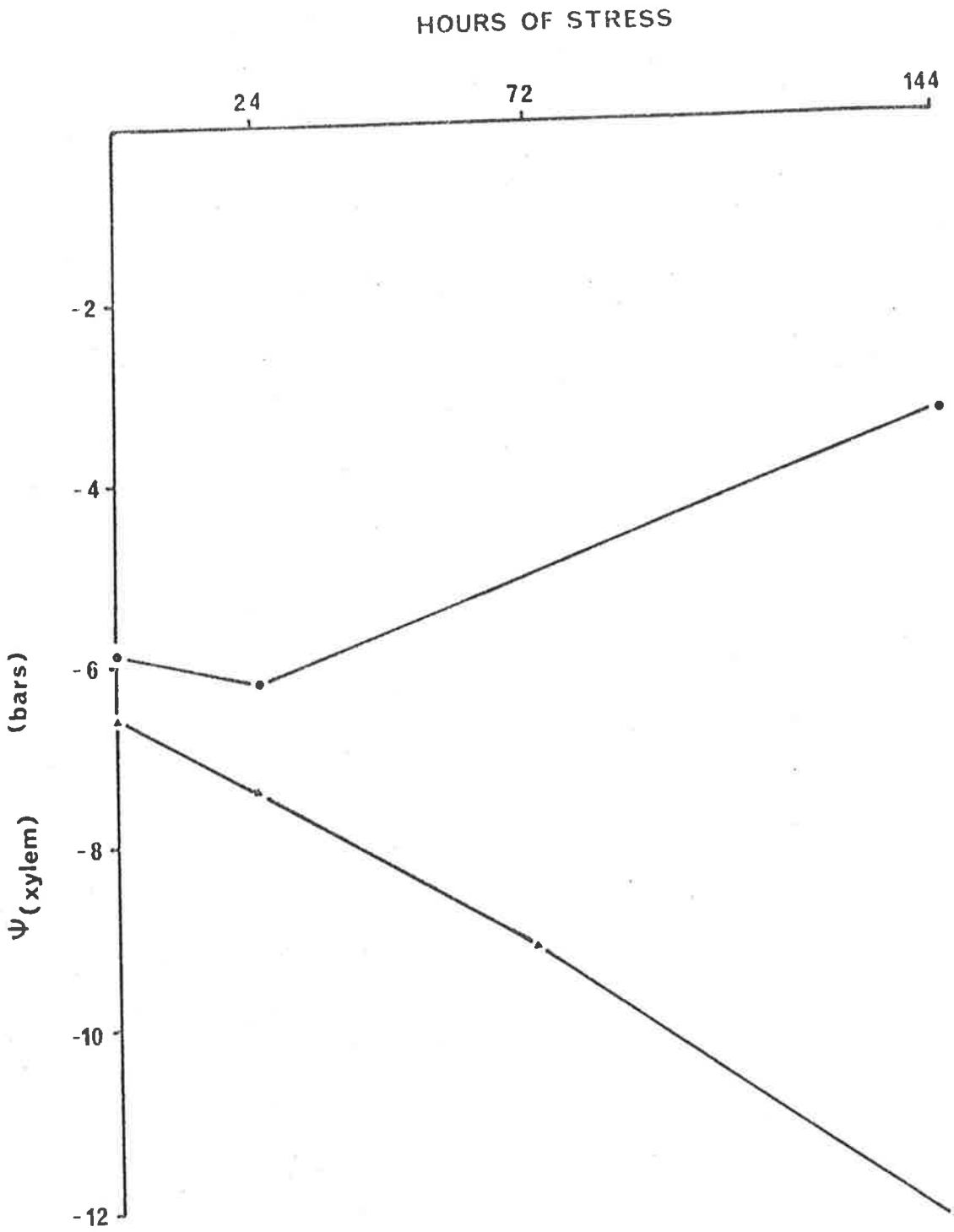

HOURS OF STRESS
24
72
144
-2
-4
-6
-8
-10
-12
Ψ(xylem) (bars)

Table 8.

Xylem water potential (- bars) in the first leaf of 20 barley genotypes stressed with PEG (-20 bars osmotic potential) measured 0, 24, 72 and 144 hours after stress imposition. Mean of 3 replicates.

Genotype	Time (hours)				
	0	24	72	144	Mean
Arivat	6.6	8.1	9.0	11.2	8.7
Bankuti Korai	6.2	7.5	9.3	12.5	8.9
BR 1239	7.1	6.7	8.5	11.8	8.5
CI 3576	7.4	7.9	10.0	13.2	9.6
Clipper	6.5	7.2	9.0	11.1	8.5
CPI 18197	6.6	7.3	9.0	11.3	8.5
Cyprus Black	6.5	8.1	9.5	12.1	9.0
Dore	5.7	6.6	8.0	12.9	8.3
Excelsior	6.2	7.6	9.1	11.8	8.6
Greenough	5.2	6.8	9.6	12.4	8.5
Hiproly	6.1	7.4	10.5	13.1	9.3
Ketch	6.9	6.9	7.6	11.1	8.1
Maraini	6.7	7.4	8.8	12.3	8.8
Mona	6.8	7.8	9.2	11.5	8.8
Princess	6.4	6.6	8.7	13.1	8.7
Prior A	6.7	8.2	8.8	12.6	9.1
Proctor	6.8	7.1	8.5	11.8	8.6
Stewart	6.8	7.6	8.9	12.2	8.9
Velvon II	6.3	7.3	8.4	11.2	8.3
Zephyr	6.7	7.4	8.8	12.3	8.8
Mean	6.6	7.4	9.1	12.1	
LSD ($p=.05$)	n.s.	n.s.	n.s.	n.s.	0.9

Combined Analysis:	Source of Variation	LSD (p=0.5)
	Genotype	0.9*
	Time	0.4**
	Genotype x Time	n.s.

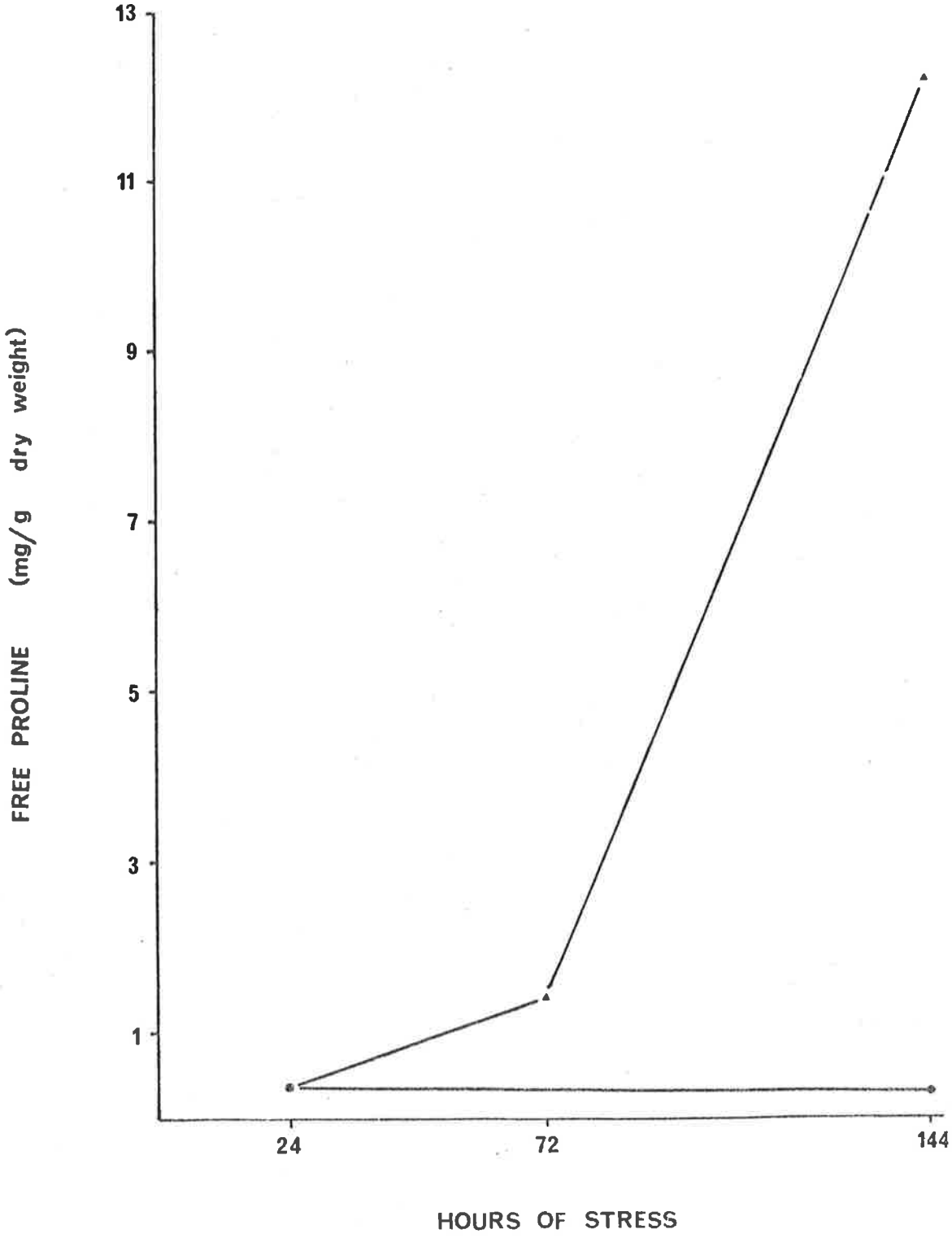
Table 9.

Xylem water potential in the unstressed controls at the times corresponding to 0, 24 and 144 hours after stress imposition. Mean of two replicates and 20 genotypes.

Time of Stress (hours)	Xylem Water Potential (- bars)
0	5.9
24	6.2
144	3.3
Mean	5.2
LSD (p=.05)	0.4**

Figure 4.

Free proline (mg/g dry weight) in the first leaf of stressed (▲) and unstressed (●) plants of 20 barley genotypes 24, 72 and 144 hours after stress imposition.


13
11
9
7
5
3
1
FREE PROLINE (mg/g dry weight)
24
72
144
HOURS OF STRESS

accumulation of free proline after the imposition of stress was neither as rapid nor as consistent as expected and after 72 hours many genotypes had not accumulated significant amounts. After 144 hours, however, proline had accumulated to levels approaching those reported by Singh *et al.* (1972) (Table 10).

Proline level after 144 hours of stress was not related to the xylem water potential of each genotype. Neither was there a relationship between proline accumulated in this experiment and in that reported by Singh *et al.* (1972) for the 10 common genotypes. They exposed seedlings to PEG of -20 bars osmotic potential for 72 hours but found that water potential fell more quickly and proline accumulated more rapidly than occurred here.

Leaf survival was measured by the percentage of the first leaf that remained green and obviously healthy 72 hours after the relief of stress. There was no measurable senescence in the unstressed plants of any genotype but a proportion of the first leaf was necrotic in all previously stressed plants. The genotypes varied significantly in the percentage of leaf which remained viable, varying from 48% in Greenough to 75% in CPI 18197 (Table 11).

The number of tillers, number of leaves, and plant height were all significantly reduced by stress (Table 11). The genotypes were significantly different for all three characters in both the well watered and stress treatments. There was also a significant genotype x stress interaction, indicating that the genotypes differed in their reaction to the stress and recovery treatment. It is this interaction that is of particular interest in this study.

To compare the genotypes for their reaction to the stress but allowing for differences between them in their non-stress values, the stressed means for each genotype were expressed as a percentage of their

Table 10.

Free proline (mg/g dry weight) in the first leaf of twenty barley genotypes, 72 and 144 hours after stress imposition. Mean of three stressed replicates.

	Time after stress imposition (hours)	
	72	144
Genotype	Free proline	
Arivat	1.12	7.7
Bankuti Korai	0.92	15.1
BR 1239	1.63	16.4
CI 3576	1.62	8.2
Clipper	5.21	11.9
CPI 18197	1.70	12.0
Cyprus Black	0.99	6.8
Dore	2.43	21.1
Excelsior	0.41	6.3
Greenough	2.68	10.5
Hiproly	0.62	11.1
Ketch	0.40	16.0
Maraini	0.62	9.4
Mona	0.60	13.6
Princess	2.67	18.3
Prior A	0.29	11.8
Proctor	0.66	16.2
Stewart	0.32	6.3
Velvon II	0.56	11.4
Zephyr	2.97	14.2
Mean	1.42	12.2
LSD (p=.05)	n.s.	6.6*

Table 11.

Leaf survival in stressed leaves and number of tillers, number of leaves and plant height in the stressed and unstressed plants of twenty barley genotypes, measured 72 hours after stress relief. Mean of two replicates.

Genotype	Leaf survival %	Tillers/plant		Leaves/plant		Plant height (cm)	
		Control	Stress	Control	Stress	Control	Stress
Arivat	66	3.6	3.3	10.2	9.4	44.4	29.4
Bankuti Korai	61	3.6	2.3	11.4	7.3	56.5	34.6
BR 1239	70	3.1	2.2	8.0	7.2	48.9	30.7
CI 3576	67	3.8	2.9	11.4	8.9	46.1	31.5
Clipper	60	2.6	2.7	10.3	9.1	52.6	29.2
CPI 18197	75	3.4	3.0	10.9	9.1	48.0	32.6
Cyprus Black	70	3.7	2.9	11.6	9.1	52.8	31.5
Dore	66	4.6	3.0	13.5	9.0	46.3	31.0
Excelsior	61	2.7	3.0	8.8	9.1	52.9	31.1
Greenough	48	3.1	1.5	10.0	6.3	56.0	36.4
Hiproly	66	3.3	2.7	10.3	7.2	52.7	32.2
Ketch	59	3.0	2.5	11.7	9.1	48.8	29.8
Maraini	72	3.2	3.4	9.3	9.9	36.8	26.6
Mona	56	4.2	2.4	13.4	8.4	52.6	31.4
Princess	60	4.1	2.5	13.1	7.6	50.3	29.2
Prior A	55	3.6	2.8	11.2	9.7	49.3	30.7
Proctor	64	3.5	2.7	10.8	8.1	48.3	29.7
Stewart	70	3.9	3.5	10.3	10.9	49.4	29.2
Velvon II	65	3.5	2.8	10.7	7.6	49.3	30.2
Zephyr	59	3.2	2.0	9.9	7.6	48.4	30.5
Mean	63	3.5	2.7	10.8	8.5	49.6	30.9
LSD (p=.05)	11.9*	0.6**	0.6**	1.6**	1.5**	4.1**	3.5*

Combined Analysis of variance:	LSD	LSD	LSD
Source of variation:			
Between genotype means	0.4	1.05**	2.57**
Between stress and control	0.12**	0.33**	0.81**
Genotype x stress	0.56**	1.49**	3.64**

unstressed control means (Table 12). Thus Tiller Percent (TP) was defined as

$$\text{Tiller Percent} = \frac{\text{Tiller number (mean of 2 reps.) of ith genotype when stressed}}{\text{Tiller number (mean of 2 reps.) of ith genotype when not stressed}} \times 100$$

Leaves Percent (LP) and Height Percent (HP) were similarly defined.

TP, LP and HP were calculated for each genotype from the mean values for stressed and control plants rather than from the individual replicates. Pairing of replicates from control and stressed treatments for such calculations would have been necessarily arbitrary and could not be used to provide an estimate of error for these derived quantities. The values given were intended to be used as a guide for further work.

Leaf survival (63%) (Table 11) and height (62%) (Table 12) were more influenced by the stress than were tiller number (77%) and leaf number (77%). There appeared to be some compensatory growth on the relief of stress in tiller number and leaf number, particularly for some genotypes where the stressed treatments had more tillers and leaves than did the controls.

A correlation matrix for all four characters, together with free proline and xylem water potential after 144 hours of stress revealed only four significant correlations (Table 13).

The high correlation between TP and LP is indicative of the relationship between leaf number and tiller number in such young plants. Percentage leaf survival was significantly correlated with TP and positively, although not significantly, related to LP and HP. This would indicate, but not prove, that tiller production or survival and leaf survival were similarly affected by the stress treatment. The significant negative interactions between free proline and TP or LP

Table 12.

Tiller Percent (TP), Leaves Percent (LP) and Height Percent (HP) in 20 barley genotypes measured 72 hours after relief of stress.

Genotype	TP	LP	HP
Arivat	92	92	66
Bankuti Korai	64	64	61
BR 1239	71	90	63
CI 3576	76	78	68
Clipper	104	88	56
CPI 18197	88	83	68
Cyprus Black	78	78	60
Dore	65	67	67
Excelsior	111	103	59
Greenough	48	63	65
Hiproly	82	70	61
Ketch	83	78	61
Maraini	106	106	73
Mona	57	63	60
Princess	61	58	59
Prior A	78	87	63
Proctor	77	75	62
Stewart	90	106	59
Velvon II	80	71	61
Zephyr	63	77	64
Mean	77	77	62

Table 13.

Correlation matrix for TP, LP, HP and leaf survival 72 hours after stress relief and Ψ(xylem) and free proline measured 144 hours after stress imposition. The genotype means for free proline were calculated over the two replicates used to measure the other characters.

	TP	LP	HP	Leaf Survival	Free proline
LP	.86**				
HP	.01	.16			
Leaf survival	.47*	.38	.34		
Free proline	-.50*	-.59**	-.01	-.28	
Ψ(xylem)	.24	.11	.38	.03	.12

were not consistent with the hypothesis that proline is of value to the plants either during the stress or recovery period (Singh, 1970). Similarly, there was no significant correlation between free proline after 144 hours of stress and leaf survival 72 hours after re-watering, although one was recorded by Singh *et al.* (1973d).

Experiment 2. Osmotic stress of barley seedlings - glasshouse

Cultural and stress conditions were similar to those used for Experiment 1 except that plants were grown in a glasshouse. The experiment aimed to compare the genotypes for their ability to accumulate proline after 72 hours of stress. Unstressed controls were not included as they could not provide useful additional information but there were four stressed replicates. Stress was not relieved at the conclusion of the 72 hour stress period.

Xylem water potential was again less negative than was to be expected from bathing the roots in a solution of -20 bars osmotic potential (Table 14), although mean xylem water potential after 72 hours of stress in this experiment was lower than after 144 hours in Experiment 1. Genotypes were not significantly different, however, and genotype ranking did not correlate with that in Experiment 1.

Free proline had accumulated to some extent in all genotypes although it was lower after 72 hours in this experiment (Table 14) than it was after 144 hours in Experiment 1 (Table 10). Genotypes were not significantly different for free proline content. Despite the lack of significance of the results, the free proline contents of the twenty genotypes in Experiments 1 and 2 were compared (Figure 5). The correlation coefficient ($r=0.53^*$) was not large but was significant indicating some similarity in genotype reaction.

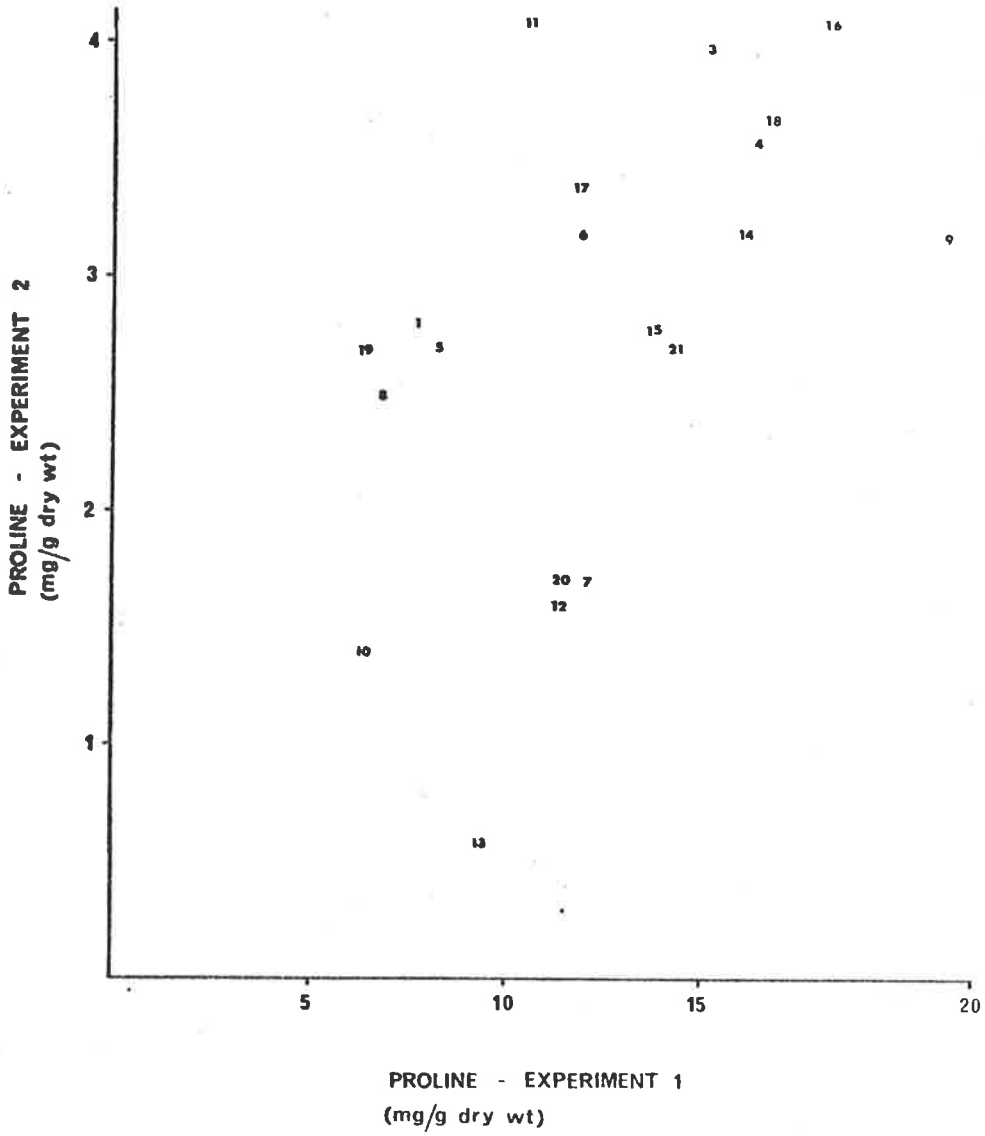
Table 14.

Xylem water potential (- bars) and free proline (mg/g dry weight) measured in the first leaf of 20 barley genotypes 72 hours after stress imposition. Mean of four replicates.

Genotype	Ψ(xylem)	Free proline
Arivat	14.1	2.8
Bankuti Korai	13.4	4.0
BR 1239	12.7	3.6
CI 3576	13.2	2.7
Clipper	13.6	3.2
CPI 18197	14.0	1.7
Cyprus Black	13.7	2.5
Dore	12.9	3.2
Excelsior	12.3	1.4
Greenough	12.1	4.1
Hiproly	13.6	1.6
Ketch	13.0	3.2
Maraini	12.8	0.6
Mona	13.8	2.8
Princess	13.0	4.1
Prior A	12.5	3.4
Proctor	12.8	3.8
Stewart	11.9	2.7
Velvon II	12.0	1.7
Zephyr	14.0	2.7
Mean	13.1	2.9
LSD (p=.05)	n.s.	n.s.

Figure 5.

Free proline (mg/g dry weight) accumulated by 20 barley genotypes when stressed with Polyethylene glycol for 144 hours in Experiment 1 compared with the free proline they accumulated after 72 hours in Experiment 2. For the key to genotypes see Table 2, p. 41. The correlation coefficient for the relationship is $r = 0.53^{*}$.


PROLINE - EXPERIMENT 2
(mg/g dry wt)
4
3
2
1
5
10
15
20
PROLINE - EXPERIMENT 1
(mg/g dry wt)
11
16
3
18
4
17
6
14
9
1
19
5
15
21
8
20
7
12
10
13

These results did not agree with those of Singh *et al.* (1972). The results were not comparable, however, as Singh and co-workers achieved more severe levels of stress and did not record differences in Ψ_w at the conclusion of the stress period. Stress was not severe in Experiment 1 of this study but roots were exposed to PEG over a longer period and uptake of the osmoticum cannot be discounted. Variability between replicates of the same genotype were high for Ψ(xylem) and free proline in both experiments and there were differences between genotypes for Ψ(xylem) in Experiment 1 which probably also contributed to variability in free proline. These experiments did not provide sufficient evidence to determine if the variability measurement of free proline was due to differences between genotypes in exclusion of the osmoticum, differences in Ψ_w, problems in sampling or variability in the analysis of proline. The experiments therefore prompted further work where stress was applied by withholding water.

Experiment 3. Seedlings stressed by withholding water - growth cabinet

This was a preliminary experiment to examine the water potential of the leaves and the free proline accumulated by the 20 genotypes used in the previous experiment in response to a stress imposed by withholding water. The experiment was conducted in a growth cabinet but was limited by available space to three replicates all of which were subjected to water stress.

Ψ(xylem) and free proline results are summarised in Table 15. Five days after withholding water mean Ψ(xylem) of the twenty genotypes had fallen to -19.1 bars. One day later mean Ψ(xylem) was -22.4 bars. There were no unstressed controls for comparison but the Ψ(xylem) of control plants grown under similar conditions in Experiment 1 varied

Table 15.

Xylem water potential (- bars) and free proline (mg/g dry weight) measured in the first leaf of 20 barley genotypes five and six days after withholding water. Mean of three replicates.

Genotype	Ψ(xylem)			Free Proline		
	Day 5	Day 6	Mean	Day 5	Day 6	Mean
Arivat	19.9	24.6	22.3	6.7	19.4	13.0
Bankuti Korai	18.9	23.3	18.9	6.8	17.8	12.3
BR 1239	18.4	21.9	18.4	7.1	17.8	12.5
CI 3576	20.3	24.7	22.5	11.2	14.7	12.9
Clipper	20.1	21.2	20.6	10.6	16.3	13.4
CPI 18197	22.4	24.0	23.2	8.2	14.0	11.1
Cyprus Black	20.9	23.8	22.3	9.0	14.9	12.0
Dore	18.1	21.6	19.9	6.6	15.5	11.1
Excelsior	21.2	29.4	25.3	8.2	17.7	13.0
Greenough	17.9	20.6	19.2	5.3	14.9	10.1
Hiproly	14.6	18.6	16.6	5.7	15.4	10.6
Ketch	19.7	20.9	20.3	7.1	17.2	12.2
Maraini	17.1	22.8	20.0	4.2	12.3	8.3
Mona	19.2	20.7	19.9	7.8	17.4	12.6
Princess	19.4	21.5	20.4	6.8	15.3	11.1
Prior A	19.8	23.5	21.6	9.7	17.8	13.8
Proctor	18.9	21.3	20.1	9.6	16.6	13.1
Stewart	19.1	21.8	20.4	5.6	15.7	10.6
Velvon II	16.8	20.6	18.7	5.4	18.4	11.9
Zephyr	19.0	21.8	20.4	8.0	20.5	14.3
Mean	19.1	22.4	20.8	7.5	16.5	12.0
LSD (p=.05) (for analysis within days)	n.s.	n.s.	2.5**	n.s.	n.s.	n.s.

Combined Analysis (LSD p=.05)

Source of Variation		
Genotype	2.5**	n.s.
Time	0.8**	1.4**
Genotype x time	n.s.	n.s.

between -3.3 and -6.2 bars. Genotypes differed for Ψ(xylem) in a combined analysis but there was no significant interaction with time. Genotype differences for Ψ(xylem) could reflect differences in the rate of water removal from the small pots or differences in water potential at the same soil water status but there was insufficient evidence to determine which mechanism was most important. The differences between genotypes for Ψ(xylem) in this experiment were not related to those in Experiment 1.

Free proline content of the stressed plants five days after withholding water averaged 7.5 mg/g dry weight. There were no unstressed controls for comparison but it is usual for proline content to be less than 1 mg/g dry weight in leaves of unstressed plants. Free proline had increased to 16.5 mg/g dry weight by the sixth day. The genotypes did not differ significantly for free proline content but variability between replicates of the same genotype was high.

Five days after withholding water there was a highly significant positive correlation between free proline content and xylem water potential when calculated over the three replicates and twenty genotypes, reflecting a relationship between the two parameters. There was no such relationship on the subsequent day, however. There is no strict control over the timing of stress imposed by withholding water. Proline accumulation would most likely have commenced in each plant when water potential (or the osmotic component of potential) fell below a critical level (Chu *et al.*, 1974). A correlation between Ψ(xylem) and free proline would be obtained if genotypes did not vary significantly for this characteristic. No relationship would be expected on the sixth day at greater stress levels, however, if genotypes accumulated proline at different rates after the critical water potential. Genotypes remained not significantly different on the sixth day when free proline was analysed after adjustment for differences in Ψ(xylem). Similarly,

a comparison of the accumulation from day five to day six did not reveal a significant trend.

The experiment did demonstrate the general ability of a range of barley genotypes to accumulate proline when stressed by withholding water and the free proline levels in this experiment were similar to those reported by Singh *et al.* (1973d) for seedlings stressed osmotically. The data was not sufficiently accurate, however, to allow a comparison of the proline accumulated between the two days of measurement and a glasshouse experiment was designed to further investigate possible genotypic differences in response to stress.

Experiment 4. Seedlings stressed by withholding water - glasshouse

Ten genotypes were stressed by withholding water at the three-leaf stage. The experiment was conducted in a glasshouse to provide sufficient plants of each genotype for measurements of leaf water status, proline accumulation and plant performance. The pots were re-watered five days after withholding water and the plant characteristics were recorded after a further five days. Despite the warning of Corletto and Laude (1974) that a recovery period of five days may not be sufficient, recovery was limited to this period by the small pot size which would have restricted growth if the experiment had been extended.

A consistent nomenclature has been used to describe the water treatment, sampling time combination used in this experiment. Thus; 0N is the unstressed control plants on day 0, just prior to withholding water;

2S, 3S, 4S and 5S - stressed plants sampled, 2, 3, 4 and 5 days after withholding water;

5N and 10N - unstressed controls sampled on the days corresponding to 5 and 10 days after withholding water in the stressed treatments;

10R - previously stressed plants sampled 10 days after withholding water but 5 days after re-watering.

Xylem Water Potential

The change in xylem water potential in response to the stress and recovery treatment is illustrated in Figure 6. The Ψ(xylem) of the stressed plants was significantly lower than that of the controls two days after withholding water. It decreased rapidly for the next three days and five days after withholding water was -29.3 bars compared to -7.2 bars in the control pots. Five days after re-watering however, the Ψ(xylem) of previously stressed plants was not significantly different from that measured in the controls.

Genotypes were significantly different in separate analyses for 4S but not 5S (Table 16). There was some tendency for the leaves to break within the seal of the pressure chamber during measurement on the fifth day and this resulted in greater error of measurement. The combined data was significantly skewed and was transformed to logarithmic values before analysis. Genotypes and water treatments were different but there was no interaction between them. Genotypes therefore tended to maintain ranking for Ψ(xylem) whether stressed or not. This is supported by a significant correlation between the genotype means for Ψ(xylem) in the stressed and the unstressed pots ($r=0.78^{**}$ between the genotype means calculated over treatments 2S, 3S, 4S and 5S against those calculated over 0N, 5N and 10N).

In addition, means for Ψ(xylem) in the stressed treatments of this experiment and in Experiment 3 were significantly correlated ($r=0.7^{*}$) for the nine common genotypes which suggests that genotype differences were a real effect. This may be due to real differences in water status or due to an interaction between genotype and method of measurement.

Figure 6.

Xylem water potential in the first leaf of stressed (▲) and unstressed plants (●) over a five day period after withholding water and five days after re-watering. Mean of eight genotypes and three replicates.

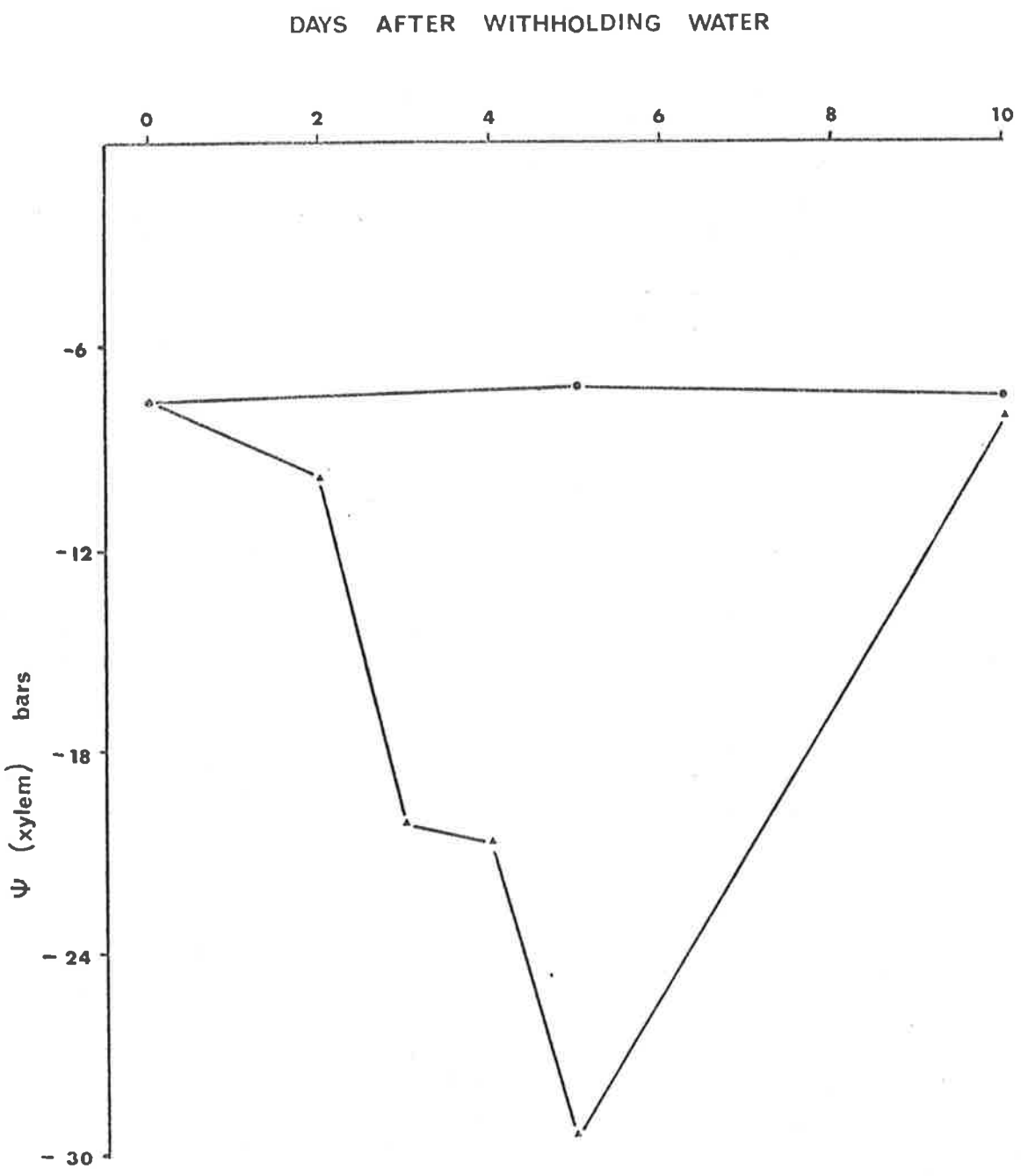

DAYS AFTER WITHHOLDING WATER
0
2
4
6
8
10
-6
-12
-18
-24
-30
Ψ (xylem) bars

Table 16.

