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Title: GLUTAMINE SYNTHETASE AND α-AMYLASE ACTIVITY IN GERMINATING WHEAT UNDER WATER STRESS

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An integrated study of growth, enzyme activity, and water potential of wheat seeds germinated under mild water stress conditions was conducted to observe the relationship among the three kinds of parameters studied. From the relationship, the cause(s) or mechanism(s) of water stress in plant growth may be enlightened.

Two important Pacific Northwest commercial cultivars, Hyslop and Wanser were studied under three mild stress conditions which often prevail at planting time in dryland areas of the Pacific Northwest. The stress conditions were simulated by mannitol solutions to provide -3, -6, and -9 bars of water potential. Seeds were germinated at 20-25°C in the dark and samples were taken daily. For seedling axis, water content, fresh and dry weights, soluble protein content, glutamine synthetase (GS) activity and total water potential were determined. For endosperm, α-amylase activity and total water potential were measured.

Experimental results reveal that water potential, water content, fresh weight, dry weight, and soluble protein content of the seedling axis were progressively decreased with increasing stress. GS activity was reduced by the stress during the first two days of germination, then adaptation processes occurred resulting in comparable GS activity in all treated materials. The adaptive mechanism apparently occurs at the transcriptional level in producing more efficient GS in compensating the mild environmental stress.

In endosperm, α-amylase activity was decreased by the stress treatments while water potential was increased by stress. As the treatments continued, a differential reduction expressed by the two cultivars occurred with the drought resistant Wanser being less affected or able to produce relatively more enzyme under stressed conditions.

Based on the close relationship of water potential and soluble protein content in seedling axis, protein synthesis probably is the most sensitive system in plants to be affected by water stress. Under continuous mild stresses adaptive mechanism and genetic ability of adjustment seems to take effect and allow plants to cope with adverse conditions.

Glutamine Synthetase and α-Amylase Activity in Germinating Wheat Under Water Stress

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GLUTAMINE SYNTHETASE AND α-AMYLASE ACTIVITY IN GERMINATING WHEAT UNDER WATER STRESS

INTRODUCTION

Water is required for biological activity. All forms of life including plants are dependent on water for their survival. In plants, water serves as a reactant in photosynthesis and in hydrolytic processes such as starch digestion, as an aqueous environment for metabolic processes and enzyme reactions, as a solvent for minerals and nutrients, and as a physical force in maintaining turgidity for cell enlargement and growth (Kramer, 1963).

Seldom are plants free from water stress during their growth cycle in the field. Worldwide, a deficit of water is one of the most limiting factors in crop production. The degree, duration and timing of water stress during the growing season often determines the crop yield and quality (Begg and Turner, 1976).

In wheat seeds, water is absorbed gradually in the first few hours of germination due to the bulk of endosperm which contains about 80% by weight of not very hygroscopic starch. Once the monolayer of water is sufficed the multi-layer and capillary water uptake is rapid and thereafter gradual increases in water uptake are observed (Ching, 1972; Mayer and Poljakoff-Mayber, 1975).

This imbibition process allows the quiescent seed to awaken and to reactivate pre-existing macromolecules and organelles formed during seed maturation. Continued water uptake over the germination period allows both catabolic and anabolic reactions to occur which ultimately result in growth of the seedling (Ching, 1972). During seed germination and subsequent growth stages water stress can modulate these metabolic processes which in turn affect normal growth pattern. Presently, how the growth and developmental pattern are affected is not clear.

In the Pacific Northwest wheat producing areas, the wheat crop is dependent on stored soil moisture from rainfall during the summer fallow period and the fall and winter months of the crop year. Soil moisture is often limiting during seed germination and emergence time, thus adequate stand establishment in these areas is difficult. Several workers have shown the interrelationship between seed germination, emergence, and increased soil water potential. Lindstrom (1973) reported that 80% emergence of two wheat varieties, McCall and Nugaines, was achieved at soil water potential of -10.0 bars. However, the rate of emergence was greatly affected as soil water potential decreased from 0.4 bars to -4.0 bars. Gul and Allan (1976) observed that the time required for wheat seed germination and emergence at soil water potentials of -2.2 bars to -14.4 bars doubled with decreases of -4.0 bars increments.

These studies reveal that the primary response to water stress during germination is a reduction of early embryo growth. However, little work has been done on how water stress modulates metabolic processes during seed germination and subsequent growth. This study was instigated to determine some of the effects of three mild water stress conditions on the growth of germinating Hyslop and Wanser wheat over a four day period.

The specific objectives were to determine the effect of simulated water stress on:

1. Total water potential in the seedling axis and endosperm during germination.

2. Seedling fresh weight, dry weight, and percent water content.

3. Activity of glutamine synthetase, an important anabolic enzyme responsible for the synthesis of nucleotides, nucleic acids, and aromatic nitrogenous compounds and N-assimilation and transport.

4. Activity of α-amylase, an important catabolic enzyme involved in wheat reserve hydrolysis thereby providing carbon backbones for growth activities during germination and subsequent development.

From these experimental data it is hoped that some causes or

mechanisms of water stress on plant growth may be discerned or some leads may be provided for further studies into the mechanisms of water stress in plants.

MATERIALS AND METHODS

Certified seed of Hyslop and Wanser, two winter wheat varieties in commercial production in Oregon, were used for this study. Hyslope is a soft, white, semi-dwarf, winter wheat recommended for dryland areas with about 400 mm of rainfall. Wanser is a hard, red, medium height, winter wheat well adapted to dryland areas with 300 mm of rainfall (Conway, 1977).

Each seed sample was sized with a 6 1/2 (64 x 3/4 inch) slotted screen. Discolored and cracked seeds were removed from the screened sample by hand.

Water Stress Treatments

Water stress during seed germination and subsequent growth was imposed by germinating seeds in an osmotium. Solutions of mannitol with osmotic potentials of -3, -6, -9 bars were prepared using Helmerick and Pfeifer formula (1954)

$$\text{grams mannitol} = \frac{PVm}{RT}$$

where

P = osmotic potential

V = volume in liters

m = molecular weight of mannitol, 182.17

R = .0825 liter atmospheres per degree per mole

T = absolute temperature

The control treatment 0-bar used distilled water as the wetting agent.

Fifty seeds per variety per treatment were placed in 10x10x3 cm plastic boxes between four layers of Whatman No. 1 filter paper wetted with 10 ml of water or mannitol solutions. Two boxes per treatment were put into plastic bags to reduce evaporation. The plastic wrapped boxes were then randomly placed in a germinator.

Seeds were germinated in the dark at 20-25°C for four days. Samples for growth, water potential or enzyme assays were taken every 24 hours. To compensate for the uptake of water by the seeds and growing seedlings glass distilled water was added to each box on the second and third day. For Hyslop, 0.6, 0.4, 0.3, and 0.2 ml water were added on the second day for 0, -3, -6 and -9-bar treatments, respectively; for Wanser 0.9, 0.5, 0.4, and 0.3 ml, respectively, were watered. On the third day, water addition was 1.3, 1.3, 0.8 and 0.8 ml for Hyslop materials and 2.0, 1.4, 0.9, and 0.8 ml for Wanser, respectively. Germination counts were not recorded because 90% or more were observed in four days under all mild stress treatments with materials from 0 and -3 bar reaching their maximum on the second day.

Plant Water Status

Water status in germinating Hyslop and Wanser seeds was measured psychrometrically by a sample chamber psychrometer (Wescor C-51) in conjunction with a dewpoint microvoltmeter (Wescor H-33).

Samples for total water potential determination were placed in a deep, small sample chamber. Three minutes were allowed for vapor pressure equilibration within the chamber. A cooling current was then passed through the thermocouple junction for 10 seconds. After cooling, the dewpoint of the sample was detected at the thermocouple by the microvoltmeter. The reading from the microvoltmeter was then converted into bars from a standard curve of KCl solutions with known concentrations.

Total Water Potential

For first and second day samples, six germinating seeds were selected from each treatment. Embryo and endosperm were separated and sectioned. The individual sections were placed in the sample chamber for total water potential measurement.

For third day samples, 18 germinating seeds each were selected from the 0-bar and -3-bar treatments. Shoots were excised and a mid-shoot section (1 mm in thickness) was used for

the water potential measurement. Three mid-shoot sections were used for each water potential measurement.

The endosperms of the selected seedlings were sectioned. Three endosperm sections were then used for each water potential measurement.

Due to a slower rate of germination observed in the more severe stress treatments, six germinating seeds each were selected from the -6-bar and -9-bar treatments. The embryo and endosperm were separated and sectioned. An individual section was placed in the sample chamber for water potential measurement.

For fourth day samples, 18 germinating seeds were selected from each treatment. Shoots were excised and mid-shoot sections were obtained. Three mid-shoot sections were used for each water potential measurement.

The endosperm of the selected seedlings were sectioned. Three endosperm sections were used for each water potential measurement.

Osmotic water potential of the experimental material was not determined because of the difficulties in extracting sap from limited material.

Growth Analysis

The fresh weight, dry weight, and percent water content of seedling axis were determined. Four replications of 10 germinating seeds per variety were randomly taken from each treatment every 24 hours for four days. Seed embryo and endosperm were separated on a moist filter paper, then embryos or seedling axes were put into glass weighing bottles and the fresh weight of the embryos or seedlings was recorded. The fresh materials were then oven dried at 100°C for 1 hour and at 70°C for 23 hours. After drying, the dry weights were recorded.

The percent water content was calculated:

$$\text{percent water content} = \left(\frac{\text{Fresh weight - Dry weight}}{\text{Fresh weight}}\right) \times 100$$

The embryo dry weight and percent moisture content of air dried seeds were also determined. Four replications of 10 seeds per variety were soaked in water for 4 hours at 5°C. After soaking, embryo and endosperm were separated on a moist filter paper. The dry weight and percent water content were determined as previously described.

The water content, fresh, and dry weights were not measured for endosperm material because the lose of material is unavoidable during dissection and accurate data are difficult to obtain.

Enzyme Analysis

Ten germinating seeds were randomly taken from each treatment. Axis and endosperm were separated on a moist filter paper. The axis fresh weight was recorded. The endosperms were dropped into tubes containing 6 ml of cold grinding buffer of Ca-acetate 10 mM, pH 6.0 and stored in an ice bath for α-amylase determination later that day.

Glutamine synthetase activity in the embryos was determined by the method of O'Neal and Joy (1973). The assay reaction is:

$$\text{L-Glutamate} + \text{Hydroxylamine} + \text{ATP} \xrightleftharpoons{Mg^{++}} \text{glutamyl hydroxymate} + \text{ADP} + \text{Pi}$$

The reaction product, glutamyl hydroxymate yields a characteristic brown color with ferric chloride and is read spectrophotometrically.

Ten dissected embryos were ground in 10 ml of grinding buffer containing 0.1 M N-2 hydroxyethylpiperazine, N'-α -ethanesulfonic acid (HEPES), 4 mM $MgAc_2$, 0.1 M sucrose, and 10 mM 2-mercaptoethenol pH 7.5, in a cooled mortar and pestle. The homogenate was centrifuged at 20,000 g for 10 minutes. The supernatant was collected and assayed for glutamine synthetase activity.

The reaction mixture consisted of 0.4 ml of enzyme extract and 0.5 ml of reaction buffer containing 0.1 M HEPES, 0.04 M $Mg\ SO_4$, 0.16 M L-glutamic acid, pH 7.5 with or without 0.02 M

ATP. The reaction mixtures were preincubated at 37°C for 2 minutes, then 0.1 ml of 0.2 M hydroxylamine, pH 7.5 was added. After a 10 minute incubation at 37°C, the reaction was stopped by an addition of 1 ml of color reagent (0.18 M $FeCl_3 \cdot 6\ H_2O$ dissolved in 0.67 M HCl and 5% TCA). After 10 minutes, 1 ml of glass distilled H_2O was added, to bring the final volume to 3 ml. The mixture was centrifuged at 5000 g for 1 minute to remove protein.

The amount of reaction product (glutamyl hydroxymate) formed in 10 minutes was determined colormetrically at 540 nm against a blank of enzyme extract and all other assay components except ATP. The total activity was defined as nanomoles of product per minute per seed. The specific activity was defined as nanomoles of product per minute per milligram of extracted protein. Soluble protein in the enzyme extract was determined by the method of Lowery et al. (1951) after trichloroacetic acid precipitation. Two to three replications per variety per day were assayed for glutamine synthetase activity.

α-Amylase activity in germinating wheat endosperms was determined by a modification of the Mitchell (1972) method as

Starch	$\xrightarrow{\alpha\text{-amylase}}$	glucose	+	dextrine
$+ I_2$		$+ I_2$		$+ I_2$
blue complex		no color complex		reddish complex

Ten dissected endosperms were ground in 10 ml of extracting buffer in a polytron (Brinkmann, Inc.) set at 5.5 speed for 30 seconds. The homogenate was transferred to a water bath and heated at 70°C for 20 minutes. After heating, the homogenate was centrifuged at 20,000 g for 10 minutes. The supernatant was collected and assayed for α-amylase activity.

