EVALUATION OF FREE LEAF PROLINE CONCENTRATION AS A PRACTICAL METHOD FOR MEASURING DROUGHT STRESS IN PLANTS

by

RICHARD PAUL WALDREN

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Approved by:

Ivan D Teare
Major Professor

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INTRODUCTION

The yield potential of a crop cannot be fully attained if its water needs are not met at all times. When irrigating a crop it is difficult to always know when to apply water. Many present methods of irrigation scheduling use soil moisture tension or percent of field capacity as a basis for determining when to apply water. However, each soil is different in amount and availability of its water supply and each crop is different in its ability to extract soil water, which can lead to problems.

The irrigator is really interested in when his crop needs water, not the moisture status of his soil. If plant indicators could be used to determine the water status of the crop, coupled with a soil moisture inventory system based on soil moisture tension, the irrigator would know when to irrigate and how much water to apply.

When the crop shows visible signs of water stress it is already too late for maximum yields. Therefore, it is necessary to detect when only a slight water deficiency is present in a crop.

Recent studies by various workers have shown that the free proline content of leaves increases markedly with only slight water stress. This applies to many important agricultural crops such as ladino clover, barley, corn, wheat, and many others. It may also be valuable for selecting drought resistant and frost resistant varieties. In both cases, higher accumulation of proline indicates increased resistance.

Most of the previous research on proline and drought stress has been conducted in the laboratory with excised leaves and used analytical techniques not suited for field studies.

The purpose of this project was to develop a simple, inexpensive method of proline analysis, and then evaluate the effectiveness of free proline accumulation as a sensitive indicator of drought strss and drought resistance in both the laboratory and the field.

CHAPTER I

RAPID DETERMINATION OF FREE PROLINE FOR WATER STRESS STUDIES

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L. S. Bates, R. P. Waldren, and I. D. Teare

SHORT COMMUNICATION

RAPID DETERMINATION OF FREE PROLINE FOR WATER STRESS STUDIES

SUMMARY

Proline, which increases proportionately faster than other amino acids in plants under water stress, has been suggested as an evaluating parameter for irrigation scheduling and for selecting drought resistant varieties. The necessity to analyze numerous samples from multiple replications of field grown materials prompted the development of a simple, rapid colorimetric determination of proline. The method detected proline in the 0.1 to 36.0 µmoles/g range of fresh weight leaf material.

INTRODUCTION

Severe water stress induces numerous metabolic irrigularities in plants. A tremendous free proline accumulation (up to 100 times the normal) is one of the most dramatic stress characteristics (1); it has been used as a single parameter to measure physiological dryness (5). The necessity to quickly sample and analyze field grown materials prompted us to develop a simple, colorimetric determination of proline suitable for field laboratories.

Chinard (2) described an acid-ninhydrin method for proline which subsequently was studied for the effects of various interferences (3,4 7,8). Although several free amino acids can interfere with such proline determinations, the free amino acid levels reported in stressed plants (1,6) were low compared with proline. Color yields of interfering amino acids also were low. The techniques described by Chinard

(2) and Troll and Lindsley (7) work well with purified or semi purified proline samples, but did not work with the simple fractionation and filtration techniques we needed for rapid field analysis.

Concentration and color yield differences suggested a simplified determination of proline for field studies.

MATERIALS AND METHODS

Samples. Fully expanded "sun" leaves from field-grown soybean and sorghum plants were sampled. Purified proline was used to standardize the procedure for quantifying sample values.

Reagents. Acid-ninhydrin was prepared by warming 1.25 g ninhydrin in 30 ml glacial acetic acid and 20 ml 6M phosphoric acid, with agitation, until dissolved. Kept cool (stored at $4^{\circ}C$), the reagent remains stable 24 hours (7).

Procedure. 1) Approximately 0.5 g of plant material was homogenized in 10 ml of 3% aqueous sulfosalicylic acid and the homogenate filtered through Whatman #2 filter paper. 2) Two ml of filtrate was reacted with 2 ml acid-ninhydrin and 2 ml of glacial acetic acid in a test tube for 1 hour at $100^{\circ}C$, and the reaction terminated in an ice bath. 3) The reaction mixture was extracted with 4 ml toluene, mixed vigorously with a test tube stirrer for 15-20 sec. 4) The chromophore containing toluene was aspirated from the aqueous phase, warmed to room temperature, and the absorbance read at 520 nm using toluene for a blank. 5) The proline concentration was determined from a standard curve and calculated on a fresh weight basis as follows:

$[(\mu g \text{ proline/ml} \times \text{ml toluene})/115.1\ \mu g/\mu mole]/[(g\ \text{sample})/5] = \mu moles$ proline/g of fresh weight material.

RESULTS AND DISCUSSION

Field studies of water stress, requiring numerous samples from multiple replications, have been limited greatly by the absence of rapid, simple techniques for determining plant stress conditions. Proline, which increases proportionately faster than other amino acids in stressed greenhouse-grown plants (1), has been used to evaluate controlled-environment stress studies. We selected proline to evaluate similar field studies.

Practicality dictated compromising between absolute accuracy and time-consuming manipulations. We recognized that certain amino acids, notably glutamine, would increase the apparent baseline level of proline. Under stress conditions, the increase of glutamine and other interfering ninhydrin positive compounds should be negligible in relation to the many-fold proline increase (1,6). The color yield of glutamine, the major interference, yields less than 1.5% of an equivalent amount of proline (3). Comparisons between stressed and unstressed individuals should range slightly less than is based upon absolute proline values, but the relative values should indicate the degree of plant stress. Therefore we accepted the acid-ninhydrin reagent of Troll and Lindsley (7) as sufficiently accurate.

Preparation of free proline was simplified by using 3% sulfosalicylic acid. It is colorless, an effective protein precipitant in aqueous solution, and does not interfere with the acid-ninhydrin reaction. Additional interfering materials, which normally raised the baseline at 520 nm, were removed presumably by adsorption to the protein-sulfosalicylic acid complex.

Extraction of the proline-ninhydrin chromophore was accomplished in toluene, a less noxious and more effective solvent than the commonly used benzene. Extraction of naturally-occurring free proline and of added proline was rapid and quantitative with complete conformity to Beer's Law.