Xylem water potential (- bars) in all water treatments and genotypes in Experiment 4. Mean of three replicates. Water treatment 0N is the measurement before imposition of the treatments: 2S, 3S, 4S and 5S are the stressed treatments 2, 3, 4 and 5 days after withholding water respectively; 5N and 10N are the unstressed treatments corresponding to 5 and 10 days after withholding water; 10R is the re-watered treatment 5 days after re-watering.

Combined analysis (after log. transformation)

Source of Variation	LSD (p=0.5)
Genotype	0.041**
Water Treatment	0.046**
Genotype x Water Treatment	n.s.

Table 16.

Genotype	ON	5N	10N	10R	2S	3S	4S	5S	Mean	Mean (log)
Asahi 2	7.7	7.7	8.5	9.4	12.2	23.5	23.2	30.8	15.4	1.114
Bankuti Korai	6.9	6.3	6.9	6.7	7.8	18.3	21.7	26.0	12.6	1.022
CI 3576	8.1	7.9	8.6	8.8	12.4	20.0	19.9	28.8	14.3	1.102
Clipper	8.2	6.8	6.9	7.3	8.7	20.7	18.8	31.1	13.6	1.055
Cyprus Black	7.5	7.2	7.5	8.0	11.4	19.9	17.9	29.9	13.7	1.070
Dore	7.3	7.6	6.4	9.3	9.0	17.4	18.2	31.2	13.3	1.056
Excelsior	8.3	7.2	7.7	7.9	10.3	23.6	24.6	33.3	15.4	1.106
Maraini	8.0	7.2	6.7	7.9	9.0	17.7	20.2	26.0	12.8	1.043
Proctor	7.9	7.5	7.3	8.0	9.5	20.2	23.7	31.0	14.4	1.081
Stewart	7.2	6.4	7.6	7.2	8.1	19.0	17.0	24.9	12.3	1.027
Mean	7.7	7.2	7.4	8.1	9.8	20.1	20.6	29.3	13.8	
LSD (p=.05)	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	4.7*	n.s.		
* Mean (log)	0.884	0.853	0.866	0.900	0.977	1.289	1.307	1.465		1.068

* Mean (log) refers to the means of the data when logarithmically transformed i.e. the means relating to the analysis of the logarithmically transformed data.

Bankuti Korai and Stewart tended to have higher, while Excelsior, Asahi 2 and Cyprus Black tended to have lower xylem water potential. The results of Hansen *et al.* (1977) suggest that Proctor should have been more stressed than Excelsior but this was not the case.

Relative Water Content (RWC)

RWC was measured on selected days to provide further information on water status. It followed a similar pattern to Ψ(xylem) (Table 17). Mean RWC (over all genotypes) was reduced over the stress period to 62.9% five days after withholding water. Five days after re-watering, however, RWC of previously stressed plants had returned to the level of the unstressed controls.

The genotypes varied significantly for RWC in the combined analysis but only at 5S in individual analyses. There was not a significant interaction between genotype and water treatment. The correlation between the genotype means for the stress and control treatments was not significant but there may have been greater error associated with the control estimate as it was measured only once.

Relationship Between Ψ(xylem) and RWC

The relationship between Ψ(xylem) and RWC, termed the moisture release curve (Jones and Turner, 1978), of Figure 7 was constructed using all available genotype and replicate points for the treatments 2S, 3S, 4S, 5S and 10N. The 10R results were excluded as they may have introduced additional variability due to incomplete catabolism of osmotic components. There was considerable spread in the points at high stress levels but variation in RWC explained 88% of the variation in Ψ(xylem). This does not eleminate the possibility of genotype differences in the nature of the curve, however.

Table 17.

Relative Water Content (%) measured in 10 genotypes and 6 water treatments in Experiment 4. Mean of 3 replicates.

	Water Treatment						
Genotype	10N	10R	2S	3S	4S	5S	Mean
Asahi 2	95.3	96.9	95.1	71.4	71.5	55.1	79.0
Bankuti Korai	95.4	97.0	95.1	83.1	73.8	61.7	83.7
CI 3576	94.1	96.6	97.0	77.6	74.6	62.5	82.9
Clipper	97.1	97.4	96.1	83.0	78.6	69.4	85.7
Cyprus Black	97.8	97.5	94.4	79.2	78.4	62.6	84.2
Dore	94.0	96.1	96.9	80.4	80.8	59.0	83.8
Excelsior	95.7	95.4	93.1	81.0	73.6	61.9	82.7
Maraini	95.5	97.5	95.1	80.1	76.0	67.3	85.2
Proctor	94.1	96.0	94.1	79.4	68.7	60.6	81.4
Stewart	95.5	97.1	96.8	84.3	84.0	68.8	86.3
Mean	95.4	96.7	95.2	80.1	75.7	62.9	83.5
LSD (p=.05)	1.5*	n.s.	n.s.	n.s.	n.s.	8.9*	

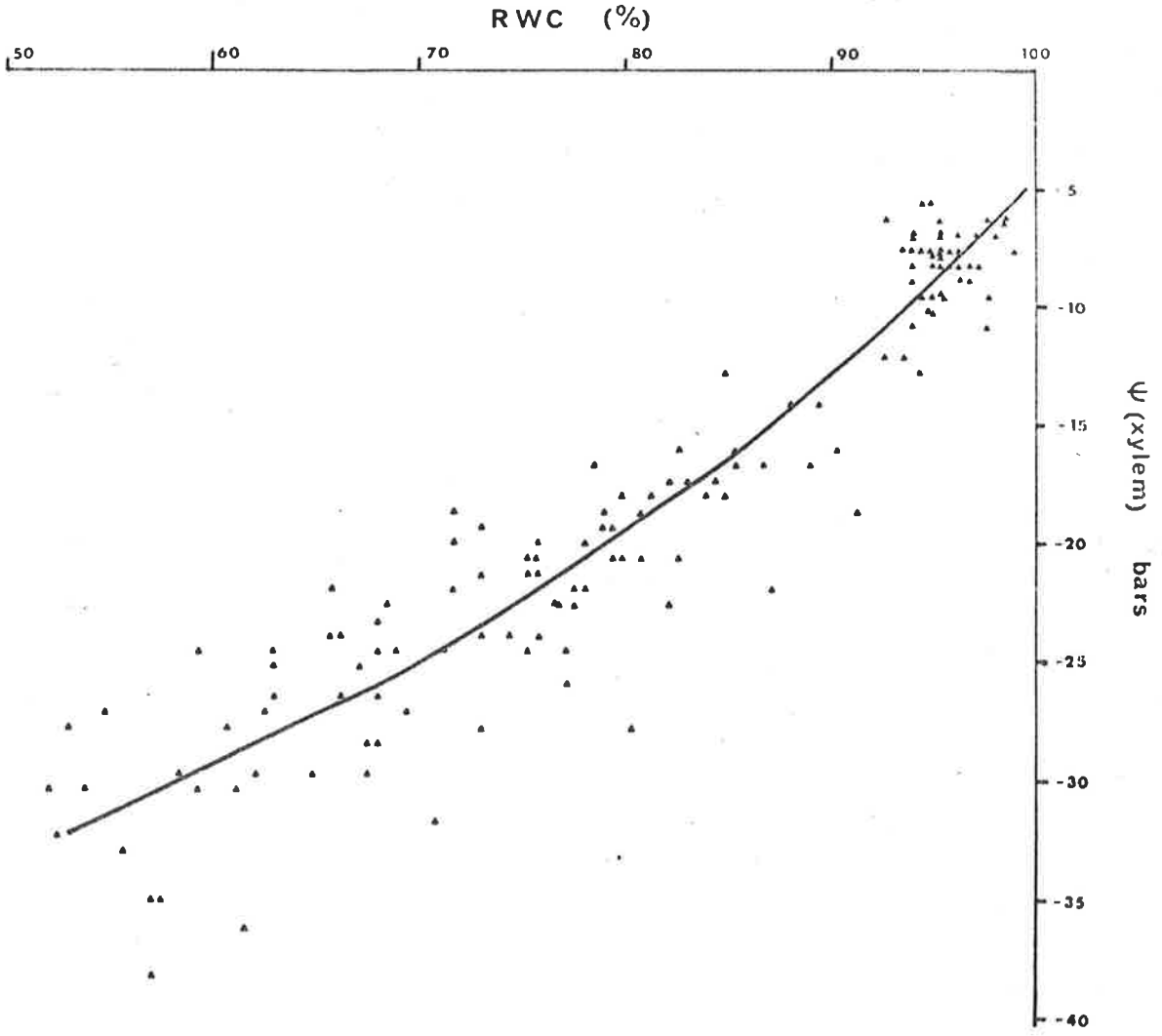
Combined analysis

Source of variation	LSD (p=.05)
Water treatment	2.8**
Genotype	2.7**
Genotype x water treatment	n.s.

Figure 7.

Relationship between xylem water potential (bars) and Relative Water Content (%) for n = 140 from treatments 2S, 3S, 4S, 5S and 10N in Experiment 4.

$\Psi(\text{xylem}) = + 0.0068\ RWC^2 - 0.477\ RWC - 24.96\ (R^2 = 0.88)$.


RWC (%)
50
60
70
80
90
100
-5
-10
-15
-20
-25
-30
-35
-40
ψ (xylem) bars

There were only 15 possible points (3 replicates and 5 water treatments) for comparing RWC and Ψ(xylem) within genotypes and the failure to achieve a reading for Ψ(xylem) at 5S for all replicates of all genotypes reduced the number of points.

It was possible to demonstrate a non-linear relationship for only four of the genotypes. To fully define the curve it would have been necessary to take readings at higher Ψ(xylem) values (e.g. pre-dawn) but the objective of this comparison was to compare the two parameters at greater stress levels.

The genotypes did not vary significantly for the linear regression coefficient (Table 18) and the lines were not significantly displaced. That there was no interaction between genotype and method of water status measurement is supported by the highly significant correlation between the genotype means for RWC and Ψ(xylem) measured five days after withholding water (r=0.79**).

The results do indicate that the genotype differences in water status measured in this experiment (Tables 16 and 17) were a real effect and not associated with the method of measurement. These differences may indicate stress avoidance but it is noteworthy that genotype ranking tended to be maintained even in the absence of stress.

Water Use

Genotypes differed significantly in the quantity of water used during the stress period as measured by the change in pot weight (Table 19). This crude measure includes evaporation from the soil surface as well as transpiration losses from the leaves. No attempt was made to mulch the surface so evaporation may have been high. In addition, the change in pot weight would have been affected by the gain in plant dry weight but this was small relative to water lost. The

Table 18.

Number of points (n), linear regression coefficient (b), linear correlation coefficient (r), significance of fitting a non-linear equation and $100R^2$ for the relationship between Ψ(xylem) and RWC in 10 barley genotypes.

Genotype	n	b	r	Signif. non-linear	$100R^2$
Asahi 2	14	0.51	0.94**	n.s.	89
Bankuti Korai	14	0.55	0.94**	**	95
CI 3576	15	0.56	0.96**	n.s.	92
Clipper	15	0.66	0.95**	n.s.	90
Cyprus Black	14	0.59	0.96**	n.s.	93
Dore	15	0.63	0.98**	n.s.	95
Excelsior	14	0.63	0.90**	*	90
Maraini	15	0.57	0.94**	**	95
Proctor	15	0.62	0.94**	n.s.	88
Stewart	12	0.58	0.96**	*	96
All	143	0.59	0.94**	**	90

Table 19.

Water used (ml) by pots of 10 barley genotypes during a stress period of 5 days. Mean of 3 replicates. The pots contained 5 plants.

Genotype	Water Used (ml)
Asahi 2	61.0
Bankuti Korai	73.7
CI 3576	71.0
Clipper	82.0
Cyprus Black	69.3
Dore	62.7
Excelsior	66.7
Maraini	72.7
Proctor	73.0
Stewart	79.5
Mean	70.9
LSD (p=.05)	10.9*

quantity of water lost during the five day stress period ranged from 61.0 ml for Asahi 2 to 82.0 ml for Clipper.

Stomatal Frequency and Size

Stomatal characters may influence water relations or water loss during the stress period (Miskin *et al.*, 1972). Leaf impressions for measurement of stomatal frequency and size were taken from the first leaf of three plants and the second and third leaf of one plant on day 0, just prior to the stress period.

The stomatal frequency varied between the leaves of the same plant but not between the same leaves of different plants (Table 20).

The overall correlation (3 reps x 10 genotypes x 5 leaves) between stomatal frequency and stomatal length was highly significant but not large ($r = -0.57^{**}$). The correlation was higher when calculated on the genotype means ($r = -0.76^{*}$), thus supporting the finding by Miskin and Rasmusson (1970) that the frequency and length of stomata were negatively correlated in another set of barley genotypes. The correlation in this experiment was established despite the fact that the genotypes were not significantly different for either frequency or length (Table 21). There was no significant interaction between genotype and leaf (either the same leaves of different plants or different leaves of the same plant).

Stomatal frequency or length was not related to water used during the stress period. Other relationships will be discussed later.

Proline Accumulation

Free proline accumulated in the first leaf of all genotypes

Table 20.

Stomatal frequency (number per microscope field and per mm^2) and stomatal length (ocular units and mm) on the first, second and third leaf of barley plants. Mean of 3 replicates and 10 genotypes.

Plant	Leaf	Frequency		Length	
		per field	per mm^2	ocular units	mm
1	1	72.3	29.0	17.8	0.045
1	2	67.3	27.0	20.2	0.051
1	3	58.6	23.5	19.1	0.048
2	1	72.6	29.2	17.7	0.044
3	1	74.2	29.8	17.9	0.045
LSD (p=.05)		5.2**	2.1**	0.7**	0.002**

Table 21.

Stomatal frequency and length of 10 barley genotypes. Mean of 3 replicates and 5 leaves.

Genotype	Frequency		Length	
	per field	per mm^2	ocular units	mm
Asahi 2	70.9	28.5	17.6	0.044
Bankuti Korai	69.5	27.9	18.1	0.045
CI 3576	68.6	27.6	18.3	0.046
Clipper	71.4	28.7	18.9	0.047
Cyprus Black	69.4	27.9	18.6	0.047
Dore	71.9	28.9	17.8	0.046
Excelsior	78.0	31.3	18.2	0.046
Maraini	64.8	26.0	19.9	0.050
Proctor	63.3	25.4	18.5	0.046
Stewart	56.3	22.6	20.8	0.052
LSD (p=.05)	n.s.	n.s.	n.s.	n.s.

over the five day stress period. Accumulation had commenced after the second day and by the fifth day had reached levels nearly one hundred times those in the unstressed controls (Figure 8). It was surprising, however that the free proline content (mean of all genotypes) had increased rather than decreased five days after re-watering.

When analysed within sampling days, the genotypes did not differ significantly at any time other than the fifth day after withholding water and five days after re-watering (Table 22).

The data was transformed to logarithmic values to induce normality prior to performing a combined analysis on treatments 2S, 3S, 4S, 5S and 10R. The genotypes and water treatments were both highly significant but there was no significant interaction. Proline content in the stressed treatments was significantly greater each day than the previous day.

The significant differences in proline accumulated by the genotypes as a result of stress could be influenced by the difference in water status. If this were true, the relationship between free proline and water status may have been the same for all ten genotypes. This possibility was examined by calculating the regression between proline and water status, measured as either RWC or Ψ(xylem), for each genotype, ignoring the replicate structure and the influence of sampling on different days.

There was a significant linear relationship between proline and RWC within all ten genotypes (Table 23), the variation in RWC accounting for between 58% and 90% of the variation in proline. The regression coefficients ranged between -0.3 (Maraini) to -1.002 (Cyprus Black (Figure 9). The regression coefficients were highly significantly different, indicating that the differences in free proline accumulation in the genotypes were not solely due to differences in water status.

Figure 8.

Free proline (mg/g dry weight) in the first leaf of barley seedlings 0, 2, 3, 5 and 10 days after withholding water (▲) and in the control plants corresponding to days 0, 5 and 10 (●). Stressed plants were re-watered on day 5. Mean of eight genotypes in three replicates.

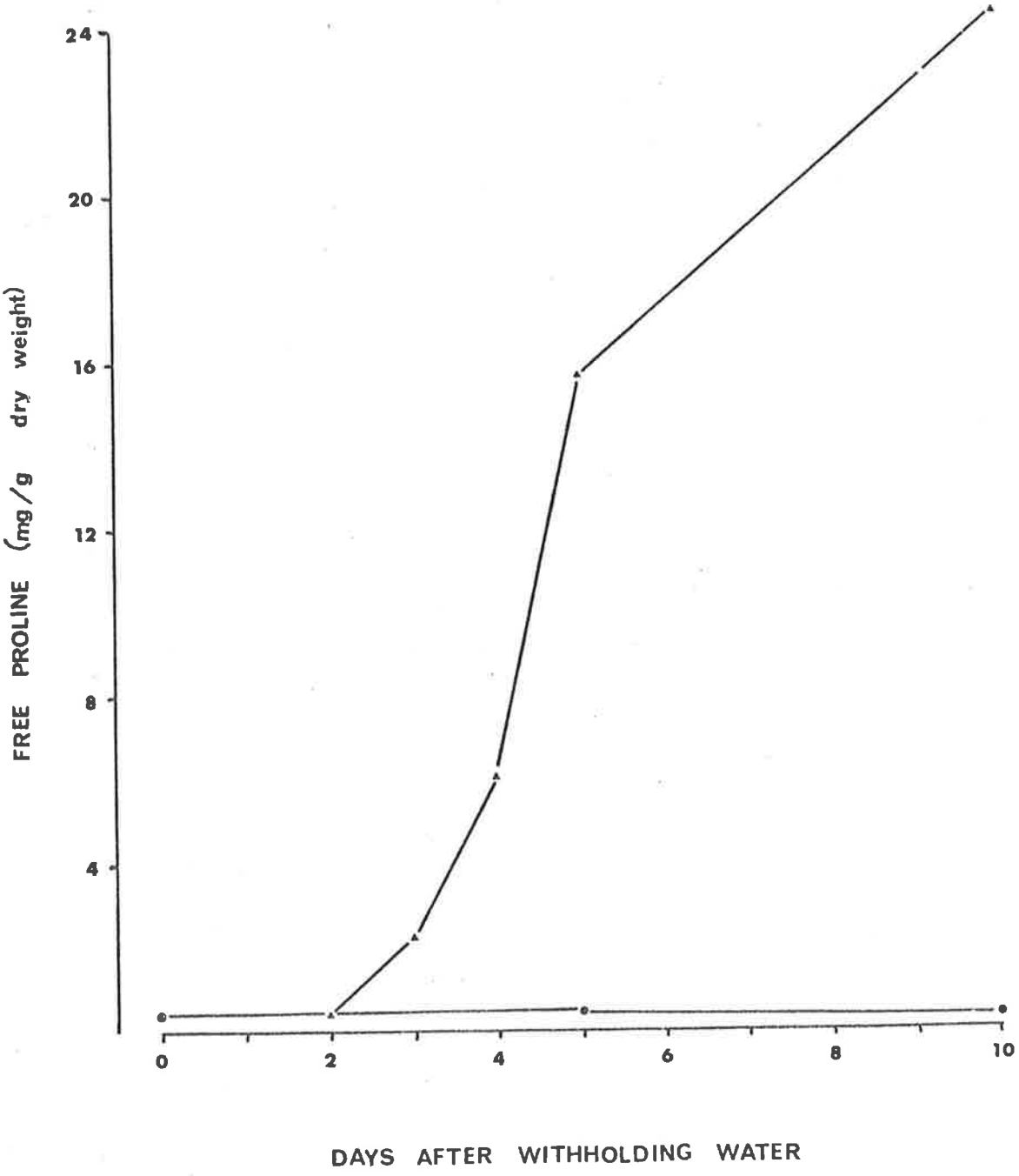

24
20
16
12
8
4
FREE PROLINE (mg/g dry weight)
0
2
4
6
8
10
DAYS AFTER WITHHOLDING WATER

Table 22.

Free proline (mg/g dry wt.) in the first leaf of 10 barley genotypes $2^{(2S)}$, $3^{(3S)}$, $4^{(4S)}$ and $5^{(5S)}$ days after withholding water and 5 days after re-watering (10R). Mean of 3 replicates. Also the genotype and treatment means after logarithmic transformation.

	Treatment						
Genotype	2S	3S	4S	5S	10R	Mean	Mean (log)
Asahi 2	.32	3.26	6.63	13.20	24.32	9.55	0.81
Bankuti Korai	0.38	3.18	12.49	22.01	33.05	14.22	0.94
CI 3576	0.39	4.26	7.83	12.97	27.30	10.55	0.85
Clipper	0.45	2.65	4.66	16.90	17.46	8.42	0.74
Cyrpus Black	0.44	2.66	4.21	19.52	36.99	12.76	0.85
Dore	0.44	1.80	3.22	22.03	19.50	9.40	0.75
Excelsior	0.40	1.33	5.85	15.16	24.06	9.36	0.78
Maraini	0.45	1.60	5.74	10.28	18.95	7.40	0.72
Proctor	0.41	0.64	7.48	15.11	22.76	9.28	0.76
Stewart	0.35	0.66	1.54	10.00	19.58	6.43	0.63
Mean	0.40	2.26	6.12	15.72	24.40	9.78	
LSD (p=.05)	n.s.	n.s.	n.s.	8.26*	11.70*	-	-
Mean (log)	0.15	0.44	0.75	1.19	1.39		0.78

Combined Analysis (after logarithmic transformation)

Source of Variation	LSD (p=.05)
Genotype	0.12**
Treatment	0.16**
Genotype x Treatment	n.s.

* Mean (log) is the mean of the logarithmically transformed data i.e. the mean relating to the analysis of logarithmically transformed data. It is not the logarithm of the raw data mean.

Table 23.

Regression equations for free proline (y) and RWC (x) where y = a + b

a = intercept on proline axis

c = intercept on RWC axis

b = regression coefficient

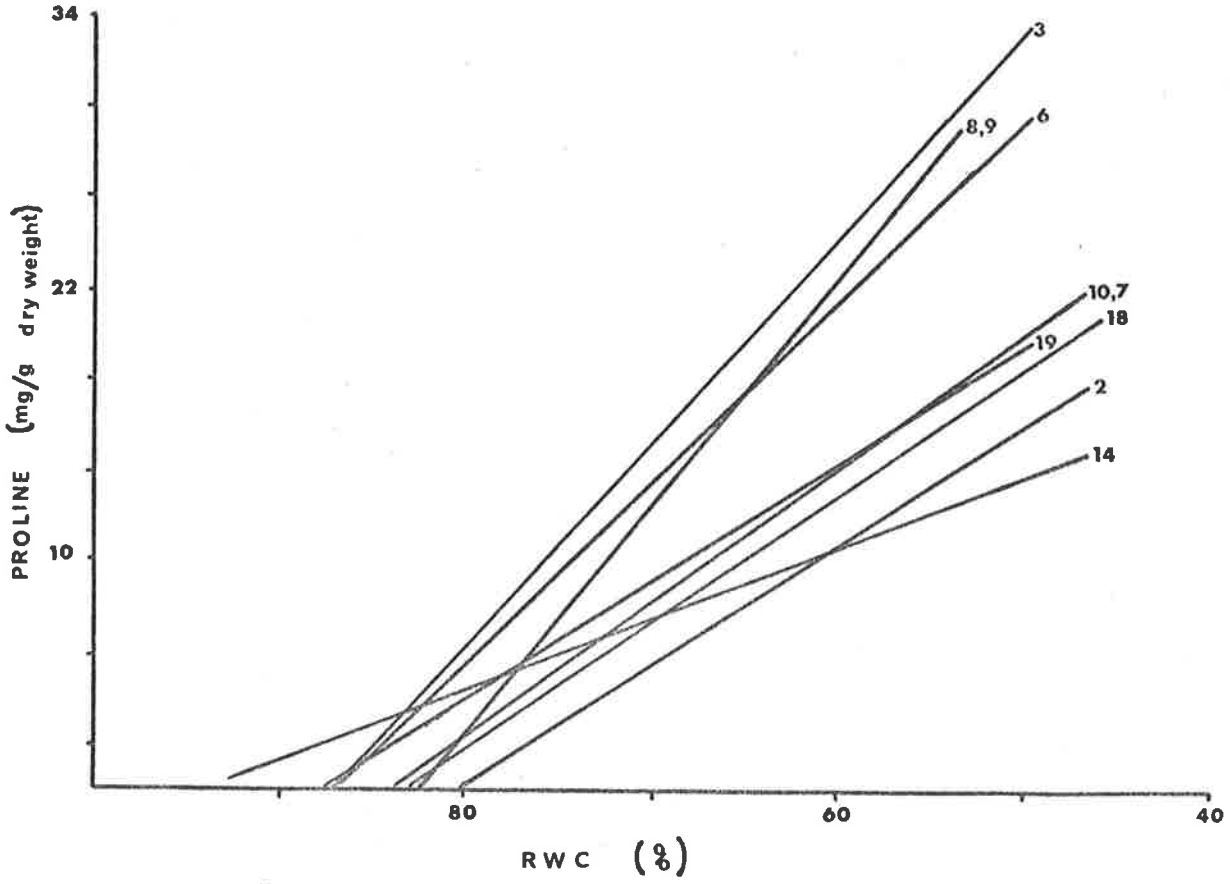
r = correlation coefficient

n = number of points used to calculate regression.

Genotype	a	c	b	r	n
Asahi 2	42.3	80.7	-0.524	-0.83**	9
Bankuti Korai	76.9	87.1	-0.883	-0.95**	9
CI 3576	44.1	88.2	-0.500	-0.87**	9
Clipper	69.4	87.2	-0.796	-0.86**	9
Cyprus Black	82.3	82.2	-1.002	-0.94**	9
Dore	79.3	82.7	-0.958	-0.94**	9
Excelsior	48.7	84.6	-0.575	-0.93**	9
Maraini	28.6	95.1	-0.301	-0.76*	9
Proctor	46.9	83.3	-0.563	-0.72*	9
Stewart	46.4	86.8	-0.535	-0.92**	7

Figure 9.

The relationship between Relative Water Content - RWC (%) and free proline (mg/g dry weight) measured in the first leaf of ten genotypes in Experiment 4. For the key to genotypes see Table 2, p. 41.


34
22
10
PROLINE (mg/g dry weight)
3
8,9
6
10,7
18
19
2
14
80
60
40
RWC (%)

The trend was similar for the relationship between proline and Ψ(xylem) (Table 24). With the exception of Asahi 2, which had a correlation coefficient of 0.58, all regressions were significant and the regression coefficients, including the one for Asahi 2, were highly significantly different.

Genotype values for three measures of proline accumulation were correlated (Table 25). The three measures were the regression coefficient between free proline and RWC, the regression coefficient between free proline and Ψ(xylem) and mean free proline content measured on day 5 (5S). Such a relationship is expected because the mean proline content on day 5 is a component of all three quantities. The relationship between proline and measures of water status provide an explanation for the variability of proline response at any particular sampling time but mean proline content in treatment 5S is still the best available measure of proline at that time.

Five days after re-watering, the proline content in the first leaf of all genotypes was high (Table 22), despite water status of the leaves having returned to normal levels. In most genotypes the proline content was higher in 10R, five days after re-watering than in 5S, at the end of the stress period. In one genotype (Dore) the proline content did marginally decrease but the increase in other genotypes ranged from 0.56 mg per g dry weight in Clipper to 14.33 mg per g dry weight in CI 3576. The percentage increase over this period ranged from -11% to 110%. This increase on the relief of stress does not agree with previous reports on the change in free proline on the relief of stress (Singh, 1970; Blum and Ebercon, 1976) and was not expected. It would have been valuable to follow the change in proline over a longer period but the experiment was terminated due to the small pot size. There are a number of possible explanations for this effect. It is possible

Table 24.

Regression coefficient (b) and correlation coefficient (r) for the relationship between free proline and Ψ(xylem) in ten barley genotypes. (n) is the number of points used to calculate the regression.

Genotype	b	r	n
Asahi 2	-0.72	n.s.	9
Bankuti Korai	-1.76	-0.79*	9
CI 3576	-0.88	-0.87**	9
Clipper	-1.41	-0.87**	9
Cyprus Black	-1.51	-0.94**	9
Dore	-1.45	-0.86**	9
Excelsior	-1.03	-0.84**	9
Maraini	-0.70	-0.84**	9
Proctor	-1.01	-0.78*	9
Stewart	-1.01	-0.78*	7

Table 25.

Correlation coefficients for three measures of proline accumulation in Experiment 4. $b_{RWC/proline}$ is the regression coefficient between RWC and free proline, $b_{\Psi(xylem)/proline}$ is the regression coefficient between Ψ(xylem) and free proline, free proline is the content measured in 5S.

	$b_{\Psi(xylem)/proline}$	free proline
$b_{RWC/proline}$	0.92**	-0.91**
$b_{\Psi(xylem)/proline}$		-0.89**

that the accumulation of proline continued for some time after stress relief and actually did fall after a lag period. It is also possible that plants did not metabolise the proline because there was no recovery, either due to unrecognised tissue mortality or a limitation of some other environmental factor.

Chlorophyll Loss

Total chlorophyll content was measured five days after withholding water in the first leaf of stressed plants and on the same day in unstressed plants. Plants in different pots of each genotype were not at the same water potential after stress and the considerable variability in total chlorophyll content included this variable. For this reason the chlorophyll content was adjusted for differences in water status prior to analysis (Steel and Torrie, 1960 - page 315) and it is these adjusted values which are presented in Table 26.

Genotypes did differ in total chlorophyll content in the absence of stress and the five days of stress reduced the content to an average of 66% of the control level. Genotypes did not differ significantly in their reaction to stress, however, with total chlorophyll content being reduced from 63.3% in Bankuti Korai to 68% in Asahi 2.

These results did differ slightly from those of Singh *et al.* (1973d) in that genotypes in this study did differ in chlorophyll content when not stressed. The results do agree, however, in showing no differences between genotypes in their reaction to stress.

Table 26.

Chlorophyll content (mg/g dry weight) measured in stressed and well watered plants of 10 barley genotypes five days after withholding water. Stressed results are presented before and after adjustment for water potential differences but means and analysis are presented only after adjustment.

Genotype	Control	Stressed		Mean
		Not Adjusted	Adjusted	
Asahi 2	13.53	10.10	9.20	11.37
Bankuti Korai	13.93	6.63	8.82	11.38
CI 3576	14.59	10.49	9.71	12.15
Clipper	15.77	12.45	11.28	13.53
Cyprus Black	13.25	7.65	8.40	10.83
Dore	15.69	6.90	10.30	13.00
Excelsior	13.88	6.32	9.11	11.50
Maraini	12.91	8.23	8.53	10.72
Proctor	15.70	10.29	10.59	13.14
Stewart	14.46	9.07	10.11	12.29
Mean	14.24	8.81	9.61	11.99

Analysis

Source of Variation	LSD (p=.05)
Genotype	1.55**
Treatment	2.32**
Genotype x treatment	n.s.

Effect of Stress and Recovery

Dry weight increase was retarded by stress (Table 27), so that the mean dry weight following five days of stress was 0.492g per five plants compared with 0.794g per five plants in the well watered treatment at the same time. Five days after re-watering, the previously stressed treatment averaged 0.715g per five plants compared with 1.078g per five plants in the controls. The water treatments were highly significantly different as were the genotypes and the interaction between genotypes and water treatment.

The relative growth rate (RGR) (Table 28) of the stressed plants over the five days of stress averaged 0.013 g/g/day compared with 0.110 g/g/day in the controls over the same period. In both cases the genotypes were significantly different. The apparent negative relative growth rate during stress could be a real effect or due to experimental error. The genotype means for RGR during stress were significantly correlated with their unstressed growth rate (r=0.75*) indicating some degree of relativity in stress and non-stress performance. This, however, does not eliminate the possibility of subtle genotypic differences during stress.

When plants were re-watered, the mean relative growth rate over the five day recovery period was 0.075 g/g/day compared with 0.060 g/g/day in the controls over the same period. This suggests some compensatory growth over the recovery period but this may have been confounded with the initial difference in size of the plants in the two treatments. When the growth rate of the stressed plants over this period of recovery (RGR10S) is compared with that of control plants of comparable initial dry weight (RGR5N) the previous stress is seen to have inhibited growth during this time.

Table 27.

Dry weight (g/5 plants) in stressed and control treatments of 10 barley genotypes 0, 5 and 10 days after stress imposition. Stressed plants re-watered on day 5. Mean of 3 replicates.

	Treatment					
Genotype	0N	5S	5N	10R	10N	Mean
Asahi 2	.483	.433	.730	.613	.853	.623
Bankuti Korai	.463	.633	.880	.933	1.573	.897
CI 3576	.423	.486	.800	.740	1.093	.709
Clipper	.460	.486	.810	.613	.973	.669
Cyprus Black	.453	.476	.793	.763	1.247	.747
Dore	.503	.493	.726	.650	1.045	.681
Excelsior	.433	.456	.760	.616	.903	.634
Maraini	.500	.500	.823	.750	1.110	.737
Proctor	.440	.466	.750	.660	.843	.632
Stewart	.490	.486	.866	.813	1.127	.757
Mean	.464	.492	.794	.715	1.078	.708
LSD (p=.05)	n.s.	n.s.	n.s.	.139**	.229*	

Combined Analysis

Source of variation	LSD (p=.05)
Genotype	0.063**
Treatment	0.056**
Genotypes with N treatments	0.141**
Treatments with N genotypes	0.143**

Table 28.