Enzyme extract was added to an incubation mixture composed of 1 ml of reaction buffer (10 mM Ca-acetate, pH 4.8) and 1 ml of substrate starch solution (200 mg soluble starch in 100 ml of reaction buffer). The final incubation volume was 3 ml. After 0 and 5 minute incubation at 0°C a 0.5 ml alliquot was taken from the reaction tube and combined with 0.5 ml of color reagent (60 mg KI and 60 mg I_2 in 100 ml of 0.05 N HCl). Five milliliters of glass distilled H_2O was added to the mixture. The optical density was read at 620 nm against a blank of 0.5 ml color reagent, 0.5 ml reaction buffer and 5.0 ml glass distilled H_2O. The total activity was defined as milligrams starch hydrolized per minute per seed. The specific activity was defined as milligram starch hydrolized per minute per milligram extracted protein. Soluble protein in the extract was determined by the method of Lowery et al. (1951) after trichloroacetic acid precipitation. Two replications per variety per day were assayed for α-amylase activity.

LITERATURE REVIEW

Concept of Water Status in Plants (Brown and Van Haveren, 1972)

The water status in plants is best explained in terms of free energy. In the plant tissue, the free energy of water is expressed as the difference between the free energy of pure water and the free energy of water in the system at the same temperature and pressure. The resulting net free energy is termed water potential (Ψw). Total water potential is affected by three component forces that change the free energy of water. They are: 1) osmotic potential, $\Psi \pi$, 2) turgor or pressure potential, Ψp, and 3) matric potential, Ψm.

Osmotic potential is the primary component of total water potential in cell vacuoles and the cytosol. It lowers the free energy of water because the dissolved solutes bind water molecules and thus reduce the total mobility of water in the cell.

Turgor potential is an important component of total water potential in the cytoplasm, vacuole, and especially phloem elements. Turgor potential results from forces of hydrostatic pressure in excess of atmosphere pressure acting outwardly on the cell walls and inwardly on the tonoplast. It generally raises the free energy of water by confining water molecules and thus making them more available for work.

Matric potential is a component of total water potential in cell walls and membranes because of the absorbability of biocolloids in these structures. It originates from forces of capillarity, adsorption, and hydration acting on the surrounding water molecules. The matric potential usually lowers the free energy of water. In well watered plants and fleshy tissue the matric potential is near zero and is not biologically significant until much of the tissue water, for example 50 percent, is lost (Wiebe, 1966).

Generally, the status of water in plant tissue can be defined as the sum of the various components:

$$\Psi w = \Psi\pi + \Psi p$$

As water stress is imposed on a plant, the total water potential decreases in response to a reduction in water supply. Concurrently, there is a linear decline in the turgor potential, with the osmotic potential decreasing at a slower rate. Eventually total water potential is equal to osmotic potential and the plant loses its turgidity. Any further change in total water potential is due to osmotic potential alone (Hsiao et al., 1976; Morgan, 1977).

Plant Responses to Water Stress

There is volumnous information concerning plant responses to water stress. This review will summarize only the effects of water

stress on the physiological and metabolic processes during seed germination and seedling growth.

Growth

Growth can be defined as the irreversible enlargement of the cell. This enlargement is accompanied by differentation, proliferation of membranes and organelles, and increases in protein and cell wall material per cell (Hsiao, 1973).

Water deficits impair plant growth. Reductions of 10 to 20 percent in dry weight and yield have been reported under soil water potentials of -0.51 to -0.71 bars. For example, growth or yield reductions have been observed in potato (-0.51 bars), maize (-0.71 bars), sugarcane (-0.66 bars), and alfalfa (0.51 bars (Sands and Rutter, 1959).

Woodhams and Kozlowski (1954) showed an average 25 percent reduction of vegetative growth (foliage, stem, and root) in 8 week old bean and tomato plants under fluctuating soil moisture contents of 20.50 (field capacity) to 13.50% (permanent wilting point). They further observed reductions in plant height of 32.13% in bean and 22.65% in tomato at these same stress levels.

Duysen and Freeman (1974) showed that the growth rate of water stressed 11 day old wheat seedlings was reduced nearly 90% over that of non-stressed wheat seedlings. At a leaf water potential

of -5.0 bars (non-stress condition) the average growth rate of the shoot was 22.3 mm per day, whereas at leaf water potentials of -9.0 to 14.0 bars (stressed condition) the average growth rate of the shoot was 2.4 mm per day.

Conway (1977) observed that shoot length and seedling dry weight of seven wheat cultivars decreased with increasing water stress. At soil water potential of -6.0 bars average shoot length was decreased 31% and seedling dry weight was reduced 11% than the control of 0 bars.

Water stress not only reduces total growth but also alters the pattern of growth (Kramer, 1963). For example, root-shoot ratios are often increased by water deficits. Morphologically, thickness of cell walls and the amount of cutinization and liginification increases under water stress.

Kramer (1963) noted that the essential factor in plant water relations was the internal water balance or degree of turgidity of the cell. This factor in turn would mediate physiological processes which determine quantity and quality of growth.

Turgor potential is considered the source of the physical push or pressure necessary for cell enlargement. Kirkham et al. (1972) observed that in isolated radish cotyledons incubated in growth medium plus graded concentrations of mannitol (-1.0 to -16.0 bars), cell enlargement was inhibited 11% at turgor potential below +3.0 bars.

Several workers have shown the critical role turgor potential plays in plant growth under mild water stress conditions (-3.0 to -5.0 bars). Boyer (1968) observed that leaf growth in sunflower decreased 66% at leaf water potentials from -1.5 bars to -3.0 bars and no leaf growth occurred at leaf water potentials greater than -4.0 bars. He suggested that sunflower leaves require a minimum turgor potential of about +6.5 bars for growth. Low leaf water potentials at night (-1.9 bars) favor leaf growth and maintain maximum turgor potential whereas leaf water potentials during the day (-3.5 bars) reduce growth through a lowered turgor potential. Barlow et al. (1976) monitored leaf elongation rates in water stressed corn seedlings. After 3 hours of water stress, the leaf elongation rate of stress plants (Ψw = -5.0 bars) was 10% of the control plants (Ψw = -0.2 bars). The turgor potential of the control plants was +3.7 bars and +0.3 bars for the stressed plants indicating that turgor potentials are proportional to leaf elongation rate not total water potential.

Seed Germination and Emergence

Water stress reduces seed germination and delays seedling emergence. Doneen and MacGillivray (1943) observed the effect of decreasing soil moisture content on germination (emergence) of vegetable seeds. They showed that rapid germination and emergence

occurred at soil moisture content of 15.7% (field capacity) but declined as soil moisture content reached 8.0% (permanent wilting point). However they noted that vegetable seeds differed in their ability to germinate at a soil moisture content of 9%, for example germination percentages for cabbage, 94%; carrot, 57%; lima bean, 23%; and lettuce 0% were observed. These results suggest that species differ in their sensitivity to a particular water stress level and that some species for example, cabbage, are relatively drought tolerant.

Uhvits (1946) found that alfalfa seed germination and rate of germination progressively decreased with increased water potential of the germination substrata. The stress treated seeds (Ψw = 12.0 atms) germinated 4% of the control (Ψw = 0.1 atms) on the second day of germination, however on the sixth day of germination the stressed seeds germinated 60% of the control and on the tenth day the stressed seeds germinated 80% of the control.

Kaufmann and Ross (1970) reported that wheat germinated 90% at a soil water potential of -8.0 bars but no germination occurred at a soil water potential of -14.0 bars. They observed that with increasing soil water potentials the time requied for 50% wheat germination increased for example 35 hours at 0 bars and 140 hours at -8.0 bars. Gul and Allan (1976) observed that selected wheat lines differed in their germination and emergence capability at soil water potentials of

-2.2 bars to -10.2 bars. For example, 5 days after initial emergence 93 wheat lines achieved 80% emergence at -2.2 bars, 62 wheat lines achieved 80% emergence at -6.0 bars, and 45 wheat lines achieved 80% emergence at -10.2 bars. This differential response suggest that wheat lines can be selected that will establish well and rapidly under relatively low soil water potentials.

Carbohydrate Metabolism

Most studies on the effect of water stress on carbohydrate metabolism have looked primarily at photosynthesis, particularly on carbohydrate composition under water stress conditions.

Woodhams and Kozlowski (1954) studied the distribution of carbohydrates in tomato and bean plants subject to fluctuating soil moisture contents of 20.50% (field capacity) to 13.50% (permanent wilting point). After 8 weeks of growth they observed a marked decrease in sugars and starch in the root, stem, and leaves of both species. For example, in tomato stems reducing sugars were decreased 20%, non-reducing sugars 25%, and starch 27%. The carbohydrate distribution in the leaves and roots followed a similar trend. Woodhams and Kozlowski suggested that decreases in total carbohydrates were primarily due to water stress effects on photosynthesis.

Stewdart (1971) observed in wilted bean leaves (desiccated to 75%

of their original fresh weight) kept in the dark for 12 hours, a 47% reduction in starch content over that of fully turgid leaves. This starch loss was accompanied by a similar increase in free (alcohol-soluble) sugars. He noted that at any given time during increased starch hydrolysis in wilted leaves, the additional amount of carbohydrate lost from starch was recovered in free sugars. He measured the sucrose content of wilted and turgid leaves and observed a 50% increase in sucrose in wilted leaves at the same time and stress level when starch breakdown accelerated. He postulated that bean leaves kept in the dark can convert starch into sucrose under periods of brief moisture stress.

Maranville and Paulsen (1970) observed decreases in starch content of 3 week old corn seedlings grown under a photoperiod of 16 hours light and 8 hours dark. Under three watering regimes control (no water withheld), moderate (4 days water withheld) and severe (7 days water withheld), leaf starch content was reduced 45% and 50%, respectively. They observed a 63% increase in α-amylase activity and a 80% decrease in invertase activity in the leaves of severely stressed seedlings. Sucrose levels stayed relatively constant across all three water stress levels. They inferred that changes in carbohydrate metabolism under these stress conditions were mediated primarily through reduced photosynthesis. Increased starch hydrolysis, increased α-amylase activity, and decreased invertase

activity were as the authors postulated plant adaptions to brief periods of moisture stress.

Protein Content and Synthesis

Shah and Loomis (1965) observed decreases in soluble and total protein in water stressed sugar beet leaves. Barnett and Naylor (1966) reported that soluble protein in two bermuda grass varieties, Common and Coastal, was decreased 31% and 36%, respectively, as shoots were stress to -10.0 to 37.0 bars leaf water potentials. Stutte and Todd (1969) found protein content decreased to 58% of the control in wheat leaves as relative water content reached 60% or greater. He postulated that changes in protein content of wheat seedlings under water stress were due either to an inhibition of protein synthesis or an acceleration of protein degradation.

Hsiao (1970) monitored polyribosome content in corn seedlings to infer reduction of protein synthesis under mild water stress (Ψw = -7.0 to -10.0 bars). He observed a 27% shift of ribosomes from the polymeric to monomeric form after 30 minutes stress at tissue water potential of -7.0 bars.

Four hours later at tissue water potential of -10.0 bars, 65% of the ribosomes were in a monomeric form. Rewatering of seedlings stressed at -10.5 and -11.0 bars for four hours, recovered polyribosomes and water potential after 2.5 hours. The shift of

polysomes to monosomes brought about by water stress was blocked by cycloheximide. Because cycloheximide inhibits protein synthesis by preventing the release of nascent polypepitides from the polysome, Hsiao concluded that a lack of chain initiation may be the cause of polyribosome reductions under brief moisture stress. Lin et al.(1967) suggested that under water stress, protein synthesis may be controlled only at the translation level because of the rapidity and reversibility of the protein synthesis response. Dhindsa and Cleland (1975) showed not only the quantity of protein synthesis was inhibited under water stress but the quality was altered. In sectioned defoliated oat coleoptiles, 3 hour incubation at mannitol concentration of 0.01 M to 0.5 M inhibited incorporation of ^{14}C-leucine, with maximum inhibition (about 80%) occurring at 0.3 M mannitol concentration. Qualitative effects of these stress conditions on protein synthesis were illustrated by separation of soluble proteins from stressed tissue on polyacrylamide gels. Dhindsa and Cleland concluded that under moisture stress of 0.1 M to 0.5 M mannitol, protein synthesis in oat coleoptiles was differentially inhibited, with the synthesis of some proteins being affected to a greater extent than the synthesis of others.