The spectrum of the chromophore was determined on a Beckman DB-G spectrophotometer. An absorbance maximum at 520 nm was obtained in contrast to maxima of 515 and 517 nm reported under other conditions. The spectrophotometer was calibrated with a didynium standard.

We were able to quantify proline in a range of 0.1 to 36.0 μmoles/g of fresh-weight leaf material. The rapid assay required only 2-2.5 hours per set of 30 samples.

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L. S. Bates
Department of Grain Science
and Industry
R. P. Waldren and I. D. Teare
Evapotranspiration Laboratory
Kansas State University
Manhattan, Kansas, USA

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CHAPTER II

FREE PROLINE ACCUMULATION IN DROUGHT STRESSED PLANTS UNDER LABORATORY CONDITIONS

Submitted to Plant and Soil.

R. P. Waldren and I. D. Teare

SHORT COMMUNICATION

FREE PROLINE ACCUMULATION IN DROUGHT STRESSED PLANTS UNDER LABORATORY CONDITIONS

SUMMARY

Free proline accumulation was measured in leaves of intact sorghum (*Sorghum bicolor* L. cv. Pioneer 846) and soybean (*Glycine max* L. cv. Calland) grown in growth chambers and subjected to "normal" drought stress. Stomatal diffusive resistance and leaf water potential were used to determine the degree of stress at the time of proline analysis.

Free proline did not accumulate markedly in either species until each was severely stressed, indicating that proline is not a sensitive indicator of drought stress. Free proline accumulated under less stress in soybean than in sorghum. Since soybean is less drought resistant than sorghum, proline accumulation may be an indicator of drought resistance or susceptibility.

INTRODUCTION

Most previous studies of free proline accumulation have been with excised leaves and changes that occurred after excision (1,6,7,11, 12), with little work on intact plants subjected to normal water deficiency (8,9).

Stomatal diffusive resistance and leaf water potential have been suggested as good parameters to measure drought stress (3,5) but no one has compared the two parameters with free proline accumulation.

We measured free proline accumulation in the leaves of intact sorghum and soybean plants subjected to drought stress and used leaf water potential and stomatal diffusive resistance to determine the degree of stress where proline begins to accumulate.

METHODS AND MATERIALS

Soybean (Glycine max L. cv. Calland) and sorghum (Sorghum bicolor L. cv. Pioneer 846) were planted in 6-inch clay pots containing a mixture of silt loam soil and sphagnum moss peat (2:1). They were placed in growth chambers under 30°C and 50% relative humidity with a 16-hour photoperiod and grown without drought stress for four weeks. The pots were saturated with nutrient solution (Miracid[1/], 19 g/1) once a week, and were watered as needed.

After four weeks the pots were watered until saturated, allowed to drain 1 hour, and then covered with plastic bags secured to the bases of plants to prevent soil water evaporation.

Stomatal diffusive resistance was measured with a diffusion porometer (4) on the upper and lower surfaces of the uppermost fully expanded leaf of both species. Average leaf stomatal resistance (R_s) was calculated by

$$\frac{1}{R_s} = \frac{1}{R_u} + \frac{1}{R_b}$$

where R_u and R_b are the resistances of the upper and lower leaf surfaces, respectively. Leaf water potentials of the same leaves were measured with the pressure-bomb method (10). Then the leaves were analyzed for free proline using a spectrophotometric technique (2).

Four plants were sampled three times a day during the stress period until severe wilting occurred.

1/ Stern's Garden Products, Geneva, N.Y.

RESULTS AND DISCUSSION

Figure 1 compares free proline in the leaves of each species with stomatal diffusive resistance. When stomatal diffusive resistance (R_s) became too high to measure with the porometer, resistance was defined as infinite (∞). Stomata were then completely closed and plants showed severe signs of wilting. With soybean, R_s was about 37 sec/cm before free proline began to accumulate. Soybean was then visibly drought stressed, i.e., loss of turgor, trifoliates folding, etc. With sorghum, R_s was infinite (stomata completely closed) before free proline began to accumulate. Again, the plants were visibly drought stressed, i.e., loss of turgor and rolled leaves. The presence of extremely high resistance before proline accumulated indicates that proline accumulation is not as sensitive an indicator of drought stress as stomatal resistance

Figure 2 compares free proline with leaf water potential (Ψ_{leaf}). As with R_s, leaf water potential had to become high (about -14 bars in soybean and -24 bars in sorghum) before any significant free proline accumulated. Both values were high enough to cause visible signs of drought stress.

Soybean (a more drought-susceptible crop) began to accumulate proline under less stomatal diffusive resistance and leaf water potential than sorghum (a more drought-resistant crop), so the amount of stress necessary to trigger proline accumulation may be a function of a plant's resistance to drought stress. Contrary to our findings, Palfi and Juhasz (7) report that under equally water deficient conditions, drought resistant varieties synthesize more proline than less resistant varieties. The difference may have resulted from their working with