Relative growth rate (g/g/day) from days 0 to 5, the stress period and days 5 to 10, the recovery period for stressed and control treatments.

Genotype	RGR5S	RGR5N	RGR10S	RGR10N	Mean
Asahi 2	-.023	.081	.071	.033	.040
Bankuti Korai	.061	.127	.079	.117	.096
CI 3576	.027	.127	.085	.064	.076
Clipper	.009	.113	.049	.038	.052
Cyprus Black	.010	.112	.093	.089	.076
Dore	-.004	.073	.054	.084	.052
Excelsior	.011	.113	.059	.034	.054
Maraini	0	.100	.082	.060	.060
Proctor	0.011	.107	.070	.023	.053
Stewart	.114	.103	.053	.053	.067
Mean	.013	.110	.075	.060	.064
LSD (p=.05)	.041*	.036*	n.s.	.035**	

Combined Analysis

Source of Variation	LSD (p=.05)
Between Treatments	.036**
Between Genotypes	.021**
Interaction (Genotype x treatment)	n.s.

The genotypes did not differ significantly for RGR following stress relief and this is a reflection of the between replicate variability. It is interesting, however, that there was still a significant correlation (r=0.61*) between the variety means for RGR5N and RGR10S (from about the same dry weight).

The highly significant interaction for dry weight indicated that the genotypes differed in their response to the stress treatment. This interaction was investigated by calculating the Dry Weight Percentage (DWP) for each genotype. This allowed a comparison of the genotype performance under stress relative to its performance when not stressed.

DWP was defined as:

$DWP5 = (DW5_{is.} / DW5_{ic.}) \times 100$, where

$DW5_{is.}$ is the dry weight of the ith genotype (mean of three replicates) on day 5 when stressed, and

$DW5_{ic.}$ is the dry weight of the ith genotype (mean of three replicates) on day 5 when not stressed.

DWP10 was similarly defined using the dry weights for day 10.

DWP5 (Table 29) of the genotypes varied from 56.1% for Stewart to 75.3% for Bankuti Korai, most genotypes being around 60% while the DWP10 of the genotypes varied between 59.3% for Bankuti Korai to 78.3% for Proctor. There was not a significant relationship between DWP5 and DWP10 (r= -0.41 n.s.) but the trend was for the best performing genotypes during the stress period to be relative worse after the recovery period.

Leaf area, number of tillers, number of leaves and plant height were also measured on the last day of the experiment and the results are presented in Table 30. The leaf area was also measured

Table 29.

DWP5 and DWP10 of ten barley genotypes (DWP defined on p. 96).

Genotype	DWP5	DWP10
Asahi 2	59.3	71.9
Bankuti Korai	75.3	59.3
CI 3576	60.8	67.7
Clipper	60.0	63.0
Cyprus Black	60.0	61.2
Dore	67.9	62.2
Excelsior	60.0	68.2
Maraini	60.8	67.6
Proctor	62.1	78.3
Stewart	56.1	72.1

Table 30.

Leaf area cm^2/pot measured before stress imposition and in previously stressed(10R) and control(10N) pots on day 10. Number of tillers, number of leaves and plant height (cm) measured in treatment 10R and 10N. LAP10, TP10, LP10 and HP10, are the 10R means expressed as a percentage of 10N for Leaf Area, Tiller Number, Leaf Number and Height respectively.

	Leaf Area (cm^2)				Tiller Number			Leaf Number			Plant Height (cm)		
	0N	10R	10N	LAP10	10R	10N	TP10	10R	10N	LP10	10R	10N	HP10
Asahi 2	160.7	190.0	232.8	81.6	9.7	10.3	94.2	31.0	37.0	83.8	24.4	28.5	85.6
Bankuti Korai	140.6	236.9	272.0	87.1	7.7	5.3	145.3	33.7	34.7	97.1	35.1	47.6	73.7
CI 3576	123.9	200.9	239.1	84.0	7.7	8.3	92.8	25.3	28.7	88.2	30.6	33.1	92.4
Clipper	138.5	182.0	223.0	81.6	6.0	6.7	89.6	23.3	29.3	79.5	31.8	34.5	92.2
Cyprus Black	129.4	195.2	244.4	79.9	8.7	8.3	104.8	31.3	33.7	92.9	31.3	36.1	86.7
Dore	117.6	182.8	227.4	80.4	11.7	10.5	111.4	34.0	36.5	93.2	27.5	33.8	81.4
Excelsior	128.0	192.8	222.7	86.6	9.7	10.7	90.7	27.3	33.3	82.0	28.9	33.7	85.8
Maraini	157.9	218.6	265.4	82.4	7.7	8.3	92.8	27.3	32.3	84.5	29.3	35.8	81.8
Proctor	130.3	183.3	209.2	87.6	8.0	8.3	96.4	30.0	32.7	91.7	32.2	36.4	88.5
Stewart	187.3	240.0	294.6	81.5	9.7	9.3	104.3	33.0	32.0	103.1	30.1	36.7	82.0
Mean	139.8	202.3	243.6	83.0	8.6	8.6	100.0	29.6	32.9	90.0	30.1	35.7	84.3
LSD (p=.05)	n.s.	38.8*	33.7**		2.4**	1.5**		4.3**	3.7**		4.0**	3.3**	

LSD (p=.05) in Combined Analysis of Variance

Source of Variation				
Genotype	24.25**	1.36**	2.8**	2.44**
Treatment	13.9**	n.s.	2.2*	2.76*
Genotype x Treatment	n.s.	n.s.	n.s.	3.46*

just prior to the imposition of the stress treatments and these results are also included in this table.

The leaf area of previously stressed plants remained below that of the control plants five days after stress relief. There was no significant genotype x treatment interaction although the genotype and treatment effects were significant. It can be concluded that the genotypes did not differ in the reaction of their leaf area to stress.

The number of tillers on previously stressed plants was similar to the number on unstressed plants and, on the average, there were less than two tillers per plant, even in the unstressed control. Although the genotypes differed in the number of tillers per pot, there was no evidence of genotype differences in response to the stress for this character.

There were fewer leaves in the previously stressed plants than in the controls but the differences were not great. As was the case for tiller number, however, the genotypes were significantly different but there was no evidence for differences between genotypes in reaction to the stress treatment.

There was a significant interaction between genotype and treatment for plant height. The height of Bankuti Korai was more influenced by stress than was the height of the other genotypes. Bankuti Korai is a very early genotype and had entered the reproductive phase of growth and commenced to elongate in the well watered treatment just prior to the conclusion of the experiment thus contributing to the significant interaction.

Relationships between Characters

The experiment was confined to the seedling stage in the hope that there would be no differences between genotypes in water status at

the completion of the stress period. This was not the case, however and both the importance of the differences and their influence on the response to stress must be considered.

It is possible that differences in root pattern or efficiency were responsible for the differences in the water status of the leaves but neither characteristic was measured in this experiment. Xylem water potential was correlated with stomatal frequency (r= -0.73*). The genotypes with fewer stomata were less stressed five days after withholding water. The possibility that low stomatal frequency was related to low leaf area and this was the basis of the lower levels of stress was not supported by the results. Stomatal frequency was correlated with only one measure of leaf area (Table 31) and the sign of this correlation indicated that the genotypes with lower stomatal frequency tended to have greater leaf area. Similarly, the correlation between Ψ(xylem) and the various measures of leaf area (Table 31) were positive, so that those genotypes with higher leaf area were less stressed than those with a lower leaf area and this relationship was reinforced throughout the stress period.

Xylem water potential of the stressed and control treatments were related (p. 75). Stomatal frequency may be responsible for small differences in Ψ(xylem) (Miskin *et al.*, 1972) which may have small cumulative effects on leaf area as was suggested by Fischer (1970). This, however, must remain speculation with this limited data.

RWC was not as closely related to either leaf area or stomatal frequency as was Ψ(xylem) (Table 31), but there was a high correlation with water used during the stress period (r=0.88**, Figure 10). Field experiments with barley have shown that genotypes with greater leaf area use more water and can become more stressed (Gardiner, 1971) but in this experiment those genotypes that used

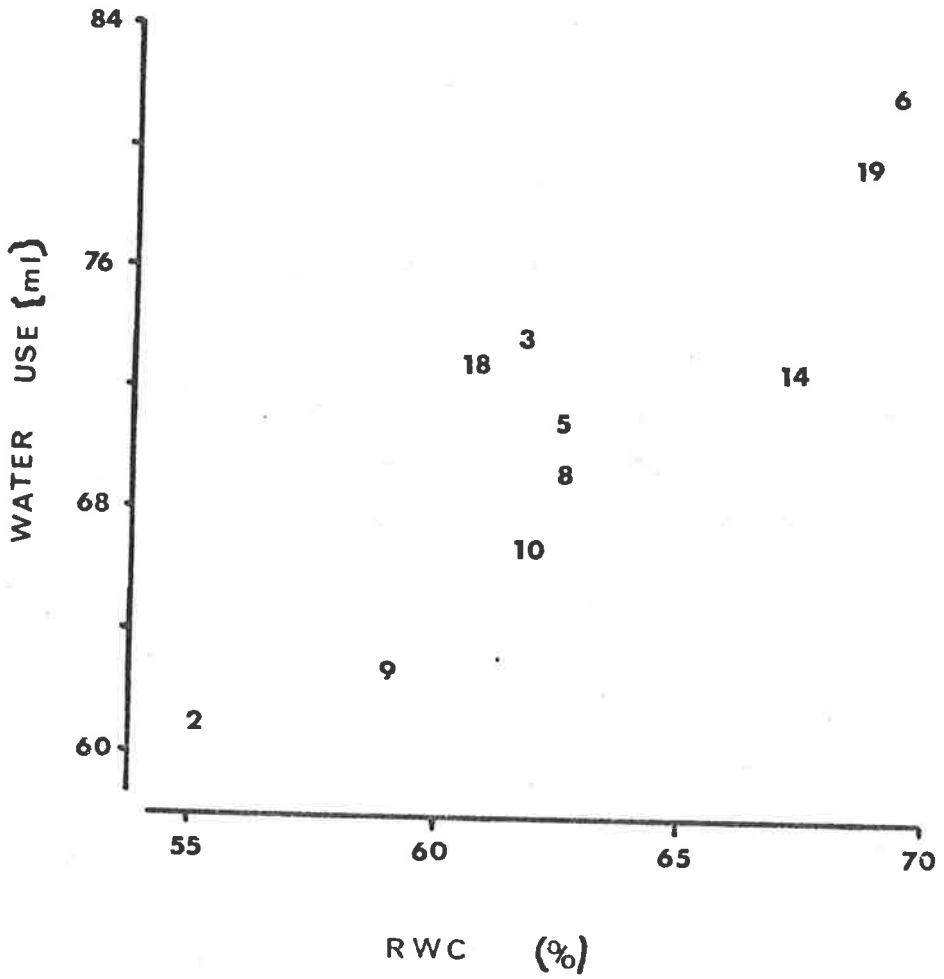
Table 31.

Correlation coefficients over the 10 genotype means for stomatal frequency (SF), Ψ(xylem), relative water content (RWC), relative growth rate of the previously stressed treatment from day 5 to day 10 (RGR10S) and the various measures of leaf area. LAO is the leaf area prior to stress, LA10S and LA10N are the leaf areas of the stressed and control plants five days after re-watering.

	LAO	LA10S	LA10N
SF	-.67*	-.55 n.s.	-.60 n.s.
Ψ(xylem)	.65*	.91**	.92**
RWC	.37 n.s.	.39 n.s.	.45 n.s.
RGR10S	.49 n.s.	.78**	.80**

Figure 10.

The relationship between Relative Water Content (%) and water used during the five day stress period in Experiment 4 ($r = 0.88^{**}$, $n = 10$). For the key to genotypes see Table 2, p. 41.


84
76
68
60
WATER USE (ml)
55
60
65
70
RWC (%)
6
19
3
18
14
5
8
10
9
2

least water during the stress period were most stressed (i.e. had the lowest RWC). A similar trend between Ψ(xylem) and water used was not significant (r=0.44 n.s.).

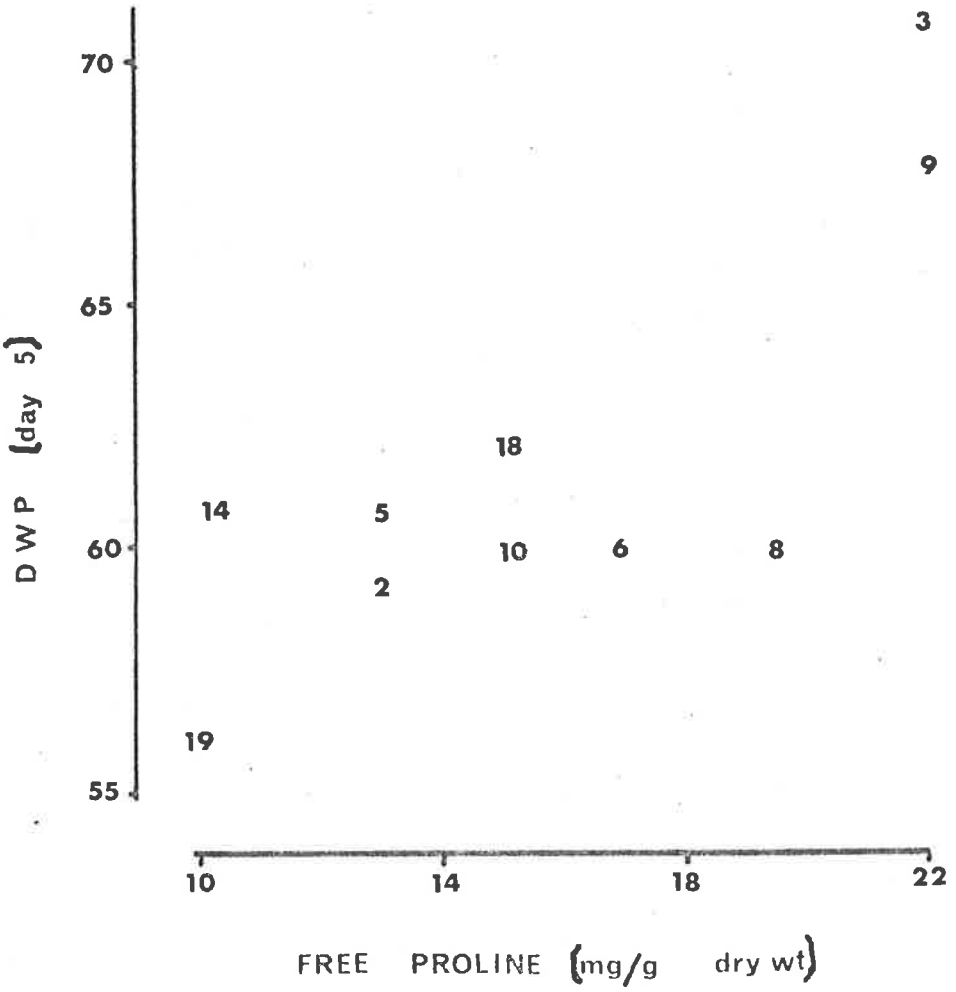
The experiment did not provide evidence of the mechanism of this relationship. Root exploration of the pots and differences in root efficiency were not studied but would be unlikely to influence water use in the limited pot volume. Early stomatal closure may have contributed to this response as this would conserve water but allow growth to continue at night.

The character derived to rank genotypes for resistance to the stress period was DWP5, dry weight in the stressed treatment expressed as a percentage of that in the control (defined on p.). There was no relationship between DWP5 and either measure of water status at the conclusion of stress (r RWC. DWP5 = -0.35 n.s.; r Ψ(xylem). DWP5 = 0.07 n.s.). DWP5 was significantly correlated with amount of free proline accumulated during the stress period (r=0.76**, Figure 11). There was therefore a tendency for genotypes that accumulated most proline to perform better during the stress period relative to their own controls.

In contrast to these results there was a negative correlation between DWP10 and proline accumulated during the stress period (r= -0.69*). DWP10 is the dry weight of the previously stressed plants on day 10 expressed as a percentage of the dry weight in the unstressed controls on the same day. It was not related to either measure of water status at the conclusion of stress. It depended more on the growth rate of the control plants from day 5 to day 10 (r= -0.66*) than on the growth of the recovering plants over the same period (r=0.08 n.s.). Genotypes that accumulated most proline during the stress period tended to be those that, unstressed, grew most rapidly

Figure 11.

The relationship between free proline in the first leaf of ten barley genotypes five days after withholding water and DWP5 (dry weight when stressed relative to dry weight in the unstressed control plants of the same genotype at the same time) ($r = 0.76^*$, $n = 10$). For the key to genotypes see Table 2, p. 41.


70
65
60
55
DWP (day 5)
3
9
18
14
5
10
6
8
2
19
10
14
18
22
FREE PROLINE (mg/g dry wt)

between days 5 and 10 although the correlation was not significant (r=0.60 n.s.). Ability to accumulate proline was related to TP, number of tillers in the previously stressed treatment expressed as a percentage of the control tiller number. This correlation is of dubious value however, as it is very dependent on the Bankuti Korai result.

This experiment demonstrated a relationship between leaf area, water relations and post stress recovery. In similar young plants the dependence of relative growth rate on leaf area was shown by Singh *et al.* (1973d). Ability to accumulate proline was related to performance during the stress period but not to post stress recovery. It did not influence leaf area as there was no measurable leaf senescence and proline was not catabolised during the post stress recovery period so it could not contribute to post-stress performance.

An additional treatment was added to this experiment to measure the proline accumulated by an extended range of genotypes in response to the water stress. Eleven genotypes were added to treatment 5S within the experiment and a further replicate of the 21 genotypes was also added. Proline content and xylem water potential were measured in the first leaf of each treatment five days after withholding water.

The genotypes varied significantly for Ψ(xylem) at the conclusion of the stress period and also in the amount of free proline accumulated by that time (Table 32). There was no correlation between genotype means for Ψ(xylem) and free proline (r=0.01 n.s.), indicating that genotype differences in proline accumulation were not solely due to differences in water status. The genotype values for Ψ(xylem) were significantly correlated with the mean values in Experiment 3 (r=0.62*) indicating that genotypes reacted similarly for this character. There was, however, no relationship between the two experiments for free proline accumulated.

Table 32.

Xylem water potential (- bars) and free proline mg/g dry weight in 21 genotypes, five days after withholding water. Mean of four replicates.

Genotype	Ψ(xylem)	Free proline
Arivat	31.1	14.1
Asahi 2	33.5	13.3
Bankuti Korai	26.0	22.0
BR 1239	30.4	12.1
CI 3576	29.1	13.6
Clipper	30.9	17.2
CPI 18197	37.3	11.2
Cyprus Black	29.7	19.1
Dore	31.1	21.6
Excelsior	33.6	15.3
Greenough	26.7	16.0
Hiproly	23.8	13.9
Ketch	32.1	17.3
Maraini	28.5	10.3
Mona	30.7	13.3
Princess	34.7	17.8
Prior A	32.6	17.0
Proctor	31.5	15.3
Stewart	24.9	10.5
Valvon II	32.5	13.0
Zephyr	32.2	12.9
Mean	31.1	15.1
LSD (p=.05)	4.5**	5.8**

Twenty genotypes were common to Experiments 1, 2, 3 and the additional treatment of Experiment 4. Genotypes were not different in Experiment 3 but there were significant (but not high) correlations between genotype means for proline accumulation in Experiments 1, 2 and 4 (Table 33). The errors associated with proline estimation in these experiments, probably associated with differences in Ψ_w, would have contributed to the poor correlation between experiments.

These experiments did not correlate well with those of Singh *et al.* (1973d) in ranking genotypes for ability to accumulate proline. They did, however, suggest that proline may have been related to survival and growth of seedlings during stress.

Experiment 5. Genotypes stressed at specific development stages

The response of a range of genotypes to a moisture stress has not often been studied later than the seedling stage except for an estimate of yield under field conditions. It is often difficult to make comparisons at the later stages. The development pattern of different genotypes are rarely synchronised and they are therefore stressed at different ontogenetic stages which may affect their response (Chinoy, 1962; Fischer, 1973). It is also difficult to ensure similar stresses for genotypes with different growth habits and leaf areas.

The seedling experiments (Experiments 1 to 4) showed that genotypes apparently varied in their response to stress at that stage. This experiment aimed to extend those results by comparing a range of genotypes in their response to water stress at later stages of development. Imposition of equitable stress is not possible under field conditions so the experiment was conducted in large pots and the genotypes were stressed at similar stages of development but not necessarily at the same time. The use of pots eliminated differences

Table 33.

Correlation matrix of genotype means of proline accumulated by 20 genotypes under stress in Experiments 1, 2 and 4.

	Experiment	
Experiment	2	4
1	0.53*	0.45*
2		0.55*

between genotypes due to rooting depth and it was hoped that differences in drought escape could also be minimised by stressing at specific development stages.

The experiment compared 18 genotypes in four water treatments. Soil water potential was maintained between -0.1 and -0.33 bars in pots of all water treatments except during stress imposition. Stress was not applied in Water Treatment 1 (control) at any time. Water was withheld in Water Treatment 2 (vegetative stress) to reduce soil water potential to -15 bars just prior to jointing when the pots were re-watered. In Water Treatment 3 (pre-anthesis stress) water was withheld so that soil water potential fell to -15 bars just prior to anthesis when pots were re-watered. Water was withheld after anthesis in Water Treatment 4 (post-anthesis stress) until the soil water potential reached -15 bars. Pots were re-watered after sampling for Ψ(xylem) and free proline but water was again withheld for the duration of the experiment.

Water Use

The record of water used each week (Figure 12), demonstrates the way in which the stresses were applied and also the very different growth patterns of the genotypes under study.

The vegetative and pre-anthesis stresses delayed maturity and prolonged water use. This was largely due to the development of later tillers following stress relief.

Anthesis in the early genotypes (e.g. Bankuti Korai and Stewart) occurred before the period of maximum water use and the grain filling period was prolonged. Anthesis coincided with maximum water use in mid-maturing genotypes like Clipper but for the later genotypes such as Maraini, anthesis followed after maximum water use and the

Figure 12.

Water use pattern (water used each week) by four representative genotypes in the four water treatments of Experiment 5 over the 16 week recording period. The vertical arrow indicates, for each genotype, the anthesis date of the main shoot in the unstressed control treatment. The four water treatments are:

■———■ Water Treatment 1 - unstressed control

□—— —— □ Water Treatment 2 - vegetative stress

○—·—·○ Water Treatment 3 - pre-anthesis stress

●- - - - -● Water Treatment 4 - post-anthesis stress.

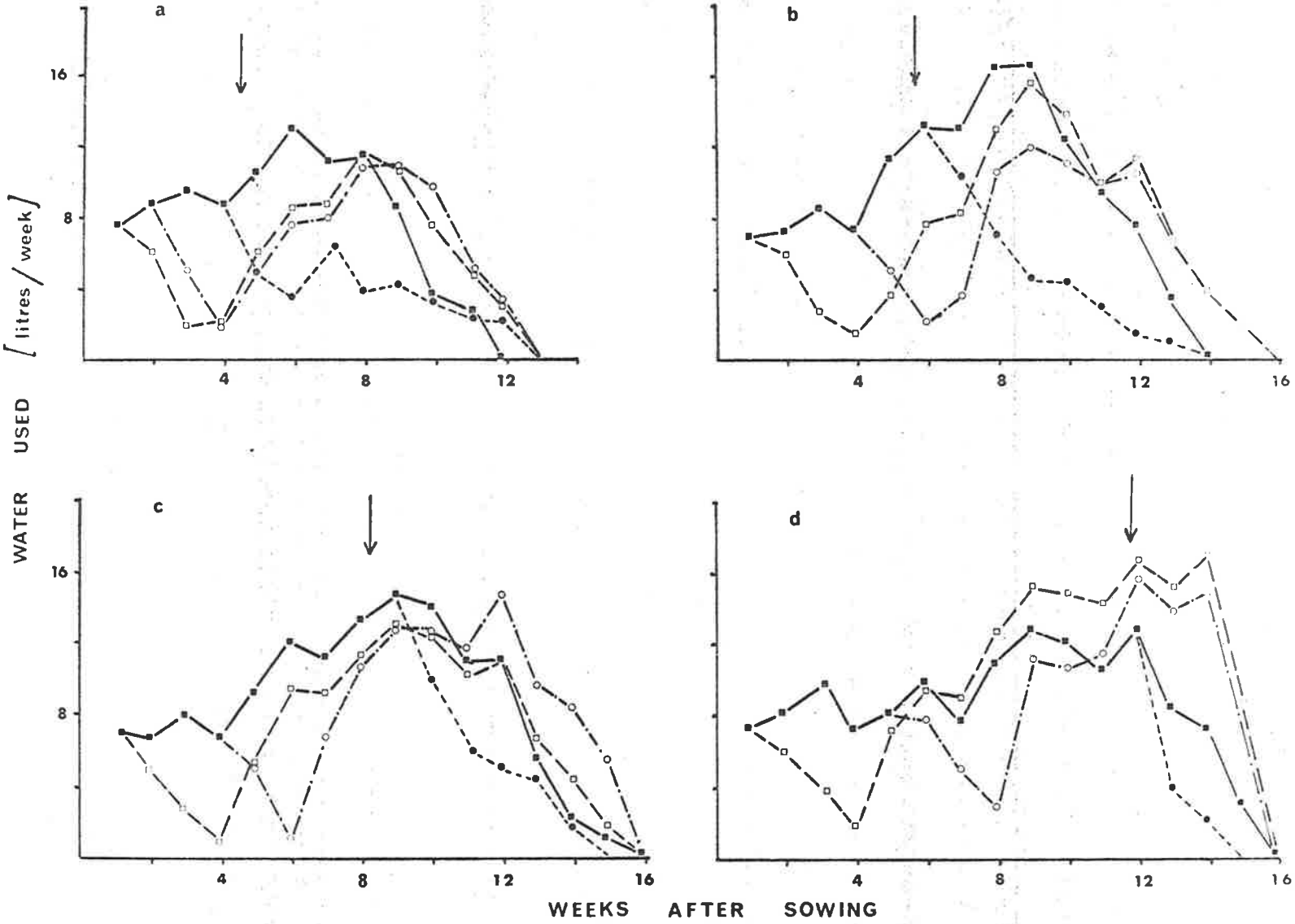
The genotypes are:

a) Bankuti Korai:- very early maturing, two-row.

b) Stewart:- early maturing, six-row.

c) Clipper:- mid-maturing, two row.

d) Maraini:- very late maturing, six row.


a
b
c
d
16
8
4
8
12
16
WATER USED [litres / week]
WEEKS AFTER SOWING

grain filling period was very restricted. This may have been a real effect due to the development pattern and climatic influences (temperature or evaporative demand) (Table 34) or to the restriction on further expansion by the limited pot size.

The effects of water treatment, genotypes and their interaction on total water use (Table 35), were highly significant. Water use was greatest in the unstressed control and was restricted most by the post-anthesis stress. Genotypes varied in their water use even when not stressed and genotype means were highly significantly correlated with anthesis date (of the main tiller) (r=0.68**) with later genotypes tending to use more water.

Water Potential

Xylem water potential was measured on the youngest fully expanded leaf of one plant per pot at the end of the stress period and on the corresponding unstressed control at the same time.

The xylem water potential of the unstressed controls (Table 36) decreased throughout the growing season. This was indicative of the increasing evaporative demand as the season progressed and also the increasing leaf area of the plants. Evaporative demand increased both as a direct result of the higher temperatures and the consequent frequent operation of the evaporative coolers which increased air circulation. At no time did the Ψ(xylem) of the unstressed plants fall to levels approaching those measured in stressed plants. The genotypes varied significantly for Ψ(xylem) in the unstressed controls but there was no interaction between genotype and time of measurement.

The xylem water potential of stressed plants also decreased with later stresses (Table 36), with genotype and interaction effects significant. Genotype means in the stressed and control treatments

Table 34.

Weekly maximum and minimum temperatures in the glasshouse during Experiment 5. Temperatures in ^{o}C.

Week ended	Maximum Temperature (^{o}C)	Minimum
13.8	20.8	14.1
20.8	21.0	15.1
27.8	20.3	14.9
3.9	19.9	15.6
10.9	20.3	16.0
17.9	20.7	16.3
24.9	22.0	16.3
1.10	24.0	17.9
8.10	23.7	15.6
15.10	22.6	16.9
22.10	28.3	18.4
29.10	26.4	20.7
5.11	23.6	18.0
12.11	24.3	18.9
19.11	27.4	19.7
26.11	26.3	18.4
3.12	23.7	19.4
10.12	27.4	19.9

Table 35.

Water use (litre/pot) by 18 barley genotypes in 4 water treatments. Mean of 4 replicates. 1=control; 2=Veg.; 3=Pre-Anth.; 4=Post-Anth.

Genotype	Water Treatment				Mean
	1	2	3	4	
Arivat	15.6	13.9	13.6	11.0	13.5
Bankuti Korai	9.3	7.6	8.1	6.4	7.8
BR 1239	14.6	12.0	12.0	10.2	12.4
CI 3576	12.5	12.3	11.5	10.2	11.6
Clipper	13.1	10.9	12.5	11.1	11.9
CPI 18197	13.0	11.1	10.8	9.8	11.2
Cyprus Black	11.8	11.2	10.5	9.8	10.8
Dore	15.2	14.9	14.3	12.8	14.3
Excelsior	14.5	14.1	14.1	13.5	14.0
Greenough	12.0	10.2	10.9	8.8	10.5
Hiproly	13.3	11.0	12.2	11.4	12.0
Ketch	14.6	12.0	12.1	9.7	12.1
Maraini	13.6	15.5	14.0	14.6	14.4
Princess	14.8	12.9	10.6	13.3	12.9
Proctor	14.6	11.7	12.1	11.6	12.5
Stewart	12.9	10.9	10.2	9.0	10.8
Velvon II	17.2	14.9	15.6	13.9	15.5
Zephyr	16.9	15.1	14.4	12.8	14.8
Mean	13.9	12.4	12.2	11.0	12.3

LSD (p=.05)

Between Genotypes	0.8**
Between Water Treatments	0.4**
Genotype x Water Treatment	1.6**

Table 36.

Xylem water potential (- bars) measured in 18 genotypes when stressed at three stages of development and in unstressed plants at the same time. Mean of 4 replicates.

	Unstressed				Stressed			
Genotype	Veg.	Pre-Anth.	Post-Anth.	Mean	Veg.	Pre-Anth.	Post-Anth.	Mean
Arivat	12.1	12.2	14.3	12.8	21.9	28.3	37.4	29.2
Bankuti Korai	11.0	13.9	14.2	13.0	21.5	22.6	24.1	22.7
BR 1239	10.8	9.6	13.3	11.2	21.5	29.8	40.0	30.4
CI 3576	11.3	14.5	17.3	14.4	24.8	26.9	34.8	28.8
Clipper	12.3	13.3	14.8	13.4	23.5	28.8	33.1	28.5
CPI 18197	12.2	11.6	14.0	12.6	23.2	29.8	41.1	31.4
Cyprus Black	12.4	11.9	17.0	13.7	23.2	28.0	42.2	31.1
Dore	9.4	11.3	14.2	11.6	20.9	26.6	31.9	26.5
Excelsior	11.1	13.4	15.0	13.1	19.9	28.6	35.8	28.1
Greenough	10.4	11.9	14.6	12.3	23.6	24.1	31.5	26.4
Hiproly	12.2	15.6	15.5	14.4	23.3	27.4	32.5	27.7
Ketch	11.0	11.8	16.0	12.9	24.0	27.1	35.6	28.9
Maraini	11.2	11.5	16.7	13.1	21.1	27.6	41.3	30.0
Princess	10.9	11.4	14.8	12.3	22.2	23.3	34.1	26.5
Proctor	10.2	12.4	13.2	11.9	21.0	26.6	36.7	28.1
Stewart	10.3	15.2	15.6	14.6	23.5	29.6	41.4	31.5
Velvon II	9.7	11.8	17.3	12.9	22.1	25.7	41.5	29.8
Zephyr	11.9	11.5	14.9	12.7	22.5	26.3	36.8	28.5
Mean	11.1	12.5	15.3	13.0	22.4	27.1	36.2	28.6

LSD (p=.05)		
Between Genotypes	1.61**	2.66**
Between Stages	0.66**	1.09**
Genotype x Stage	n.s.	4.61**

were not correlated (r=0.2 n.s.) nor was there a significant correlation between genotype means for xylem water potential in this experiment and Experiment 4. Genotype differences in xylem water potential at the same soil water potential may be due to many factors including stress history, timing and completeness of stomatal closure, leaf area, differences in rooting pattern or differences in environmental conditions at the time of measurement. The fact that genotypes differed for Ψ(xylem) at the conclusion of stress, however, must be considered in any discussion of responses to the stresses in this experiment.

Free Proline

Free proline content of the leaves was measured in both the stressed treatment and the corresponding unstressed control at the conclusion of the stress period.

The free proline content of the leaves of unstressed plants remained at a very low level at all times and there were no significant differences between the times of measurement or genotypes. Only the genotype means are presented in Table 37.

Free proline increased in the leaves of all stressed plants at all stress stages, the levels being higher after the vegetative stress and lower after the post-anthesis stress when the leaves were approaching maturity. Leaves sampled in the vegetative stress treatment had been stressed for a longer period and this may be the reason for the higher free proline levels, rather than a greater potential for accumulation.

The genotypes varied significantly in the amount of proline they accumulated during the stress and the interaction between genotype and stress treatment was also highly significant. There was no relationship between the free proline levels and xylem water potential for each genotype at any stress stage. The correlation coefficients

Table 37.

Free proline content (mg/g dry weight) in youngest fully expanded leaf in plants of 18 genotypes stressed at three stages. Also mean of unstressed plants at same stage. Mean of 4 replicates.