Rhodes and Matsuda (1976) observed that growth rate reductions in pumpkin and pea seedlings were proportional to reductions in polyribosome levels during brief moisture stress (30 minute incubation in various concentrations of NaCl). For example, they found a 1%

reduction in polyribosome percentage was associated with a 0.18 and 0.20 cm per day reduction in pumpkin and pea seedling growth rates. They postulated that a good correlation exists between reduction in polyribosomes and growth rates under brief water stress and that monitoring the polyribosome rates of drought tolerant and drought sensitive species would provide a basis for predicting water stress effects on growth.

Enzyme Activities

Todd (1972) stated that under water stress release or activation of degradative enzymes occurred and levels of anabolic enzymes decreased while others increased. Morilla et al. (1973) reported declines in nitrate reductase activity in water stressed corn seedlings. Enzyme activity decreased when leaf water potential reached -2.0 bars and was 25% of the control at a leaf water potential of -13.0 bars. They concluded that the inhibition of nitrate reductase activity under water stress was due to a decline in the rate of synthesis of the enzyme rather than an increase in the rate of enzyme degradation.

Bardzik et al. (1971) observed that L-phenylalanine ammonia-lyase (PALase) and nitrate reductase were reduced 45% and 50% in maize seedlings stressed to water deficits of 20%. Since PALase and nitrate reductase have relatively short half lives of 4 hours (Durst and Mohr, 1966) and 6 hours (Schrader et al., 1968),

Bardzik et al. (1971) postulated that the activity of short lived enzymes during mild dessication is markedly reduced. Huffaker et al. (1970) observed the effect of mild water stress in barley seedlings on enzymes of the carboxylative phase of photosynthesis. They concluded that under leaf water potentials of -7.0 to -8.0 bars ribulose-1, 5-diphosphate carboxylase and phosphoribulokinase activity were relatively insensitive with 10.8% and 0% decreases in enzyme activity from the control. Phosphoenolpyruvate carboxylase activity decreased 21% at similar leaf water potentials. They suggested that the reduction in phosphoenolpyruvate carboxylase activity could be the result of a biochemical adaptation to mild water stress causing a decrease in synthetic capabilities so as to reduce the overall energy requirements of the plant.

Among the enzymes whose activities are increased under moderate to severe water stress (Ψw = -10.0 bars to -15.0 bars) are ribonuclease and α-amylase in leaves (Todd, 1972).

Morilla et al. (1973) observed that in dessicated corn seedlings (Ψw = -15.0 bars) RNase activity increased 50% over that of the control plants (Ψw = -3.0 bars). Tvorus (1970) indicated increases in RNase activity in response to water stress was due to de novo synthesis of the enzyme, since the response to dessication could be prevented by chclohexamide. Maranville and Paulsen (1970) observed a 63% increase in α-amylase activity in corn seedlings subject to

severe water stress. They concluded that the observed decline in leaf starch content of water stressed corn seedlings was due indirectly to the stimulation of α-amylase activity. In contrast, Wilson (1971) observed that in crested wheatgrass a water potential of 20 atmospheres resulted in an 80% inhibition of α-amylase activity in germinating seeds. He postulated that α-amylase synthesis in crested wheatgrass was a relatively drought intolerant process. Jones (1969) reported that in germinating barley seed, 0.6 M concentrations of polyethyleneglycol and mannitol inhibited GA-induced α-amylase activity by 80%. Jones and Armstrong (1971) postulated that inhibition of α-amylase production by sugars is mediated through the osmotic modulation of hydrolytic products from the starchy endosperm.

Osmotic Adjustment

Growth is very sensitive to water stress. The ability of plants to maintain steady-state growth under water stress by reducing osmotic potential and thereby maintaining turgor potential is termed osmoregulation (Hsiao et al., 1976; Morgan, 1977).

Kauss (1967) observed that in the algae Ochromonas malhamensis increasing the osmotic pressure of the growth medium caused cells to first shrink and then regain their volume through the accumulation of α-galactopyranosyl glycerol (IF) in concentrations high enough to compensate directly for the osmotic stress. Kauss (1973) later

reported that a 10 minute osmotic stress of 30 mOsmole resulted in a ten fold increase of IF formed from exogenously supplied glucose-^{14}C. He postulated that the accumulation of IF in Ochromonas malhamensis under osmotic stress was due to enhanced synthesis rather than reduced degradation of IF.

In higher plants, osmoregulation has been eluded to by observed increases in soluble sugars (Stewdart, 1971) and amino acids (Barlow et al., 1976) under water stress conditions. Malate and potassium might participate in osmoregulation. Dhindsa et al. (1975) showed that a combined malate and potassium concentration of 0.15 M accounted for more than 55% of the total osmotic component in elongating cotton fiber. Culter and Rains (1978) noted that concentrations of malate increased in water stressed cotton leaves. They observed a two-fold increase in malate concentrantions at leaf water potentials of -10.0 bars to -16.0 bars. They suggested that malate may be an important osmotic agent in cotton leaves.

Meyer and Boyer (1972) observed osmotic adjustment in water stressed soybean seedlings. As water potentials decreased from -3.0 bars to -7.0 bars in the hypocotyls, the osmotic potential rose from -8.0 bars to -11.0 bars and the turgor potential remained constant at +5.0 bars. The growth rate of the hypocotyls decreased 57% upon reducing water potential from -3.0 bars to -7.0 bars. Removal of the cotyledons resulted in a 100% growth inhibition at

water potentials of -5.0 bars to -6.0 bars. Meyer and Boyer postulated that under water stress conditions the osmotic adjustment in elongating cells of soybean hypocotyls appeared to be dependent on the transfer of solutes from the cotyledons to the growing hypocotyl and these solutes were necessary in maintaining turgor potential and growth.

Morgan (1977) reported genetic variation among wheat genotypes for the ability of osmoregulation. He observed osmoregulation at both pre- and post-anthesis stage. He reported that turgor potential was maintained sufficiently for growth up to leaf water potentials of -15.0 bars. Morgan postulated that the presence of osmoregulation in promising wheat lines would be a reliable criterion in selecting for drought resistance.

Glutamine Synthetase Activity in Plants

The amide glutamine is formed by the amination of glutamate catalysed by glutamine synthetase. The reaction is as follows:

$$\text{L-glutamate} + NH_3 + \text{ATP} \xrightleftharpoons[\text{enzyme}]{Mg^{++}} \text{L-glutamine} + \text{ADP} + \text{Pi}$$

No investigations have been instigated to eludicate the effect of water stress on glutamine synthetase activity in higher plants. Regulatory studies on glutamine synthetase activities reveal that this enzyme is subject to feedback inhibition of glutamine metabolic end products

and more importantly by energy charge of the cell.

O'Neal and Joy (1973) reported that glutamine synthetase isolated from pea leaves was inhibited competively by histidine and ornithine and non-competively by alanine, glycine, and serine. However, this inhibition was significant only when Mn^{++} served as a co-factor in the reaction and the concentrations of metabolites required for 50% inhibition of enzyme activity were rather high, 1.7 to 6.5 mM for histidine, 7.8 mM for ornithine and over 40 mM for glycine, serine, and alanine. They suggested most likely that glutamine synthetase activity in pea leaves is regulated by the energy charge of the cell. They showed with Mg^{++} serving as a co-factor ADP, 5'-AMP and Pi were signigicantly inhibitory and the relative activity of glutamine synthetase was 53% and 20% at energy charges of 0.8 and 0.5.

Weissman (1976) reported that the activity of glutamine synthetase of sunflower roots after the roots were incubated in the following culture solutions: 9 day NO_3, 7 day NO_3, 2 days NH_4, 3 or 5 days zero N and 3 or 5 days darkness increased with increasing cellular energy charge. A significant correlation coefficient of 0.72 was obtained for all experimental data. Weissman postulated that the relationship between glutamine synthetase activity and energy charge provides the cell with a highly sensitive control mechanism under conditions of rapid consumption or limited supply of adenylate energy. He further showed that in the 3 or 5 day zero N and 3 or 5 days

darkness treatments, high concentrations of alanine modulated the glutamine synthetase activity-energy charge relationship. Lignowski et al. (1971) studied the changes in glutamine synthetase activity in germinating pumpkin seeds. They observed low levels of activity during the first two days of germination, 0.04 umoles per min. per cotyledon pair. Enzyme activity increased rapidly thereafter and reached a maximum of 0.35 umoles. By the eighth day of germination, glutamine synthetase activity decreased to 0.25 umoles. They reported no appreciable change in ATPase activity in the enzyme preparation during the germination period. ATPase activity from 2 to 8 days of germination was 4.5 to 5.0 umoles PO_4 released per glutamine synthetase assay. The observed maximum activity of glutamine synthetase at the sixth day of germination paralleled the period of greatest axis growth and nitrogen transport from the cotyledons of pumpkin seedlings (Lignowski and Splittstoesser, 1971; Splittstoesser and Stewdart, 1970).

RESULTS AND DISCUSSION

Total Water Potential

A literature search indicated that no detailed studies have been conducted on the total water potential of water stressed germinating seeds. This study therefore attempted to measure the total water potential of embryo, endosperm, and shoot of water stressed Wanser and Hyslop seedlings throughout the four day germination period (Tables 1-2).

The water potential of the seedling axis and endosperm were high at the beginning of germination, then decreased with progressive water uptake (Figure 1a-b) growth (Figures 2a-b and 3a-b) and hydrolysis of stored food reserves (Figures 7a-b and 8a-b).

After one day in germination, the general trend showed that the seedling axis and endosperm of the 0-bar treatment had the lowest water potential for both varieties than those of the water stress treatments. In Hyslop material, the water potential of the seedling axis and endosperm increased with greater stress treatment. The range in total water potential was -23 bars (axis) and -25 bars (endosperm) for the 0-bar treatment and -38 bars (axis and endosperm) for the -9-bar treatment. A similar trend was observed in Wanser seedlings of -29 and -25 bars (axis, endosperm) for 0-bar treated material and -31 and -33 bars for -9-bar treated material.

TABLE 1. Total water potential (-bars) of seedling axis and endosperm of seeds of Hyslop germinated at 20-25°C in dark under various water stressed conditions.

Treatment		Days of Germination 1 Day	2 Day	3 Day	4 Day
0-bar	axis	22.6 ± 1.9	22.3 ± 2.9*	19.3 ± 0.9*	15.9 ± 1.8*
	endosperm	24.7 ± 0.5	26.5 ± 1.3	22.4 ± 1.0	19.3 ± 1.3
-3-bar	axis	31.2 ± 1.4	28.7 ± 0.9*	28.7 ± 0.9*	22.0 ± 2.8*
	endosperm	24.8 ± 1.2	21.3 ± 2.0	23.6 ± 1.3	24.5 ± 0.6
-6-bar	axis	30.9 ± 2.0	29.7 ± 2.3	27.2 ± 2.0*	30.5 ± 2.8*
	endosperm	30.8 ± 3.3	26.9 ± 0.9	26.3 ± 2.3	27.5 ± 0.1
-9-bar	axis	38.0 ± 4.0	27.8 ± 2.7	31.3 ± 1.1*	29.1 ± 2.6*
	endosperm	38.0 ± 5.9	30.9 ± 1.8	28.7 ± 0.5	29.8 ± 1.2

* shoot was used.

TABLE 2. Total water potential (-bars) of seedling axis and endosperm of seeds of Wanser germinated at 20-25°C in dark under various water stressed conditions.

Treatment		Days of Germination 1 Day	2 Day	3 Day	4 Day
0-bar	axis	28.9 ± 2.2	25.5 ± 1.2*	24.3 ± 1.7*	16.7 ± 1.3*
	endosperm	24.6 ± 4.2	20.3 ± 0.6	22.1 ± 0.4	14.0 ± 2.0
-3-bar	axis	31.0 ± 0.7	25.6 ± 0.4*	27.1 ± 2.1*	24.4 ± 0.7*
	endosperm	20.3 ± 3.3	20.7 ± 0.4	22.0 ± 3.6	21.3 ± 0.5
-6-bar	axis	27.6 ± 1.0	24.1 ± 0.1	32.0 ± 0.9*	28.5 ± 1.6*
	endosperm	29.1 ± 0.8	25.2 ± 0.2	29.1 ± 1.7	25.7 ± 0.4
-9-bar	axis	30.6 ± 1.1	30.2 ± 0.5	34.8 ± 0.6*	33.6 ± 0.7*
	endosperm	33.1 ± 1.5	30.0 ± 0.4	30.8 ± 3.5	30.7 ± 1.8

* shoot was used

Total water potential in the axis and endosperm decreased in most material as germination progressed to the second day. In Hyslop material total water potential of the stressed material decreased to -29, -30, and -28 bars in the axis and -21, -27, -31 bars in the endosperm. However, the 0-bar treatment resulted in a constant axis water potential of -22 bars and an increased endosperm total water potential of -27 bars. Wanser material had similar axis water potentials of -25 to -24 and -30 bars under 0, -3, -6, and -9 bar treatments and endosperm water potentials of -20, -21, -25, and -30 bars, respectively. On the third day of germination the 0-bar treated Hyslop material had a low axis and endosperm total water potential of -19 and -22 bars, respectively whereas all other treatments resulted in higher total water potentials of -29, -27, and -31 bars (axis) and -24, -26, and -29 bars (endosperm) for the respective treatments. All axis water potential increased with greater stress treatment whereas in the endosperm, water potentials were similar at -22 bars for 0 and -3 bar treated material and higher at -32 and -35 bars for the -6 and -9 bar treatments. In both varieties the greatest decrease in axis and endosperm water potential occurred on the fourth day of germination. Under the 0-bar treatment, Hyslop had axis and endosperm total water potentials of -16 bars and -19 bars, respectively, Wanser material responded similarly with -17 and -14 bars, respectively. In all other treatments axis and

endosperm water potential increased with greater degree of water stress.