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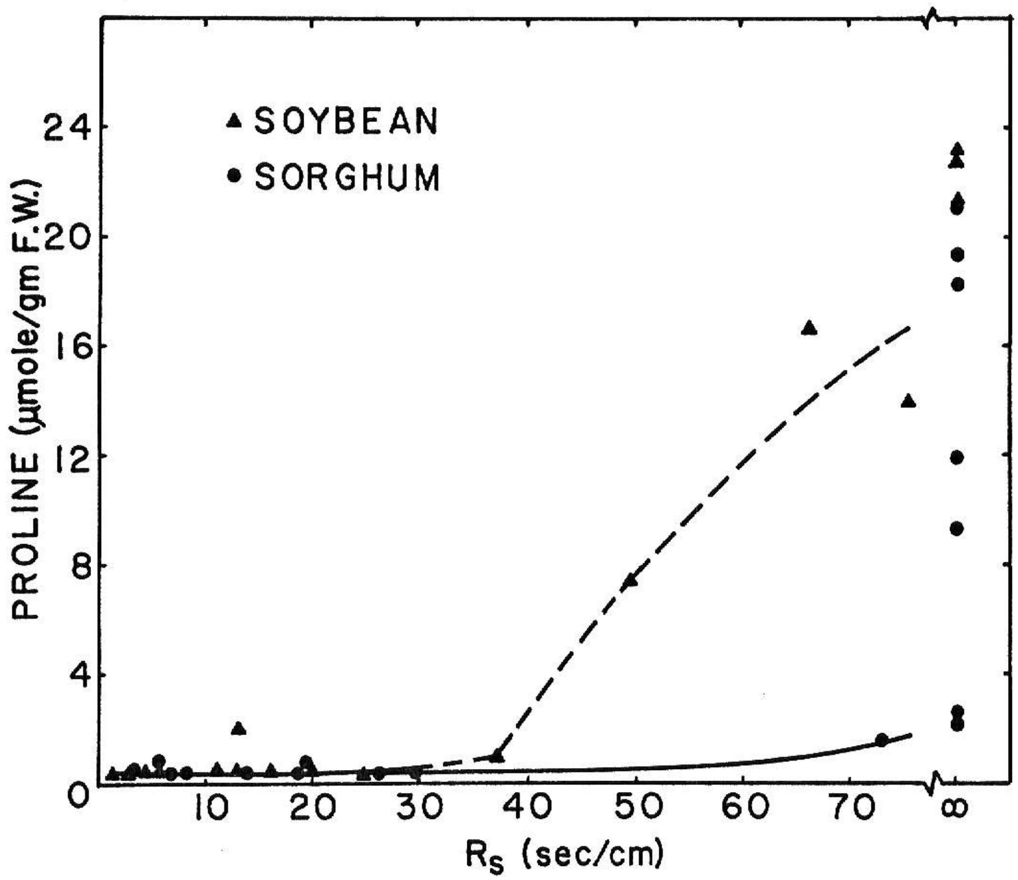

Figure 1. Free proline accumulation and stomatal diffusive resistance in drought stressed sorghum and soybean leaves.

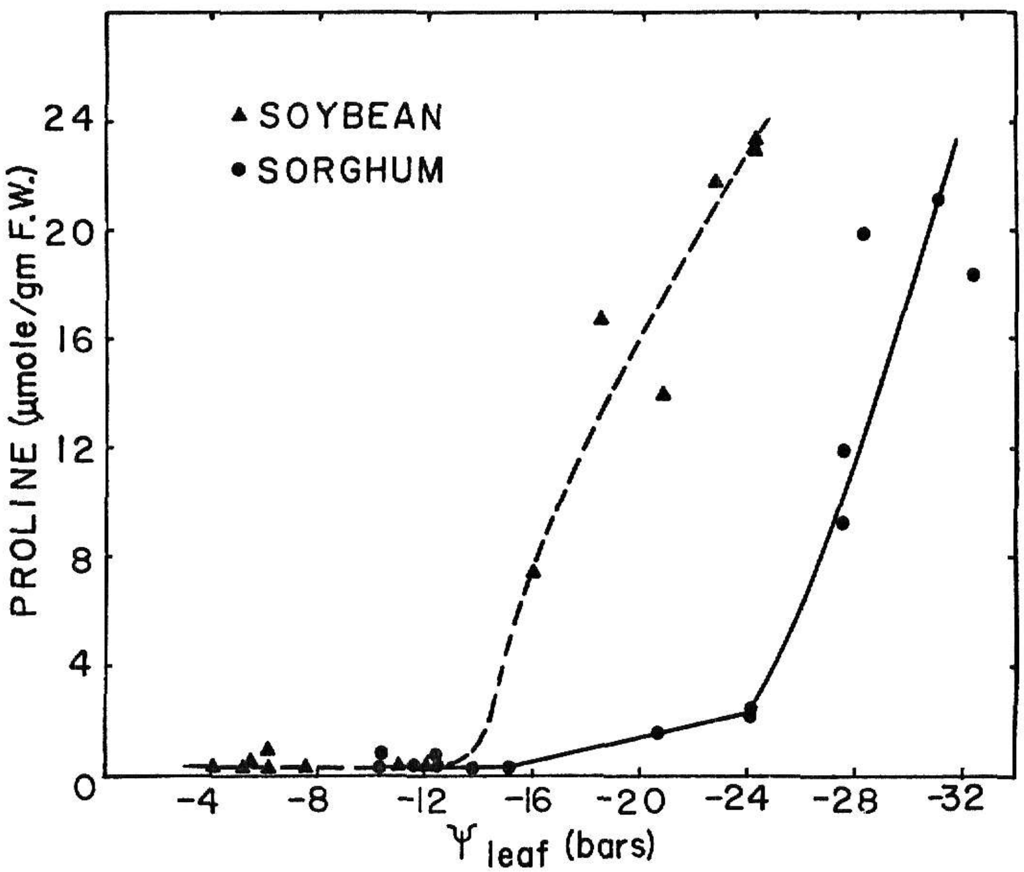

Figure 2. Free proline accumulation and leaf water potential in drought stressed sorghum and soybean leaves.

excised leaves of the same species subjected to drought stress, while we worked with two different species and subjected the intact plant to drought stress. Reaction to drought stress obviously differed between the two species.

Free proline increased more than 200-fold in sorghum; more than 60-fold in soybean, but free proline's sensitivity to drought stress appears to be quite low. Therefore, free proline accumulation does not appear to be a good indicator of drought stress because the signal does not occur until after there is a critical need for water.

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Contribution no. 1379, Evapotranspiration Laboratory, Agricultural Experiment Station, Manhattan, Kansas 66506.

R. P. Waldren and I. D. Teare
Evapotranspiration Laboratory
Kansas State University
Manhattan, Kansas, USA 66506

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CHAPTER III

FREE PROLINE CONCENTRATION IN SORGHUM AND SOYBEAN PLANTS UNDER FIELD CONDITIONS

Submitted to Crop Science

R. P. Waldren, I. D. Teare, and S. W. Ehler

FREE PROLINE CONCENTRATION IN SORGHUM AND SOYBEAN PLANTS UNDER FIELD CONDITIONS[1/]

R. P. Waldren, I. D. Teare, and S. W. Ehler[2/]

ABSTRACT

Free proline in the leaves of sorghum (*Sorghum bicolor* (L.) Moench cv.Pioneer 846) and soybean (*Glycine max* (L.) cv. Calland) was measured in the field under drought stress and adequate soil moisture. Leaf water potential and stomatal diffusive resistance were the measures of drought stress in the plants.