	Unstressed	Stressed			
Genotype	Mean	Veg.	Pre-Anth.	Post-Anth.	Mean
Arivat	0.5	19.7	8.9	9.8	12.8
Bankuti Korai	0.8	18.5	16.7	13.6	16.3
BR 1239	0.7	13.1	7.4	9.6	10.0
CI 3576	0.5	18.9	13.3	12.0	14.8
Clipper	0.7	11.6	11.4	6.4	9.8
CPI 18197	0.8	18.0	9.7	10.5	12.7
Cyprus Black	0.7	16.3	10.7	11.5	12.8
Dore	0.9	11.6	7.8	8.5	9.3
Excelsior	0.5	10.3	8.0	7.1	10.6
Greenough	0.6	12.1	9.9	5.5	9.2
Hiproly	0.8	13.5	10.0	5.9	9.8
Ketch	0.8	11.1	10.9	9.4	10.5
Maraini	0.5	8.4	5.4	1.3	5.0
Princess	0.8	16.3	8.0	3.1	9.1
Proctor	0.7	7.2	10.4	7.6	8.4
Stewart	0.3	5.4	13.6	10.9	10.0
Velvon II	0.9	9.5	8.0	7.1	8.2
Zephyr	0.6	10.8	8.7	8.5	9.3
Mean	0.7	12.9	10.0	8.2	10.4

LSD (p=.05)

	Unstressed	Stressed
Between genotypes	n.s.	2.0**
Between water treatments	n.s.	1.0**
Genotype x water treatment	n.s.	4.1**

for this comparison for the vegetative, pre-anthesis and post-anthesis stresses were 0.20, 0.22 and 0.10 respectively. There was a significant correlation between the date of anthesis (of the main tiller in the unstressed control) and free proline accumulated by the end of the stress period. The correlation coefficients for the 18 genotypes means for the vegetative, pre-anthesis and post-anthesis stresses were -0.50*, -0.78** and -0.72** respectively. Thus earlier genotypes tended to accumulate more proline during stress than did the later ones.

There was no significant relationship between free proline measured in this experiment and for the same 18 genotypes in Experiment 4 (r=0.36 n.s.) nor was there a significant relationship with the results published by Singh *et al.* (1972) for the nine common genotypes. It is possible that proline accumulated in this experiment represents stress history modified by genotype potential to accumulate it. If proline is useful during stress as suggested by the seedling experiments, then differences in accumulation may be reflected in performance.

Shoot Dry Weight

Shoot dry weight is here defined as the weight of all dry matter excluding roots but including the weight of the infertile tillers which remained at the time of harvest. Water treatment, genotype and interaction effects were all significant for this character.

All water treatments (averaged over genotypes) were significantly different from each other and all stress treatments were lower than the unstressed control (Table 38). Post-anthesis stress reduced dry weight more than stress at the earlier stages. If dry weight of the main tiller alone is considered, however, the pre-anthesis stress had the greatest effect. The main tiller was at the stage commonly regarded as most critical during this period while other

Table 38.

Shoot dry weight (g/plant) of the whole plant and of the main tiller only in four water treatments. Mean of 18 genotypes and 4 replicates.

Character	Water Treatment				
	Control	Vegetative	Pre-anthesis	Post-anthesis	LSD (p=.05)
Plant dry weight	10.9	10.0	9.8	9.1	0.3**
Main tiller dry weight	4.1	3.6	3.3	3.7	0.2**

tillers of the plant were at this critical stage during the post-anthesis stress.

The dry weight of the unstressed controls varied from 6.5g per plant to 13.8g per plant (Table 39) and there was a tendency for the dry weight of the later genotypes to be greater (r=0.64** for the correlation between the dry weight of the unstressed control plants and the date of anthesis of the main tiller in those plants).

The genotype x water treatment interaction for dry weight indicated that genotypes differed in response to stress. There was no interaction, however, if the unstressed controls were excluded from the analysis i.e. the analysis was performed only using water treatments 2, 3 and 4. Genotypes therefore differed in their overall reaction to stress but not necessarily differently to stress at separate stages.

Since genotypes differed for total dry weight even when not stressed, little additional information on their resistance to stress can be achieved by examining their stress performance directly. For this reason Dry Weight Percentage (DWP) was derived to compare genotype response to stress after correction for unstressed performance.

Dry Weight Percentage (DWP) was derived for each water treatment, genotype, replicate combination by the formula

$DWP_{ijk} = (DW_{ijk}/DW_{ilk}) \times 100$, where DW_{ijk} is the shoot dry weight of the ith genotype in the jth water treatment (j= 1 is the unstressed control) in the kth replicate. DWP was calcualted only for j = 2 to 4.

Genotypes varied significantly for DWP (Table 39) and comparisons of the genotype means are appropriate as there was no significant interaction between genotype and water treatment. The DWP of the various genotypes varied from 74.9% for Bankuti Korai to 105.3% for Excelsior. Values greater than 100% are possible when stress

Table 39.

Total dry weight (g/plant) of 18 barley genotypes when not stressed and DWP (dry weight when stressed as a percentage of control dry weight) for three stress treatments. Mean of 4 replicates.

Genotype	Dry Weight	DWP of Stress Treatments			
	Control	Veg.	Pre-Anth.	Post-Anth.	Mean
Arivat	11.0	97.7	94.1	71.3	87.7
Bankuti Korai	6.5	80.2	77.2	67.3	74.9
BR 1239	12.1	86.0	85.8	73.1	82.4
CI 3576	10.2	92.2	94.6	88.5	91.8
Clipper	9.7	94.0	102.9	95.2	97.4
CPI 18197	11.0	82.9	79.4	81.0	81.1
Cyprus Black	10.3	98.5	94.0	84.6	92.4
Dore	12.1	100.1	98.6	88.1	95.6
Excelsior	10.9	109.5	103.8	102.6	105.3
Greenough	9.1	90.0	92.6	73.5	85.4
Hiproly	10.4	91.2	92.3	89.4	91.1
Ketch	11.4	81.3	85.4	74.1	80.3
Maraini	10.3	108.8	96.5	101.6	102.3
Princess	12.5	98.7	83.0	95.6	92.4
Proctor	11.3	87.4	91.3	90.3	89.7
Stewart	11.2	79.1	73.9	71.9	75.0
Velvon II	13.8	87.5	92.3	79.8	87.1
Zephyr	11.7	94.9	96.9	85.8	92.5
Mean	10.9	92.2	90.8	84.2	89.2

LSD (p=.05)	4 Water Treatments	3 Stress Treatments	
	DW	DW	DWP
Between Genotypes	0.6**	0.8**	9.1*
Between Water Treatments	0.3**	0.4**	3.7**
Genotype x Water Treatment	1.2**	n.s.	n.s.

performance exceeds control performance. This may be a real effect where stress induces improved performance; due to depressed control performance when stresses other than those imposed are operating or due to an accumulation of errors in deriving the character.

DWP values represent a measure of resistance to the stresses imposed in the experiment. The differences between genotypes could represent the effect of escape, avoidance or tolerance. The experiment was planned to minimise differences in escape by stressing at specific development stages but the highly significant correlation between the genotype means for DWP and the date of anthesis (of the main tiller in the unstressed control ($r=0.71^{**}$)) suggests that this may not have been successful.

A correlation matrix constructed on the genotype means for each stress stage revealed a complex relationship between anthesis date, Ψ(xylem), free proline accumulated and DWP (Table 40).

DWP was positively related to anthesis date within each stress treatment. Free proline was also related to DWP after the pre- and post-anthesis stress but those genotypes accumulating most proline were apparently less resistant to stress. This, however, was influenced by the negative relationship between anthesis date and free proline which was much greater for the pre- and post-anthesis stress. There was also a small negative correlation between anthesis date and Ψ(xylem) for the post-anthesis stress. Only anthesis date was important in explaining the variability associated with DWP. When its effects were excluded, neither Ψ(xylem) nor free proline contributed significantly to the between-genotype variability as indicated by the partial regression coefficients (Table 40).

Table 40.

Correlation matrix and partial regression coefficients for DWP (dependent variable) and anthesis date (of the main tiller in the unstressed control treatment), Ψ(xylem) and free proline. Calculated for the three stress treatments using the genotype means.

	Anthesis Date	Ψ(xylem)	Free Proline
VEGETATIVE STRESS			
DWP	0.61**	0.46	-0.02
Anthesis Date		0.36	-0.51*
Ψ(xylem)			-0.24
β	0.54	2.15	0.88
t	3.30**	1.64	2.02
PRE-ANTHESIS STRESS			
DWP	0.50*	-0.12	-0.47*
Anthesis Date		-0.33	-0.78**
Ψ(xylem)			0.22
β	0.26	0.17	-0.59
t	0.97	0.17	-0.51
POST-ANTHESIS STRESS			
DWP	0.75**	-0.10	-0.63**
Anthesis Date		-0.49*	-0.72**
Ψ(xylem)			0.09
β	0.84	0.81	-0.02
t	3.29**	1.80	-0.03

Grain Yield

Post-anthesis stress reduced grain yield per plant more than stress at any other stage but the pre-anthesis stress reduced the grain yield of the main shoot more than did the post-anthesis stress (Table 41). This was similar to the result for shoot dry weight.

There was a tendency for the earlier genotypes to be lower yielding (Table 42) but, in contrast to the results for shoot dry weight, late genotypes did not yield as well as those of intermediate maturity (Figure 13).

Hiproly has been excluded from all discussion of yield and its components. Low yield in all treatments was due to infertility and its naked grain. Yield did not vary with water treatment, suggesting that its inherent inferfility dominated the water treatment effect.

Since genotypes varied for yield in the unstressed control, the significant water treatment interaction with genotype was investigated by calculating the Yield Percentage (YP) by:

$YP_{ijk} = (Y_{ijk}/Y_{ilk}) \times 100$, where Y_{ijk} is the grain yield of the ith genotype in the jth water treatment (j = 1 is the unstressed control) in the kth replicate. YP_{ijk} was calculated for j = 2, 3 and 4.

There was no genotype x water treatment interaction for YP although the genotype and water treatment effects were highly significant. The analysis was performed both with and without the results for Hiproly but this had little bearing on the conclusions.

The genotype differences for YP represent differences in genotype reaction to the stress and, as for shoot dry weight, there was a relationship between YP and anthesis date. The correlation over the 17 genotypes was positive and highly significant (r=0.63**), the later genotypes yielding relatively more when stressed than did the earlier ones.

Table 41.

Grain yield/plant and grain yield/main shoot in four water treatments. Mean of 18 genotypes and four replicates.

Water Treatment	Grain Yield g/plant	Grain Yield g/main shoot
Control	4.15	1.52
Vegetative	3.99	1.41
Pre-anthesis	3.64	1.16
Post-anthesis	2.19	1.21
LSD (p=.05)	0.16**	0.07**

Table 42.

Grain yield (g/plant) of 18 barley genotypes when not stressed and YP (yield when stressed as a percentage of control yield) for the three stress treatments. Mean of four replicates.

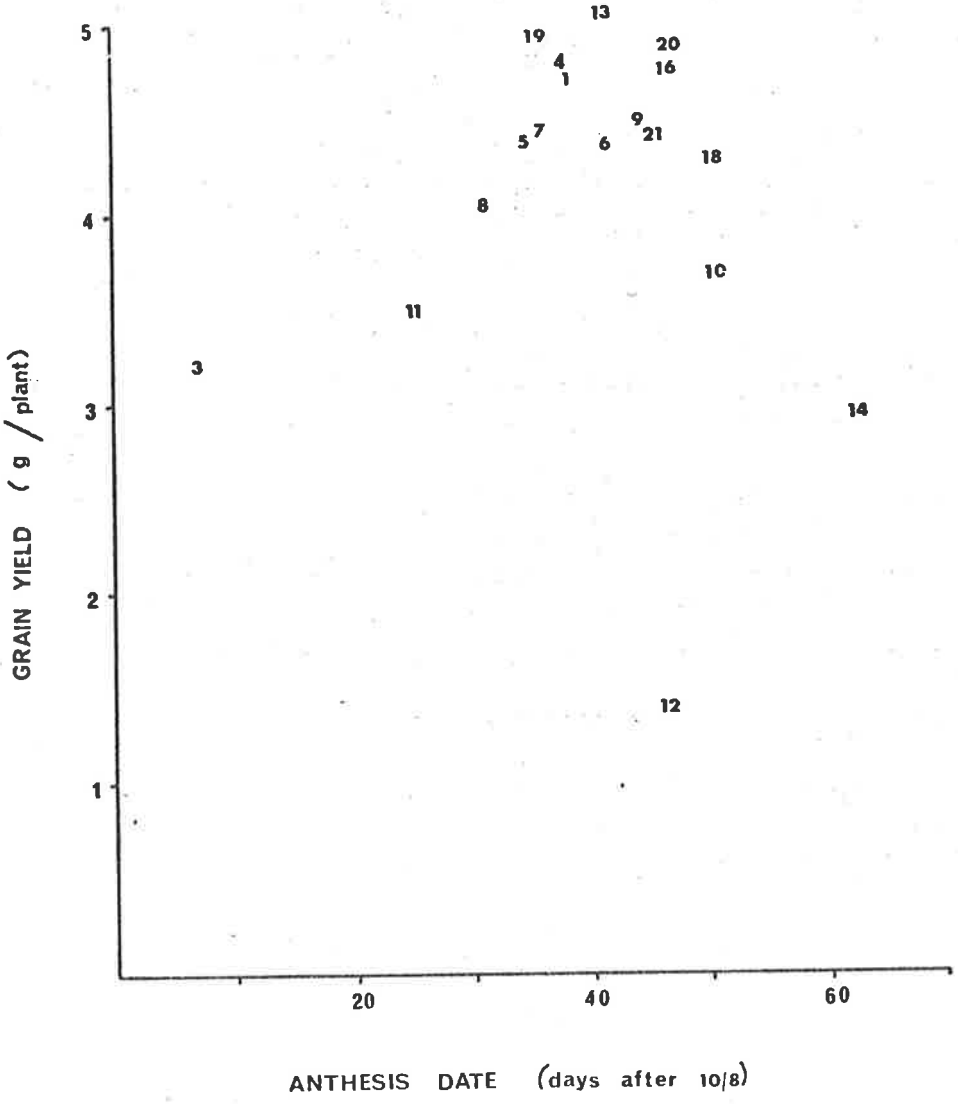
	Yield	YP of Stress Treatments			
Genotype	Control	Veg.	Pre-Anth.	Post-Anth.	Mean
Arivat	4.73	107.1	89.4	46.6	80.1
Bankuti Morai	3.23	80.8	76.6	63.3	73.6
BR 1239	4.82	94.2	81.4	55.0	78.8
CI 3576	4.39	101.5	95.3	79.8	92.2
Clipper	4.38	91.8	101.9	80.7	91.5
CPI 18197	4.45	89.8	75.9	74.4	80.1
Cyprus Black	4.07	112.1	88.1	73.2	91.1
Dore	4.51	112.3	115.3	69.8	99.1
Excelsior	3.69	118.5	108.5	99.0	108.5
Greenough	3.48	88.8	89.7	55.7	78.1
Hiproly	1.42	100.2	99.6	103.4	100.9
Ketch	5.11	87.1	77.5	57.4	74.0
Maraini	2.95	106.7	100.3	96.1	101.0
Princess	4.78	96.9	67.9	82.4	82.4
Proctor	4.33	92.8	91.3	77.6	87.2
Stewart	4.97	86.0	64.5	57.5	69.3
Velvon II	4.90	94.0	95.9	62.9	86.2
Zephyr	4.43	101.9	97.3	72.0	90.4
Mean	4.15	97.9	89.8	72.5	86.9

Analyses

Source of Variation	LSD (p=.05)		
	Yield	YP	
		Include Hiproly	Exclude Hiproly
Genotype	0.34**	15.5**	13.1**
Water Treatment	0.16**	6.3**	5.5**
Genotype x Water Treatment	0.69**	n.s.	n.s.

Figure 13.

The relationship between grain yield (g/plant) and anthesis date (days after 10/8) of the main shoot in the unstressed control treatment in 18 genotypes in Experiment 5. Mean of four replicates. The key to genotypes see Table 2, p. 41.


GRAIN YIELD (g / plant)
5
4
3
2
1
20
40
60
ANTHESIS DATE (days after 10/8)
13
19
20
4
16
1
9
5
7
6
21
18
8
10
11
3
14
12

A correlation matrix (Table 43) for YP, anthesis date, Ψ(xylem) and free proline was constructed for the 17 genotypes excluding Hiproly. YP was associated with anthesis date and the other relationships were similar to those for DWP (see Table 40). Multiple regression confirmed as it did for dry weight, that anthesis date was apparently the only character associated with the genotypic differences in reaction to the stress treatments in terms of grain yield.

Yield Components

Grain yield is often considered as the end product of the multiplicative action of various components. The components measured in this experiment were:

Grain Yield (Y) = Number of fertile tillers per plant (X1) x
Number of florets per tiller (X2) x
Percentage of florets to set seed (X3) x
Grain weight (X4).

The influence of the components on yield is always of interest but they assume greater significance in this study because the stress was aimed at specific stages of development and was expected to influence the components differently at each stage. The final grain yield would then be the end result of stress on a particular component and the importance of later component compensations. The average response of all genotypes will be discussed before comparing individual genotype responses to the imposed stresses.

The responses of the whole plant and the main shoot only are summarised in Table 44.

Relief of the two stresses applied before anthesis resulted in the formation of late tillers. This is much more obvious in the

Table 43.

Correlation matrix and partial regression coefficients for YP (dependent variable) and anthesis date (of the main shoot in the unstressed control treatment), Ψ(xylem) and free proline. Calculated for the three stress treatments on the genotype means.

	Anthesis Date	Ψ(xylem)	Free Proline
VEGETATIVE STRESS			
YP	0.80**	0.11	-0.42
Anthesis date		0.15	-0.47
Ψ(xylem)			-0.46
β	1.45	-0.04	-0.14
t	4.10**	-0.24	-0.40
PRE-ANTHESIS STRESS			
YP	0.43	-0.09	-0.41
Anthesis date		-0.33	-0.79**
Ψ(xylem)			0.22
β	0.36	0.36	-0.89
t	0.76	0.21	-0.44
POST-ANTHESIS STRESS			
YP	0.56*	-0.05	-0.46
Anthesis date		-0.53*	-0.71**
Ψ(xylem)			0.14
β	0.94	1.09	0.20
t	2.02	1.29	0.13

Table 44.

Stress means for yield and its components expressed as a percentage of the non-stress mean. For all tillers and the main shoot only. Y is grain yield, X1 is tillers per plant, X2 is florets per tiller, X3 proportion of the total florets that set seed and X4 is the grain weight.

Treatment	Y	X1	X2	X3	X4
MEAN OF ALL TILLERS					
Vegetative	96.1	102.3	93.5	99.0	102.2
Pre-anthesis	87.7	110.7	90.0	95.3	95.3
Post-anthesis	70.1	91.9	98.8	86.3	89.9
MAIN SHOOT ONLY					
Vegetative	92.8		93.3	101.5	99.5
Pre-anthesis	76.3		89.9	92.3	94.8
Post-anthesis	79.6		98.0	93.7	88.1

results for the pre-anthesis stress than for the vegetative stress but observation suggested that the earlier stress resulted in tiller mortality with increased tillering when the stress was relieved. Stress after anthesis decreased tiller number through increasing the number of tillers which were sterile.

The total number of florets per spike was influenced by stress at all stages but more particularly by stress prior to anthesis. This is reinforced by the negative statistical association after the tillering response. There was little difference between main shoot response and the whole plant response for this character.

Floret fertility was depressed by the pre-anthesis stress and also by the post-anthesis stress, with the latter most important for the whole plant and slightly less important for main shoots. Many reports (e.g. Salter and Goode, 1969; Fischer, 1973) have indicated that floret fertility is reduced by stress prior to anthesis and this is supported by these results. Stress was timed on the stage of development of the main shoot. This shoot would have been at the most critical stage for the pre-anthesis stress while the other tillers of the plant, which were slower to develop, were at the critical stage for the post-anthesis stress.

Grain weight was most reduced by the post-anthesis stress in both the whole plants and the main shoots, while the pre-anthesis stress restricted grain filling to some extent. This may have been due to the compensatory effects resulting from the increased tillering rather than a direct effect of stress on the component.

It has often been stated that the component most affected by stress is that growing most rapidly at the time (Aspinall *et al.*, 1964). This statement is largely supported by these results. Post-anthesis stress reduced fertile tiller number, however, and this was due to higher tiller mortality.

Genotypes varied in the expression of the various yield components and to compare their response to stress, the character percentage values were calculated for the three stress stages by:

$X(A)P_{ijk} = (X(A)_{ijk} / X(A)_{ilk}) \times 100$, where A can take the value 1 to 4 indicating the yield component X1 to X4 and $X(A)_{ijk}$ is the yield component of the ith genotype in the jth water treatment (j - 1 is the unstressed control) in the kth replicate.

Two-rowed genotypes produced more tillers in the unstressed control treatment than did the six-rowed types. There was no relationship between the number of tillers and anthesis date. The tillering response of the different genotypes to stress was complicated (Table 45). Some genotypes, particularly Ketch, Maraini and Clipper responsed to the relief of the vegetative stress by the production of a greater number of tillers than they did when not stressed and many genotypes, including Ketch, Clipper and CPI 18197 responded in this way after the relief of the pre-anthesis stress. Tiller mortality was apparently greater after the post-anthesis stress, particularly in BR 1239 and Ketch. The tillering response of the various genotypes was not related to either Ψ(xylem) or free proline.

Genotypes had different numbers of florets per tiller even when not stressed (Table 46). This was largely due to the difference between the two- and six-rowed types but there was also variation within head type. There was no clear distinction between the two head types for X2P (total florets when stressed as a percentage of control performance) but it was negatively associated with X1P (tiller number when stressed as a percentage of the control). The correlation coefficients in the vegetative stress and pre-anthesis stress were $r= -0.64^{**}$ and $r= -0.65^{**}$ respectively. This relationship was probably

Table 45.

Number of tillers/plant (X1) of 18 barley genotypes when not stressed and X1P - the number of tillers/plant when stressed as a percentage of the unstressed control for the three stress treatments. Mean of 4 replicates.

Genotype	Head Type	X1	X1P at Stress Stage			
		Control	Veg.	Pre-Anth.	Post.Anth.	Mean
Arivat	6	3.1	96.7	135.6	80.7	104.3
Bankuti Korai	2	3.3	112.1	106.8	85.0	101.3
BR 1239	6	2.7	100.8	87.3	81.3	89.7
CI 3576	2	4.1	108.2	109.3	93.5	103.7
Clipper	2	4.1	111.5	129.5	94.3	111.7
CPI 18197	2	3.7	93.5	125.3	96.9	105.2
Cyprus Black	6	3.3	101.7	99.0	89.8	96.8
Dore	2	4.4	106.2	112.7	94.1	104.3
Excelsior	6	3.0	109.4	108.8	96.9	105.0
Greenough	6	2.0	110.7	109.5	83.1	101.1
Hiproly	2	3.8	78.5	83.9	86.8	82.7
Ketch	2	4.3	123.4	145.3	83.9	117.5
Maraini	6	1.8	120.6	103.8	115.7	113.4
Princess	2	4.1	103.2	101.9	106.2	103.8
Proctor	2	4.4	103.4	112.7	94.3	103.5
Stewart	6	2.5	112.4	113.3	103.7	109.8
Velvon II	6	2.9	96.3	111.9	91.6	100.7
Zephyr	2	4.8	85.1	106.3	91.6	94.3
Mean (excluding Hiproly)		3.4	105.6	105.6	92.9	103.9

Analyses (Exlcuding Hiproly)

Source of Variation	LSD (p=.05)	
	X1	X1P
Genotypes	0.5**	n.s.
Water Treatment	0.2**	6.0**
Genotype x Water Treatment	0.9**	24.8*

Table 46.

Total florets per tiller (X2) of 18 barley genotypes when not stressed and X2P (florets per tiller as a percentage of the unstressed control) for three stress treatments. Mean of four replicates.

Genotype	Head Type	X2 Control	X2P for Stress Treatments			
			Veg.	Pre-Anth.	Post-Anth.	Mean
Arivat	6	37.0	103.2	74.6	105.6	94.4
Bankuti Korai	2	19.0	88.7	83.0	107.0	92.9
BR 1239	6	50.9	105.3	108.2	106.9	106.8
CI 3576	2	19.3	90.2	92.4	104.1	95.6
Clipper	2	23.0	79.2	86.5	102.5	89.4
CPI 18197	2	22.2	93.1	80.2	100.1	91.1
Cyprus Black	6	34.1	93.4	88.8	99.3	93.8
Dore	2	24.6	94.4	99.2	103.9	99.1
Excelsior	6	34.1	109.5	94.6	103.3	102.5
Greenough	6	53.3	93.4	97.2	89.9	93.5
Hiproly	2	16.0	104.6	106.2	102.6	104.6
Ketch	2	22.9	75.3	76.2	101.1	84.2
Maraini	6	52.7	87.7	95.8	98.3	93.9
Princess	2	26.5	96.4	91.6	101.3	96.4
Proctor	2	28.8	89.3	87.5	101.2	92.7
Stewart	6	39.4	80.2	66.3	87.9	78.1
Velvon II	6	46.2	96.6	92.9	101.6	96.6
Zephyr	2	27.7	101.7	91.4	103.6	98.9
Mean (excluding Hiproly)		33.0	92.8	88.6	101.0	94.1

Analyses (excluding Hiproly)

Source of Variation	LSD (p=.05)	
	X2	X2P
Genotype	2.3**	8.4**
Water Treatments	1.1**	3.5**
Genotype x Water Treatment	4.6**	14.5*

due to later formed tillers having fewer florets as the correlation coefficient for the post-anthesis stress was not significant (r=0.28 n.s.).

The percentage of fertile florets (X3) is rarely measured in experiments and yet it is regarded as the most sensitive component to stress (Fischer, 1973). The effects due to genotype, water treatment and interaction were all significant for this component (Table 47). Hiproly was less fertile than the other genotypes with only 58.5% of the spikelets fertile when not stressed. The six-rowed genotypes tended to have a lower percentage of fertile florets but this was not universal as Stewart (Six rowed) was highly fertile and Zephyr (two-rowed) was not as fertile as many of the six-rowed types.

Genotype, water treatment and interaction effects were all significant for X3P (floret fertility when stressed expressed as a percentage of control performance). Genotype means for this character were correlated with the date of anthesis (of the main shoot in the unstressed control) (r=0.67**), indicating that the later genotypes withstood stress better for this character. The percentage of fertile florets (X3) in the unstressed control, however, was negatively related to anthesis date (r= -0.57*). Thus the genotypes that were most fertile when not stressed appeared to be the most sensitive to stress for the character. The later genotypes flowered at a time when water use, and probably leaf area, was declining in the well watered controls. The resultant stress on the plants may have reduced fertility. Stress prior to anthesis delayed senescence of these later genotypes resulting in higher fertility relative to their own controls than in the earlier maturing types.

Mean thousand grain weight varied between the genotypes but there was no consistent pattern (Table 48). Neither grain weight nor X4P (grain weight when stressed as a percentage of control performance) were significantly related to the other yield components or to anthesis

Table 47.

Percentage of total florets that produce grain (X3) in 18 barley genotypes when not stressed and X3P (fertility as a percentage of the unstressed control) for the three stress treatments. Mean of four replicates.

	X3	X3P for Stress Treatments			
Genotype	Control	Veg.	Pre-Anth.	Post-Anth.	Mean
Arivat	80.0	110.1	101.8	70.1	94.0
Bankuti Korai	94.2	88.3	88.1	77.4	84.6
BR 1239	82.7	99.1	94.0	74.2	90.4
CI 3576	97.4	98.7	85.9	86.5	90.4
Clipper	91.4	101.2	100.6	96.2	99.3
CPI 181967	95.1	100.5	91.2	90.5	94.1
Cyprus Black	84.4	101.7	86.3	78.0	88.6
Dore	90.9	103.7	105.5	83.1	97.4
Excelsior	80.6	99.0	105.0	100.2	101.7
Greenough	78.3	82.0	81.9	76.3	80.1
Hiproly	58.5	108.5	111.8	110.2	110.2
Ketch	94.8	94.3	83.7	75.7	84.5
Maraini	62.0	86.6	92.5	99.6	92.9
Princess	91.4	99.2	90.4	92.5	94.0
Proctor	75.2	103.6	107.9	96.8	102.8
Stewart	91.4	99.3	91.8	66.1	85.7
Velvon II	73.5	105.0	101.4	99.0	102.0
Zephyr	73.5	111.2	111.7	94.6	105.9
Mean (excluding Hiproly)	84.5	99.0	95.3	85.7	93.4

Analysis (Excluding Hiproly).

Source of Variation	LSD (p=.05)	
	X3	X3P
Genotypes	5.2**	7.6**
Water Treatments	2.5**	3.2**
Genotype x Water Treatment	10.4**	13.1**

Table 48.

Thousand grain weight (g) (X4) in 18 barley genotypes when not stressed and X4P (grain weight when stressed as a percentage of the unstressed control) for the three stress treatments. Mean of four replicates.

Genotype	X4	X4P for Stress Treatments			
	Control	Veg.	Pre-Anth.	Post-Anth.	Mean
Arivat	54.0	100.9	82.9	93.0	92.3
Bankuti Korai	55.0	93.4	97.7	91.6	94.3
BR 1239	40.9	95.9	100.8	96.4	97.8
CI 3576	56.3	108.1	110.1	96.0	104.7
Clipper	49.8	108.5	96.8	90.6	98.7
CPI 18197	57.2	105.9	81.9	91.1	93.0
Cyprus Black	43.9	112.5	112.4	99.8	108.2
Dore	44.5	105.2	100.6	89.3	98.4
Excelsior	44.2	102.7	101.7	96.9	100.4
Greenough	41.4	105.9	107.0	97.3	103.4
Hiproly	41.0	104.8	106.8	102.3	104.8
Ketch	51.8	94.3	88.2	90.1	90.9
Maraini	52.3	115.1	104.5	83.9	101.2
Princess	48.3	100.1	80.4	85.9	88.8
Proctor	45.1	97.6	88.6	85.5	90.6
Stewart	55.5	95.8	87.8	84.4	89.4
Velvon II	50.0	95.7	89.3	70.5	86.5
Zephyr	45.2	108.2	93.5	80.3	94.0
Mean (Excluding Hiproly)	49.1	102.7	95.5	89.6	96.0

Analysis (excluding Hiproly)

Source of Variation	LSD (p=.05)	
	X4	X4P
Genotypes	2.8**	9.2**
Water Treatments	1.3**	3.9**
Genotype x Water Treatment	5.6**	n.s.

date.

Yield Components - genotype effects

The importance of the various yield components in determining the response of the genotypes to the various stresses was tested by multiple regression where YP (yield when stressed as a percentage of control yield) was the dependent variable and component percentage values were the independent variables. The importance of the component response was tested by the magnitude of the standardised partial regression coefficient (Table 49) which allows comparison at equivalent units.

The relative response of all components was highly significantly related to relative yield response in all water treatments. The relative response of no component, however, was uniquely responsible for genotype response in terms of YP. The two earlier formed components were more important for the vegetative stress but there was little difference in importance between any component for the pre-anthesis stress, the two later formed components being slightly more important. Floret number per tiller (X2) was relatively unimportant in determining yield response after post-anthesis stress while thousand grain weight was slightly less important than the two remaining components.

The relative importance of the yield components at each stress stage indicates that the stress affect of yield is not mediated by the response of particular components but reflects underlying plant responses. This is particularly illustrated for the post-anthesis stress where timing was definitely equivalent for all genotypes and yet response in terms of yield was influenced to some extent by all components (after adjustment for the relationships between them) with tiller number and floret fertility only slightly more important.

Table 49.

Standardised partial regression coefficients for the yield component percentage values when regressed on YP at three stress stages.

	Water Treatment		
Component	Vegetative	Pre-anthesis	Post-anthesis
X1P	0.89**	0.59**	0.61**
X2P	0.94**	0.46**	0.14**
X3P	0.69**	0.68**	0.63**
X4P	0.54**	0.65**	0.43**
R^2	0.90	0.92	0.98

Weater Use Efficiency and Harvest Index

Dry weight was highly correlated with water used. When calculated on the genotypes means the correlation was 0.90**. The correlations between water use and dry weight were also high within each water treatment (Figure 14). It is not surprising that these two characters should be related as, to some extent, each would be dependent on the other; stress would restrict dry weight expansion which on the relief of stress would restrict water use. The high correlation does not eliminate the possibility of genotypic differences in the relationship and to examine this possibility, the Water Use Efficiency for dry weight production was calculated. It was derived from:

$$\text{WUE (dry weight)} = \frac{\text{Shoot dry weight per pot (g)}}{\text{Water use per lot (litre)}}$$

Water Use Efficiency for dry weight was increased by stress and particularly so for the post-anthesis stress (Table 50). Genotypes also varied for WUE (dry weight) but there was no significant interaction with water treatment. Water Use Efficiency for dry weight varied from 3.38 for Bankuti Korai to 4.64 for Princess but the genotype differences were not significantly related to dry weight, anthesis date, Ψ(xylem) or free proline.

There was also a significant correlation between water use and grain yield for all water treatments (Figure 15) although not as close as those between water use and dry weight.

Water Use Efficiency for grain yield WUE (yield) was calculated as for WUE (dry weight) with grain yield per pot replacing dry weight as the numerator. All effects were significant (Table 51). The vegetative and pre-anthesis stress both increased WUE (yield) while the post-anthesis stressed decreased it relative to the control. Despite the statistical interaction, the correlation coefficients between

Figure 14.

The relationship between dry weight (g/pot) and water used (litres/pot) by 18 genotypes in four water treatments in Experiment 5. For the key to genotypes see Table 2, p. 41.

a) Water Treatment 1 (unstressed control) - r=0.87**

b) Water Treatment 2 (vegetative stress) - r=0.90**

c) Water Treatment 3 (pre-anthesis stress) - r=0.88**

d) Water Treatment 4 (post-anthesis stress) - r=0.91**

e) The calculated regression lines for figures (a), (b), (c) and (d).