The general trend showed that as growth rate, glutamine synthetase, and α-amylase activity reached their maximum on the fourth day of germination total water potential decreased greatly in Hyslop and Wanser seedlings under 0 and 3-bar water stress. However, under the more severe water stress treatments of -6 and -9-bar, seedling axis and endosperm total water potential changed slightly throughout the germination period. This indicates that at water stress conditions of -9.0 bars or greater, changes in water potential proceed slowly with germination time resulting in reduced metabolic activities and in general slower growth rate.

Large variations were observed between treatments and days of germination and noticeable trends over germination time were difficult to conclude. This suggests that the methodology needs improvement. The location of watering in the germination box on the second and third day could account for the large observed variations. The sectioning of the material for the readings, the sample size, the atmospheric temperature and humidity, and lastly the electrical power supply may all contribute to the variability of the water potential of the experimental materials. The general trend in reference to stress and to germination stage was nevertheless obvious, and the range of water potential in germinating wheat was

of value as no information has been reported.

Growth Analysis

Experimental data indicated that all growth parameters such as seedling axis water content, fresh weight, and dry weight were progressively reduced over the four day germination period with increasing water stress. The degree of growth reduction varied somewhat with varieties, duration or days of germination, and water stress treatments (Figures 1a-b to 3a-b).

Percent Water Content of Embryo or Seedling Axis (Figure 1a-b)

After one day in germination there was a nine-fold increase in water content by both varieties under all water stress treatments. The percent water content for Hyslop material in the 0-bar treatment was 60% with other stress materials at lower water contents of 56, 50, and 52%, respectively. Wanser material responded similarly at 67, 58, 59, and 59%, respectively. These trends were continued to the second day of germination but with smaller increments.

On the third day of germination, Hyslop seedling axis had closer water contents of 81% and 78%, respectively, under 0 and -3-bar treatments while the -6-bar and -9-bar treated material had lower water contents of 72% and 69%. Wanser seedlings responded differently to stress treatments. The 0-bar and -3-bar treated

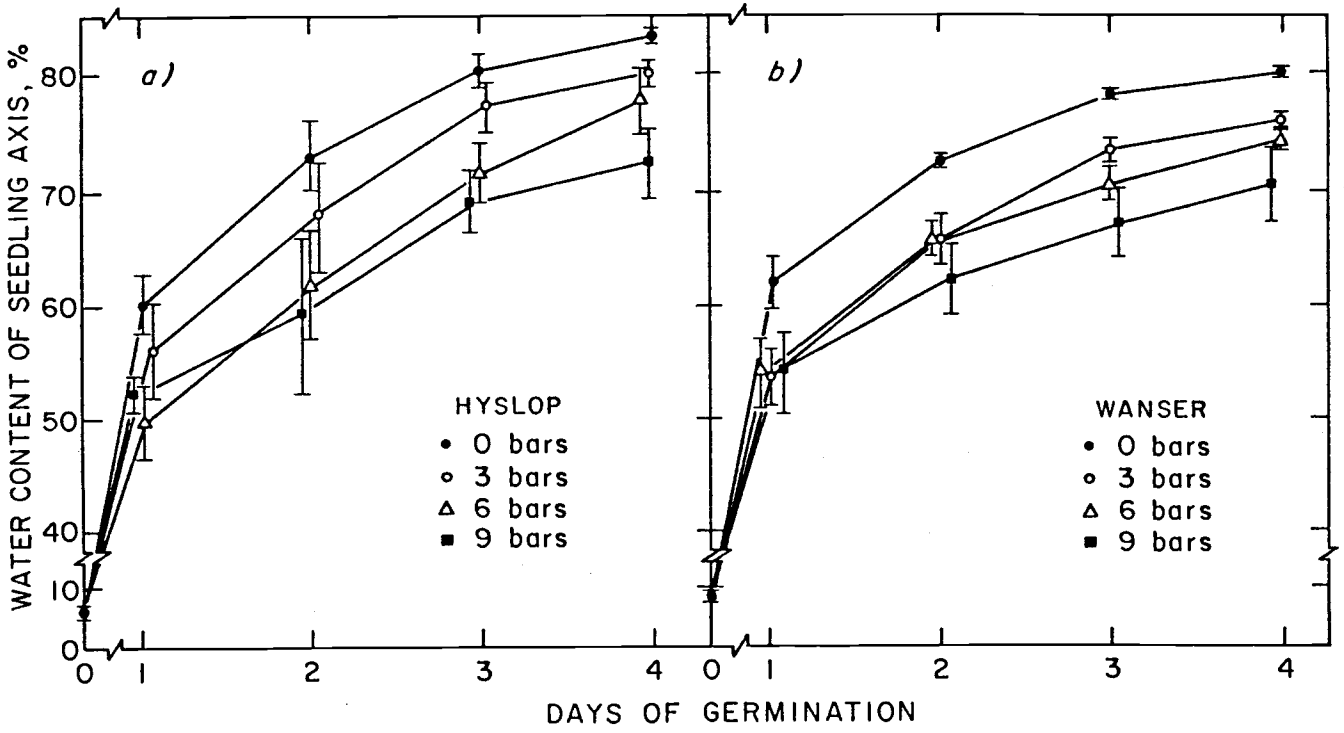

Figure 1a-b. Water content (% fresh weight) of embryo or seedling axis of Hyslop (a) or Wanser (b) seeds germinated under different water stresses at 20-25°C in dark.

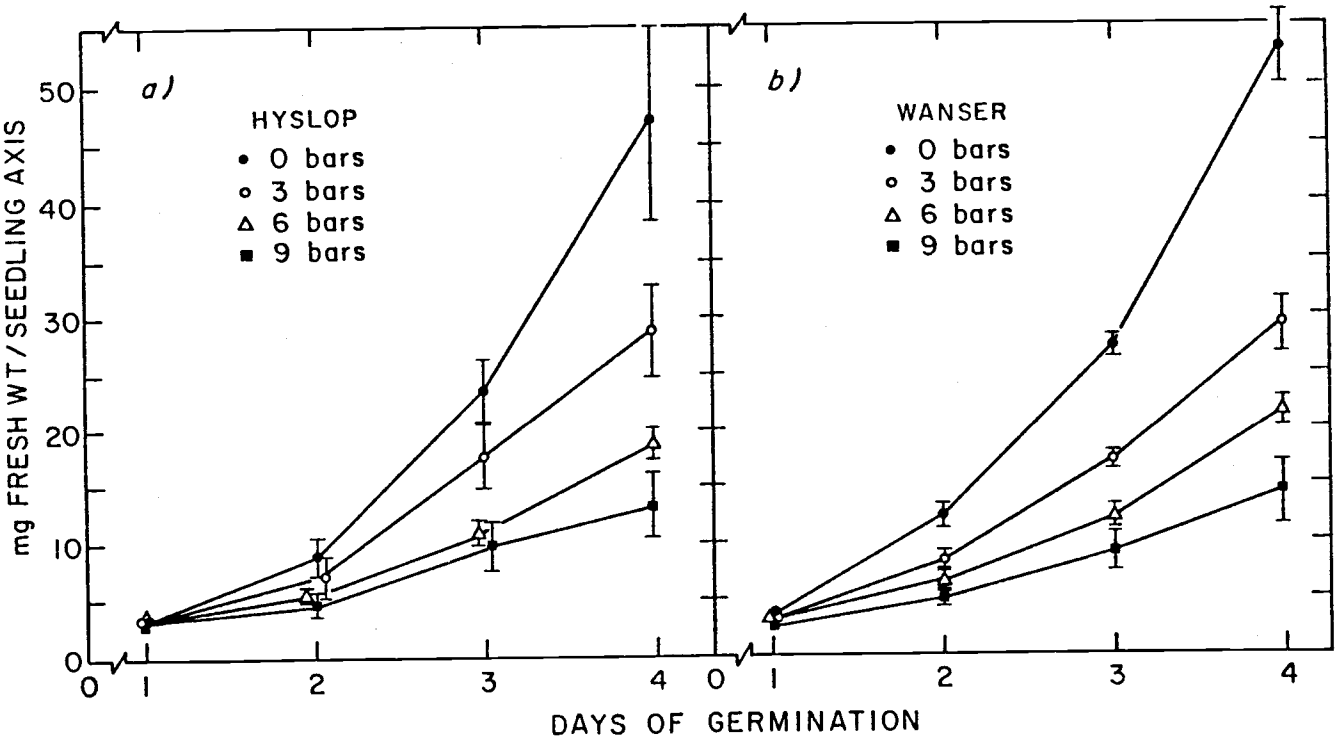

Figure 2a-b. Fresh weight of embryo or seedling axis of Hyslop (a) or Wanser (b) seeds germinated under different water stresses at 20-25°C in dark.

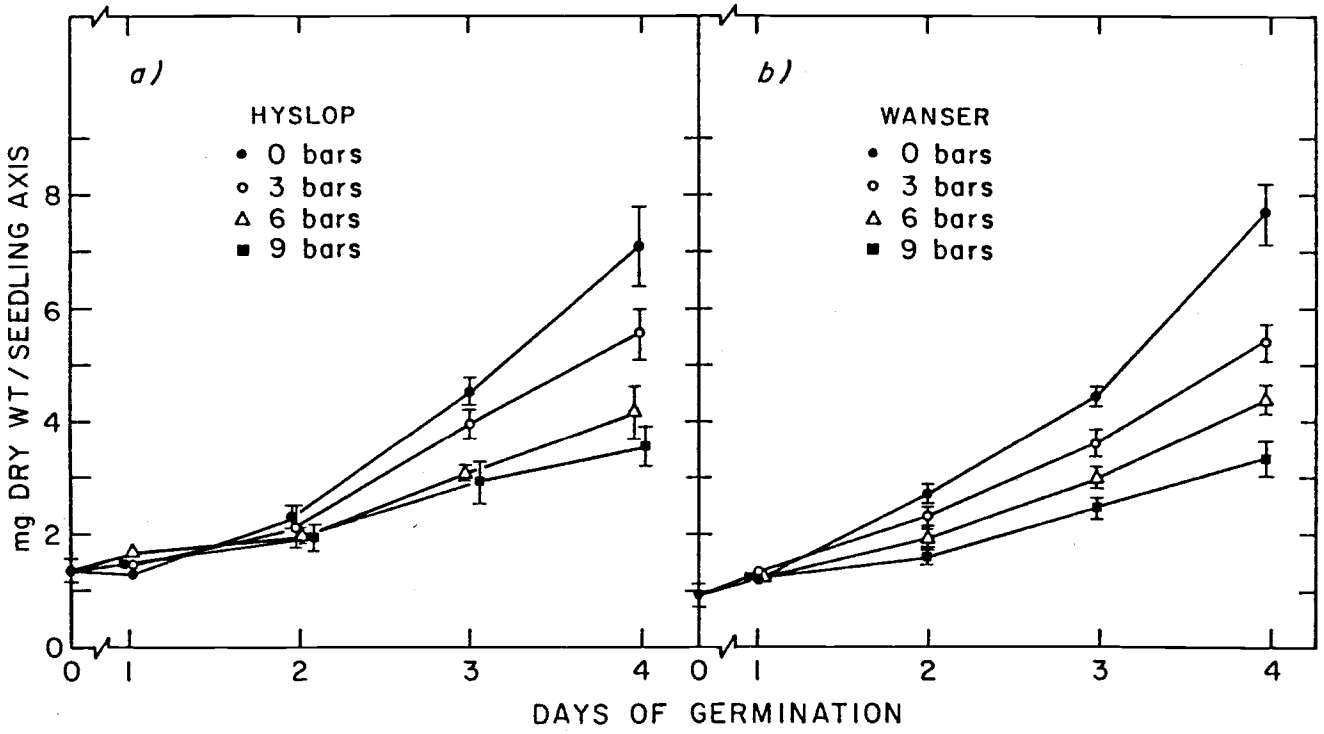

Figure 3a-b. Dry weight of embryo or seedling axis of Hyslop (a) or Wanser (b) seeds germinated under different water stresses at 20-25°C in dark.

material had high but deviating water contents of 84% and 79%. Lower water contents were obtained in the -6-bar and -9-bar treatments at 76% and 72%. A slight increase in water uptake by all seedlings was shown on the fourth day of germination. The Hyslop material had water contents of 84, 81, 78, and 73% for 0, -3, -6, and -9-bar treatments, respectively. Wanser seedlings showed the same trend as noted on the previous day. Throughout the germination period Wanser had somewhat higher water content in embryo or seedling axis than that of Hyslop under all experimental conditions.