Free leaf proline in nonstressed plants changed during the day and during the growing season, although the changes were not great. Free proline increases with light intensity and it varies widely in middle-leaves that are shaded or receive sunlight.

In drought-stressed plants, free proline did not accumulate significantly until the plants were severely stressed and visibly wilting (about -20 bars leaf water potential in soybean). Variation was high in both stressed and nonstressed plants. However, factors that affect transpiration demand also can affect free proline accumulation.

Free proline was not a good indicator of drought stress in the

Additional index words: drought stress, leaf water potential, stomatal diffusive resistance

[1/]Contribution no. 1388, Department of Agronomy, Evapotranspiration Laboratory, Kansas Agricultural Experiment Station, Kansas State University, Manhattan, Kansas 66506.

[2/]Assistant Instructor, Associate Professor, and Assistant Professor, Department of Agronomy, Kansas State University, Manhattan, Kansas 66506

field for irrigation scheduling because it increased after the critical time to irrigate for maximum yeild.

INTRODUCTION

A good plant indicator of drought stress to predict irrigation scheduling has been needed for many years. Such an indicator would have to be reliable under variable field conditions and sensitive enough to function when or before the critical need for water to maximize yield. Ideally, such an indicator would be simple and inexpensive.

Recent studies by various workers have shown that the free proline contents of excised leaves of many important agricultural crops increase markedly with drought or temperature stress. Palfi and Juhasz (12) have suggested using proline concentration as the sole parameter to measure drought stress, and that proline accumulation could be used as a measure of drought resistance. Le Saint (9, 10) suggested that high proline might indicate frost resistance.

Several studies have shown that proline formation requires available carbohydrates, and that high light intensity and high temperature enhance its accumulation (6). Goas et al. (3) reported that proline accumulation indicated a disturbance in nitrogen metabolism but they did not speculate how the changes occurred. Morris et al. (11) showed that proline is formed directly from glutamic acid and/or N-acetylglutamic acid in drought-stressed higher plants. Kudrev(7, 8) suggested that proline accumulation is caused by decomposition of such other products as protein, not the conversion of products of current photosynthesis.

Most research previously conducted on proline accumulation has been with plants in greenhouses or with detached leaves. We used sorghum (a drought-resistant crop), and soybean (a drought-susceptible crop) to determine how free proline responds to drought stress and adequate soil moisture under field conditions.

METHODS AND MATERIALS

The experiment was conducted in 1972 and 1973 at the Evapotranspiration Research Field, 14 km south of Manhattan, Kansas, on an alluvial silt loam soil. Soybeans (*Glycine max* L. cv. Calland) were planted May 17, 1972, and June 6, 1973, and sorghum (*Sorghum bicolor* (L.) Moench cv. Pioneer 846), June 30, 1972, and June 1, 1973.

The 1972 sorghum plots were double cropped by planting sorghum after winter wheat (*Triticum aestivum*) was harvested and the plots were irrigated. The soybean plots received no irrigation or were irrigated to maintain soil moisture at 20, 40, 60, or 80% field capacity.

The 1973 sorghum was planted on the same site but it was not irrigated. The soybeans were planted in and around drainage lysimeters under a rainout shelter (18). The lysimeters were maintained at 40, 60, 80, and near 100% moisture depletion.

In 1972, drought stress occurred during three weeks of hot, dry weather in August. In 1973, rainfall prevented drought stress except under the rainout shelter.

Proline content was determined by the Bates et al. method (2), on fully expanded, upper leaves in both species. Sampling times were 10:00 to 14:00 hr, and all samples were replicated 4 times.

Soil moisture was determined gravimetrically (top 25 cm) and with a neutron attenuation meter[3/] (25 to 150 cm depth). Leaf water potential

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[3/] Troxler Model 2601.

was determined by the pressure bomb method (16). Stomatal diffusive resistance was measured with a diffusion porometer (4) on the upper and lower surfaces of uppermost fully expanded leaves of both species. Average leaf stomatal resistance (R_s) was calculated by

$$\frac{1}{R_s} = \frac{1}{R_u} + \frac{1}{R_b}$$

where R_u and R_b are resistances of the upper and lower leaf surfaces, respectively.

RESULTS AND DISCUSSION

Canopy Profile Changes. Figure 1 shows free proline in leaves at different heights in the plants canopy during low transpirational demand in both an open and a closed canopy (1973). In an open canopy, where light was not limiting, proline remained fairly constant throughout the profile, but in the closed canopy, there was significant reduction (5% level) in free proline -- related to age and light. Widest variations in both sorghum and soybean were in the middle of the canopy where both sun drenched and shaded leaves were present. In general, free proline was lower and varied less in the bottom, shaded leaves than in upper, nonshaded leaves.

Figure 2 shows canopy profile differences in stressed and nonstressed plants during high transpiration demand (1972). Stressed soybean plants showed significantly higher free proline concentration in upper leaves than in nonstressed leaves, with small differences among leaves in the middle and lower parts of the canopy. Upper leaves of nonstressed sorghum plants were slightly higher in free proline. We do not know why.