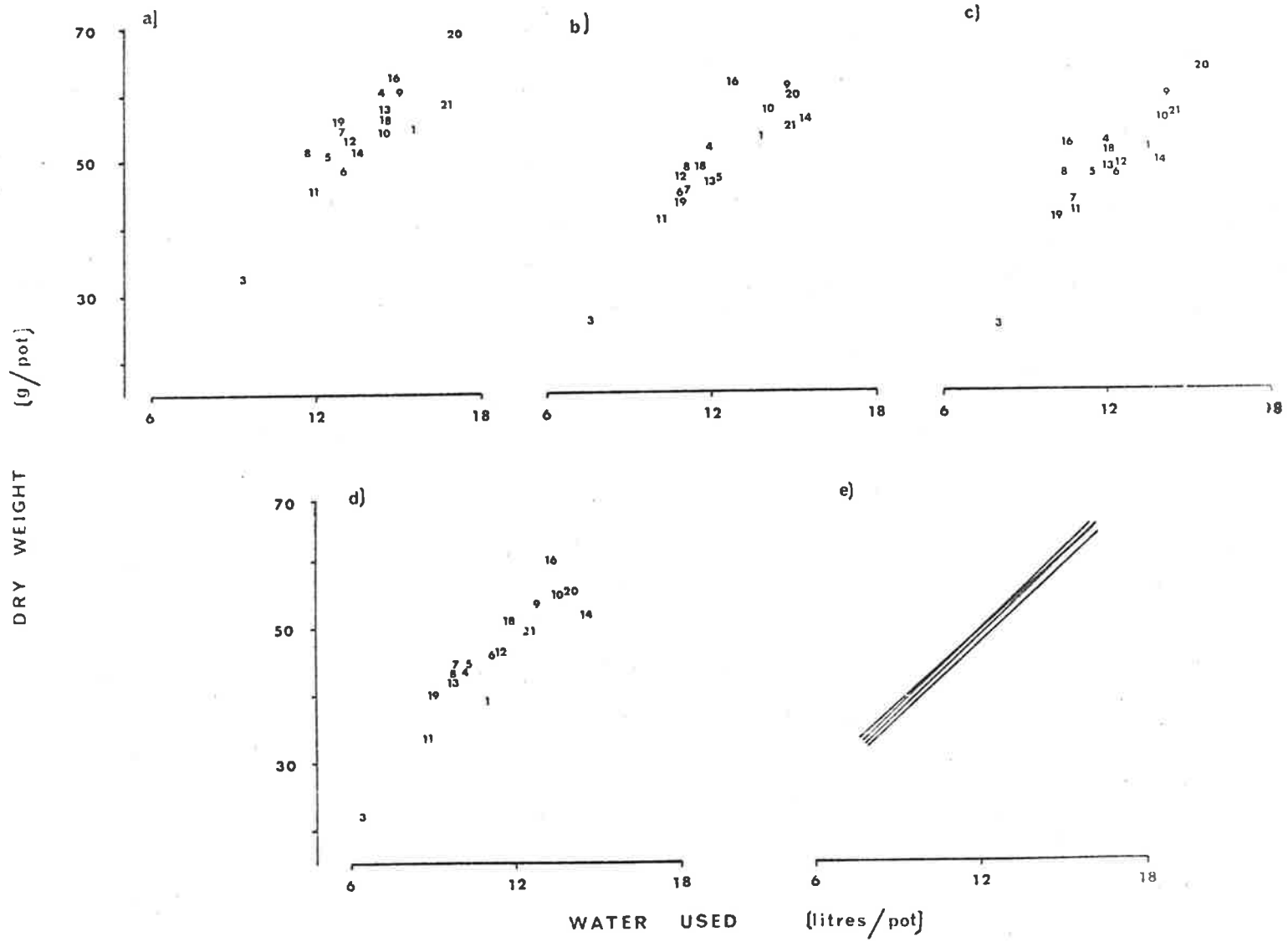

a)
b)
c)
d)
e)
70
50
30
6
12
18
DRY WEIGHT (g/pot)
WATER USED (litres/pot)

Table 50.

Water Use Efficiency for dry weight production in 18 genotypes in the unstressed control and at three stress stages. Mean of four replicates.

Genotype	Water Treatment				
	Control	Veg.	Pre-anth.	Post-anth.	Mean
Arivat	3.50	3.83	3.81	3.57	3.68
Bankuti Korai	3.51	3.45	3.13	3.44	3.38
BR 1239	4.16	4.32	4.31	4.31	4.27
CI 3576	4.09	3.81	4.14	4.38	4.10
Clipper	3.70	4.13	3.92	4.18	3.98
CPI 18197	4.24	4.07	4.10	4.57	4.24
Cyprus Black	4.39	4.44	4.53	4.36	4.43
Dore	4.02	4.06	4.17	4.17	4.10
Excelsior	3.82	4.18	4.03	4.08	4.00
Greenough	3.83	3.99	3.90	3.81	3.88
Hiproly	3.90	4.30	3.92	4.01	4.03
Ketch	3.92	3.87	4.02	4.42	4.19
Maraini	3.82	3.59	3.56	3.59	3.64
Princess	4.24	4.79	5.01	4.52	4.64
Proctor	3.88	4.24	4.25	4.41	4.19
Stewart	4.35	3.96	4.02	4.42	4.19
Velvon II	4.03	4.06	4.08	3.94	4.04
Zephyr	3.46	3.65	3.91	3.84	3.72
Mean	3.94	4.04	4.05	4.11	4.04

Analysis

Source of Variation	LSD (p=.05)
Genotype	0.22**
Water Treatment	0.11*
Genotype x Water Treatment	n.s.

Figure 15.

The relationship between grain yield (g/pot) and water used (litres/pot) by 17 genotypes (Hiproly excluded) in the four water treatments of Experiment 5. For the key to genotypes see Table 2, p. 41. The water treatments are:

a) Water Treatment 1 (unstressed control) - r=0.55*

b) Water Treatment 2 (vegetative stress) - r=0.55*

c) Water Treatment 3 (pre-anthesis stress) - r=0.73**

d) Water Treatment 4 (post-anthesis stress) - r=0.57*

e) The calculated regression lines for figures (a), (b), (c) and (d).

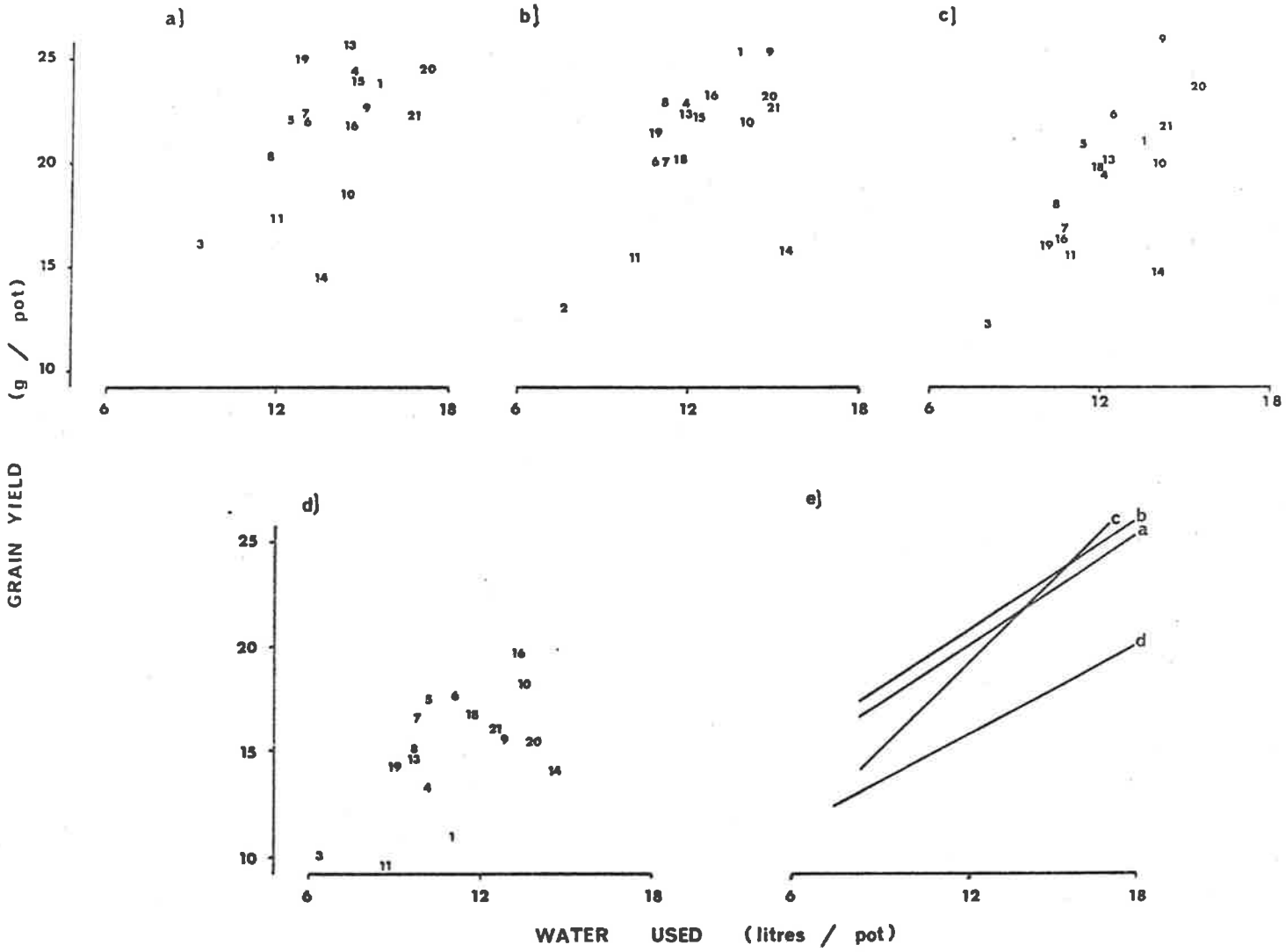

a)
b)
c)
d)
e)
GRAIN YIELD (g / pot)
WATER USED (litres / pot)
25
20
15
10
6
12
18
a
b
c
d

Table 51.

Water Use Efficiency for grain yield - WUE (yield) - in 17 barley genotypes (Hiproly excluded) when not stressed and when stressed at specific developmental stages. Mean of four replicates.

	Water Treatment				
Genotype	Control	Veg.	Pre-anth.	Post-anth.	Mean
Arivat	1.51	1.79	1.54	1.01	1.46
Bankuti Korai	1.75	1.72	1.54	1.61	1.66
BR 1239	1.65	1.88	1.62	1.29	1.63
CI 3576	1.75	1.77	1.76	1.67	1.74
Clipper	1.67	1.82	1.76	1.60	1.71
CPI 18197	1.71	1.78	1.57	1.68	1.69
Cyprus Black	1.72	1.95	1.64	1.50	1.70
Dore	1.49	1.69	1.80	1.23	1.55
Excelsior	1.29	1.51	1.40	1.31	1.38
Greenough	1.46	1.51	1.44	1.11	1.38
Ketch	1.75	1.86	1.63	1.52	1.69
Maraini	1.09	0.99	1.05	0.99	1.04
Princess	1.62	1.79	1.57	1.48	1.61
Proctor	1.48	1.72	1.60	1.43	1.56
Stewart	1.95	1.93	1.57	1.57	1.75
Velvon II	1.43	1.54	1.50	1.12	1.42
Zephyr	1.31	1.48	1.48	1.18	1.36
Mean	1.57	1.69	1.56	1.38	1.55

Analysis Source of Variation	LSD (p=.05)
Genotype	0.11**
Water Treatment	0.06**
Genotype x Water Treatment	0.23**

the genotype values for all treatments were significant (Table 52) so genotype ranking tended to be maintained in all water treatments.

The greater complexity of the response in terms of WUE (yield) compared to WUE (dry weight) is a reflection of the response of Harvest Index to stress. Harvest Index in this experiment is defined as:

HI = Grain Yield/Total dry weight (shoot dry weight only),

and it links the two measures of water efficiency directly = i.e.

WUE (yield) = WUE (dry weight) x Harvest Index.

Genotypes differed in Harvest Index in this experiment (Table 53) while the interaction between genotype and water treatment was also significant. The mean of all genotypes also varied over the four water treatments with the post-anthesis stress reducing the index below that of the other treatments. The straw weight component of harvest index had already been determined by the time of stress and only the grain yield component was significantly affected.

Genotype values for Harvest Index were negatively correlated with the anthesis date of the unstressed control. The correlation coefficient of the control treatment was r= -0.78** with the later genotypes having lower harvest indices. There was also a significant correlation between harvest index and X3' (percentage of fertile florets). The correlation coefficient for the unstressed control treatment was r=0.74** indicating that the more fertile genotypes also had the higher harvest indices. There was also a correlation for the vegetative stress (r=0.73**) and a weak one for the pre-anthesis stress (r=0.45 n.s.). Thus, when not stressed, the later flowering genotypes tended to have a higher percentage of sterile florets and to have a lower harvest index than the earlier genotypes but, if stress occurred after anthesis, other components became more important.

Table 52.

Correlation coefficients between the four water treatments for Water Use Efficiency for grain yield calculated over 17 genotype means (Hiproly excluded).

Water Treatment	Vegetative stress	Pre-Anthesis Stress	Post-Anthesis Stress
Control	0.88**	0.68**	0.79**
Vegetative stress		0.82**	0.76**
Pre-anthesis stress			0.58*

Table 53.

Harvest Index of 18 genotypes when not stressed and when stressed at specific developmental stages. Mean of four replicates.

Genotype	Water Treatment				
	Control	Veg.	Pre-anth.	Post-anth.	Mean
Arivat	0.43	0.47	0.41	0.28	0.40
Bankuti Korai	0.50	0.49	0.39	0.36	0.42
CI 3576	0.43	0.47	0.43	0.38	0.43
Clipper	0.45	0.44	0.45	0.38	0.43
CPI 18197	0.40	0.44	0.38	0.37	0.40
Cyprus Black	0.39	0.44	0.36	0.34	0.39
Dore	0.37	0.42	0.43	0.29	0.38
Excelsior	0.34	0.36	0.35	0.32	0.34
Greenough	0.38	0.38	0.37	0.29	0.36
Hiproly	0.14	0.14	0.15	0.14	0.14
Ketch	0.45	0.48	0.41	0.35	0.42
Maraini	0.29	0.28	0.30	0.27	0.28
Princess	0.38	0.38	0.31	0.33	0.35
Proctor	0.38	0.41	0.38	0.33	0.37
Stewart	0.45	0.49	0.39	0.36	0.42
Velvon II	0.36	0.38	0.37	0.28	0.35
Zephyr	0.38	0.41	0.38	0.31	0.37
Mean	0.35	0.41	0.38	0.31	0.37

Analysis

Source of Variation	LSD (p=.05)	
	Include Hiproly	Exclude Hiproly
Genotype	0.03**	0.02**
Water Treatment	0.01**	0.01**
Genotype x Water Treatment	0.05**	0.05**

Significance of Experiment 5 Results

Performance of genotypes in response to stress was estimated by DWP, the dry weight when stressed relative to the unstressed control. DWP suggested that later genotypes performed relatively better than the early ones. A single cycle of stress was applied at the vegetative and pre-anthesis stages and the later genotypes were stressed for a shorter proportion of their growing period. Similarly, later genotypes were stressed for a shorter period after anthesis (Figure 12) and this may account for the relationship.

Dry weight was significantly related to water use in all water treatments but genotypes did differ for Water Use Efficiency (for dry weight production). For the control and two stresses before anthesis it is likely that the dry weight differences (i.e. leaf area) dictated the quantity of water used. This does not explain why the relationship held for the post-anthesis stress where water was not supplied to satisfy demand.

Grain yield is related to dry weight and WEU (yield) to WEU (dry weight) by Harvest Index. Harvest Index was affected by stress but was reduced by stress only after anthesis. It was significantly related to X3 (percentage of fertile florets) for the control and two stresses before anthesis but not for the post-anthesis stress. The late maturing genotypes were not using water as quickly by anthesis and leaf area was probably falling by that time. It could be expected, then, that Harvest Index would be lower in these genotypes and that the component most important in determining this response would be percentage of fertile florets. Post-anthesis stress reduced Harvest Index more than any other treatment but it was not determined by the percentage of fertile florets.

These relationships suggest that the difficulty in realistically ranking genotypes for resistance to stress were associated with

a) the proportion of the growth period that was stressed for the genotypes of different maturity and

b) the later genotypes in the control treatment being subjected to stress before anthesis as demonstrated by a declining leaf area at that time. The stress may have been due to temperature, evaporative demand, nutrient availability or pot size.

Experiment 6. Genotypes stressed for specific development periods

The genotypes in Experiment 5 were stressed at specific development stages. This meant that they were stressed at different times, under different environmental conditions and for differing proportions of their development periods. The stress was not severe and ample opportunity was allowed for recovery, particularly after the early stresses.

Experiment 6 was designed to complement these results by applying stress throughout specific development periods and to minimise differences due to the environment, particularly those due to temperature, by growing the plants in a small glasshouse in which temperature was maintained at $22^{o}C \pm 2^{o}C$. The soil was dried prior to the experiment and the water treatments were

Water Treatment 1 (HHH): High regime from sowing to harvest.

2 (LHH): Low regime from sowing to jointing and high thereafter.

3 (LLH): Low regime from sowing to anthesis and high thereafter.

4 (HHL): High regime from sowing to anthesis and low thereafter.

5 (LLL): Low regime from sowing to maturity.

Soil water potential was allowed to fluctuate between -0.1 and -0.33 bars in the high regime. Water was required twice each day to maintain the regimes when the plants were actively growing. Water was added to the pots on the low regime when soil water potential had fallen to -15 bars as calculated by pot weight. Sufficient water was added to raise the average soil water potential in the pot to -1 bar but, in reality, the surface of the soil varied from field capacity to -15 bars while the soil at depth would have been permanently dry. Water was required twice each week to maintain this regime when the plants were actively growing.

Eight genotypes were used in this experiment. Mona was used as an early maturing two-row type to complement Bankuti Korai. Unfortunately seed for this cultivar proved to have been mixed and some late maturing plants dominated the pots in which they occurred. For this reason Mona results have been excluded from many of the analyses and this will be stated where it has occurred. Cyprus Black and Stewart were included as early-mid maturing six-row types; Clipper and Dore as mid-maturing two-row types and Princess and Proctor as late maturing two-row types.

Water Use

Figure 16 illustrates the water use pattern of four of the genotypes in the five water treatments. Water use was extremely high in this experiment as a result of the large volume of air movement required to maintain the selected temperature in the confined glasshouse space. The grain filling period of the earlier genotypes was longer than in Experiment 5 and took up a greater proportion of the total growing period. Unlike that experiment, however, anthesis occurred in all genotypes before the period of most rapid water use.

Figure 16.

Water use pattern (water used each day) by four representative genotypes in the five water treatments of Experiment 6. The vertical arrow for each genotype indicates anthesis date of the main shoot in the unstressed control treatment. The five water treatments are:

■——————■ Unstressed control (HHH)

△—— ——△ Water Treatment 2 (LHH)

▼——·—— ·▼ Water Treatment 3 (LLH)

□– – – – –□ Water Treatment 4 (HHL)

●——————● Water Treatment 5 (LLL).

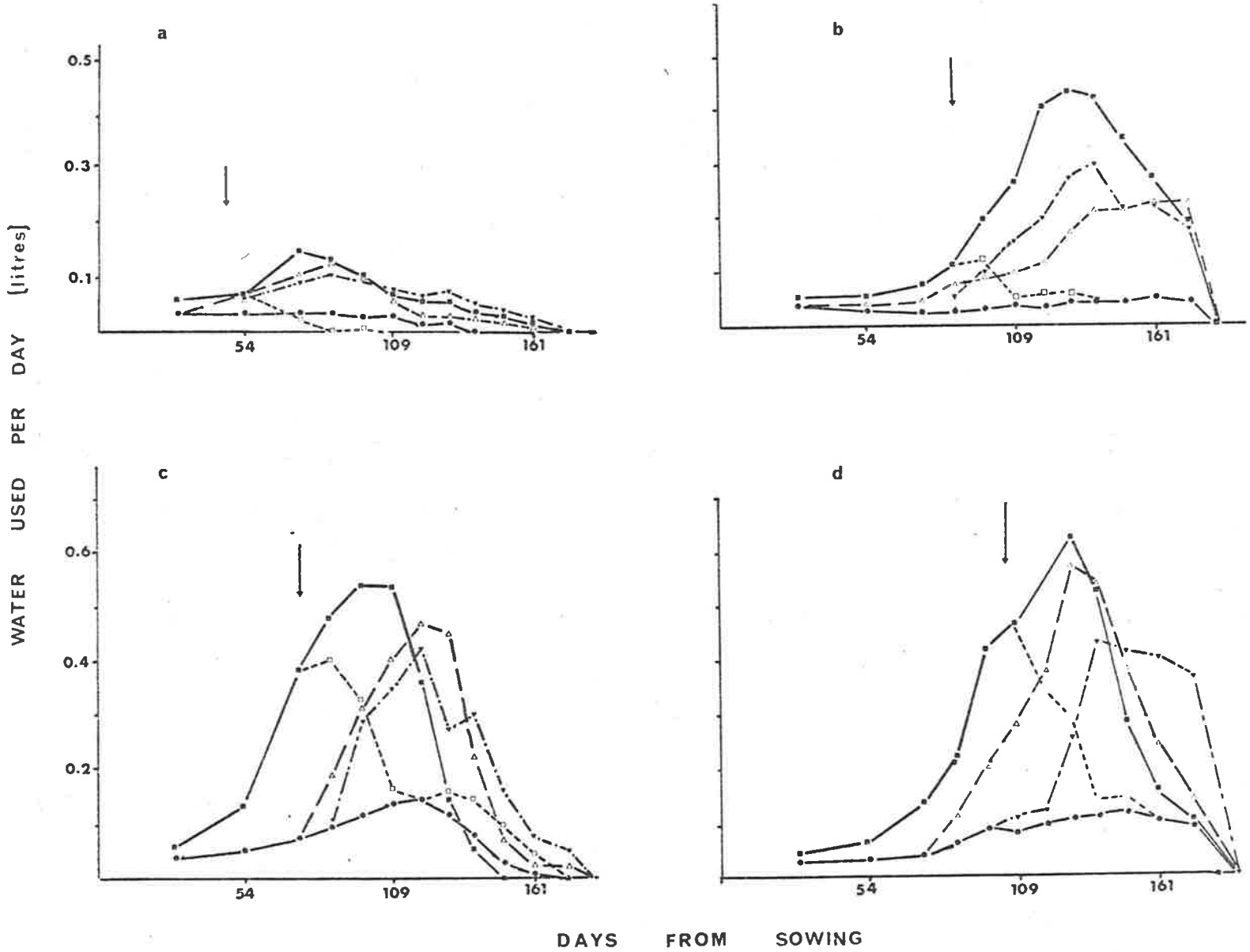
The genotypes are:

a) Bankuti Korai:- very early maturing, two-row.

b) Clipper:- mid maturing, two-row.

c) Stewart:- early maturing, six-row.

d) Proctor:- late maturing, two-row.


a
b
c
d
0.5
0.3
0.1
0.6
0.4
0.2
54
109
161
WATER USED PER DAY (litres)
DAYS FROM SOWING

Water use increased quickly when the pots were transferred from the low to the high regime (i.e. at jointing in treatment 2 (LHH) and at anthesis in treatment 3 (LLH)). The rate of increase was equivalent to that of the control pots but maximum water use was never as great. Similarly, water use fell when pots were transferred from the high to the low regime so that it stabilized at about the same level as in pots of the same genotype in treatment 5 which were maintained on the low regime throughout the experiment.

Genotype, water treatment and interaction effects on water use were all statistically significant (Table 54). Treatment 2 (LHH) and 3 (LLH) restricted total water use to 78.1% and 68.9% of the control respectively while treatment 4 (HHL) and treatment 5 (LLL) reduced total water use to 59.1% and 29.7% respectively. Genotypes varied in the amount of water they used when not stressed, varying from 12.4 litres per pot for Bankuti Korai to 50.6 litres per pot by Dore. These differences were an indication of the extreme differences in growth. The water treatment x genotype interaction was significant for water use but these results were related to differences in dry weight and will be discussed under the section on Water Use Efficiency.

Xylem Water Potential

Measurement of xylem water potential was not as clearly a measure of imposed stress in this experiment as in Experiment 5. Plants were subjected to cyclic stresses for specific periods and xylem water potential would vary with water status of the soil. It was measured either on the same day for plants of all genotypes in Treatment 1 (HHH) and 5 (LLL) or at the same stage of development but not on the same day. In the first case Ψ(xylem) represents the effect of the treatments because soil in all pots on the same regime was not necessarily at the

Table 54.

Water used (litre/pot) by eight barley genotypes in five water treatments. Mean of three replicates.

Genotype	Maturity*	Water Treatment HHH	LHH	LHH	HHL	LLL	Mean
Bankuti Korai	VE	12.4	9.1	10.5	4.1	4.0	8.0
Mona	VE	23.1	24.1	19.5	8.0	6.5	13.3
Cyprus Black	E	32.8	26.2	26.4	21.4	13.6	24.4
Stewart	E	36.7	27.6	25.7	28.6	12.0	25.8
Clipper	M	32.5	18.6	30.7	10.6	5.9	17.4
~~Clipper~~ Dore	M	50.6	41.7	29.5	31.3	14.2	33.5
Princess	L	45.9	33.2	27.8	31.6	13.5	30.4
Proctor	L	41.8	33.3	27.3	29.0	12.7	27.9
Mean		34.7	27.1	23.9	20.5	10.3	23.0

Analysis

Source of Variation	LSD (p=.05)
Water Treatments	2.9**
Genotypes	2.4**
Genotypes within water treatments	5.2**

* VE = Very Early, E = Early, M = Midseason, L = Late.

same Ψ(soil). When measured at the same critical stage of developmemt, Ψ(xylem) reflected the plant response at similar levels of Ψ(soil) and was more like the measurements in Experiment 5. It was still not a complete measure of the water treatment however, because stress was cyclic after Ψ(xylem) measurement.

Water potential was first measured one week after the two earliest genotypes (Bankuti Korai and Mona) were transferred from the low to the high regime at jointing. At this time Ψ(xylem) of all genotypes was recorded in Water Treatments 1 (HHH) and 5 (LLL). The differences between water treatments were significant but not large (Table 55). Plants maintained on the low regime were smaller than at the first time of Ψ(xylem) measurement in Experiment 5 and this is reflected in higher Ψ(xylem) values in this experiment. Xylem water potential measurements were made on four other occasions to sample critical times of development for all genotypes. On each occasion Ψ(xylem) in treatment 5 (LLL) was significantly but only slightly below treatment 1 (HHH).

Xylem water potential was also measured for each genotype in each water treatment one week after plants were transferred from high to low water status but Ψ(xylem) of plants in treatment LLH by this time was not significantly different from that of the controls. When plants were transferred from high to low status (Treatment 4 - HHL), however, xylem water potential fell much lower than in the treatment maintained on the low water regime (LLL) (Table 56). Genotypes varied significantly at this stage but the differences between them were not related to those measured in other experiments.

Free Proline

Free proline was measured in the youngest fully expanded leaf at the time of each sampling for Ψ(xylem). The first sample was taken

Table 55.

Xylem water potential (- bars) in plants maintained on the high and low regime. Measured in all plants when Bankuti Korai and Mona were one week after jointing. Mean of three replicates.

		Water Treatment		
Genotype	Maturity	High Regime (HHH)	Low Regime (LLL)	Mean
Bankuti Korai	VE	18.1	24.8	21.5
Mona	VE	16.3	20.1	18.2
Cyprus Black	E	12.8	18.0	15.4
Stewart	E	9.9	16.6	13.3
Clipper	M	12.4	17.7	15.1
Dore	M	11.0	14.8	12.9
Princess	L	10.0	16.3	13.2
Proctor	L	10.4	16.8	13.6
Mean		12.6	18.1	15.4

Analysis	
Source of Variation	LSD (p=.05)
Water Treatment	2.3**
Genotype	1.8**
Genotype within water treatment	n.s.

Table 56.

Xylem water potential (- bars) in eight genotypes and five water treatments one week after transferrence from high to low water status in Water Treatment 4 (HHL). This corresponds to one week after anthesis of the main shoot in the unstressed control.

Genotype	Maturity	Water Treatment HHH	LHH	LLH	HHL	LLL	Mean
Bankuti Korai	VE	19.0	20.1	19.0	37.1	24.7	23.9
Mona	VE	16.0	17.8	18.5	33.3	23.4	21.8
Cyprus Black	E	17.0	17.2	16.0	40.2	26.2	23.3
Stewart	E	17.7	18.0	17.4	38.1	25.1	23.3
Clipper	M	20.1	23.5	25.3	35.0	27.6	26.3
Dore	M	12.6	13.0	13.7	38.0	22.6	20.0
Princess	L	10.0	11.5	12.4	34.5	19.5	17.6
Proctor	L	12.4	14.3	14.1	35.2	21.0	19.4
Mean		15.6	16.9	16.6	36.4	23.7	

Analysis

Source of Variation	LSD (p=.05)
Water Treatments	1.7**
Genotypes	1.5**
Genotypes within water treatments	3.0**

when Bankuti Korai and Mona were sampled one week after jointing (corresponding to the Ψ(xylem) sample of Table 55). All genotypes were sampled on the same day but it followed that they were at different stages of development. Proline had accumulated in those plants maintained on the low regime by this time (Table 57) but the genotypes were not found to differ significantly. Soil in the pots maintained on the low regime was not necessarily at -15 bars at the time of sampling, however, and genotypes had different stress histories which may have influenced the response by increasing variability (Singh *et al.*, 1973d).

Only those plants transferred from the high to the low regime had accumulated significant amounts of proline when plants were measured at equivalent developmental stages (Table 58). The free proline of previously stressed plants had returned to levels which were equivalent to the controls and the plants in the treatment maintained on the low regime (LLL) had relatively low free proline levels. These plants were small at the time of measurement, were relatively unstressed (Table 56) and therefore had lower free proline levels than those in treatment HHL which were recently transferred to the low status and had high leaf area but with low leaf water potential.

The genotypes were significantly different for free proline one week after transferrence from the high to low water regime in Treatment 4 (HHL) (Table 59) but variability was very high. There was no relationship between proline accumulated by the different genotypes in the experiment with that of the same genotypes in other experiments, nor were free proline and Ψ(xylem) related.

Shoot Dry Weight

Shoot dry weight varied from 7.7 g per pot to 150 g per pot over the genotypes and water treatments with effects due to genotype,

Table 57.

Free proline (mg/g dry weight) in eight genotypes in two water treatments on the same day. Measurements made one week after jointing in Bankuti Korai and Mona, the two earliest genotypes. Mean of three replicates.

		Water Treatment		
Genotype	Maturity	HHH	LLL	Mean
Bankuti Korai	VE	0.7	3.4	2.1
Mona	VE	0.5	2.2	1.4
Cyprus Black	E	0.3	4.1	2.2
Stewart	E	0.6	4.8	2.7
Clipper	M	0.4	3.2	1.8
Dore	M	0.2	2.8	1.5
Princess	L	0.5	3.4	2.0
Proctor	L	0.7	5.2	3.0
Mean		0.5	3.6	3.0

Analysis

Source of Variation	LSD (p=.05)
Water Treatments	2.5*
Genotypes	n.s.
Genotypes within water treatments	n.s.

Table 58.

Free proline (mg/g dry weight) in the youngest fully expanded leaf in five water treatments measured one week after anthesis of the main shoot in the unstressed control. Genotypes not measured on the same day. Mean of eight genotypes and three replicates.

Water Treatment	Free proline
1 (HHH)	0.9
2 (LHH)	0.7
3 (LLH)	1.1
4 (HHL)	6.9
5 (LLL)	2.8
Mean	2.4
LSD (p=.05)	1.6**

Table 59.

Free proline (mg/g dry weight) in eight genotypes in water treatment 4 (HHL). Measured in the flag leaf one week after transferrence from the high to the low water regime. Mean of three replicates.

Genotype	Maturity	Free proline
Bankuti Korai	VE	8.4
Mona	VE	5.6
Cyprus Black	E	9.1
Stewart	E	6.2
Clipper	M	7.4
Dore	M	6.8
Princess	L	6.5
Proctor	L	4.8
Mean		6.9
LSD (p=.05)		3.9*

water treatment and interaction all highly significant (Table 60). Ranking for dry weight accumulation of the water treatments was 1 (HHH) greater than 2 (LHH) and 3 (LLH) which were equal to 4 (HHL) and all treatments greater than 5 (LLL). If Mona results are excluded from the analysis, however, treatment 2 (LHH) produced significantly more dry weight than 3 (LLH).

Bankuti Korai produced only 25.9 g per pot when not stressed and this contrasts with the 149.6 g per pot produced by Dore. There was some tendency for the later genotypes to produce more dry weight but the significant correlation was solely due to the extreme result of Bankuti Korai (i.e. this genotype was very early and produced very low dry matter yields).

The significant interaction between genotype and water treatment for dry weight suggested that genotypes varied in response to the water treatments but they varied substantially for total dry weight when not stressed. Their response to the water treatments was examined by calculating Dry Weight Percentage (DWP), the dry weight when stressed as a percentage of that in the control treatment, as defined on p. 116. There was a genotype x water treatment interaction for DWP (Table 60) and genotype responses to the various stresses was complex. Proctor, Princess and Cyprus Black performed relatively better over all treatments but genotype ranking for DWP varied with water treatment. There was not a relationship between DWP and anthesis date for any treatment in contrast to the results in Experiment 5. Nor was there a relationship between DWP and either free proline or xylem water potential.

Grain Yield

Water treatment, genotype and interaction effects on yield were all highly significant (Table 61). Stress after anthesis limited

Table 60.

Total shoot dry weight (g/pot) in water treatment 1 (HHH) and the mean of all treatments. Also DWP (dry weight percentage) for water treatments 2, 3, 4 and 5. Mean of three replicates.

		Dry Weight			Dry Weight Percent			
Genotype	Maturity	HHH	Mean	LHH	LLH	HHL	LL	Mean
Bankuti Korai	VE	25.9	16.3	68.8	80.6	33.9	29.7	53.2
Mona	VE	65.2	32.1	64.2	112.7	41.1	28.8	61.7
Cyprus Black	E	106.5	74.5	80.6	68.8	62.6	38.1	62.5
Stewart	E	118.2	80.3	67.7	63.2	75.8	33.1	60.0
Clipper	M	79.3	46.8	64.1	67.4	46.7	22.5	50.2
Dore	M	149.6	98.6	75.8	61.4	60.6	32.6	57.6
Princess	L	141.1	99.8	78.5	64.1	73.8	37.5	63.5
Proctor	L	112.6	81.2	89.5	80.7	71.2	42.2	70.9
Mean		100.8	69.7	73.6	74.8	58.2	33.1	59.9

Analysis

Source of Variation	LSD (p=.05)	
	DW	DWP
Water Treatment	10.4**	6.8**
Genotype	8.3**	6.8**
Genotype x Water Treatment[a]	18.6**	16.9*

[a] LSD values for the interaction only appropriate for comparisons of genotypes within water treatments.

Table 61.

Grain yield (g/pot) in the unstressed control (HHH) and the mean yield of all treatments. Also YP for the four stress treatments. YP (defined on p. 120) is the yield when stressed expressed as a percentage of control yield. Mean of three replicates.