In both varieties water uptake proceeded rapidly on the first day of germination and gradually thereafter. The control material consistently had a higher embryo or seedling axis water content than stressed materials throughout the germination period. The greatest reduction in water content occurred in the -9-bar treatment, which resulted in an average reduction of 16% of the control in Hyslop seedlings and 14% in Wanser seedlings after four days. This demostrates that under these mild water stress conditions of -3 to -9 bars the ability of water absorption by Hyslop and Wanser seedlings from their surroundings is not greatly impaired. However, as water stress is increased to greater than -9 bars as often happens in Pacific Northwest dryland areas during late summer and early fall, (Russelle, 1978) water uptake by the seeds can be limited. Water content of embryo or seedling axis is thus the least sensitive indicator

of mild water stress effect on wheat germination and seedling growth (next section) under these experimental conditions.

Fresh Weight of Embryo or Seedling Axis (Figure 2a-b)

Embryo fresh weights after one day of germination were 3.4 mg to 3.0 mg in Hyslop and 3.6 mg to 3.0 mg in Wanser. Fresh weights of Hyslop in the 0 and -3, -6, -9-bar treatments increased steadily to 8.8, 5.1, and 4.9 mg, respectively on the second day of germination. A trend was also indicated in Wanser, embryo fresh weights of all treated material were reduced from 12.2 mg for the control to 8.3, 6.8, and 5.0 mg, respectively for the three water stress treatments. On the third day of germination seedling axis fresh weight in the 0-bar treatment of both varieties was 2.5 to 3 times higher than the -9-bar treated material. Fresh weight reductions between the -3, -6, and -9-bar treated Hyslop material were noted to be 18.0, 11.1, and 9.7 mg, respectively. The fresh weight of Wanser seedling axis were progressively reduced by the three treatments from 27.2 mg for the control to 17.0, 12.4, and 9.2 mg, respectively. There was a rapid increase in fresh weight of Hyslop and Wanser seedling axis on the fourth day of germination. Fresh weights for Hyslop seedling axis in 0, -3, -6, and -9- bar treatments were 47.5, 28.8, 18.9, and 13.3 mg, respectively and for Wanser material were 53.7, 29.1, 21.4, and 14.4 mg, respectively. All

these weights were statistically significantly different from control as shown by t-test in Appendix. Comparison between the two varieties revealed that Wanser seedlings had slightly higher fresh weights under all treatments than Hyslop seedlings.

In general, fresh weight was reduced progressively by the increasing stress treatments from the second day of germination and thereafter. Fresh weight gains appeared to be the most sensitive indicator of mild water stress effect on wheat seed germination and subsequent growth under these experimental conditions. The percent fresh weight reduction from the control rose with increasing water stress and germination time. On the fourth day of germination, the fresh weight of -9-bar treated material was reduced by 72% of the control for Hyslop and by 73% for Wanser. Throughout the germination period, Wanser seedlings fresh weight under all treatments were decreased more than Hyslop seedlings. Hyslop seedlings appeared to be less sensitive to the mildest water stress treatment of -3-bar than Wanser. The fresh weight is the best indicator of water stress effect on growth because it is the composite of water absorption capacity and assimilation (Figures 1a-b and 3a-b).

Dry Weight of Embryo or Seedling Axis (Figure 3a-b)

In both varieties there was a small increase in dry weight after one day of germination. Embryo dry weight rose steadily in all

material as germination progressed to the second day. The 0, -3, -6, and -9-bar treatments, respectively resulted in 2.3, 2.2, 1.9, and 1.9 mg axis dry weight in Hyslop. In Wanser, dry weight was reduced with respective treatments to 2.7, 2.4, 2.0, and 1.6 mg. This trend was observed consistently with further germination time. On the third day of germination, Hyslop seedling axis dry weights in the 0 and -3-bar treatments were high at 4.5 and 4.0 mg whereas the other stress material had smaller dry weight gains of 3.2 and 2.9 mg, respectively. Under 0, -3, -6, and -9-bar treatments Wanser material was reduced resulting in 4.5, 3.6, 3.0, and 2.5 mg, respectively. Dry weight increased greatly on the fourth day of germination. Hyslop material had seedling dry weights of 7.1, 5.5, 4.1, and 3.6 mg in the 0, -3, -6, and -9-bar treatments, respectively, while Wanser material increased to 7.7, 5.4, 4.4, and 3.4 mg, respectively. The dry weights of stressed material were statistically significantly different from the control (Appendix). The general effect of water stress on seedling axis dry weight was similar in both varieties.

Dry weights of seedling axis under the -9-bar treatment were reduced to 17% to 49% of the control for Hyslop on different germination days and 40% to 56% for Wanser. The dry weight of Wanser embryo or seedling axis was more sensitive to all treatments than Hyslop. This is in agreement with similar trends observed for fresh weight (Figure 2a-b). Seedling dry weight was a better indicator

of mild water stress effect than water content but not as sensitive as the fresh weight.

In this study, decreases in embryo or seedling axis water content fresh weight, and dry weight under mild stress conditions of -3 to -9-bars reflect similar trends in growth parameter reductions observed by other investigators (Boyer, 1968; Kirkham et al., 1972; Hsiao, 1973; Duysen and Freeman, 1974; and Barlow et al., 1976). Seedling dry weight reductions in Hyslop and Wanser followed a similar decreasing trend as observed by Conway (1977) under soil water potentials of -3 to -6-bars. In this study, the degree of dry weight reductions on the fourth day of germination under the -3 and -6-bar stress levels was greater, 23% and 42% for Hyslop and 30% and 43% for Wanser, respectively, than Conway's reported reductions of 7% and 11%. The difference in dry weight reduction between these two experiments was due to methodology used for simulating water stress treatment.

Soluble Protein in Seedling Axis (Figure 4a-b)

In both varieties, the control material had the highest protein content per individual seedling axis throughout the four day germination period. The protein content of treated materials were reduced proportionally to water stress on the third and fourth day of germination. The low protein content of stressed materials on these

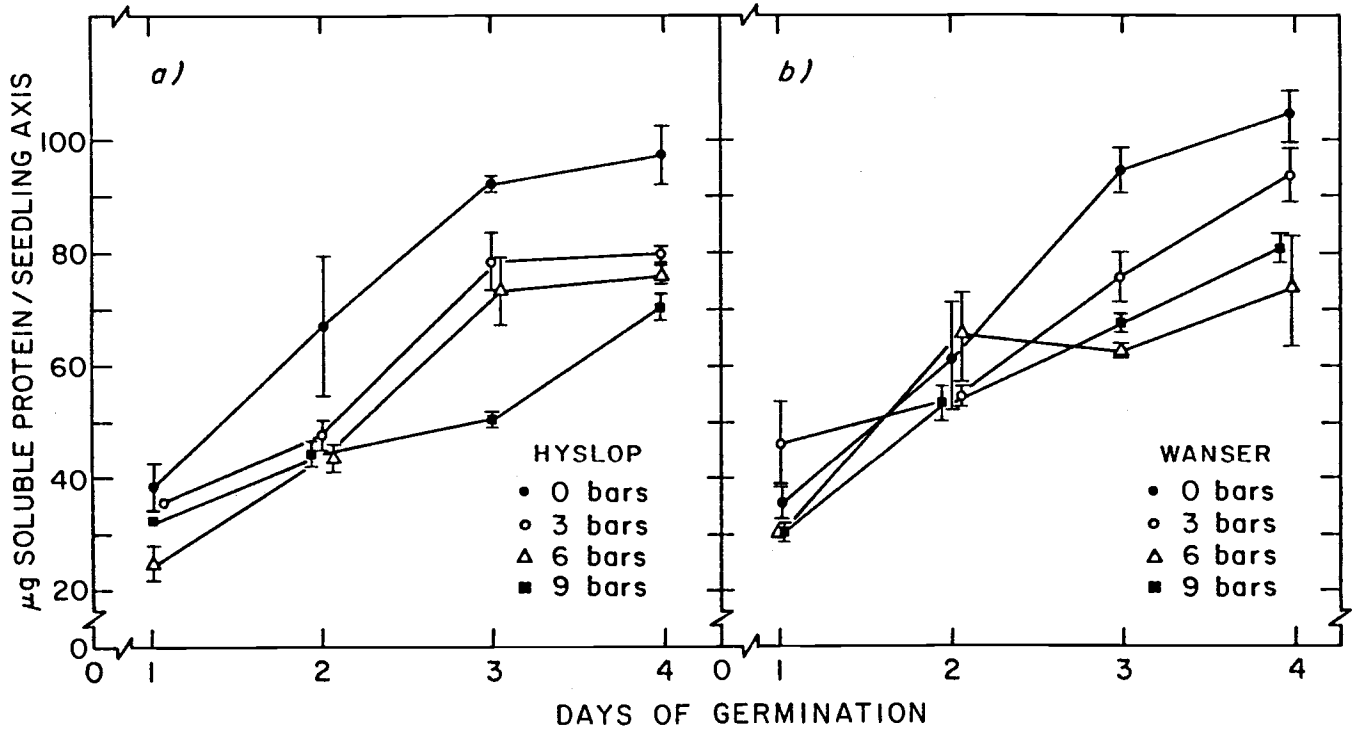

Figure 4a-b. Soluble protein per seedling axis of Hyslop (a) or Wanser (b) seeds germinated under different water stress conditions at 20-25°C in dark.

germination days apparently resulted in higher specific activity of glutamine synthetase (Figure 6a-b).

Variations in soluble protein content of seedling axis were observed after one day under various stress treatments (Figure 5a-b). A general trend of increased protein content with decreased water stress was indicative in both varieties. The range of protein content for Hyslop was 39 μg per seedling axis (0-bar treatment) to 34 μg (-9-bar treatment). In Wanser the amount of extractable protein was less 26 μg per seedling axis (0-bar treatment) to 21 μg (-9-bar treatment).

The protein content of both varieties increased rapidly on the second day of germination and thereafter. The rate of protein content increase was reduced under water stressed conditions. In Hyslop the control material had a higher protein content of 67 μg per seedling axis than the water stressed materials of 48, 44, and 45 μg for -3, -6, and -9-bar, respectively. On the third day of germination an average of 92, 79, 73, and 51 μg of soluble protein was extracted from one seedling axis of Hyslop seeds germinated at 0, -3, -6, and -9-bar, respectively. A slight increase was observed to 97, 80, 76, and 70μg per seedling axis of these treatments on the fourth day of germination. In Wanser, the differences were less obvious. The protein content ranged from 52 μg per seedling axis in the 0-bar treated seeds to 43 μg in the -9-bar treated material. The

soluble protein content of seedling axis increased to 85, 66, 53, and 58 μg on the third day and to 95, 83, 63, and 71 μg on the fourth day for 0, -3, -6, and -9-bar treatments, respectively. The soluble protein content of stressed materials was statistically significantly different from the control (Appendix). The soluble protein content of water stress seedling axis was reduced with water stress on the second day of germination and thereafter with protein content of the -9-bar treated material reduced to 28% of the control for Hyslop and 25% for Wanser on the fourth day. These trends suggest that protein synthetic capacity is limited under mild water stress. This correlates well with parallel decreases in fresh and dry weights (Figures 2a-b and 3a-b). The observed trends in soluble protein with progressive water stress are similar to those reported by Shah and Loomis (1965) for sugar beet and Barnett and Naylor (1966) for bermuda grass. The sensitivity of protein synthesis to mild stress of -9.0 bars is in agreement with Hsiao's (1970) reported 27% shift from polyribosomes to monoribosomes in corn seedlings under mild leaf water potential of -7.0 bars.

Enzyme Activities

Glutamine Synthetase (GS)

Experimental data indicates that glutamine synthetase activity

in embryo or seedling axis of germinating wheat seeds varied with cultivars, days of germination, and stress treatments (Figures 5a-b and 6a-b). The general trend observed in both varieties and overall stress treatments was a rapid increase in enzyme activity up to the third day of germination followed by a slight decrease in Hyslop and a leveling in Wanser on the fourth day. Difference in specific activity among seedling axis of various stress treatments occurred on the first and second day of germination then converged on the third day and deviated again on the fourth day resulting in an increased in vitro glutamine synthetase activity in seedling axis with increasing stress (Figure 6a-b). Maximum GS activity on the third and fourth day of germination coincide with large increases in water uptake and growth (Figures 1a-b to 3a-b). Similar trend of maximum GS activity occurring with greatest seedling axis growth and N transport was observed by Lignowski et al. (1971), Splittstoesser and Stewdart (1970), and Lignowski and Splittstoesser (1971) in pumpkin seedling.