Under conditions of low transpirational demand (nonstressed), middle

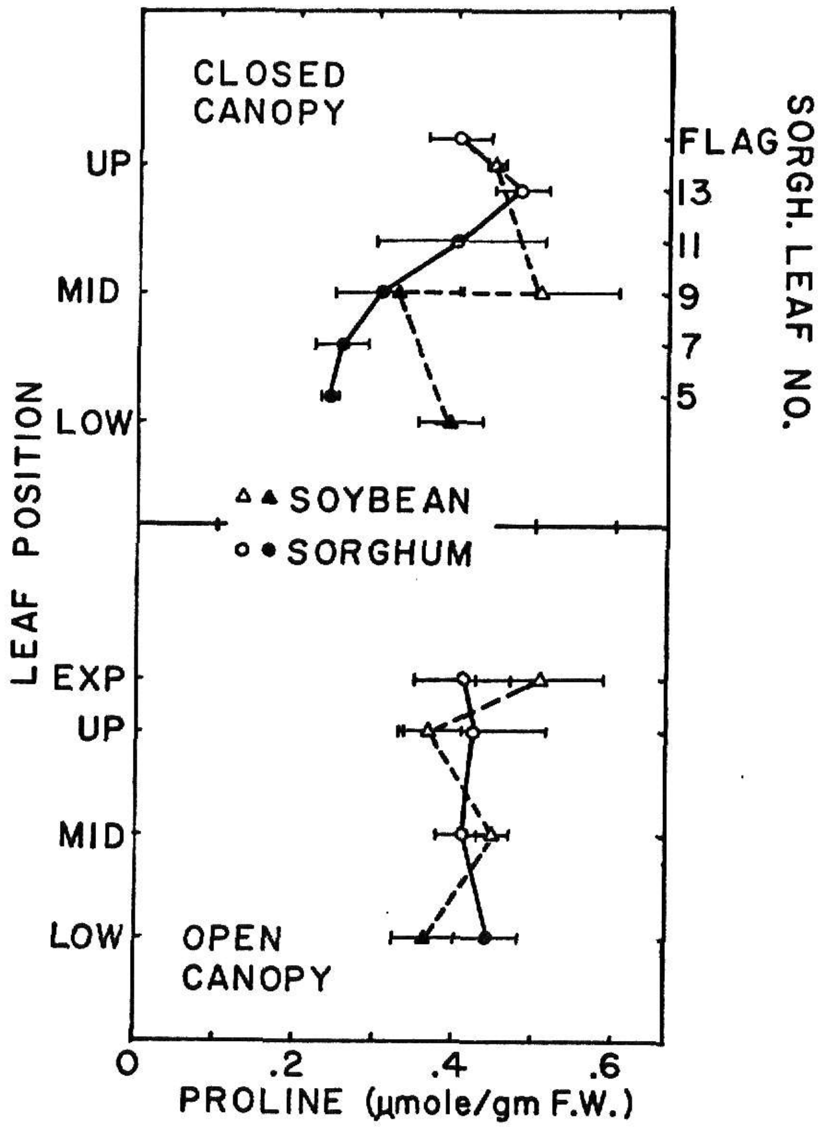

Figure 1. Free proline concentration in sorghum and soybean in open and closed canopy profiles at midday under conditions of low transpiration demand (Open symbols: sun leaves; closed; shaded leaves). Up - upper fully expanded leaf; mid - middle of profile; low - bottom of profile; exp - expanding and/or not collared.

shaded leaves of sorghum and soybean were lower in free proline than were middle nonshaded leaves, which supports Kliewer and Kider findings (6) that high light intensity increases free proline concentration.

Seasonal and Diurnal Changes. Figure 3 shows free proline changes in nonstressed leaves through the growing season under conditions of low transpirational demand (1973). Free proline in sorghum leaves remained fairly constant until anthesis then rose slightly and persisted to maturity. Extreme caution was necessary to wash the pollen off the leaves at anthesis because it caused extremely high proline readings. Free proline concentration of soybean varied so widely during the season that seasonal changes could not be determined, except for a probable slight increase at the pod filling stage.

Figure 4 shows changes in free proline in nonstressed leaves during 12 hours (7:00 to 19:00 hr) under low transpirational demand (1973). Standard deviations of the samples were high at times, expecially afternoon in soybean. Statistical analysis showed significant differences (5% level) for both sorghum and soybean. It appears that sampling time as well as amount of sunlight affects free proline concentration in nonstressed leaves; the differences, though not great, were statistically significant.

Free Proline Accumulation in Drought-stressed Plants. We found that visible signs of severe stress (not recovering from wilting during the night) occurred before proline accumulated. Figure 5, comparing proline concentration and leaf water potential (Ψ_{leaf}) in soybeans, shows that proline did not accumulate until about -20 bars. We did not measure leaf water potential on field sorghun under stress in 1972, but growth chamber

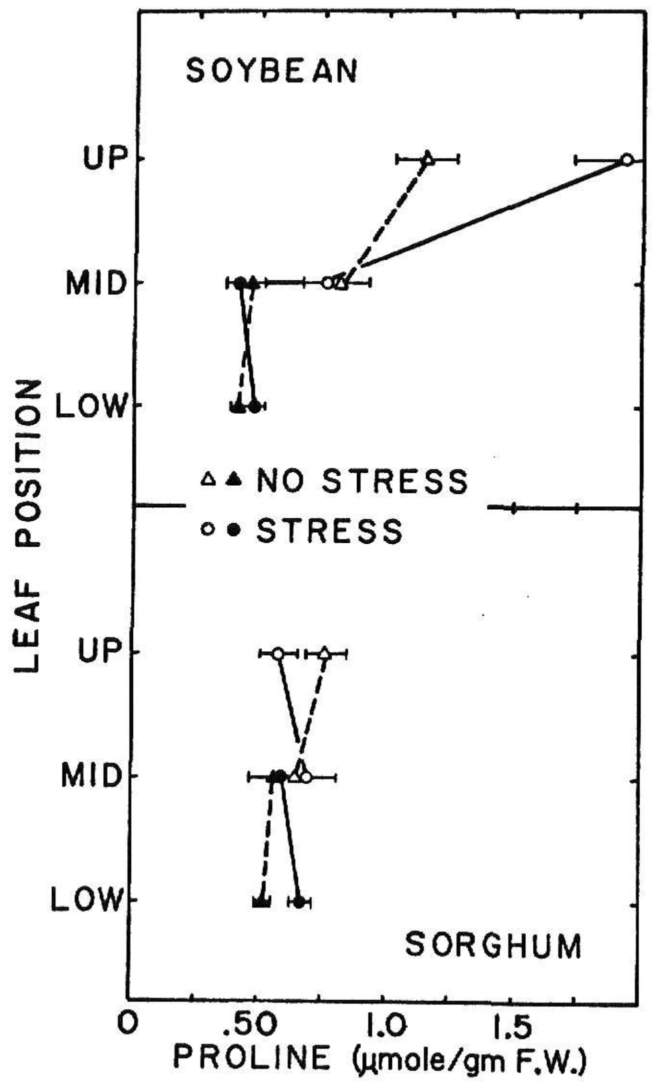

Figure 2. Free proline concentration in drought stressed and non-stressed sorghum and soybean canopy profiles at midday under conditions of high transpirational demand (open symbols: sun leaves; closed: shade leaves). Up - upper fully expanded leaf; mid - middle of profile; low - bottom of profile.