		Water Treatment						
		Yield		Yield Percent (YP)				
Genotype	Maturity	HHH	Mean	LHH	LLH	HHL	LLL	Mean
Bankuti Korai	VE	14.16	8.39	66.9	76.0	26.1	26.3	48.8
Mona	VE	25.62	11.72	76.6	100.4	26.1	33.7	59.2
Cyprus Black	E	38.35	27.02	104.8	91.1	31.8	30.1	64.5
Stewart	E	48.51	32.76	82.5	74.6	47.3	33.3	59.4
Clipper	M	34.65	19.75	59.9	72.7	36.0	24.2	48.2
Dore	M	54.44	36.04	84.1	71.4	35.0	39.9	57.6
Princess	L	46.89	32.60	83.5	74.4	44.9	45.6	62.1
Proctor	L	35.28	25.74	104.9	94.0	45.6	44.5	72.2
Mean		37.86	24.83	82.9	81.8	36.6	34.7	59.0

Analysis

Source of Variation	LSD (p=.05)		
	Yield	YP	
		+ Mona	- Mona
Water Treatment	3.92**	n.s.	12.6*
Genotype	3.57**	11.25**	8.5**
Genotypes within water treatments	8.78**	n.s.	n.s.

grain yield more than stress before this stage so that the grain yield in the stress treatment applied only after anthesis was not significantly different from that in the treatment where stress was applied throughout the total experiment.

Genotypes varied in their yield in the absence of water stress with Bankuti Korai producing only 14.2 g per pot at one extreme and Dore with 54.4 g per pot at the other. There was no relationship between anthesis date and grain yield in this data.

The significant interaction between the effects of the water treatments and genotypes suggest that the genotypes differed in response to stress. This was examined by calculating Yield Percentage (YP) - the yield when stressed as a percentage of the control yield (defined on p. 120). The significant interaction for grain yield when only the four stress treatments were used in the analysis was lost when the analysis was performed on YP. This was probably due to the increased variability associated with combining the two variables in calculating the derived character.

Proctor yielded more when stressed relative to its own control than did the other genotypes, although it was not significantly different from Cyprus Black or Princess. Bankuti Korai and Clipper performed relatively poorly. There was no apparent relationship between YP and anthesis date, Ψ(xylem) or proline.

Yield Components

The general pattern of yield component response was similar to that for Experiment 5. In general, stress at any stage influenced the component formed during that stage (Table 62). As for Experiment 5, however, relief of pre-anthesis stress resulted in the formation of late tillers which modified the result at harvest, while post-anthesis

Table 62.

Yield component percentages (yield component when stressed as a percentage of control performance) over all genotypes when stressed for various development stages. Mean of three replicates and eight genotypes. X1 is tiller number (fertile only), X2 is florets/tiller, X3 is the percentage of florets that set seed and X4 is thousand grain weight.

	Yield Component Percentage			
Water Treatment	X1P	X2P	X3P	X4P
2 (LHH)	85.1	93.5	111.8	100.1
3 (LLH)	104.2	84.4	101.2	95.8
4 (HHL)	57.9	97.6	78.3	87.7
5 (LLL)	58.7	74.3	91.0	89.1

stress inhibited tillers from forming grain.

Tiller number was the component most affected when plants were maintained on low water status to jointing. It was reduced more during stress than is apparent in Table 62, but the relief of stress induced late tiller development. Total floret number per tiller was also reduced by this treatment despite stress having been relieved at the time of its determination. Floret fertility was higher than in the controls, however, while grain weight was little different. Tiller number was greater in the treatment maintained on the low water regime to anthesis than in the unstressed controls, but floret number per tiller was lower. The later formed components were little affected by the stress treatment.

Transfer of plants from the high to the low water regime after anthesis (Treatment 4) resulted in lower floret fertility and lower grain weight but the component most affected was the number of fertile tillers.

All yield components were reduced below those of the controls in plants maintained on the low water regime from sowing to maturity. The first formed components were the most affected, however, with the number of fertile tillers reduced relatively more than the other components. Observations suggested that fertile tiller number was reduced by suppression rather than failure to develop after initiation.

Yield Components - genotype effects

The number of fertile tillers per plant was much greater in this experiment than in Experiment 5. There was no clear distinction between two-row and six-row genotypes in number of tillers but only Bankuti Korai of the two-row types had a lower tiller number than Cyprus Black and Stewart (Table 63). The genotype x water treatment interaction for tiller number was not significant which provides little evidence for

Table 63.

Number of tillers per plant (X1) of eight genotypes in the unstressed control and the mean of all water treatments. Also X1P (number of tillers as a percentage of control performance) for the water treatments 2 to 5. Mean of three replicates.

		X1			X1P			
Genotype	Maturity	HHH	Mean	LHH	LLH	HHL	LLL	Mean
Bankuti Korai	VE	20.2	11.7	92.9	117.0	47.1	47.1	76.0
Mona	VE	38.0	31.7	37.0	140.7	54.6	66.7	70.0
Cyprus Black	E	33.1	29.3	114.2	140.2	55.3	83.1	97.5
Stewart	E	33.9	26.0	94.6	121.5	94.3	74.7	96.2
Clipper	M	36.6	17.3	62.4	89.0	47.6	30.6	54.6
Dore	M	51.5	38.0	87.9	86.7	50.6	55.8	70.3
Princess	L	39.5	29.0	77.2	77.5	51.6	53.5	64.9
Proctor	L	44.6	37.0	81.1	68.9	67.2	61.7	69.7
Mean		37.1	27.5	85.1	104.2	57.9	58.7	75.7

Analysis

Source of Variation	LSD (p=.05)			
	X1		X1P	
	+ Mona	- Mona	+ Mona	- Mona
Water Treatment	2.99**	2.7**	6.2**	5.3**
Genotype	3.14**	3.0**	7.2**	7.0**
Genotype x water treatment	n.s.	n.s.	14.5*[a]	14.0*[a]

[a] Appropriate only for comparisons of genotypes within water treatments.

a difference between genotypes in their response to stress. Analysis of X1P (tiller number when stressed as a percentage of control performance - defined on p. 127), however, revealed a significant interaction. Tillering in Clipper was reduced by each of the stress treatments which was unlike its performance in Experiment 5. The two six-row genotypes, Cyprus Black and Stewart, responded to the relief of early stresses by producing later tillers and they were less affected by the low regime throughout growth. Bankuti Korai responded to the relief of stress before anthesis by the production of late tillers but fertile tiller number was reduced by stress after anthesis.

The two six-rowed genotypes produced more florets per tiller than the others (Table 64) and since there was a significant interaction between genotype and water treatment, differences in response for this character were indicated. There was no genotype x water treatment interaction for X2P (number of florets per tiller when stressed as a percentage of control performance - defined p. 127) but Bankuti Korai, Stewart and Cyprus Black had lower values than the others. There was a negative correlation between X1P and X2P when calculated on the genotype means for Water treatment 3 (LLH) (r= -0.91**) but not for the other water treatments, suggesting that those genotypes that formed late tillers on the relief of stress had reduced numbers of florets per tiller, probably because the later formed tillers had few florets.

Stress after anthesis generally reduced the percentage of fertile florets in all genotypes but there was no genotype x water treatment interaction (Table 65). The percentage of fertile florets (genotype means for all water treatments) was related to mean YP (yield percent). The correlation (r= -0.84*) suggested that the most fertile genotypes yielded less when stressed relative to their own control performance.

Table 64.

Number of florets per tiller (X2) in eight genotypes in the unstressed control and the mean of all water treatments. Also X2P (florets per tiller when stressed as a percentage of the unstressed control performance) for water treatment 2, 3, 4 and 5. Mean of three replicates.

		X2		X2P				
Genotype	Maturity	HHH	Mean	LHH	LLH	HHL	LLL	Mean
Bankuti Korai	VE	15.1	10.8	76.1	71.2	98.6	71.7	79.4
Mona	VE	16.3	8.8	108.7	91.0	86.1	56.5	85.6
Cyprus Black	E	41.2	29.2	85.6	73.9	109.4	70.9	85.0
Stewart	E	35.0	22.0	81.4	68.0	81.8	63.1	73.6
Clipper	M	15.5	13.2	95.1	86.2	100.9	84.8	91.7
Dore	M	20.3	15.8	94.9	93.4	94.9	78.5	90.4
Princess	L	23.9	20.9	101.1	90.8	107.7	87.4	96.8
Proctor	L	21.7	17.7	105.1	100.7	101.2	81.5	97.1
Mean		23.6	21.0	93.5	84.4	97.6	74.3	87.4

Analysis

Source of Variation	LSD (p=.05)			
	X2		X2P	
	+ Mona	- Mona	+ Mona	- Mona
Water Treatment	0.95**	1.25**	3.03**	5.43**
Genotype	1.70**	1.62**	10.08**	9.59**
Genotype x water treatment	3.80**[a]	3.66**[a]	n.s.	n.s.

[a] Appropriate only for comparisons of genotypes within water treatments.

Table 65.

Percentage of fertile florets (X3) of eight genotypes in the unstressed control treatment and the mean of all water treatments. Also X3P (fertile florets when stressed as a percentage of the control performance) for water treatments 2, 3, 4 and 5. Mean of three replicates.

		X3				X3P		
Genotype	Maturity	HHH	Mean	LHH	LLH	HHL	LLL	Mean
Bankuti Korai	VE	84.2	74.6	93.8	89.5	65.9	93.8	85.7
Mona	VE	63.4	57.2	186.0	96.0	71.6	96.0	102.8
Cyprus Black	E	51.2	48.6	127.5	109.2	74.9	69.1	95.2
Stewart	E	70.4	68.0	116.5	106.6	73.8	87.7	96.1
Clipper	M	82.2	78.9	99.7	105.8	84.8	93.2	95.0
Dore	M	82.4	78.6	102.1	97.3	83.8	94.7	94.5
Princess	L	71.8	69.4	97.4	99.8	86.1	102.5	96.4
Proctor	L	57.9	59.1	125.3	104.9	86.4	93.3	102.5
Mean		71.3	67.2	111.8	101.2	78.3	91.0	95.3

Analysis

Source of Variation	LSD (p=.05)			
	X3		X3P	
	+ Mona	- Mona	+ Mona	- Mona
Water Treatment	3.3**	3.2**	6.9**	6.2**
Genotype	3.0**	2.8**	n.s.	n.s.
Genotype within water treatment	n.s.	n.s.	n.s.	n.s.

This relationship held for only the two pre-anthesis stress treatments, however. The correlation coefficients between X3 and YP for Water Treatments 2, 3, 4 and 5 were r= -0.90**, r= -0.88**, r= -0.31 n.s. and r= -0.30 n.s. respectively. As for Experiment 5, there was a tendency for genotypes which were most fertile when not stressed to be relatively more affected by stress. Thus for Water Treatment 2 (LHH) there was a highly significant negative correlation between X3 and X3P of r= -0.91**. The trend was similar but the correlation coefficient not significant (r= -0.70 n.s.) for Water Treatment 3 (LLH). There was no relationship for the two post-anthesis stress treatments (r= -0.10 n.s. for Water Treatment 4 and r=0.72 n.s. for Water Treatment 5). It is not possible to determine if the relationship between YP and X3 is due to either

(a) the genotypes with the highest proportion of fertile florets when not stressed being more sensitive in terms of floret fertility to the stress treatments, or the less likely explanation that

(b) some genotypes were stressed even in Water Treatment 1 (HHH) and thus appeared more resistant to further stress.

Genotype and Water treatment effects were statistically significant for thousand grain weight (X4) (Table 66). Bankuti Korai and Clipper had lighter grains in this experiment, unlike their performance in Experiment 5.

There was no statistical interaction between genotypes and water treatments for thousand grain weight in this experiment but the genotype differences for X4P (Grain weight when stressed as a percentage of grain weight in the unstressed control) suggested that Cyprus Black and Stewart had heavier grains when not stressed but were relatively more affected by the stress treatments. Princess, however, was less

Table 66.

Thousand grain weight (X4) of eight genotypes in the unstressed control treatment and the mean over all water treatments. Also X4P (grain weight when stressed as a percentage of control performance) for water treatments 2, 3, 4 and 5. Mean of three replicates.

		X4		X4P				
Genotype	Maturity	HHH	Mean	LHH	LLH	HHL	LLL	Mean
Bankuti Korai	VE	44.7	41.9	102.2	101.8	80.7	83.5	92.0
Mona	VE	43.7	41.9	109.9	116.6	97.2	92.2	100.9
Cyprus Black	E	54.7	46.2	86.2	81.6	76.6	77.5	80.5
Stewart	E	56.3	50.0	91.6	86.1	83.5	82.2	85.8
Clipper	M	46.9	45.8	104.8	93.1	94.4	93.4	96.8
Dore	M	48.1	46.2	100.7	93.7	90.6	96.7	95.4
Princess	L	49.5	50.7	110.7	106.9	95.2	99.4	103.0
Proctor	L	46.8	44.3	101.5	101.3	86.5	89.1	94.4
Mean		49.2	46.0	100.1	95.8	87.7	89.1	93.0

Analysis

Source of Variation	LSD (p=.05)			
	X4		X4P	
	+ Mona	- Mona	+ Mona	- Mona
Water Treatment	1.9*	1.8*	2.7*	2.6*
Genotype	1.8*	1.8*	3.4**	3.4**
Genotypes within water treatments	n.s.	n.s.	n.s.	n.s.

affected by stress in terms of grain weight. Performance in terms of X4P was not related to the other yield components or to YP for any water treatment. The component is often considered the most important in determining response to stress in an environment where stress becomes increasingly severe throughout growth. While it was influenced by post-anthesis stress in this experiment, genotype response in terms of fertile tiller number appeared to be more important in determining relative response of grain yield to the water treatments imposed.

Additional information on the importance of the response of various yield components to the water treatments in determining yield response may have been gained from multiple regression but the data was too limited to realistically use the method.

Water Use Efficiency and Harvest Index

There was a close correlation between total water used and total dry weight when calculated on genotype means over all water treatments (r=0.99**). It was also high when calculated within water treatments (Figure 17), the correlation coefficients being r=0.97**, r=0.93**, r=0.94**, r=0.99** and r=0.98** for treatments 1 to 5 respectively, when Mona data was excluded.

Water treatments were not significantly different for Water Use Efficiency for dry weight production - WUE (dry weight) but the genotypes did vary (Table 67). Bankuti Korai had a low efficiency while Princess was more efficient than the other genotypes. The genotypes did not respond similarly to all water treatments and this is reflected in the highly significant genotype x water treatment interaction. Clipper, for example, had the highest WUE (dry weight) in Treatment 4 (HHL) while Proctor did so in Treatment 5 (LLL).

Figure 17.

The relationship between dry weight (g/pot) and the water used (litres/pot) by eight genotypes in the five water treatments of Experiment 6. For the key to genotypes see Table 2, p. 41. The water treatments are:

a) Water Treatment 1 (HHH) - r=0.97**

b) Water Treatment 2 (LHH) - r=0.86**

c) Water Treatment 3 (LLH) - r=0.98**

d) Water Treatment 4 (HHL) - r=0.98**

e) Water Treatment 5 (LLL) - r=0.97**

f) The regression lines for all water treatments.

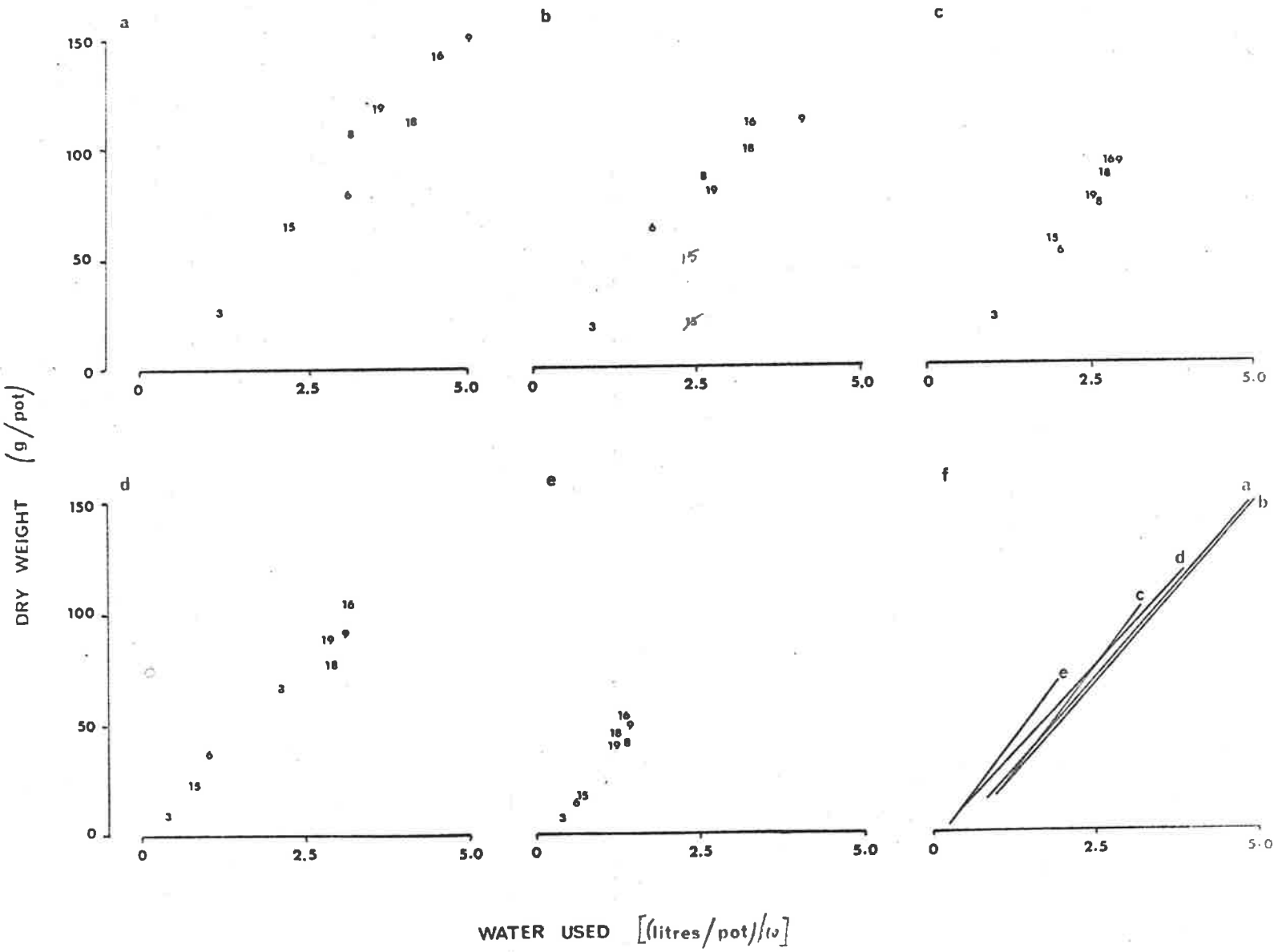

a
b
c
d
e
f
150
100
50
0
0
2.5
5.0
DRY WEIGHT (g/pot)
WATER USED [(litres/pot)/w]

Table 67.

Water Use Efficiency for dry weight - WUE (dry weight) (g/litre) in eight genotypes in five water treatments. Mean of three replicates.

Genotype	Maturity	Water Treatment HHH	LHH	LLH	HHL	LLL	Mean
Bankuti Korai	VE	2.08	1.94	2.01	2.05	1.91	2.00
Mona	VE	2.66	~~0.81~~ 1.73	2.87	2.81	2.38	2.46
Cyprus Black	E	3.25	3.19	2.78	3.12	2.99	3.06
Stewart	E	3.23	2.90	2.93	3.14	3.26	3.09
Clipper	M	2.45	2.79	2.47	3.50	2.96	2.86
Dore	M	2.96	2.70	3.08	2.94	3.40	3.02
Princess	L	3.09	3.34	3.24	3.30	2.93	3.18
Proctor	L	2.70	2.89	3.18	2.68	3.55	3.02
Mean		~~2.18~~ 2.80	~~2.73~~ 2.68	2.83	2.94	~~3.05~~ 2.92	2.88

Analysis

Source of Variation	LSD (p=.05) + Mona	- Mona
Genotype	0.24**	0.19**
Water Treatment	n.s.	n.s.
Genotypes within water treatments	0.53**	0.42**

WUE (dry weight) was higher for Experiment 5 than for Experiment 6. This reflected the different environmental conditions for the two experiments, particularly the high evaporative demand and water use in Experiment 6. Despite the absolute difference, mean WUE (dry weight) of the seven common genotypes were correlated for the unstressed control treatment in the two experiments (r=0.99**). Three other water treatments were approximately equivalent in the two experiments (Treatments 2, 3 and 4) and the genotype means for WUE (dry weight) were related in these treatments (Figure 18).

There was also a high correlation between water used and grain yield in Experiment 6 (Figure 19). The correlation coefficient was 0.88** over the genotype means for all water treatments and the correlations were equally high within water treatments. The slope for Water treatment 4 (HHL) was apparently different from the other treatments and this was reflected in the lower Water Use Efficiency for grain yield - WUE (yield) in this treatment (Table 68). Genotypes also varied for WUE (yield), the most efficient being Clipper and Stewart. There was a significant interaction between the effect of genotype and water treatment, however, so genotypes did not respond similarly to all water treatments. Clipper, for example, was little affected by post-anthesis stress while most genotypes were very inefficient in this treatment.

The correlation between genotype means for WUE (yield) in the unstressed control (Treatment 1) in Experiments 5 and 6 was, for the seven common genotypes, r=0.87** (Figure 20). The correlation for Treatment 2 (LHH) was also significant (r=0.95**) but not the two other approximately equivalent treatments. The absence of a relationship for Treatment 4 (HHL) was influenced by Cyprus Black which performed differently in the two experiments.

Figure 18.

The relationship between Water Use Efficiency for Dry Weight production in Experiment 5 and Experiment 6 for the seven genotypes common to the two experiments in the approximately equivalent treatments. For the key to genotypes see Table 2, p. 41. The correlation coefficients for the water treatments are:

a) Water Treatment 1 - r=0.98**

b) Water Treatment 2 - r=0.95**

c) Water Treatment 3 - r=0.84**

d) Water Treatment 4 - r=0.76*

e) The regression lines for all water treatments.

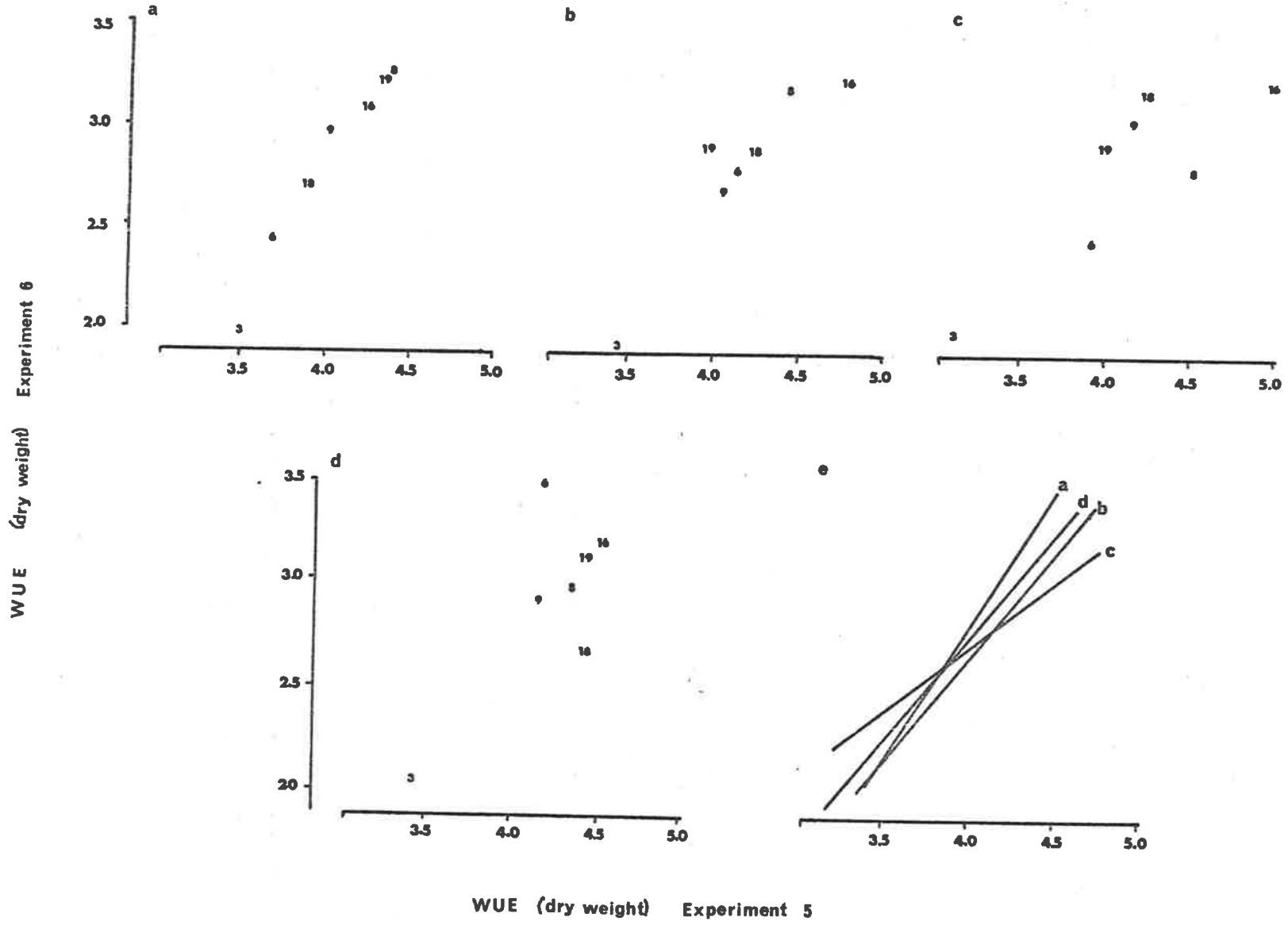

a
b
c
d
e
3.5
3.0
2.5
2.0
3.5
4.0
4.5
5.0
WUE (dry weight) Experiment 6
WUE (dry weight) Experiment 5

Figure 19.

The relationship between grain yield (g/pot) and water used (litres/pot) by eight genotypes in the five water treatments of Experiment 6. For the key to genotypes see Table 2, p. 41. The figures and correlation coefficients are:

a) Water Treatment 1 (HHH) - r=0.92**

b) Water Treatment 2 (LHH) - r=0.83**

c) Water Treatment 3 (LLH) - r=0.96**

d) Water Treatment 4 (HHL) - r=0.92**

e) Water Treatment 4 (LLL) - r=0.88**

f) The regression lines for all water treatments.

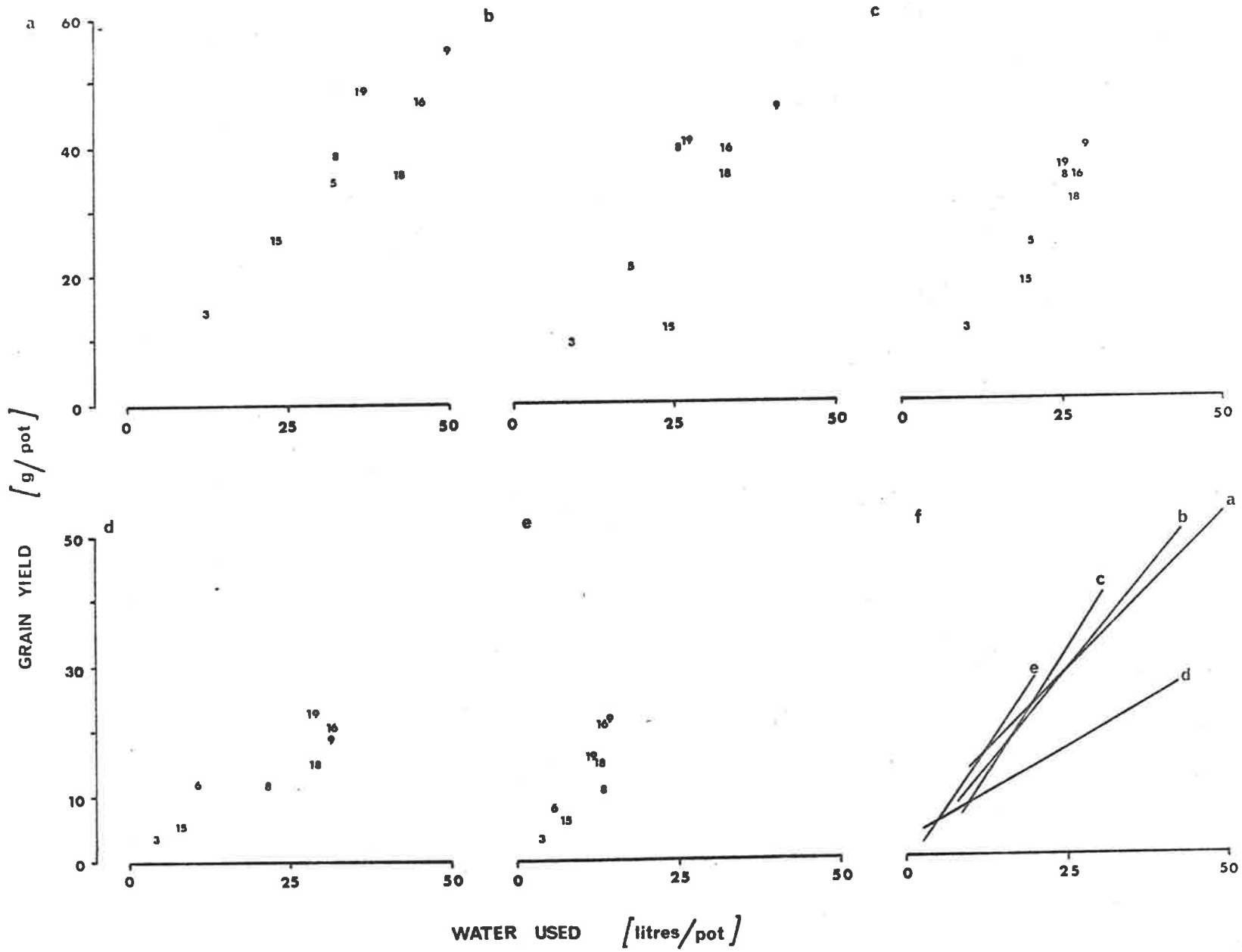

a
b
c
d
e
f
GRAIN YIELD [g/pot]
WATER USED [litres/pot]
0
20
40
60
10
30
50
25

Table 68.

Water Use Efficiency for grain yield in eight genotypes and five water treatments. Mean of three replicates.

Genotype	Maturity	Water Treatment HHH	LHH	LLH	HHL	LLL	Mean
Bankuti Korai	VE	1.14	1.03	1.04	0.85	0.93	0.99
Mona	VE	1.03	0.47	0.93	0.69	0.98	0.85
Cyprus Black	E	1.18	1.46	1.31	0.55	0.82	1.07
Stewart	E	1.32	1.45	1.41	0.80	1.35	1.27
Clipper	M	1.07	1.15	1.19	1.19	1.37	1.19
Dore	M	1.08	1.10	1.29	0.62	1.52	1.12
Princess	L	1.03	1.18	1.25	0.66	1.59	1.14
Proctor	L	0.84	1.06	1.13	0.52	1.21	0.96
Mean		1.10	1.17	1.22	0.73	1.22	1.08

Analysis

Source of Variation	LSD (p=.05) + Mona	- Mona
Water Treatment	0.15**	0.16**
Genotype	0.13**	0.13**
Genotypes within water treatments	0.30**	0.28**

Figure 20.

The relationship between Water Use Efficiency for grain yield in Experiment 5 and Experiment 6 for the seven genotypes common to the two experiments in the approximately equivalent treatments. For the key to genotypes see Table 2, p. 41.

The figures and correlation coefficients are:

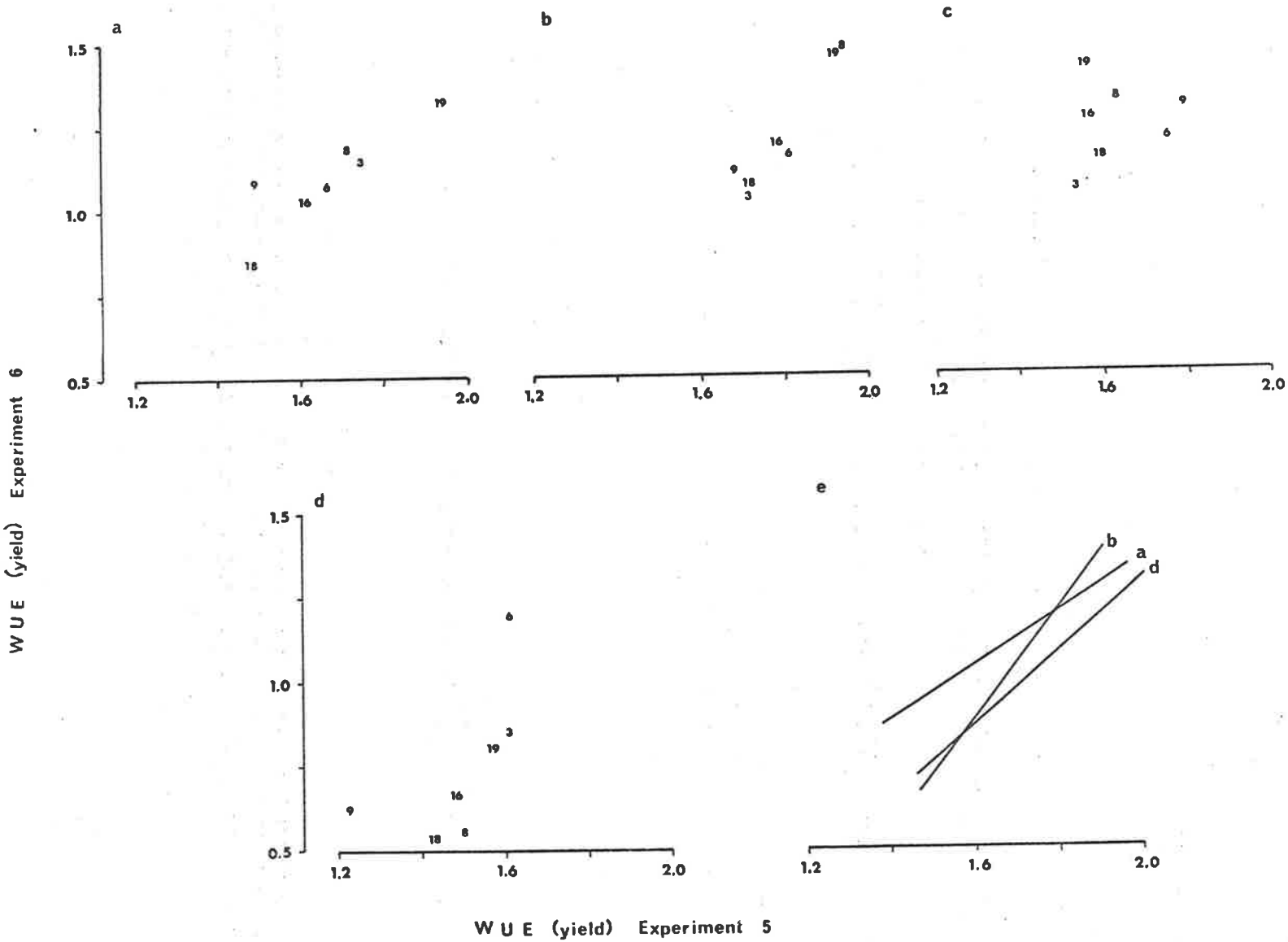
a) Water Treatment 1 - r=0.87**

b) Water Treatment 2 - r=0.95**

c) Water Treatment 3 - r=0.17 n.s.

d) Water Treatment 4 - r=0.61 n.s.

e) Regression lines for figures a, b and d.


a
b
c
d
e
1.5
1.0
0.5
1.2
1.6
2.0
WUE (yield) Experiment 6
WUE (yield) Experiment 5

Significance of Experiment 6 Results

Harvest index (calculated on the shoot dry weight only) was greater in the treatments maintained on the low regime to jointing or anthesis but was reduced by post-anthesis change from high to low water status (Table 69). Stress after anthesis affects shoot dry weight through its influence on grain yield and will therefore depress harvest index more than at other stages. Harvest index of the treatment maintained on the low regime from sowing to harvest was not significantly different from that of the control.