Total Activity of Glutamine Synthetase (Figure 5a-b)

After 1 day in germination the embryos of the control treatment had higher enzyme activity than the water stressed for both varieties. Activity in general was low, ranging from 62 nanomoles to 120 nanomoles glutamine formed per embryo per minute in Hyslop and 72 nanomoles to 128 nanomoles in Wanser.

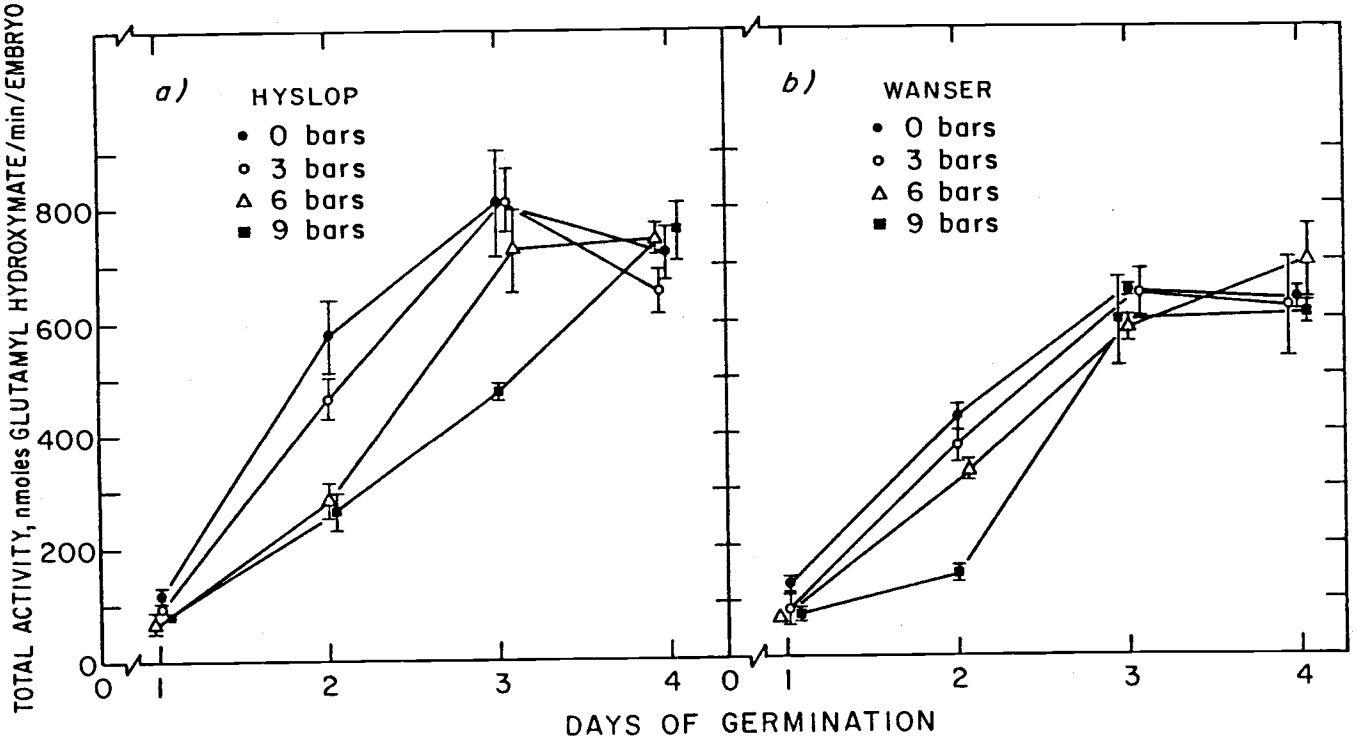

Figure 5a-b. Total activity of glutamine synthetase of Hyslop (a) or Wanser (b) seeds germinated under different water stress treatments at 20-25°C in dark.

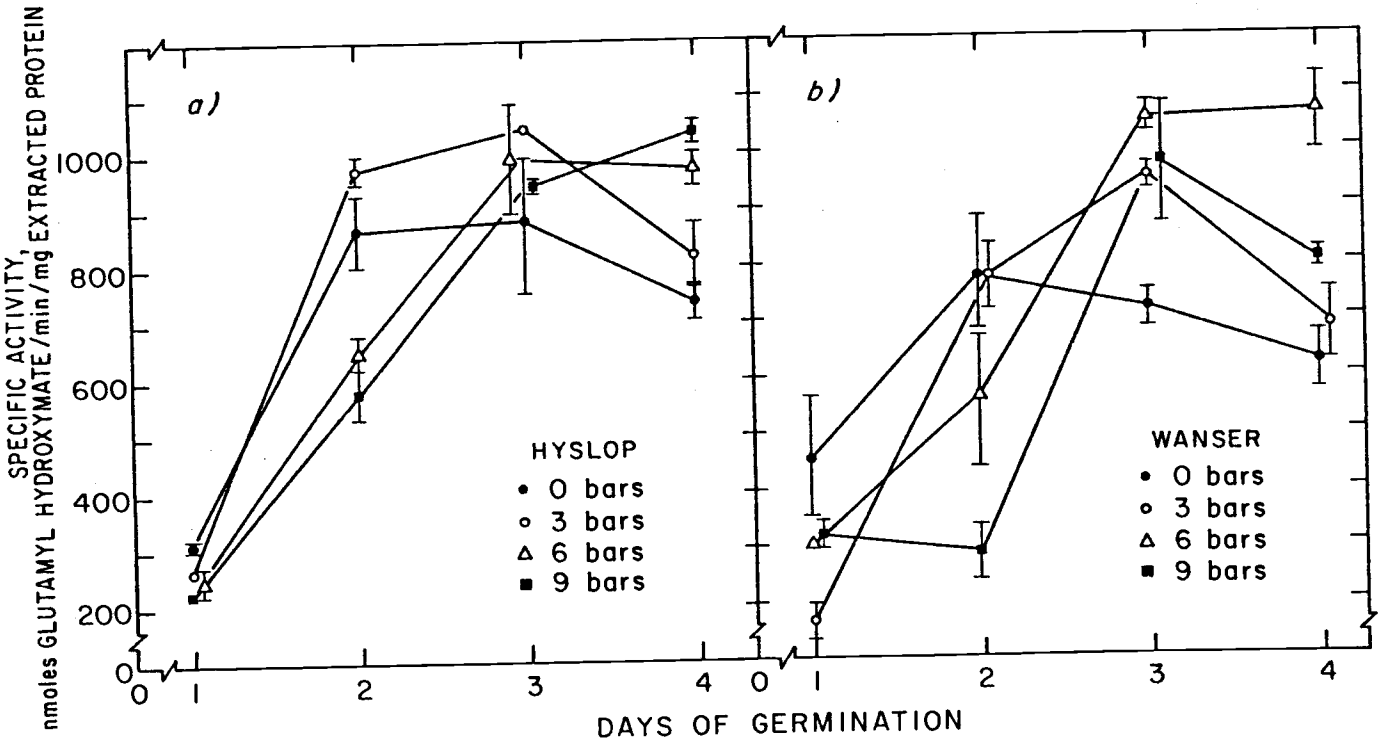

Figure 6a-b. Specific activity of glutamine synthetase of Hyslop (a) and Wanser (b) seeds germinated under different water stress treatments at 20-25°C in dark.

A rapid increase in glutamine synthetase activity in all treatments was observed on the second day of germination but the rate of increase was slower in the water stressed seedlings. In Hyslop, the seedling axis of the 0-bar treatment had the highest activity of 575 nanomoles and the -3-bar treatment resulted in 465 nanomoles. The -6-bar and -9-bar treatments rendered 284 and 259 nanomoles, respectively. In Wanser, the seedling axis of 0, -3, -6, and -9-bar treatments had enzyme activities of 424, 371, 328, and 146 nanomoles, respectively. Further increase of GS activity was observed on the third day but the differences among treatments were vanished in Wanser but observable in Hyslop. Little increase of the total activity was found in the fourth day germanants and the differences due to stress treatments was vanished. Comparison between the two varieties showed that Hyslop seedlings had somewhat higher enzyme activity across all water treatments than Wanser seedlings.

Specific Activity of Glutamine Synthetase (Figure 6a-b)

Both varieties responded similarily after one day of germination. The seedling axis of the 0-bar treatment had the highest specific enzyme activity resulting in 311 nanomoles glutamine formed per minute per mg protein at 37°C for Hyslop and 506 nanomoles for Wanser. The water stress treatments reduced the enzyme activity to that of 266 to 223 nanomoles for Hyslop and for Wanser 369 to 217

nanomoles resulting in large variations among the treatments.

After two days in germination under various water stressed conditions both varieties exhibited marked differences in reference to stress. In Hyslop seedlings the -3-bar treatment resulted in the highest specific GS activity (972 nanomoles) while lower activities of 865, 650, and 576 nanomoles were observed in seedling axis of 0, -6, and -9-bar treatments, respectively. Wanser seedlings exhibited a slightly different response, the 0, -3, and -6-bar treatments resulted in higher specific activities 823, 824, and 608 nanomoles than the -9-bar treatment (339 nanomoles). Comparison between the two varieties revealed that Hyslop seedlings had somewhat higher specific enzyme activity than Wanser seedlings.

In general, the specific GS activities of both varieties reached a maximum on the third day of germination. However, Hyslop seedlings of the 0-bar treatment increased their specific activity slightly while Wanser seedlings showed a decrease from the second day. In Hyslop seedlings the specific GS activities were close among the stress treatments, 1036, 994, and 937 nanomoles for -3, -6, and -9-bar, respectively while the 0-bar treatment resulted in the lowest specific activity of 875 nanomoles. In Wanser seedlings, all the water stress treatments resulted in higher specific GS activities (991, 1106, 1023 nanomoles for -3, -6, and -9-bar, respectively) than the 0-bar treatment (768 nanomoles).

A general trend of increased specific GS activity with increasing stress was indicated on the fourth day of germination. A reduction of specific activity from the third day was also shown in both varieties.

The total glutamine synthetase activity in germinating wheat seeds was reduced under water stress. On the second day of germination the total GS activity in the seedlings of the stress treatments was lower than that of the 0-bar treatment. Thereafter the total activity in the seedlings of the stress treatments were similar to that of the 0-bar treatment. These observations probably indicate that an attempt was made by the water stressed seedlings to overcome the unfavorable environment by producing more efficient GS for growth and biosynthetic needs. The results of GS specific activity also indicated such an attempt that more stressed treatments resulted in higher specific activity of GS.

Glutamine synthetase is widely distributed in animal, microbes, and plants. Specific reports have been published on pea (O'Neal and Joy, 1973), carrot (Caldos, 1971), pumpkin (Lignowski et al., 1971), and rice (Kanamori and Matsumoto, 1972). This enzyme plays an important role in the assimilation, storage, and translocation of reduced-N in higher plants. Glutamine is needed in many biosynthetic pathways of purine, glucosamine, p-aminobenzoic acid, phenylacetyl glutamine, diphosphopyridine nucleotide, guanosine 5'-phosphate, histidine and other amino acids (Meister, 1962). Thus glutamine

production is essential for the synthesis of nucleic acids, the reaction of many enzymes requiring NAD or NADP and growth in general. It is therefore logical to observe more or efficient GS produced under a mildly stressed condition as an adaptation measure of the wheat variety used.

O'Neal and Joy (1973) and Weissman (1976) have concluded that glutamine synthetase activity is controlled by the energy charge

$$EC = \frac{[ATP] + 1/2[ADP]}{[ATP] + [ADP] + [AMP]}$$

(Chapman et al., 1971)

of the cell. Barlow et al. (1976) showed that the ATP content of sixth leaf corn seedlings was reduced by 60% at a leaf water of -10.0 bars from -0.4 bars. Thus, if ATP content is reduced under water stress conditions and energy charge is a regulator of glutamine synthetase activity, the endogenous activity of this enzyme should be reduced proportionally to the degree of water stress imposed. However, the data on both specific and total activity on the third and fourth day of germination indicated a reversed trend. The reason for this is partly due to the in vitro assay of glutamine synthetase activity. The assay provides excess ATP in the reaction mixture that allows the enzyme to function at its maximum capacity. Measurements of endogenous glutamine synthetase activity with or without ATP and ATP content in tissue under these water stress conditions would provide evidences

to resolve this speculation but no attempt was made in this study. However, Conway (1977) has shown that under mild stress conditions similar to this study ATP content in germinating wheat was reduced 8% and 24%, respectively under soil water stresses of -3 bars and -6 bars. These reductions may suppress GS activity in vivo and eventually impair growth as shown in Figures 2a-b and 3a-b.