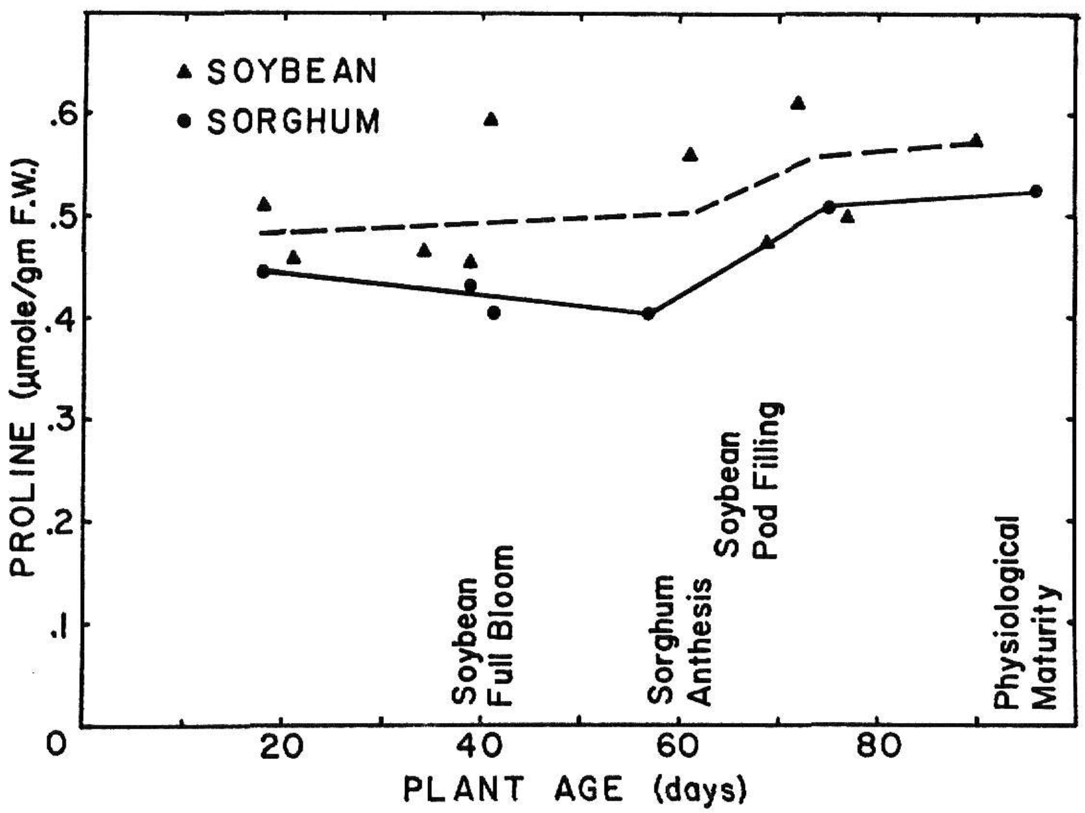

Figure 3. Seasonal changes in free proline concentration in the leaves of sorghum and soybean taken in afternoon under non-stressed conditions.

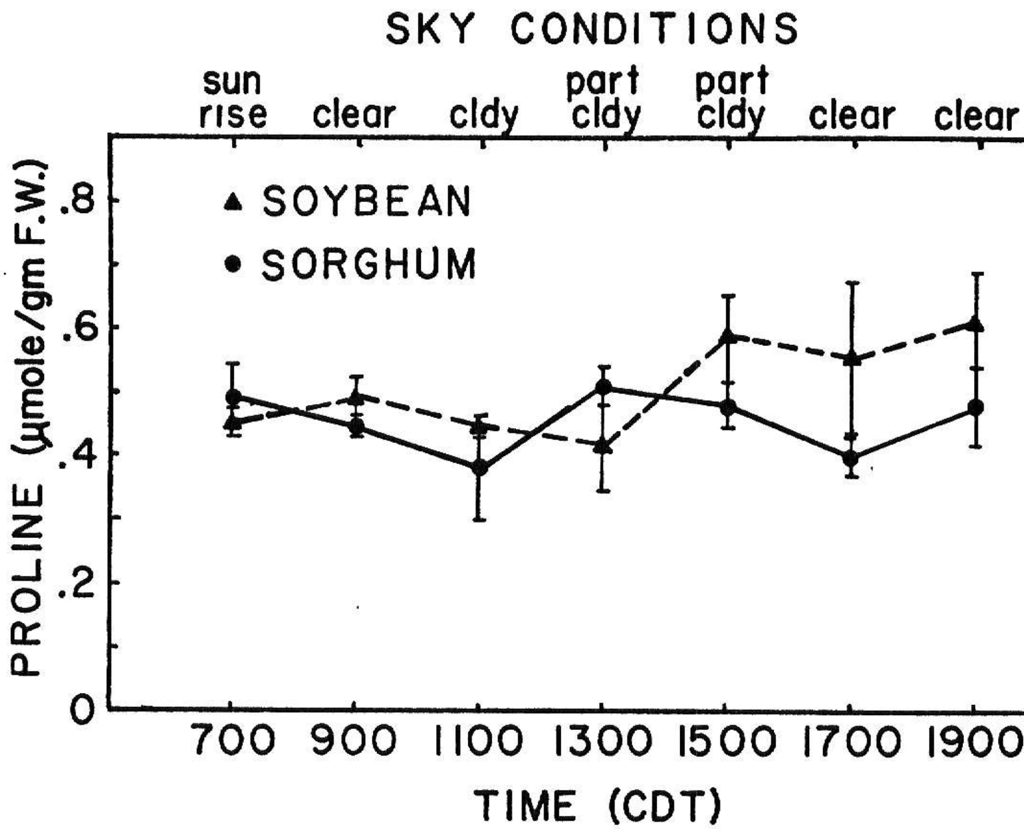

Figure 4. Diurnal changes in free proline concentration in the leaves of sorghum and soybean taken in afternoon under non-stressed conditions.

studies (20) have shown that proline accumulation in sorghum occurs at a much higher Ψ_{leaf} than in soybeans, similar to the proline-accumulation response to changes in stomatal resistance shown in Fig. 6. Stomatal closure occurs around -15 bars Ψ_{leaf} in both crops (19), so stomatal closure obviously preceded proline accumulation, which disputes the suggestion of Palfi and Juhasz (12) that proline concentration could be used as a sensitive indicator of drought stress.