There were differences between the genotypes for harvest index when not stressed, with Bankuti Korai and Clipper having higher indices. These differences were not related to those measured in Experiment 5.

Harvest index was not related to anthesis date in this experiment. It was related to X3 (percentage of fertile florets) only for treatment 5 (LLL) ($r=0.95^{**}$), the more fertile genotypes having higher harvest indices.

Correspondence between Experiment 5 and Experiment 6 was not close. Genotypes did not rank similarly for free proline, xylem water potential or any of the characters used to measure response to the stresses imposed in the experiments.

Bankuti Korai yielded less when stressed, relative to its own control than did the other genotypes in both experiments but is considered to be drought resistant. It may have been subjected to disproportionate stress in Experiment 5 but its poor performance in Experiment 6 confirms the poor response of the genotype to stress. It probably derives its apparent drought resistance from escape. The later genotypes (e.g. Proctor in this experiment) are traditionally regarded as susceptible to drought but they yielded more, relative to their own control than the other genotypes in these experiments suggesting that their poor field performance is due to their late maturity.

Table 69.

Harvest index for eight genotypes in five water treatments.

Mean of three replicates.

Genotype	Maturity	Water Treatment HHH	LHH	LLH	HHL	LLL	Mean
Bankuti Korai	VE	0.55	0.53	0.52	0.42	0.48	0.50
Mona	VE	0.39	0.47	0.35	0.25	0.46	0.38
Cyprus Black	E	0.36	0.47	0.48	0.48	0.28	0.35
Stewart	E	0.41	0.50	0.48	0.26	0.41	0.41
Clipper	M	0.44	0.41	0.47	0.33	0.47	0.43
Doré	M	0.36	0.40	0.42	0.21	0.44	0.37
Princess	L	0.33	0.35	0.39	0.20	0.40	0.33
Proctor	L	0.31	0.37	0.37	0.20	0.33	0.32
Mean		0.39	0.44	0.44	0.26	0.41	

Analysis

Source of Variation	LSD (p=.05) + Mona	- Mona
Water Treatment	0.02**	0.02**
Genotype	0.02**	0.01**
Genotypes within water treatments	0.04**	0.03**

WUE (dry weight) was a character which linked both experiments. Genotype ranking for this character was constant over experiments and water treatments. WUE (dry weight) may have been controlled by water use in response to different dry weight levels but evidence from post-anthesis stress suggests that the relationship was reinforced by dry weight change in response to water supply. While WUE (yield) was also relatively constant it was affected by differential yield response to stress, particularly if imposed after anthesis.

Ranking of genotypes for resistance to stress could not be related to either Ψ(xylem) or free proline. The complexity of the response in these experiments suggests that such a simple relationship would be unlikely.

5. DISCUSSION

5.1 Introduction

Moisture deficit limits cereal production in much of the world (Fischer and Turner, 1978). The Australian cereal belt, for example, is characterised by erratic rainfall which can be inadequate for crop growth at any time during the growing season (Sparrow, 1977). Similar conditions throughout the world are reflected in breeding programmes which aim to improve drought resistance of many cereals and programmes on barley (Eslick and Carter, 1977), wheat (Hurd, 1969) and rice (Chang *et al.*, 1974) are examples.

Resistance to drought has been defined as production in dry environments (Boyd and Walker, 1972), as a measure of resistance disassociated from production (Chinoy, 1962) or as high efficiency of water use (Ferguson, 1977) in different studies. The relationship between efficiency of water use and other measures is not well understood, although some authors have suggested that they may be unrelated (Reitz, 1974; Ferguson, 1977). The relationship was explored in this study and is discussed in a later section.

The performance of a genotype in a moisture limited environment is a function of its potential to perform in a non-limiting environment (its yield potential where grain is the character under study) and its resistance (or susceptibility) to the stress (Fischer and Maurer, 1978). It is desirable that potential and resistance be not negatively correlated although this is possible (Boyer and McPherson, 1975). In southern Australia, for example, seasons can vary from those with severe moisture deficit to those of above average rainfall that reduce yield through a surfeit of moisture (Sparrow, 1977). Drought resistant cultivars should be able to capitalise on favourable seasons while

yielding more than others when moisture is limiting.

Selection for drought resistance under field conditions is likely to be difficult except where the moisture deficit occurs with equal intensity in the same part of the growing season each year (Boyer and McPherson, 1975). There is, therefore a need for plant breeders to exploit plant morphological or physiological characters which are associated with field drought resistance (Sullivan and Eastin, 1974). Recent reviewers of the water stress literature have emphasized, however, that with few exceptions (Wright and ~~Jordon~~ Dobrenz, 1970; Hurd, 1971), plant breeders have not been able to select drought resistant cultivars by exploiting specific characteristics (Boyer and McPherson, 1975; Begg and Turner, 1976). Despite the increasing information on plant responses to water stress, the complexity of the subject has prevented a general understanding of combinations of characters which could be manipulated by breeding and selection. Many reviewers have emphasized that resistance to drought is such a complex character that no one plant attribute will be solely responsible for resistance in every environment.

Recent studies have reported tests which may be related to drought resistance (Wright and ~~Jordon~~ Dobrenz, 1970; Boyd and Walker, 1972; Singh *et al.*, 1972; Richards, 1978) but these have not generally been definitely associated with field resistance. In every study which seeks to define characters associated with resistance to moisture stress, the methods of stress imposition, measurement of stress and measurement of resistance are vital characteristics which influence the results obtained.

This study sought to explore the relationship between proline accumulation of barley genotypes and ability to perform during a period of stress or upon stress relief. The initial seedling experiments studied accumulation of proline in a range of genotypes in response

to stesss and the relationship between proline and performance. Other characters measured included stomatal frequency (Miskin and Rasmusson, 1970) and chlorophyll stability (Boyd and Walker, 1972). Experiments were also conducted where stress was imposed at later stages of growth with a view to examining genotype response to more equitable stresses than are possible under field conditions. The results from these experiments are discussed by examining methods of stress imposition, of water measurement and of resistance measurement. Plant characters associated with differences in the response of the genotypes to imposed stresses are discussed followed by a consideration of the differences in response of individual genotypes used in the study.

5.2 Imposition of Stress

Various methods have been used by breeders and physiologists to apply water stress to plants. In addition to growing plants in a dry environment (Fischer and Maurer, 1978), using rain shelters (Owen, 1958b) and diverting run-off from field grown plants (Kirby, 1970), many methods have also been used to impose stress to pot grown plants. These include withholding water, bathing roots in solutions of low osmotic potential the use of drought chambers to apply atmospheric stress, cooling roots, and the extraction of water from the rooting medium under tension. Each method has its own relevance and the choice of method will depend on the objective of the experiment. The success of the chosen method will depend on how it achieves the objective.

The first group of experiments reported in this study (Experiments 1 to 4) aimed at comparing a range of genotypes for their ability to accumulate proline at a particular leaf water potential and, in two of the experiments, to measure performance during stress or upon stress relief.

A solution of polyethylene glycol (PEG 4000) of -20 bars osmotic potential was chosen to impose stress in the first two experiments. PEG has been widely used for pot experiments and is considered by many (e.g. Lawlor, 1969; Singh *et al.*, 1973d) to be excluded by root systems and to be non-toxic. This view is not universally held, however (Begg and Turner, 1976). Growing barley seedlings in perlite to the three leaf stage and flooding the rooting medium with successive changes of PEG 4000 (osmotic potential -20 bars) as used in these experiments (1 and 2) was also used by Singh *et al.* (1972, 1973). They reported a fall in Ψ(leaf) but genotypes were not significantly different for this character after 72 hours of stress when the plants were re-watered. Such an ideal situation was not reported by Hanson *et al.* (1977), however, who found that two genotypes differed in Ψ(leaf) at the conclusion of the stress period. In neither Experiment 1 nor Experiment 2 of this study did water potential (measured with a pressure chamber) fall or free proline increase as rapidly as in the similar experiments of Singh *et al.* (1973d). This behaviour is consistent with failure to impose stress. The concentration of PEG used in these experiments which was similar to that of other studies (Singh, 1972; Hanson *et al.*, 1977), was equivalent to -20 bars osmotic potential and yet Ψ(xylem) had fallen to only -12.1 bars after 144 hours in Experiment 1 (p. 61) and -13.1 bars after 72 hours in Experiment 2 (p. 70). It is possible that PEG did not fully replace the nutrient solution in the root zone and this accounted for the slow decline of Ψ(xylem). The possibility of PEG uptake cannot be discounted, particularly over the longer exposure time in Experiment 1 ~~but, in this case, more rapid decline of Ψ(xylem) and greater accumulation of free proline would be expected~~. There were differences between genotypes in Ψ(xylem) at the conclusion of the stress period in both experiments. These differences, like those of Hanson *et al.* (1977) hinder the interpretation of

differences between genotypes in proline accumulation which may have been the result of differences in water potential and also differences in ability to accumulate proline when stressed. The two experiments were therefore not successful in either imposing the desired level of stress or in imposing equivalent stress for the different genotypes.

An additional aim of Experiment 1 was to compare genotype performance in response to the stress and recovery treatment. For the reasons above, this objective was not fully realised.

The problem encountered with the use of PEG prompted the move to experiments where seedlings were stressed by withholding water. The objective of Experiment 3 was simply to examine a range of genotypes for their ability to accumulate proline when stress was applied in this way. Seedlings were grown in sand and watered with nutrient solution which was withheld at the three-leaf stage. Five days after withholding water, Ψ(xylem) had fallen to -19.1 bars and this was reduced to -22.4 bars one day later (p. 72). Genotypes did differ in Ψ(xylem) over the stress period, however, so the absence of significant differences in proline accumulated could not be regarded as proof that they did not differ for proline accumulation potential.

Experiments 1 to 3 all failed to apply equivalent stress to the different genotypes as reflected in differences for Ψ(xylem), although the possibility of an interaction between genotype and method of measurement at the same Ψ(leaf) could not be eliminated.

Experiment 4 also applied stress by withholding water at the three-leaf stage from seedlings grown in small pots filled with soil. The objective of this experiment, however, was to examine the within-genotype relationship between water status and free proline. That genotypes would differ in water status at the conclusion of the stress period was accepted as an undesirable but inevitable consequence of the

method of imposition. The experiment also sought to explore the reasons for differences in water status at the end of the stress period and to study the performance of different genotypes during stress and recovery. Genotypes differed for Ψ(xylem) at the conclusion of the stress period. The experiment also revealed differences between genotypes in performance during stress and recovery on stress relief but, because genotypes differed in Ψ(xylem) at the conclusion of the stress period, these plant responses could not be compared directly. The small pot size restricted the recovery period which could be studied. That this may have prevented the full manifestation of the stress effect after prolonged recovery (Corletto and Laude, 1974) is supported by the failure of free proline level to fall in most genotypes over the period.

Experiment 5 and 6, where stress was imposed at later stages of growth, aimed to compare the performance of genotypes to stress applied in a way not possible under field conditions. Stress is of an intermittent nature in the field and differences between genotypes in response to the stress are dominated by stress escape so that earlier maturing genotypes often appear most resistant. These experiments aimed to compare genotypes for their response to stress applied at the same stage of development but not necessarily at the same time, thereby eliminating escape mechanisms.

Stress was imposed in Experiment 5 by withholding water so that Ψ(soil) fell to -15 bars just prior to jointing or anthesis. Stress was imposed in the same way after anthesis but plants were re-watered and another cycle of stress was imposed immediately. It would have aided interpretation of tolerance differences if Ψ(leaf) had been reduced to the same level in each pot. This could not be controlled, however, as there were insufficient leaves to allow continuous monitoring of the Ψ(leaf) and there were too many pots to use *in situ* methods.

The comparison of the response of different genotypes when stressed to the same Ψ(soil) has its own particular relevance, however. Differences in tolerance are not so easily measured but differences in avoidance can be investigated.

Experiment 6 had slightly different objectives. It sought to complement the results of the previous experiment where temperature effects and length of the stress cycle may have been different for each genotype. It compared the response of genotypes when exposed to stress for the total length of particular development periods. Stress fluctuated throughout the development period so that genotypes could not be compared directly at the same Ψ(soil) or Ψ(leaf), while the limits of Ψ(soil) were controlled. The main limitation of this technique was that Ψ(xylem) could not provide a realistic measure of the degree of stress so that performance could not be directly related to stress level. Its main advantage was, however, that differences between genotypes in stress escape were minimised.

No one method of imposing stress is uniquely better than others. Each has its own advantage and usefulness. The use of pot studies has been criticised by Owen (1958b) as the restrictive water supply in small pots does not allow time for equilibration or adjustment to stress as is possible under field conditions. There are differences between field and pot studies, mostly related to the speed of stress and in the magnitude of plant response (reviewed by Begg and Turner, 1976). Ultimately all characters must be related to field response but pot studies, like those employed in this experiment, are an important intermediate between experiments in controlled environment facilities, where there are major restrictions on experiment size and hence the number of genotypes that can be studied, and field studies where the environment cannot be controlled. It would therefore not have been

possible to study sufficient genotypes under controlled conditions, nor could stress have been imposed at will in the field. Measurement of water use is also more difficult in field studies.

Most of the experiments in this study used a single stress cycle. Plants may adapt during repeated stress cycles (Jones and Turner, 1978) and hardening responses may occur (Singh *et al.*, 1973d). These mechanisms were not studied.

5.3 Measurement of Plant Water Status

A pressure chamber was used throughout this study to estimate plant water potential. It was chosen because more measurements could be made in the one day than with other techniques.

Genotypes varied significantly for xylem water potential in Experiment 3 and whether stressed or not in Experiment 4, the experiments where stress was imposed on seedlings by withholding water. There was also a significant correlation between the genotype means in the stressed treatments of the two experiments and between the stressed and unstressed genotype means in Experiment 4. Differences between genotypes may then have been due to a real difference in water status or an interaction between genotype and method of measurement. Genotypes also differed in Relative Water Content (RWC), however, and there was a highly significant correlation between the genotype means for the two parameters ($r=0.79^{**}$ for $n = 10$ five days after withholding water). The differences between genotypes were therefore a real effect. There did not appear to be differences between genotypes in the nature of the relationship between Ψ(xylem) and RWC but, since the experiment was not specifically designed for this purpose, small genotypic differences could not have been detected.

Recent studies and reviewers (e.g. Begg and Turner, 1976; Fischer and Sanchez, 1979) have emphasized the need to measure plant water status in all studies which seek to determine differences between genotypes in resistance to moisture stress, particularly in those which seek to determine field resistance. The experiments with older plants in this study, however, have shown that measurement of water potential reached at the conclusion of a stress period is not the only parameter of importance. It is not possible to subject a plant naturally and rapidly to a particular water status. Water gradually becomes limiting to plant growth except in the case of atmospheric stress where the roots are not able to supply water quickly enough, even when the soil is at field capacity, to prevent low leaf water potential developing. In all other cases, the duration and rate of decline in plant water potential may be as important as final water potential in determining stress effect or recovery after stress.

It has been suggested that proline accumulation may be a useful measure of water status (Palfi and Juhasz, 1971). Recent evidence, and that from this study, does not support this hypothesis. Proline may not commence to accumulate until stress is moderately severe (Waldrón *et al.*, 1974; McMichael and Elmore, 1977 and Experiment 4, p. 87), the amount accumulated may depend on genotypes as well as previous stress history (Singh *et al.*, 1973d) or age of the leaf (Experiment 5, p. 113). Despite these difficulties, it is possible that proline level, if properly calibrated, could be used as a measure of water status in specific experiments. It is simple and more accurate, however, to estimate water status directly through one of the methods used to estimate Ψ_w (Begg and Turner, 1976).

5.4 Measurement of Stress Resistance

There is no one best method of measuring resistance to moisture stress. The method chosen will depend on the objectives of the particular experiment. Drought resistance may be defined as survival at a particular stress level (Levitt, 1972). This is the most easily defined and uniquely measured method. Tissue survival was measured in Experiment 1 where the aim was to compare genotypes directly for survival at a particular level of water potential. As already discussed, however, the genotypes differed in Ψ(xylem) so survival could not be directly compared. It was also planned that tissue survival should be measured at the conclusion of the recovery period in Experiment 4 but insufficient necrosis occurred five days after stress relief to provide useful data.

At the other extreme, however, survival may have little relevance in practical agriculture where production either during a period of moisture deficit or recovery on stress relief is the most important character (Laude, 1971). In some cases survival from a moisture stress may be related to production (e.g. Singh *et al.*, 1973d) while in other cases the two measures may be unrelated (Begg and Turner, 1976). Resistance measurement in terms of production can be made on growth rates or on final levels of production.

Stress resistance was measured in terms of growth rate both during and on the relief of stress in Experiment 4 where seedlings were stressed by withholding water. In this case the genotypes grew at different rates even when not stressed. High growth rate of one genotype relative to another may not then represent differences in resistance to stress but to differences in potential growth rate. This apparently occurred in the experiment as growth rate of the stressed and control plants were related ($r=0.75^*$, $n = 10$). Genotypes again grew at different

rates when stress was relieved but this was also related more to potential growth rate rather than to resistance to stress. For characters other than dry weight in Experiment 4 and for all characters in Experiment 5 and 6, stress resistance was estimated in terms of expression of the character at the conclusion of the experiment. Plants were stressed, either as seedlings or at later stages, and production measured at a specified time (after a specific period in Experiments 1 and 4 or at harvest in Experiments 5 and 6). There were differences between the genotypes for the character expression when stressed or unstressed for almost every character measured in the experiments. Performance during stress was then a function of potential performance and resistance to the stress. In this case the resistance to stress was defined as performance when stressed as a percentage of control performance. This method has been used by others (e.g. Chinoy, 1962; Fischer and Maurer, 1978) but it has the following limitations which must be recognised.

First, spuriously high values for resistance will be obtained where control performance is not a true indication of potential to perform. An overestimate of stress resistance will be obtained, for example, if the control of some genotypes are subjected to stresses not imposed on all water treatments of that genotype or on the controls of others. There was some indication that this occurred in Experiment 5. Later maturing genotypes flowered at a time of decreasing water use and probably leaf area. This was reflected in a lower percentage of fertile florets in the controls which led to lower yield than in the genotypes of intermediate maturity. Additional stress reduced the percentage of fertile florets in the earlier maturing genotypes while it had little influence on the late ones. They therefore appeared more resistant.

Second, the analysis of percentage data usually requires transformation before analysis. In these experiments, however, basic

statistics were calculated to estimate skewedness and kurtosis but in no case was transformation considered necessary.

Third, interpretation of the percentage data is not simple. It essentially compares final reduction (or increase) in a character long after the results of stress. It provides no information on the process which has been directly affected by the stress. In Experiment 4, for example, Dry Weight Percentage at the conclusion of stress was dependent on the initial dry weight, growth rate of the control plants and growth rate in the plants when stressed. Dry Weight Percentage after recovery was dependent on all of these factors plus growth rate of the controls from day 5 to day 10 and growth rate of the recovering plants. It does not therefore provide sufficient information on the importance of individual mechanisms but is a reflection of resistance in crude terms where harvest data is all that is available.

Fischer and Maurer (1978) have proposed a different method for estimating susceptibility. They calculated "drought susceptibility index" (S) by inserting a correction for stress level by calculation of an environmental index similar to that of Finlay and Wilkinson (1963). Thus, S was derived from:

$Y = Y_p (1 - SD)$, where Y is the measured yield of a genotype,

Y_p is the potential yield of that genotype and

D is the drought intensity index.

D is defined as:

$D = (1 - X/X_p)$ where X is the mean yield of all genotypes in the environment being studied and X_p is the mean yield of all genotypes in the potential environment (i.e. that environment allowing genotypes to produce maximum possible yield). They calculated S after statistically adjusting raw data for differences in escape.

The value of S will depend on the genotypes used in calculating the index and will also depend, for its validity on all genotypes having the same environment for producing potential yield. It is independent of stress level and can be used for a direct comparison between experiments. It is not very different, however, from YP (Yield Percent) as calculated in this study. If the correction factor is neglected for the different environments, the formula becomes $Y = Y_p (1 - S)$ and $1 - S$ is the same as resistance (R) which can then be defined as

$$Y = Y_p(R) \text{ or } R = Y/Y_p$$

which is the ratio used to calculate resistance in this study. (S) therefore depends on similar assumptions and has similar deficiencies as the ratio used to estimate resistance in these experiments.

The difficulties in measurement of stress resistance have led to the widespread use of such statistical manipulations. Finlay and Wilkinson (1963) did not propose their stability index specifically as a measure of drought resistance and Singh (1972) did not refer to it in this way but it has since been widely interpreted to indicate drought resistance (Hanson *et al.*, 1977; McMichael and Elmore, 1977). The stability index clearly integrates all environmental factors and, while soil moisture status may have been the chief limiting factor in Finlay and Wilkinson's study it may not have been so for all genotypes. It has been suggested, for example, that Excelsior may have appeared stable because, due to shattering, only a small proportion of its yield in favourable environments was recovered (Gardener, 1971). Stability index cannot be used to indicate drought resistance unless moisture is the only limiting factor in the environment and this is unlikely (Knight, 1970).

Resistance can be due to combinations of characters which fall under the headings of escape, avoidance and tolerance. In the seedling

experiments (1 to 4) there was no opportunity for escape because genotypes were stressed in the vegetative stage and for the same length of time. Avoidance may have been important in these experiments, however, as genotypes differed in water status at the conclusion of stress. This was explored further in Experiment 4 and will be discussed in the next section. All mechanisms may have been important in Experiment 5. The later maturing and leafier genotypes were stressed for a shorter proportion of their total growth period thus representing a form of escape not normally encountered in the field. This relationship, measured by the correlation between anthesis date and the character under study was removed where possible by multiple regression so that the other components of resistance could be studied. A similar technique was used by Fischer and Maurer (1978) for field data where early maturing genotypes escaped stress. Genotypes also differed in stress avoidance in Experiment 5. This was estimated by Ψ(xylem) at the conclusion of the stress period and, where possible, effects were removed by multiple regression to allow a measurement of tolerance. The components of escape and avoidance are important in themselves but differences in tolerance can only be compared where their effects are removed.

In Experiment 6 there were no significant correlations between anthesis date and other characters and here escape was probably not an important mechanism. The stresses were imposed in a way that did not allow the use of water potential as an estimate of avoidance since stress was imposed over a prolonged period. It was not possible, therefore, to separate avoidance and tolerance as components of resistance in that experiment.

5.5 Plant Characters and Resistance

1. Water Status

Genotypes differed in water status when stress was imposed

osmotically (p. 61 and p. 70). There did not appear to be a pattern in this response nor were the differences related to recovery after stress (Experiment 1).

Genotypes also differed in water status where stress was imposed on seedlings by withholding water. There was a relationship between the two experiments (3 and 4). Water potential in the unstressed controls was also related to that at the conclusion of stress in Experiment 4. It is possible, although unlikely, that genotypes differed sufficiently in root penetration to account for this difference in a confined pot area. This does not eliminate differences in root resistance (Passioura, 1972) as this would delay the rate of water removal. In this case, however, lower leaf area would be expected to be associated with lower water use and this was not the case. The genotypes with greatest leaf area were apparently less stressed so the hypothesis that high leaf area resulted in more rapid water use cannot be supported.

Differences between genotypes in water status were also related to stomatal frequency. Those with a higher frequency of stomata per unit of leaf area had lower leaf area but were more stressed (p. 101). A causal relationship between stomatal frequency and water status could not be established but the direction of the response was similar to that predicted by Miskin *et al.* (1972). They claimed that genotypes with fewer stomata would transpire less for the same level of photosynthesis. Jones (1975) selected barley lines for high and low stomatal density and found little effect on water relations.

The differences between genotypes in water status were not related to those in the later experiments with older plants. No causal relationship for difference in apparent avoidance could be established in these experiments but, particularly in Experiment 5, genotypes did differ in leaf water status at the same Ψ(soil). It is likely that

differences in growth rate and patterns in later experiments dominated the subtle patterns evident in the smaller plants.

The differences in water status measured in Experiment 6 cannot, for most treatments, be regarded as evidence of avoidance differences but, in reality are confounded by the level of stress at the time of measurement. In Water Treatment 4, however, when the Ψ(xylem) was measured one week after anthesis, differences between genotypes were more closely related to avoidance but there was no relationship with the results in Experiment 5.

2. Yield Components

Grafius and co-workers have published a series of papers which concentrate on the role of the yield components in estimating stress and predicting genotype response (Grafius, 1969; Grafius and Thomas, 1971). Since components are formed in sequence, they claim an oscillatory response can be expected. The existance of statistical interactions between components has long been recognised (Adams, 1967) and was basically supported in Experiment 5. The claim, however, that "the success of a genotype in an environment will depend on its pattern of deployment of resources" (Grafius and Thomas, 1971) is an oversimplification. It may be true if genotypes are stressed at similar times but different stages of development and the most successful one will be at a less sensitive stage of development. In these experiments, however, differences between genotypes in resistance tended to be maintained over different water treatments with all components being implicated in the response.

A dominant yield component response in these experiments was the production of later tillers on the relief of stress before anthesis. Not all genotypes responded in this way and the genotypes did not perform

similarly in the two experiments. Production of late tillers in this way has been noted previously (Aspinall *et al.*, 1964) but has not generally been described in field experiments although it does occur in some circumstances (Sparrow, 1977). In the environment of southern Australia however, severe stress followed by relief before anthesis (i.e. a very dry winter followed by a wet spring) is rare.

Experiment 5 and 6 were partly designed to measure the response of the yield components to stress and relief at particular stages of development. It was generally true that the component most affected was that growing most rapidly at the time of stress. Exceptions to this general rule were the response of tiller number of the relief of early stresses and tiller mortality as a result of the post-anthesis stress.

The percentage of fertile florets has not often been measured in barley experiments and yet genotypes did vary significantly for this component, particularly in Experiment 5, where it was related to harvest index and YP (the relative yield when stressed). In Experiment 6 there was also a relationship with YP. No attempt was made to determine, for these experiments, whether the percentage of fertile florets was reduced by failures in pollination, fertilization or to abortion after fertilization. It is likely that this component is important in field performance, particularly of later maturing genotypes which may be maturing at a time of increasing temperature and evaporative demand.

The total number of florets was an important component in some situations but the relationship between this and tiller number was very strong. It may be that later tillers, which were produced on the relief of stress, had lower floret number per spike or that competition for resources caused by late tillers reduced the total number of florets per tiller. This latter argument is supported by the main stem data in which the number of florets per main stem was reduced in proportion to

the increase in new tillers on the relief of stress. This, however, may have been due to the stress effect on the main stem reducing its dominance over the formation of late tillers.

Grain weight was not very responsive to the stresses imposed in these experiments. In neither Experiment 5 nor Experiment 6, however, was stress applied so that it remained severe during the period of most rapid grain growth, while this type of stress is common in the field situation (Passioura, 1977).

3. Proline Accumulation

A feature of these experiments has been the variability associated with measurement of free proline. It was possible to relate free proline accumulated with plant water status within genotypes in Experiment 4. This suggested that the variability encountered was mainly due to differences in plant water status and not directly from errors in the estimation of proline.

There was only poor agreement between experiments for proline accumulated by genotypes during stress. In most cases, however there was a significant correlation for the seedling experiments. There was no relationship between the experiments and those reported by Singh *et al.* (1973d) for proline accumulated by the nine common genotypes. Hanson *et al.* (1977) also used Proctor and Excelsior and were unable to demonstrate differences in ability to accumulate proline. They were, however, unable to compare genotypes at the same water potential. It is possible, although unlikely, that a different source of seed was used in the three experiments. All three obtained seed of Excelsior from the Waite Institute barley collection. There may be variability within this genotype, however, as Hanson *et al.* used Excelsior from two sources and one was naked, contrasting with the husked one used in these experiments.

Seed used in the seedling experiments in this study was derived by self pollination from a single plant which may account for some genotypic differences from Singh's material.

Singh *et al.* (1972) reported a high correlation between the proline accumulated by ten genotypes and the stability index of Finlay and Wilkinson (1963). This correlation is of dubious value, however, as a significant portion of the stability can be explained by stress escape of early maturing genotypes (Lewin and Sparrow, 1976) and the correlation is dependent on the result for Excelsior which may owe its stability to shattering (Gardener, 1971) and may not accumulate proline to the degree reported by Singh *et al.* (1973d) (Hanson *et al.* 1977).

In Experiment 3 there was no relationship between accumulated proline and leaf survival but plants may have been exposed to PEG for too long and differences in survival then may represent differences in exclusion of the osmoticum. In Experiment 4 there was an apparent relationship between proline accumulated and performance during the stress. There was no relationship with recovery. Recovery on the relief of stress in this experiment was related to leaf area as inferred in the report of Singh *et al.* (1973d), but there was no measurable senescence.

It has been suggested that proline may act as a source of carbon and nitrogen during the recovery period. This could not have been the case in Experiment 4 as proline level was at least as high five days after relief as on the last day of stress. It could not, therefore, have contributed to recovery over this period. No additional reason can be proposed for the high level after re-watering other than latent senescence or impaired translocation, not obvious from plant appearance.

Proline accumulated, or ability to accumulate proline as measured in seedling experiments could not be related to stress resistance in the older plant experiments. These experiments were more complex and there were environmental differences as well as differences in escape and avoidance that may have affected the apparent stress resistance.

The surest way of determining the significance of proline accumulation would be to compare near-isogenic lines which differ only for this characteristic. Selection of such lines could be achieved by seeking differences within a single cultivar or by selecting for the characteristic after backcrossing. Singh *et al.* (1972) suggested that testing genotypes by stressing osmotically in small pots would provide a useful selection tool. The population size that could be managed in a controlled environment cabinet would be limited and differences in environment can modify the accumulation of proline. Richards (1978) and Richards and Thurling (1979) found a broad sense heritability of 43% when genetic analysis for the ability to accumulate proline was made in 112 related families but narrow sense heritability was only 18%. While proline accumulation is apparently a heritable character, the variability encountered in its measurement in these experiments would need to be improved if selection were to be successful.

4. Water Use Efficiency and Harvest Index

There was a close association between water used and dry matter accumulated in Experiment 5 and Experiment 6. It cannot be assumed, however, that dry weight was governed by available water. The unstressed control and Water Treatments 2 (vegetative stress) and 3 (pre-anthesis stress) in Experiment 5 were watered to replace that used at all times except during the stress treatment. Thus water used

may have been governed by the response of dry weight to the water treatments. This does not apply to the post-anthesis stress in Experiment 5, however, where water supplied was equivalent for the different genotypes but the relationship between dry weight and water use still held. No attempt was made in Experiment 6 to supply equivalent amounts of water to the different genotypes when maintained on the low water regime. Water was added to replenish water used so that the average Ψ(soil) was reduced to -1 bar. When transferred from high to low water status after anthesis (Treatment 4 in Experiment 6), pot water use fell to become nearly equivalent to that of pots maintained on the low regime throughout the experiment. A valuable additional treatment would have been to supply all genotypes with the same amount of restricted water throughout growth. Results with wheat genotypes (Passioura, 1977) suggest that the relationship between dry weight and water use would still hold.

Despite the close relationship between water use and dry weight, genotypes did differ for Water Use Efficiency (for dry weight accumulation) - WUE (dry weight). Genotypes and water treatments varied for this character but the genotype response tended to be constant across environments. The genotype means for WUE (dry weight) were significantly correlated in all roughly equivalent treatments in Experiments 5 and 6. Passioura (1977) also reported stability for this character across environments in fourteen wheat cultivars when a fixed amount of water was supplied.

There was also good agreement between grain yield and water used in the two experiments but there was a genotype x water treatment interaction for WUE (yield). The two experiments were not equivalent for WUE (yield) except in the unstressed control and the vegetative stress treatment (Treatments 1 and 2 in both experiments). Grain yield is likely to be directly related to water used only through its

association with total dry weight. Grain yield is linked to dry weight and WUE (yield) to WUE (dry weight) through harvest index. This characteristic was most influenced by stress after anthesis. The straw component of total dry weight had been determined by this stage and only the grain component could be varied by stress. Genotypes varied for harvest index but there were differences in response to the various water treatments. In Experiment 5, harvest index response was related to the response in terms of seed set (percentage of fertile florets).

5.6 Implications for Plant Selection

The overall objective of this project was to investigate genotypic differences in response to moisture stress and, where possible, to relate these differences to characters which could be manipulated in a breeding programme to select drought resistant cultivars.

Survival when water is limiting is an important character in plant evolutionary terms but is is important in agriculture only if it affects production - either during or on the relief of stress. It is grain production that is the economically important character and breeding for drought resistance is directed to this character.

Most drought prone environments, including the cereal areas of southern Australia, are characterised by favourable as well as dry seasons and variability within seasons. Any character which contributes to drought resistance for these environments must not limit production in the absence of stress as most economic advantage can be made by capitalising on the favourable seasons.