α-Amylase in Endosperms of Germinating Wheat Seeds

α-Amylase is one of several hydrolytic enzymes involved in the degradation of starch to glucose in cereal grains. This enzyme plays an important role in wheat seed germination because it provides the necessary carbon backbones for growth and synthetic activities in seedling axis.

In this study, the general trend showed that α-amylase activity increased rapidly between the second and fourth day of germination, however, the rate of increase in total and specific enzyme activity was lower in the stressed materials (Figures 7a-b and 8a-b).

Total Activity of α-Amylase (Figure 7a-b)

Total enzyme activity was very low in both varieties after one day of germination. The control material however, had higher enzyme activity than the stressed materials. A rapid increase in total activity occurred on the second day of germination in both the

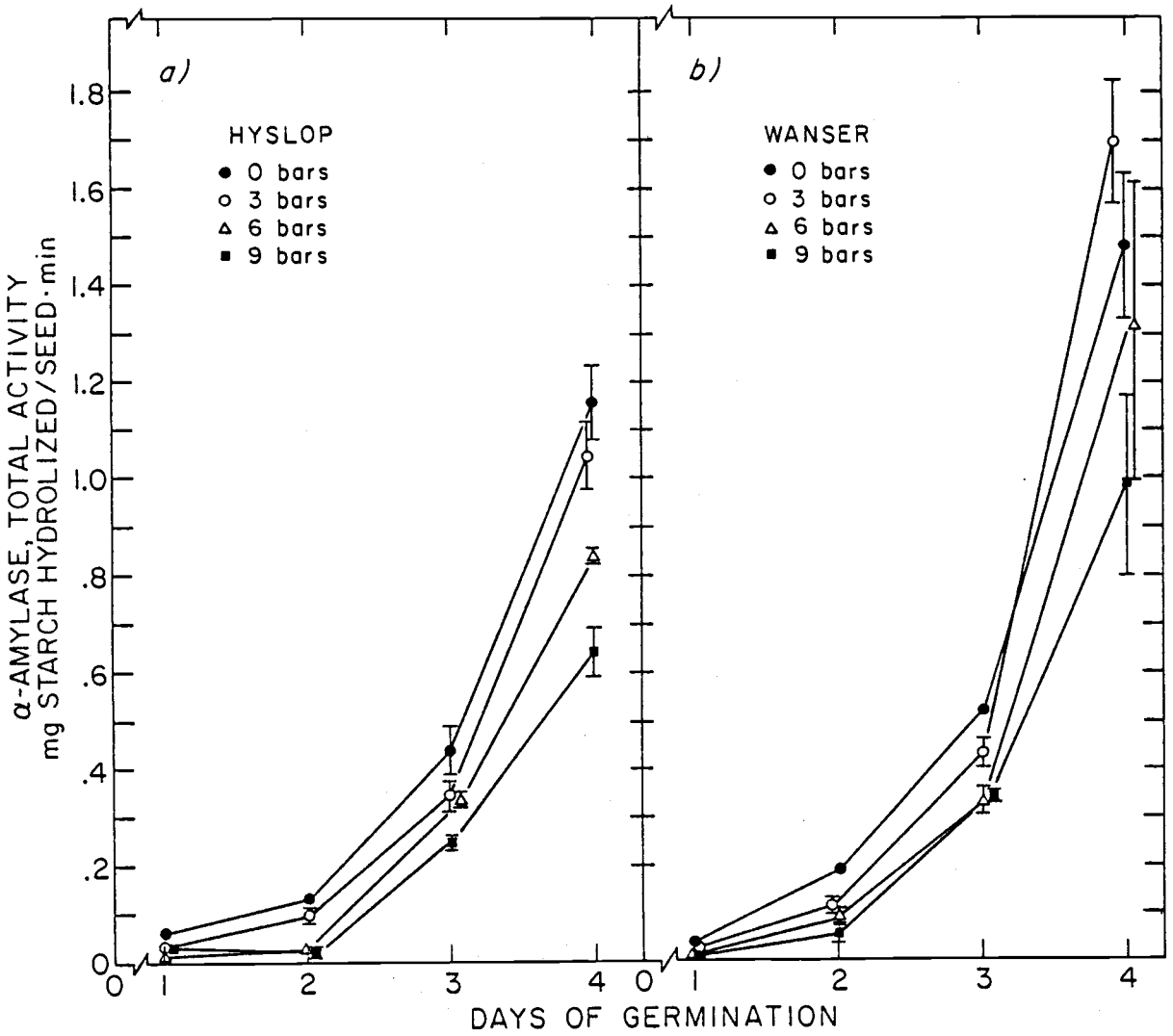

Figure 7a-b. Total enzyme activity of α -amylase in endosperms of Hyslop (a) or Wanser (b) seeds germinated under different water stresses at 20-25°C in dark.

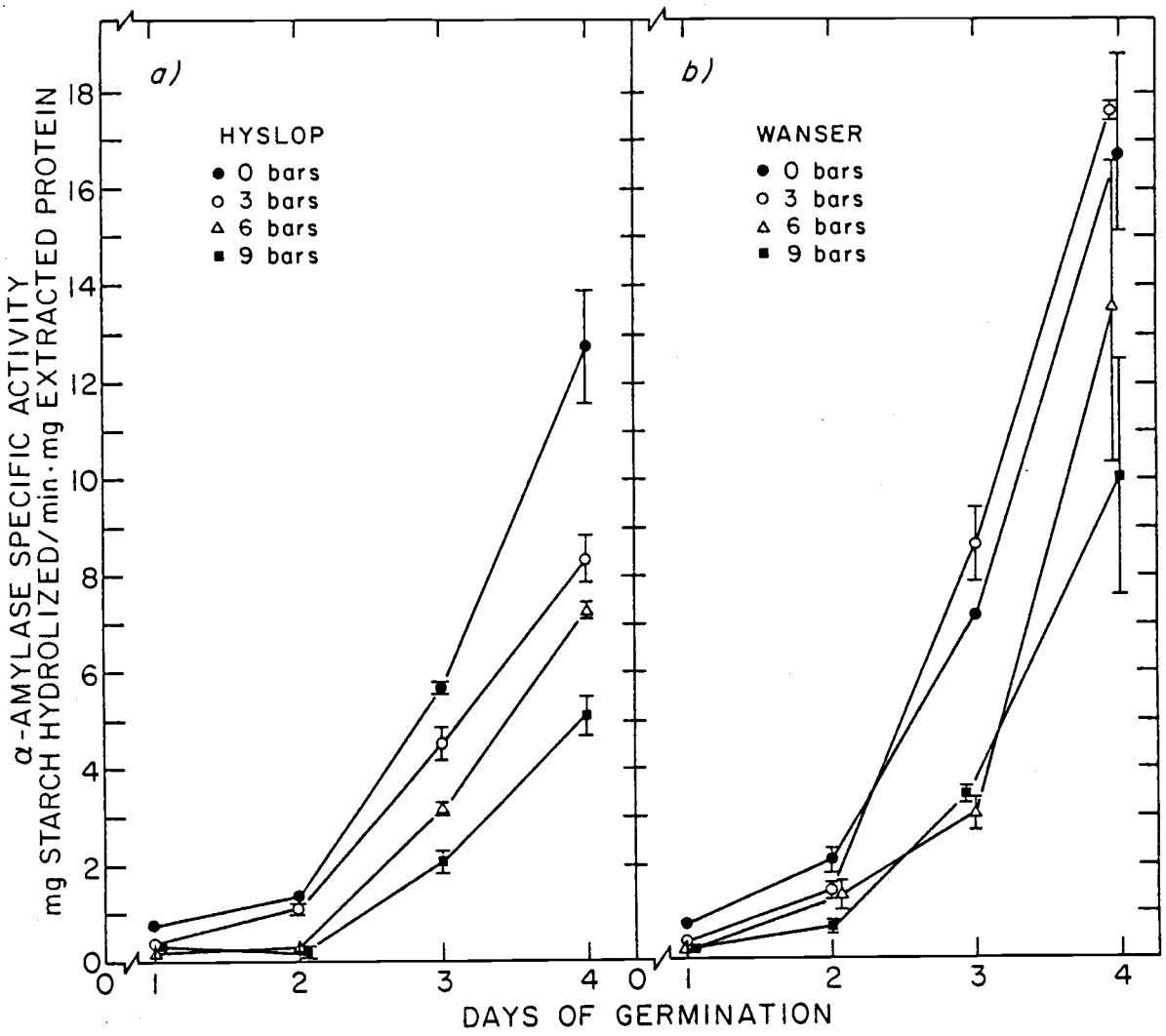

Figure 8a-b. Specific enzyme activity of α -amylase in endosperm of Hyslop (a) or Wanser (b) seeds germinated under different water stresses at 20-25°C in dark.

control and -3-bar treatment of Hyslop and under all treatments in Wanser. Hyslop endosperms had a total activity of 0.13, 0.09, 0.03, and 0.02 mg starch hydrolized per seed per minute under respective treatments. Wanser endosperm material had higher enzyme activities of 0.19, 0.11, 0.10, and 0.06 mg, respectively in the 0, -3, -6, and -9-bar treatments. On the third day of germination all treatments resulted in increased enzyme activity. Total activity in Hyslop endosperms was 0.45, 0.36, 0.35, and 0.25 mg, respectively and for Wanser material 0.52, 0.43, 0.34, and 0.34 mg, respectively. The greatest increase in starch hydrolysis occurred on the fourth day of germination. Enzyme activities in Hyslop material under 0, -3, -6, and -9-bar treatments were 1.2, 1.1, 0.86, and 0.66 mg, respectively. Wanser seedlings responded differently to the treatments. The -3-bar treated material had the highest total enzyme activity of 1.7 mg followed by lower activities of 1.5, 1.3, and 1.0 mg for the 0, -6, and -9-bar treatments, respectively. Comparison between the two varieties revealed that throughout the four day germination period Wanser seedlings had higher total enzyme activity than Hyslop under all treatments.

Specific Activity of α-Amylase (Figure 8a-b)

On the first day of germination specific activity in Hyslop and Wanser material was reduced under water stress treatments. The

control material had the highest specific activity of 0.75 mg starch hydrolized per minute per mg extracted protein for Hyslop and 0.71 mg for Wanser. Increased specific activity for Wanser material to 2.0, 1.3, 1.3, and 0.64 mg, respectively was observed on the second day of germination and to 1.4, 1.0, 0.26, and 0.17 mg for Hyslop, respectively. On the third day of germination, specific activity in Hyslop seedlings was further reduced by stresses to 5.7, 4.5, 3.2, and 2.1 mg, respectively under 0, -3, -6, and -9-bar treatments. The pattern extended to the fourth day to 12.7, 8.4, 7.2, and 5.1 mg, respectively. Wanser material responded differently to stress. After three days of germination there was a large deviation between treatments. The 0 and -3-bar treated material exhibited a large increase in activity at 7.1 and 8.6 mg, respectively. The specific activity of other treated materials increased slightly to 3.0 and 3.4 mg, respectively. By the fourth day of germination, specific activity of the α-amylase increased with the material of the -3-bar treatment having the highest specific activity at 17.5 mg others were lower at 16.7, 13.4, and 10.0 mg, respectively.

Under water stress the total and specific activity of α-amylase in germinating wheat endosperms was reduced. These results agreed with those of Jones (1969) and Wilson (1971) for barley and crested wheatgrass seed and are the reversal of the findings of Maranville and Paulsen (1970) who reported a 63% increased α-amylase activity

in leaves of corn seedlings subjected to severe water stress. Apparently water stress may stimulate or inhibit one particular enzyme according to the physiological adaptive needs of the tissue. For example, the growth of the seedling axis is reduced by water stress in seeds, so the activity or synthesis of α-amylase is suppressed in the endosperm, in order to keep pace with the limited sugar utilization by the axis. In leaves, osmoregulation is the adaptive mechanism to main tugor pressure under mild stressed conditions. Therefore, α -amylase is stimulated in leaves to produce sugars and increase osmotic potential.

The detailed changes in this experiment are as follows. Wanser hydrolized starch at a faster rate under all stress treatments than the variety Hyslop. After two days in germination α-amylase activity in both varieties was most sensitive to stress with total activity in Hyslop materials reduced by 31, 77, and 85% of the control under -3, -6, and -9-bar treatments. Wanser showed a similar trend although the degree of reduction was less to 42, 47, and 68% of the control. On the third and fourth day of germination, specific activity of -3-bar treated Wanser material increased 21% and 5%, respectively over that of the control. These results indicate that Wanser, a relative drought tolerant variety, does indeed do better under slightly stressed conditions than Hyslop by producing more or efficient α-amylase to effect an accelerated starch hydrolysis possibly resulting in faster

growth than that of Hyslop.