Palfi and others, (12, 13) have suggested that proline accumulation is an indicator of physiological dryness, but all the parameters that influence physiological dryness also influence proline accumulation. For example, Figure 7 shows changes in proline in sorghum during a change in weather conditions. The slightly stressed plot showed signs of wilting during the day but recovered at night while the severely stressed plot did not recover at night. The severely stressed plot was rapidly accumulating proline during a sunny, hot period. Then the weather turned cool and cloudy with only a sprinkle of rain (.05 cm). That change in the weather greatly reduced light intensity and transpirational demand and allowed the plants to regain turgor, which resulted in a subsequent rapid drop in proline even though soil moisture to 150 cm deep was still quite low (about 81% SMD).

Figure 7 also shows wide proline variations during periods of rapid accumulation or decline of proline. Individual plants do not all respond the same, which results in very high deviations in proline concentration under "the same conditions". Standard deviations were much lower in the afternoon than in the morning. We believe that under drought conditions the location of the root system in relation to soil moisture causes some

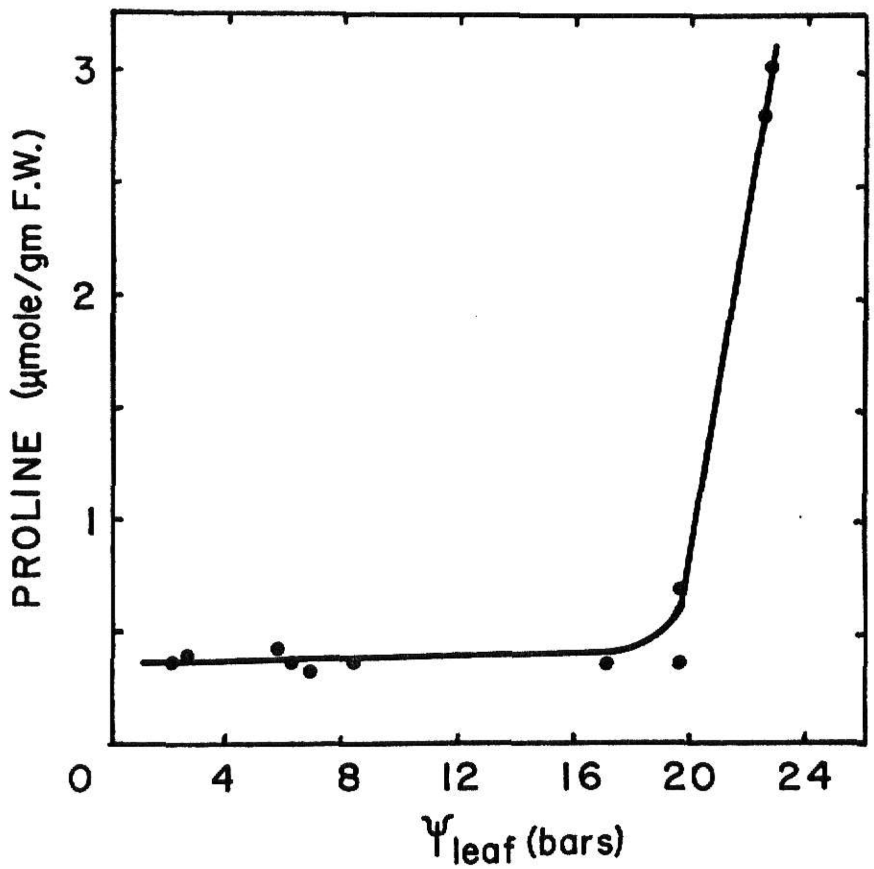

Figure 5. Free proline concentration in soybean leaves compared to leaf water potential.

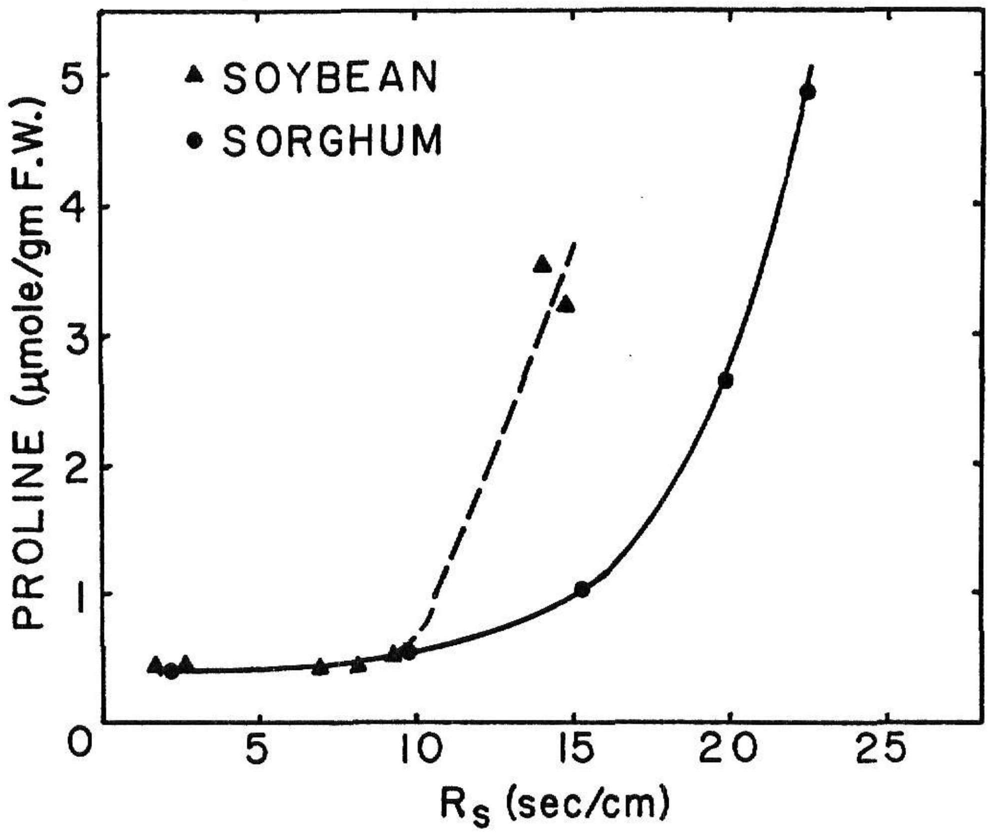

Figure 6. Free proline concentration in soybean and sorghum leaves compared to stomatal diffusive resistance.