The ability of ten genotypes to accumulate proline when stressed in the seedling stage appeared to be realted to their ability

to perform during a period of stress but not necessarily to their ability to grow on stress relief. Proline may then have an important role in survival although this has been disputed by Hanson *et al.*(1977). In the experiments of Singh *et al.* (1973d), proline was related to leaf survival which, in turn, was related to recovery on stress relief. Growth rate on stress relief in Experiment 4 was also related to leaf area but there was no measurable leaf senescence so proline was not related to either leaf survival or recovery. It has also been suggested that proline could be a valuable source of carbon and nitrogen on the relief of stress. This could not be tested in Experiment 4 because proline level had not decreased five days after re-watering.

Performance of older plants to stress was not related to either proline accumulated during stress or to the ability of the genotype to accumulate proline. Performance in these experiments (5 and 6) was, however, dominated by problems of escape and avoidance so subtle differences in tolerance would have been difficult to detect. These experiments highlighted the problem of examining the relationship of a particular character to stress resistance. The surest way to carry out such an investigation would be to develop near-isogenic lines differing only in ability to accumulate proline and compare these under similar stress conditions.

The relationship between water use and dry weight which was consistent over experiments suggests that water supply is a dominating influence on plant performance. Genotypes did differ in the efficiency with which they used water, however, and genotype ranking was maintained over two experiments grown in different seasons and under different conditions of evaporative demand. To suggest that selection for WUE (dry weight) would improve yield is an oversimplification as this would

not necessarily improve grain production. Water Use Efficiency for grain yield was more influenced by environment than was WUE (dry weight) and stress after anthesis depressed this character. While there were genotype x water treatment interactions for WUE (yield), genotype ranking tended to be maintained across water treatments and experiments.

WUE (yield) is related to WUE (dry weight) through harvest index. This character provides little additional information that can be gained from the integrated WUE (yield), but would be easier to measure.

It is postulated that the most successful genotype in the drought prone southern Australian environment will have

1. Intermediate maturity to avoid the effects of high temperature as far as possible but allow maximum use of available water
2. High WUE (yield), particularly when stressed after anthesis.

The genotypes Clipper, CI 3576 and Stewart are in this category, when judged on the results of these experiments. Clipper is widely cultivated in southern Australia and CI 3576 has been noted for its adaptability across environments (Sparrow, 1977). There is no published information on the adaptability of Stewart.

None of the plant characters measured in these experiments (except HI in experiment 6) were related to WUE (yield). Selection for this character would not be easy and could only be attempted in the selection of parents. It may well be easier to estimate WUE (dry weight) by growing plants on a known quantity of stored water and then deriving harvest index from other experiments. Harvest index is susceptible to interaction with water treatment, however, so care would need to be exercised on the environment in which selection is carried out.

5.7 Responses of Individual Genotypes

These experiments provided an opportunity to assess the response of a range of barley genotypes to particular environmental constraints at the seedling and later growth stages. The response of each genotype provides information on its worth as a parent in programmes aimed at genotype improvement.

The responses of the different genotypes in terms of many of the characters measured in these experiments were a function of the particular method of stress imposition and were not generally useful. There was however, evidence of stress avoidance by seedlings of different genotypes in Experiment 4, these differences being measured by Ψ(xylem) or RWC. This experiment also provided the best measure of differences in ability to accumulate proline while differences in growth rate in response to stress may have been more related to differences in non-stress growth rate than to a direct effect of stress.

The measures of Ψ(xylem) and proline accumulated by the genotypes in the experiments with older plants were modified by the experimental conditions and the physiological age of the tissue at the time of measurement so the differences between genotypes may not represent a real effect of response to similar stress. There were differences in performance and differences in response to stress but some of these may have been associated with maturity. The differences between genotypes in Water Use Efficiency, however, were relatively unaffected by environmental conditions and probably truly reflect the inherrent differences between the genotypes.

Table 70 summarises genotype response in terms of Ψ(xylem) and proline accumulation by seedlings in Experiment 4 and in terms of dry weight, grain yield, DWP (after adjustment for differences in anthesis date), YP (also after adjustment for anthesis date differences)

Table 70.

Key characteristics of 21 barley genotypes in Experiments 4 and 5. The characteristics included are:

Ψ - xylem water potential (- bars) five days after withholding water in Experiment 5.

proline - free proline (mg/g dry weight) measured five days after withholding water in Experiment 4.

DW - Shoot dry weight (g per plant) in the unstressed control of Experiment 5.

Y - Grain yield (g per plant) in the unstressed control of Experiment 5.

DWP - Dry Weight Percentage (%) - the mean of all water treatments. The raw data of Table 39 has been adjusted for differences in anthesis date.

YP - Yield Percentage (%) - the mean of all water treatments. The raw data of Table 42 has been adjusted for differences in anthesis date.

WUE (dw) - Water Use Efficiency for dry weight production ~~in the unstressed control treatment~~ mean of all treatments in Experiment 5.

WUE (yld)- Water Use Efficiency for grain yield ~~in the unstressed control treatment~~ mean of all treatments in Experiment 5.

Maturity - VE = very early; E = early; M = mid-maturity; L = late and VL = very late maturing.

Genotype	Maturity	Experiment 4		Experiment 5					
		Ψ	proline	DW	Y	DWP	YP	WUE (dw)	WUE (yld)
Arivat	M	31.1	14.1	11.0	4.7	91.4	81.8	3 Ø.68	1.46
Asahi 2	L	33.5	13.3						
Bankuti Korai	VE	26.0	22.0	6.5	3.2	86.4	91.3	3.38	1.66
BR 1239	M	30.4	12.1	12.1	4.8	85.0	77.8	4.27	1.63
CI 3576	E	29.1	13.6	10.2	4.4	96.6	94.9	4.10	1.74
Clipper	M	30.9	17.2	9.7	4.4	96.0	90.3	3.98	1.71
CPI 18197	E-M	37.3	11.2	11.0	4.5	84.8	82.0	4.24	1.69
Cyprus Black	E	29.7	19.2	10.3	4.1	99.8	95.6	4.43	1.70
Dore	M	31.1	21.6	12.1	4.5	98.1	96.5	4.10	1.55
Excelsior	L	33.6	15.3	10.9	3.7	104.5	102.4	4.00	1.38
Greenough	E	26.7	16.0	9.1	3.5	90.0	85.9	3.88	1.38
Hiproly	M	23.8	13.9	10.4	1.4	91.9		4.03	
Ketch	E	32.1	17.3	11.4	5.1	83.6	76.1	4.19	1.69
Maraini	VL	28.5	10.3	10.3	3.0	93.7	88.4	3.64	1.04
Mona	VE	30.7	13.3						
Princess	L	34.7	17.8	12.5	4.8	89.3	78.3	4.64	1.61
Prior A	M	32.6	17.0						
Proctor	L	31.5	15.3	11.3	4.3	87.8	81.2	4.19	1.56
Stewart	E	24.9	10.5	11.2	5.0	75.6	71.3	4.19	1.75
Velvon II	L	32.5	13.0	13.8	4.9	86.5	80.6	4.04	1.42
Zephyr	L	32.2	12.9	11.7	4.4	92.9	87.1	3.72	1.36
LSD ($p=.05$)		ns	8.3*	12.0**	0.7**	9.1**	13.1**	0.22**	0.11**

and Water Use Efficiency for both dry weight and yield in Experiment 5. This information is integrated in a description of the response for each genotype.

1. Arivat

Arivat is a six-rowed genotype of intermediate maturity. Its seedling performance was characterised by low leaf water potential when stressed and intermediate ability to accumulate proline. In Experiment 5 it produced relatively high shoot dry weight and yield levels when not stressed and intermediate levels when stressed, relative to its own control. Its response in terms of DWP and YP was characterised by very poor performance in the post-anthesis stress, where it was worse than the other 17 genotypes for both characters after adjustment for differences in anthesis date. It had relatively low WUE levels for both shoot dry weight and grain yield.

2. Asahi 2

This genotype was included only in the seedling experiments. It was included for its reported low ability to accumulate proline (Singh *et al.*, 1973d). This was confirmed in Experiment 4 where its low accumulation of proline was associated with low Ψ(xylem) after five days of stress.

3. Bankuti Korai

This genotype was included in all experiments. It is a very early maturing two-rowed type. It accumulated high levels of proline when stressed in the seedling experiments and this was combined with high levels of Ψ(xylem) when stressed.

In the experiments with older plants it was characterised by low dry matter and yield production which was associated with poor apparent resistance to stress as measured by DWP but intermediate resistance as measured by YP. This was associated by very low WUE (dry weight) but high WUE (yield).

Bankuti was a very stable variety in the studies of Finlay and Wilkinson (1963) and is considered to be drought resistant but it may owe most of its resistance to escape and relatively high WUE (yield), rather than to resistance to stress as measured by tolerance.

4. BR 1239

A six-rowed genotype of intermediate maturity, BR 1239 was included in these experiments for its relatively high potential to accumulate proline in the experiments of Singh *et al.* (1973d). This was not supported in the seedling experiments of this study.

BR 1239 had high dry weight production when stressed but relatively low harvest index. It did not perform well when stressed and had low WUE (yield) although its WUE (dry weight) was high.

5. CI 3576

This two-rowed genotype of early maturity accumulated low levels of free proline when stressed Experiment 4. Its performance in Experiment 5 was characterised by intermediate to high WUE (dry weight) but high WUE (yield). After adjustment for its early maturity, it performed relatively well when compared to its own control for both dry weight and grain yield.

5. Clipper

Clipper is a two-rowed genotype of intermediate maturity.

It accumulated high levels of free proline when stressed in Experiment 4.

Clipper responded relatively well when stressed in Experiment 5 but did not respond well in terms of either shoot dry weight or yield in Experiment 6. It had intermediate WUE (dry weight) but, due to its high harvest index, had high WUE (grain yield).

7. CPI 18197

This is a two-rowed genotype of relatively early maturity. Low accumulation of proline in the seedling experiments was associated with very low Ψ(xylem) after five days of stress.

In Experiment 5, its high dry matter and grain production when not stressed was associated with poor performance in terms of DWP and YP. It had high WUE for both dry weight and grain yield.

8. Cyprus Black

This early maturing six-rowed genotype had high proline accumulation in Experiment 4.

Cyprus Black performed well in the experiments with older plants. Relatively high production of shoot dry weight and grain yield was associated with high efficiency of water use and high apparent resistance to stress as measured by DWP and YP.

9. Dore

This two-row mid-maturing genotype also accumulated high proline levels in the seedling experiment.

In both experiments with older plants it had high production of both shoot dry weight and grain yield, associated, in Experiment 5,

with high apparent resistance to stress for both characters. It did not resist stress as well in Experiment 6. It was not as efficient in its Water Use Efficiency for either dry weight or grain yield as were many of the other genotypes.

10. Excelsior

A six-row, late maturing genotype. It was included in these experiments for its reported ability to accumulate proline associated with high stability of production when stressed (Singh *et al.*, 1973d). It did not accumulate high levels of proline when stressed in Experiment 4 but did have low Ψ(xylem) after five days of stress and this was associated with high stomatal frequency.

Its performance in Experiment 5 was characterised by relatively high dry matter production but low yield levels associated with very high apparent resistance to stress as measured by DWP and YP. It had relatively low WUE (dry weight) in Experiment 5 but very low WUE (yield).

Excelsior was regarded by Finlay and Wilkinson (1963) as a stable variety. It was the most resistant genotype in Experiment 5 as measured by DWP and YP but its apparent resistance was associated with low WUE, particularly for yield and low yield levels when supplied with ad-lib water.

11. Greenough

This is an early-maturing six-row genotype. It had high water potential when stressed in Experiment 4 and intermediate proline level. It had low dry matter production and grain yield when stressed relative to its non-stress performance and low WUE for both dry matter and grain yield.

12. Hiproly

This is a naked, two-row genotype which is known for its high lysine content. It was characterised by low floret fertility in all treatments, including the unstressed control. Hiproly had extremely high Ψ(xylem) when stressed as a seedling but accumulated low proline levels.

13. Ketch

An early maturing, two-row genotype, bred in South Australia, Ketch had low Ψ(xylem) when stressed as a seedling but had relatively high levels of free proline.

It produced high shoot dry weight levels with high efficiency in the use of water in Experiment 5 and high yield levels with high Water Use Efficiency for grain yield. It did not perform well for either dry weight or grain yield when stressed if compared with its unstressed performance.

14. Maraini

This is a very late maturing six-row genotype. It was included in these experiments for its low potential to accumulate proline and this was supported by the Experiment 4 results.

It was apparently resistant to stress as indicated by DWP and YP in Experiment 5 but this was associated with its late maturity as its apparent resistance was lost after adjustment for anthesis date. It was very inefficient for water use although this was associated with infertility.

15. Mona

Mona was included only in Experiments 4 and 6 as an early maturing comparison for Bankuti Korai. It was apparently a low accumulator of proline although there was considerable variability within the genotype and the results are not reliable.

16. Princess

A two-row genotype of relatively late maturity, Princess was similar to Ketch in many respects. It had low Ψ(xylem) in the seedling experiments but high proline accumulation when stressed.

It produced high levels of shoot dry weight and grain yield in the experiments with older plants when not stressed and did so with high Efficiency of Water Use.

17. Prior A

This two-row genotype confirmed its high potential to accumulate proline as a seedling but was not used in the experiments with older plants.

18. Proctor

A two-row genotype of late maturity, Proctor was included in these experiments for its reported low ability to accumulate proline (Singh *et al.*, 1972) and poor stability of performance in the experiments of Finlay and Wilkinson (1963). It did not accumulate high levels of proline in Experiment 4 but many genotypes accumulated less.

In the experiments with older plants it apparently resisted stress well, and this was particularly true of Experiment 6. It had only intermediate Water Use Efficiency for both shoot dry weight and grain yield.

19. Stewart

This early maturing six-row genotype proved to be a low proline accumulator in Experiment 4. This was associated with low stress levels as measured by Ψ(xylem) and very low stomatal density.

In both experiments with older plants it produced high levels of dry weight and particularly grain yield with high Water Use Efficiency (yield), associated with high harvest index. Apparent resistance to stress was low. YP, after adjustment for anthesis date, was lower in this genotype than for any other.

20. Velvon II

This late maturing six-row genotype was relatively highly stressed as a seedling in Experiment 4 and did not accumulate high proline levels.

In the experiment with older plants it produced high levels of shoot dry weight and yield, but with low efficiency.

21. Zephyr

This late maturing two-row genotype responded similarly to Velvon II in all respects except that it did not produce as much shoot dry weight or grain yield.

APPENDIX 1.

Experiment 5 - Anthesis date (days after 10/8). Main shoot only.

Genotype	Water treatment				
	Control	Veg	Pre-Anth.	Post-Anth.	Mean
Arivat	38.0	39.3	45.1	38.7	40.3
Bankuti Korai	6.7	7.9	9.1	6.7	7.6
BR 1239	37.6	40.2	38.8	36.9	38.4
CI 3576	34.2	40.4	37.0	34.9	36.6
Clipper	42.2	42.7	43.4	44.0	43.1
CPI 18197	34.2	36.4	35.7	33.9	35.1
Cyprus Black	32.6	35.2	31.6	34.3	33.4
Dore	44.6	47.3	46.7	44.2	45.7
Excelsior	50.3	50.9	54.1	50.1	51.3
Greenough	25.5	30.9	29.3	25.2	27.7
Hiproly	46.1	50.4	48.1	43.5	47.2
Ketch	36.3	41.7	36.8	35.0	37.5
Maraini	62.8	62.9	66.1	62.4	63.5
Princess	47.2	50.7	54.9	47.0	49.9
Proctor	49.8	54.2	53.7	48.9	51.6
Stewart	34.4	36.6	37.6	35.5	36.0
Velvon II	53.0	55.8	56.1	54.6	54.9
Zephyr	45.7	44.0	48.9	44.3	45.7
Mean	40.1	42.6	42.9	39.8	41.4

LSD (p=.05)

Between Genotypes	2.09**
Between Water treatments	0.97**
Genotype x water treatment	n.s.

APPENDIX 2.

Experiment 5:- Total (shoot) dry weight (g/plant)

Genotype	Water treatment				Mean
	Control	Veg	Pre-Anth.	Post-Anth.	
Arivat	11.0	10.7	10.3	7.8	9.9
Bankuti Korai	6.5	5.2	5.0	4.4	5.3
BR 1239	12.1	10.4	10.4	8.8	10.5
CI 3576	10.2	9.4	9.5	9.0	9.5
Clipper	9.7	9.0	9.8	9.2	9.4
CPI 18197	11.0	9.1	8.7	8.9	9.4
Cyprus Black	10.3	9.9	9.5	8.5	9.5
Dore	12.1	12.1	11.9	10.7	11.7
Excelsior	10.9	11.7	11.2	11.0	11.2
Greenough	9.1	8.2	8.4	6.7	8.1
Hiproly	10.4	9.5	9.6	9.2	9.7
Ketch	11.4	9.3	9.7	8.5	9.7
Maraini	10.3	11.2	9.9	10.4	10.4
Princess	12.5	12.3	10.4	12.0	11.8
Proctor	11.3	9.8	10.2	10.2	10.4
Stewart	11.2	8.7	8.2	8.0	9.0
Velvon II	13.8	12.1	12.8	11.0	12.5
Zephyr	11.7	11.0	11.2	9.9	11.0
Mean	10.9	10.0	9.8	9.1	9.9

LSD (p=.05)

Between genotypes	0.6**
Between water treatments	0.3**
Genotype x water treatment	1.2**

APPENDIX 3.

Experiment 5:- Grain yield (g/plant).

Genotype	Water treatment				Mean
	Control	Veg.	Pre-Anth.	Post-Anth.	
Arivat	4.73	5.00	4.21	2.21	4.04
Bankuti Korai	3.23	2.60	2.46	2.05	2.59
BR 1239	4.82	4.53	3.91	2.64	3.98
CI 3576	4.39	4.34	4.06	3.41	4.05
Clipper	4.38	3.97	4.42	3.57	4.09
CPI 18197	4.45	3.98	3.33	3.29	3.76
Cyprus Black	4.07	4.36	3.43	2.91	3.69
Dore	4.51	5.02	5.15	3.15	4.46
Excelsior	3.69	4.26	3.90	3.52	3.84
Greenough	3.48	3.10	3.12	1.94	2.91
Hiproly	1.42	1.28	1.37	1.31	1.35
Ketch	5.11	4.45	3.94	2.94	4.11
Maraini	2.95	3.09	2.92	2.93	2.97
Princess	4.78	4.62	3.25	3.92	4.14
Proctor	4.33	3.97	3.88	3.34	3.88
Stewart	4.97	4.22	3.21	2.84	3.81
Velvon II	4.90	4.59	4.69	3.11	4.32
Zephyr	4.43	4.47	4.27	3.06	4.06
Mean	4.15	3.99	3.64	2.92	3.68

LSD (p=.05)

Between Genotypes	0.34**
Between Water treatments	0.16**
Genotype x water treatment	0.69**

APPENDIX 4.

Experiment 5:- Tiller number/plant (X1).

Genotype	Water treatment				
	Control	Veg.	Pre-Anth.	Post-Anth.	Mean
Arivat	3.1	2.9	4.1	2.4	3.1
Bankuti Korai	3.3	3.7	3.5	2.8	3.4
BR 1239	2.7	2.7	2.3	2.1	2.5
CI 3576	4.1	4.4	4.5	3.8	4.2
Clipper	4.1	4.4	5.1	3.8	4.4
CPI 18197	3.7	3.3	4.4	3.4	3.7
Cyprus Black	3.3	3.3	3.2	2.9	3.2
Dore	4.4	4.6	4.9	4.1	4.5
Excelsior	3.0	3.2	3.2	2.9	3.1
Greenough	2.0	2.2	2.2	1.7	2.0
Hiproly	3.8	2.9	3.1	3.1	3.2
Ketch	4.3	5.3	6.2	3.6	4.9
Maraini	1.8	2.1	1.8	2.0	2.0
Princess	4.1	4.2	4.1	4.3	4.2
Proctor	4.4	4.5	4.8	4.1	4.5
Stewart	2.5	2.7	2.7	2.5	2.6
Velvon II	2.9	2.7	3.2	2.6	2.9
Zephyr	4.8	4.0	5.1	4.3	4.6
Mean	3.5	3.5	3.8	3.2	3.5

LSD (p=.05)	
Between genotypes	0.3**
Between water treatments	0.2**
Genotype x water treatment	0.7**

APPENDIX 5.

Experiment 5:- Florets/tiller (X2)

	Water treatment				
Genotype	Control	Veg.	Pre-Anth.	Post-Anth.	Mean
Arivat	37.0	38.1	27.6	38.7	35.4
Bankuti Korai	19.0	16.8	15.7	20.2	17.9
BR 1239	50.9	53.2	54.8	51.7	52.7
CI 3576	19.3	17.4	17.8	20.1	18.7
Clipper	23.0	18.2	19.7	23.4	21.1
CPI 18197	22.3	20.6	17.8	22.2	20.7
Cyprus Black	34.1	31.6	29.9	33.4	32.3
Dore	24.6	23.1	24.3	25.3	24.3
Excelsior	34.1	37.3	32.2	35.2	34.7
Greenough	53.3	49.6	51.6	47.8	50.6
Hiproly	16.0	16.7	17.0	16.9	16.6
Ketch	22.9	17.3	17.5	23.2	20.2
Maraini	52.7	46.1	50.4	52.3	50.2
Princess	26.5	25.4	24.2	26.7	25.7
Proctor	28.8	25.7	25.2	29.0	27.2
Stewart	39.4	31.1	25.9	34.2	32.7
Velvon II	46.2	44.3	42.6	47.0	44.9
Zephyr	27.7	27.0	25.3	28.6	27.4
Mean	32.1	30.0	28.9	31.4	30.7

LSD (p=.05)

Between genotypes	2.3**
Between water treatments	1.1**
Genotype x water treatment	4.6**

APPENDIX 6.

Experiment 5:- Percentage of fertile florets (X3)

Genotype	Water treatment				
	Control	Veg.	Pre-Anth.	Post-Anth.	Mean
Arivat	80.0	87.3	81.6	56.1	76.3
Bankuti Korai	94.2	83.1	82.9	72.8	83.3
BR 1239	82.7	81.7	77.6	62.2	77.0
CI 3576	97.4	96.1	83.7	84.1	90.4
Clipper	91.4	92.4	91.9	87.8	90.9
CPI 18197	95.1	95.5	82.6	84.4	90.8
Cyprus Black	84.4	85.6	72.6	66.1	77.2
Dore	90.9	94.2	95.9	75.4	89.1
Excelsior	80.6	80.4	84.6	80.6	81.6
Greenough	78.3	64.3	64.1	59.3	66.5
Hiproly	58.5	60.9	64.0	62.8	61.5
Ketch	94.8	89.3	79.3	71.7	83.7
Maraini	62.0	53.5	57.4	61.6	58.6
Princess	91.4	90.6	82.6	84.4	87.2
Proctor	75.2	77.9	80.9	72.8	76.7
Stewart	91.4	90.7	83.9	60.3	81.6
Velvon II	73.5	76.9	74.5	72.3	74.4
Zephyr	73.5	81.3	82.1	68.8	76.4
Mean	83.1	82.3	79.2	71.8	79.1

LSD (p=.05)

Between genotypes	5.2*
Between water treatments	2.5*
Genotype x water treatment	10.4*

APPENDIX 7.

Experiment 5:- Thousand grain weight (g) (X4).

Genotype	Water treatment				
	Control	Veg.	Pre-Anth.	Post-Anth.	Mean
Arivat	54.0	54.4	44.5	49.8	50.7
Bankuti Korai	55.0	51.2	53.5	49.9	52.4
BR 1239	40.9	39.0	41.0	39.2	40.1
CI 3576	56.3	60.0	60.9	53.8	57.8
Clipper	49.8	53.8	48.1	45.2	49.2
CPI 18197	57.2	60.1	47.1	51.8	54.1
Cyprus Black	43.9	48.7	48.5	42.6	45.9
Dore	44.5	46.4	44.2	39.3	43.6
Excelsior	44.2	44.9	44.7	42.5	44.1
Greenough	41.4	43.6	44.2	40.0	42.3
Hiproly	41.0	42.6	43.4	41.2	42.1
Ketch	51.8	48.8	45.6	46.6	48.2
Maraini	52.3	60.0	54.6	46.3	52.7
Princess	48.3	48.3	38.7	41.4	44.1
Proctor	45.1	43.9	39.9	38.3	41.8
Stewart	55.5	53.1	48.8	46.9	51.1
Velvon II	50.0	47.6	44.5	35.3	45.0
Zephyr	45.2	48.9	42:2	36.3	43.1
Mean	48.7	49.8	46.4	43.9	47.2

LSD (p=.05)

Between genotypes	2.8**
Between water treatments	1.3**
Genotypes x water treatment	5.6**

APPENDIX 8.

Experiment 6:- Anthesis date (days after 10/8) - main shoot only.

	Water Treatment					
Genotype	HHH	LHH	LLH	HHL	LLL	Mean
Bankuti Korai	10.3	9.0	9.0	10.3	9.0	9.5
Clipper	45.3	41.7	49.0	44.3	49.0	45.6
Cyprus Black	36.0	36.0	37.7	35.7	37.0	36.5
Dore	60.3	53.3	52.3	55.7	57.7	55.9
Mona	10.0	9.0	9.0	10.0	8.7	9.4
Princess	64.7	52.0	56.3	52.7	58.7	56.9
Proctor	57.5	56.3	61.0	67.7	61.7	61.1
Stewart	29.7	44.0	43.0	42.7	44.7	40.8
Mean	39.7	40.3	42.1	39.9	40.8	40.5

LSD (p=.05)

Between genotypes 5.1**

Between water treatments 5.5**

Genotypes within water treatments 12.4*

APPENDIX 9.

Experiment 6:- Free proline (mg/g dry wt) at time 1.

	Water treatment					
Genotype	HHH	LHH	LLH	HHL	LLL	Mean
Bankuti Korai	0.7	0.9	3.5	0.5	3.4	1.8
Clipper	0.4	2.8	3.5	0.6	3.2	2.1
Cyprus Black	0.3	3.5	3.8	0.3	4.1	2.4
Dore	0.2	2.7	3.4	0.8	2.8	2.0
Mona	0.5	0.6	3.1	0.4	2.2	1.4
Princess	0.5	3.0	3.2	0.3	3.4	2.1
Proctor	0.7	4.1	3.8	0.7	5.2	2.9
Stewart	0.6	3.2	4.1	0.5	4.8	2.6
Mean	0.5	2.6	4.8	0.5	3.6	2.4

Free Proline (mg/g dry wt) at time 2.

Bankuti Korai	1.1	0.6	1.2	8.4	2.8	2.8
Clipper	0.8	0.7	2.8	0.4	2.5	1.4
Cyprus Black	1.2	0.8	3.1	0.7	2.1	1.6
Dore	0.9	2.7	2.2	0.3	3.3	1.9
Mona	1.2	0.8	1.5	5.6	3.1	2.4
Princess	0.7	2.4	3.6	0.8	2.8	2.1
Proctor	0.6	2.2	2.8	0.7	3.9	1.8
Stewart	0.8	0.5	3.2	0.7	2.4	1.5
Mean	0.9	1.3	2.6	2.2	2.7	1.9

APPENDIX 9 cont.

Experiment 6:- Free Proline (mg/g dry wt) at time 3.

Genotype	Water treatment HHH	LHH	LLH	HHL	LLL	Mean
Bankuti Korai	0.6	1.2	0.8	-	-	0.9
Clipper	1.0	0.6	1.3	7.4	2.2	2.5
Cyprus Black	0.7	0.7	1.0	9.1	3.3	3.0
Dore	0.4	0.3	2.4	0.7	3.4	1.4
Mona	1.1	0.8	0.6	-	-	0.8
Princess	0.4	0.6	2.7	1.1	3.1	1.6
Proctor	0.5	2.5	2.1	0.6	2.8	1.7
Stewart	0.9	1.0	1.4	6.2	2.3	2.4
Mean	0.7	1.0	1.5	4.2	2.9	2.1

Free proline (mg/g dry wt) at time 4.

Bankuti Korai	0.4	0.8	0.9	-	-	0.7
Clipper	0.6	0.4	0.9	3.1	2.8	1.6
Cyprus Black	0.8	0.7	1.1	2.8	3.1	1.7
Dore	0.6	0.4	0.8	6.8	2.6	2.2
Mona	0.9	1.0	0.2	-	-	0.7
Princess	1.0	0.6	0.6	6.5	3.2	2.4
Proctor	0.9	1.1	2.5	0.7	3.0	1.6
Stewart	0.5	0.4	0.4	0.5	1.9	0.7
Mean	0.7	0.7	0.9	3.4	2.8	1.7

Free proline (mg/g dry wt) in Proctor at time 5.

Proctor	0.8	0.8	0.9	4.8	2.8	2.0

APPENDIX 10.

Experiment 6:- Total (shoot) dry weight - g/pot.

	Water treatment					
Genotype	HHH	LHH	LLH	HHL	LLL	Mean
Bankuti Korai	25.92	17.56	21.20	8.96	7.67	16.26
Clipper	79.25	51.27	50.90	36.57	17.49	46.82
Cyprus Black	106.45	85.82	73.20	66.71	40.51	74.54
Dore	149.56	112.54	91.24	91.00	48.48	98.57
Mona	65.20	19.56	55.91	22.83	15.63	32.13
Princess	141.06	110.68	90.25	104.11	52.82	99.78
Proctor	112.56	96.24	85.55	77.21	44.91	81.21
Stewart	118.23	80.06	75.09	89.39	38.95	80.34
Mean	100.77	76.46	69.87	62.10	33.61	67.75

LSD (p=.05)

Between genotypes	8.3**
Between water treatments	10.4**
Genotype x water treatment	18.6**

APPENDIX 11.

Experiment 6:- Grain Yield (g/pot).

	Water treatment					
Genotype	HHH	LHH	LLH	HHL	LLL	Mean
Bankuti Korai	14.2	9.3	10.9	3.8	3.8	8.4
Clipper	34.7	21.1	24.4	12.1	8.1	19.8
Cyprus Black	38.4	39.2	34.6	11.9	11.1	27.0
Dore	54.4	45.7	39.3	19.2	21.6	36.0
Mona	25.6	11.5	18.2	5.7	6.4	11.7
Princess	46.9	39.1	34.8	21.0	21.4	32.6
Proctor	35.3	35.1	31.0	15.2	15.2	25.7
Stewart	48.5	40.1	36.2	22.9	16.1	32.8
Mean	37.9	31.8	29.9	14.0	13.0	24.8

LSD (p=.05)

Between genotypes 3.6**

Between water treatments 3.9**

Genotypes within water treatments 8.8**

APPENDIX 12.

Experiment 6:- Total tillers/pot (X1).

Genotype	Water treatment HHH	LHH	LLH	HHL	LLL	Mean
Bankuti Korai	25.0	23.3	29.3	11.7	11.7	20.2
Clipper	57.0	36.0	50.5	29.7	17.3	36.6
Cyprus Black	34.0	37.3	46.3	18.3	29.3	33.1
Dore	68.7	59.3	57.0	34.7	38.0	51.3
Mona	56.0	20.0	76.0	25.6	31.7	38.0
Princess	55.3	42.3	42.7	28.3	29.0	39.3
Proctor	61.0	46.0	49.3	35.0	37.0	44.6
Stewart	35.0	32.7	42.6	33.0	26.0	33.9
Mean	48.1	38.7	46.6	26.7	27.5	37.1

LSD (p=.05)

Between genotypes	3.1***
Between water treatments	3.0***
Genotypes within water treatments	n.s.

APPENDIX 13.

Experiment 6:- Total florets/tiller (X2).

	Water treatment					
Genotype	HHH	LHH	LLH	HHL	LLL	Mean
Bankuti Korai	15.1	11.5	10.8	14.9	10.8	12.6
Clipper	15.5	14.7	13.4	15.6	13.1	14.5
Cyprus Black	41.2	35.3	30.4	45.1	29.2	36.2
Dore	20.3	19.3	19.0	19.3	15.9	18.8
Mona	16.3	17.7	14.8	14.0	9.2	14.4
Princess	23.9	24.2	21.7	25.7	20.9	23.3
Proctor	21.7	22.8	21.9	22.0	17.7	21.2
Stewart	35.0	28.5	23.8	28.6	22.1	27.6
Mean	23.6	21.8	15.6	23.2	17.4	20.3

LSD (p-.05)

Between genotypes	1.70**
Between water treatments	0.95**
Genotypes within water treatments	3.80**

APPENDIX 14.

Experiment 6:- Percentage of fertile florets (X3).

	Water treatment					
Genotype	HHH	LHH	LLH	HHL	LLL	Mean
Bankuti Korai	84.2	79.0	75.3	55.5	78.9	74.6
Clipper	82.2	82.0	87.0	69.5	76.6	78.9
Cyprus Black	51.2	63.7	55.3	38.3	34.7	48.6
Dore	82.4	83.7	80.0	68.9	78.0	78.6
Mona	63.4	87.7	45.4	46.9	57.2	57.2
Princess	71.8	69.6	71.4	61.1	73.2	69.4
Proctor	57.9	69.9	63.0	50.6	53.7	59.1
Stewart	70.4	81.6	74.6	52.2	61.5	68.0
Mean	71.3	76.2	70.4	55.4	64.2	67.2

LSD (p=.05)

Between genotypes	3.0**
Between water treatments	2.3**
Genotypes within water treatments	n.s.

APPENDIX 15.

Experiment 6:- Thousand grain weight (g) (X4).

	Water treatment					
Genotype	HHH	LHH	LLH	HHL	LLL	Mean
Bankuti Korai	44.7	45.6	45.5	36.0	37.5	41.9
Clipper	46.9	49.0	44.5	44.3	43.7	45.8
Cyprus Black	54.7	47.2	44.7	42.0	42.5	46.2
Dore	48.1	48.3	45.0	43.2	46.2	46.2
Mona	43.7	43.2	45.8	40.1	40.7	41.9
Princess	49.5	54.7	52.9	47.2	49.2	50.7
Proctor	46.8	47.1	46.2	39.3	43.1	44.3
Stewart	56.3	51.6	48.5	47.0	46.3	50.0
Mean	49.2	48.8	46.8	42.4	43.6	46.0

LSD (p=.05)

Between genotypes	2.0**
Between water treatments	2.3*
Genotypes within water treatments	n.s.

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