Large increases in enzyme activity under all treatments on the second and third day of germination paralleled with increases in fresh and dry weights (Figures 2a-b and 3a-b) and glutamine synthetase activity (Figures 5a-b and 6a-b) indicates a concerted effect of growth.

Jones and Armstrong (1971) have suggested that α-amylase synthesis in barley aleurone layers is under osmotic regulation. Both maltose and glucose, hydrolysis products of starch degradation inhibited α-amylase synthesis at concentrations of 0.02 M to 0.04M. This type of regulation may be related to seedling growth rate, since the rate of embryo growth or sink size governs the extent to which solutes are withdrawn from the endosperm. Thus adverse environments such as water stress which retards seedling growth and limits the utilization of C-compounds results in the accumulation of solutes in the endosperm. This accumulation of sugars causes a reduction in further hydrolytic enzyme production. This may give a plausible explanation for observed reductions in α-amylase activity as growth rate is reduced under stress treatments. No attempt was made in this study to determine the types and concentrations of solutes in water stressed wheat endosperms although such data would elucidate the regulatory mechanisms of α-amylase synthesis under water stress conditions.

Soluble Protein in α-Amylase Extracts (Figure 9a-b)

Under all treatments, large variations in soluble protein content of wheat endosperms was observed on the first day of germination. Protein content increased after two days of germination to 94, 87, 74, and 85 μg for Wanser endosperms under 0, -3, -6, and -9-bar treatments. Hyslop materials showed a reverse trend in that the -6 and -9-bar treatments had higher protein contents of 102 and 114 μg than the 0 and -3-bar treated material at 96 and 90 μg, respectively. As germination progressed to the third day and thereafter varieties responded to stress differently. In Wanser a large deviation between treatments resulted in increased soluble protein of 111 and 101 μg for -6 and -9-bar treated material and a decrease to 74 and 51 μg for the 0 and -3-bar treatments. All treatments converged on the fourth day of germination and stress effect on soluble protein vanished. In Hyslop material the soluble protein content of the 0 and -3-bar treated material decreased to 79 and 80 μg, respectively on the third day of germination. The other stress treated material had increased soluble protein contents of 108 and 120 μg, respectively. After four days of germination protein content increased to 93, 128, 119, and 130 μg for 0, -3, -6, and -9-bar, respectively.

It is difficult to assign any significance to the heat stable (70°C

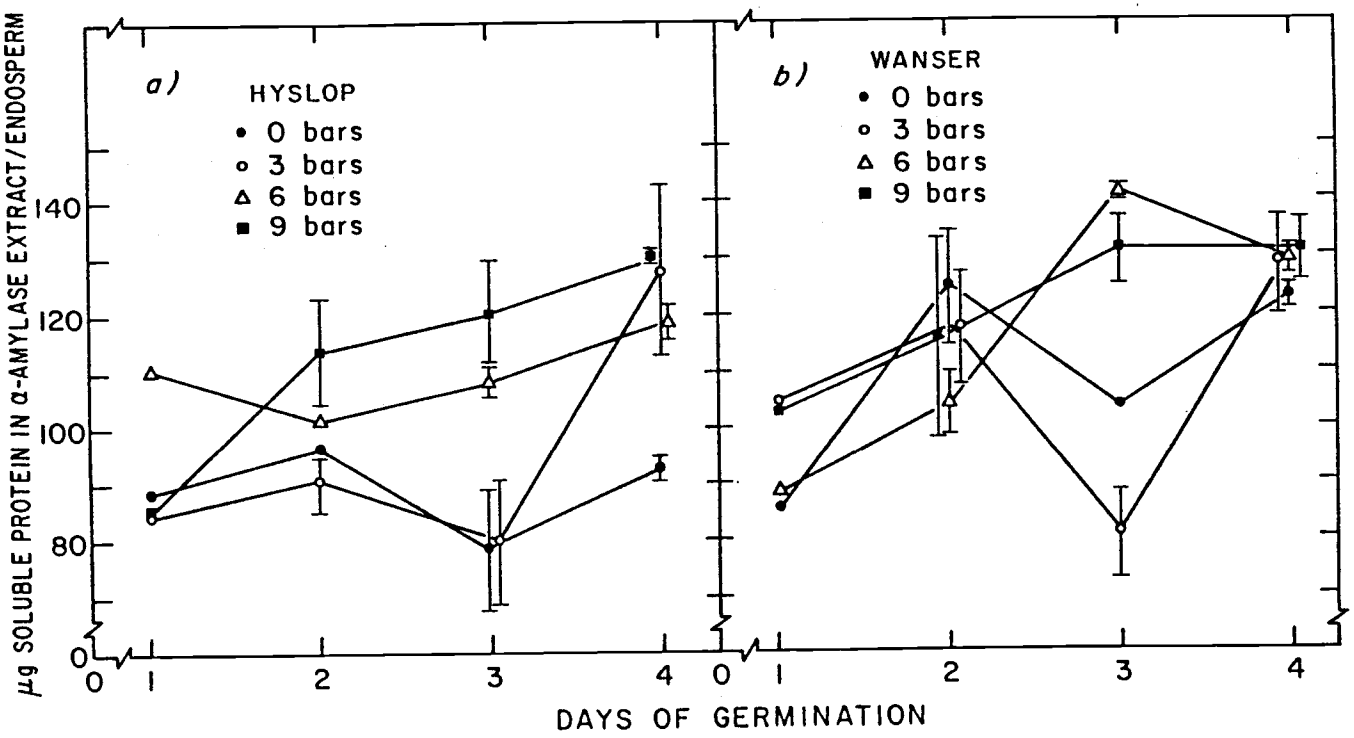

Figure 9a-b. Soluble protein per endosperm of Hyslop (a) or Wanser (b) seeds germinated under different water stresses at 20-25°C in dark.

for 20 minutes) soluble protein content in endosperm. The protein content is mainly for the calculation of specific activity of the α -amylase.

Todd (1972) observed that enzyme activity under water stress is altered depending on the degree of water stress, stage of development, and type of enzyme. He postulated that enzyme activity under water stress can be damaged by conformational changes or interactions with high concentrations of salt resulting from solvent reduction under water stressed conditions, although some enzymes can be highly tolerant to water stress by their fixed structural relationship within the cell. He further speculated that a slight reduction or increase in enzyme activity under water stress conditions may not be of importance until a critical level is attained. At this point, metabolic activities for growth are modulated by the water stress environment.

My experimental findings do not include any critical point in α-amylase activity since it was proportionally reduced with the decline of growth under increasing stresses. The total activity of glutamine synthetase on the other hand, was comparable on the fourth day of germination and the specific activity of GS, in fact, showed a reversed trend in reference to stress. Therefore a critical point of enzyme activity for growth reduction does not exist under my experimental conditions and materials.

Experimental data indicates that GS appears to be a very sensitive enzyme to water stress and it is stimulated by mild stress conditions through either more synthesis at the translational level or production of more efficient enzyme at the transcriptional level. It is known that mild water stress degrades polysomes in thirty minutes (Hsiao, 1970) therefore increasing GS activity by translational stimulation is not a likely cause. The proportional decrease of GS activity and seedling axis weight with increasing stress on the first and second day and the progressive reduction of soluble protein in stressed seedling axis throughout the four day germination period definitely denotes such polysome reduction by water stress. Transcriptional stimulation via gene activation then would be the only possibility, resulting in similar total GS activity and higher specific GS activity in stressed materials on the fourth day of germination. How the gene is activated by water stress and how the activated gene could produce more efficient enzyme is still an enigma. More sophisticated research will be required in revealing the mechanism.

The relationship between the three parameters studied growth, enzyme activity, and water potential in general is correlated and influenced somewhat by variety and more important by duration of the water stress (Table 3).

Although all criteria studied are related with the degree of stress, after two days of germination a good correlation exists

TABLE 3. Percent reduction from the control in different growth parameters on the second and fourth day of germination under water stress of -3, -6, and -9-bars.

Treatment	Seedling Axis						Endosperm		
	Ψ	H_2O%	F.wt	D.wt	G.S.	S.P.	Ψ	α-A	Sα-A
				HYSLOP					
2 Day									
-3-bar	29	8	21	4	19	28	+20	31	29
-6-bar	33	16	42	17	51	34	1	77	81
-9-bar	25	20	44	17	55	33	17	85	88
4 Day									
-3-bar	38	4	39	23	9	18	27	8	34
-6-bar	92	7	60	42	+3	22	42	28	43
-9-bar	83	13	72	49	+5	28	54	45	60
				WANSER					
2 Day									
-3-bar	+0.4	9	32	11	13	13	2	42	35
-6-bar	+5	9	44	26	23	+6	24	47	35
-9-bar	19	14	59	40	66	17	48	68	68
4 Day									
-3-bar	46	5	46	30	3	12	52	+13	+5
-6-bar	71	8	60	43	+10	33	84	13	20
-9-bar	101	13	73	56	4	25	119	33	40

Abbreviations:

Ψ = water potential
H_2O% = percent water content
F.wt = fresh weight
D.wt = dry weight
G.S. = total glutamine synthetase activity per seedlings axis
S.P. = soluble protein
α-A = total α-amylase activity per endosperm.
Sα-A = specific α-amylase activity

between protein content and embryo water potential that indicates the important role of water in maintaining the integrity of protein synthetic machinery and enzymes and the transport of substrates and products for growth during the early stages of seed germination. As assimulation increases, fresh weight becomes a sensitive indicator of stress accompanied by enzyme activities, e.g. glutamine synthetase in Hyslop and α-amylase in Wanser. This differential varietal response probably relates with varied adaptation mechanism in plants under mild stresses.

As germination progressed to the fourth day, fresh weight and water potential of seedlings axis had a close relationship. This relationship was observed in both varieties and indicates again that assimilation processes as a whole are sensitive to the amount of water available for growth.

A correlation between endosperm water potential and α-amylase activity was observed in both varieties at both stages of germination indicating that α -amylase activity is affected by water availability.

Based on the close relationship of reduction in water potential and soluble protein content in seedling axis under the three mild stressed conditions (Table 3), one may speculate that protein synthesis is the most sensitive system toward water stress. The experimental findings of Hsiao (1970) and Rhodes and Matsuda (1976) have

already proven this point. The next close relationship is the water potential and fresh weight of seedling axis (Table 3). Rhodes and Matsuda (1976) stated that the polysome percent in young seedlings could be used to predict growth rate and each 1% reduction of polysome increment equals 0.18 and 0.20 cm per day reduction in pumpkin and pea seedling growth rates. Seedling fresh weight or growth therefore is directly related with polysome content and protein synthesis. Among the reduced proteins synthesized under mild stressed conditions, there are differences in kinds and quantity. For example, GS is increased during prolonged stress (Table 3, 4-day) and the increased GS appears to be more efficient as evidenced by their specific activity (Figure 6a-b). α-Amylase on the other hand, is reduced by the water stress but the degree of reduction is lessened as the treatment is prolonged (Table 3). In addition, Wanser is less affected than Hyslop under the mild stresses (Table 3, Figures 7a-b and 8a-b) indicating the capability of this drought resistant cultivar. The lessened effect is probably due to the quantity of the α-amylase reduced by stress, since the trend of specific activity reduction had remained the same as the total activity. More detailed studies, however, will be needed to explore the mechanism of varietal differences in drought resistance.

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APPENDIX

Mean and t test results of different growth parameters of Hyslop and Wanser seedlings germinated for four days under water stresses of -3, -6, and -9-bars.

Treatment	F.wt $\bar{X}$	F.wt t	D.wt $\bar{X}$	D.wt t	S.P. $\bar{X}$	S.P. t	α-A $\bar{X}$	α-A t	S α-A $\bar{X}$	S α-A t
				HYSLOP						
0-bar	47.44		7.96		97.30		1.17		12.71	
-3-bar	28.81	4.49*	5.54	2.60	79.70	5.62*	1.07	1.40	8.38	4.98
-6-bar	18.86	7.83*	4.11	4.11*	76.10	6.67*	0.86	5.66	7.22	6.70
-9-bar	14.74	8.09*	3.84	4.35*	70.40	8.05*	0.66	7.82	5.05	8.93
				WANSER						
0-bar	54.63		7.70		94.20		1.54		16.67	
-3-bar	29.14	10.89*	5.41	7.06*	83.60	2.78	1.72	1.38	17.55	0.79
-6-bar	21.44	15.44*	4.43	10.42*	62.70	5.11*	1.32	1.09	13.42	1.32
-9-bar	14.10	16.15*	3.37	13.90*	70.90	7.67*	1.00	3.54	9.96	3.30

* Significant at 5% level.

Abbreviations:

F.wt = fresh weight
D.wt = dry weight
S.P. = soluble protein
α-A = total α-amylase activity per endosperm
Sα-A = specific α-amylase activity