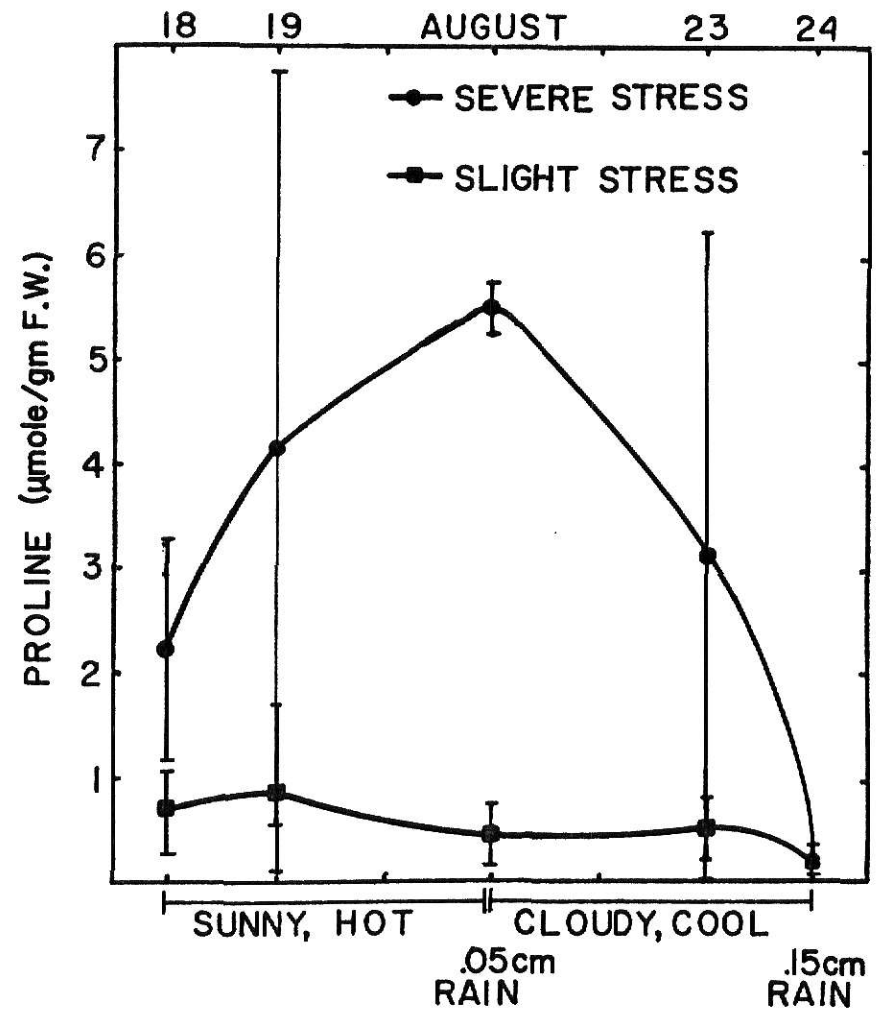

Figure 7. Free proline concentration in severely stressed and slightly stressed sorghum leaves during a period of changing transpirational demand.

plants to recover faster than others. The result is that those with access to some water have lower proline levels in the morning than those whose roots are in drier soil. By afternoon drought stress is more nearly equal so proline levels vary less then.

It appears that proline is not a sensitive indicator of drought stress in the field because it does not begin to accumulate until after stress is severe enough to adversely affect yield.

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CHAPTER IV

EVALUATION OF FREE PROLINE CONTENT IN LEAVES AS AN INDICATOR OF DROUGHT RESISTANCE IN UNSTRESSED AND HARDENED PLANTS

R. P. Waldren and I. D. Teare

EVALUATION OF FREE PROLINE CONTENT IN LEAVES AS AN INDICATOR OF DROUGHT RESISTANCE IN UNSTRESSED AND HARDENED PLANTS[1/]

R. P. Waldren and I. D. Teare[2/]

ABSTRACT

Free proline concentration was measured in sorghum (*Sorghum bicolor* (L.) Moench) and corn (*Zea mays* L.) lines having a wide range of drought resistance under drought stress. It was found that proline concentration under non-stressed conditions was not as good an indicator of drought resistance as it was under drought stress.

Soybean (*Glycine max* (L.) cv. Calland) was grown under conditions of low soil moisture that induced drought hardiness two different ways. When compared to hardening plants under the same conditions of drought stress, the already hardened plants had lower proline concentrations. This indicates that the metabolic processes that induce drought hardiness may not be the same as those that cause drought resistance.

INTRODUCTION

Free proline accumulates in the leaves of drought stressed plants and previous research has shown that the degree of proline accumulation can be a measure of drought resistance.

Palfi and Juhasz (5) have suggested that more free proline will accumulate in the leaves of drought resistant varieties than drought

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[1/] Contribution n.   , Kansas Agricultural Experiment Station, Evapotranspiration Laboratory, Manhattan, Kansas 66506.

[2/] Assistant Instructor and Associate Professor, Evapotranspiration Laboratory, Manhattan, Kansas 66506.

susceptible varieties when subjected to the same degree of drought stress. This was shown to be true by Singh, et. al. (7) with 3-week-old barley seedlings under osmotic induced drought stress without previous hardening.

Hardening can be induced in otherwise nonresistant plants by exposing them to long periods of non-lethal drought stress (3). No work has been done that shows the relationship between induced drought hardines and proline concentration.

The purpose of this experiment was to ascertain if free proline concentration in unstressed corn and sorghum leaves is an indicator of drought resistance and also to measure proline concentration in soybean (a drought susceptible crop) that was grown under conditions of low soil moisture to induce drought hardiness.

METHODS AND MATERIALS

Laboratory Experiment. Sorghum (*Sorghum bicolor* (L.) Moench) lines 298001-298024 from the Purdue-AID International Protein Quality Yield Trials and corn (*Zea mays* L.) lines WC7 (drought susceptible), W1736 (intermediate), and W3670 (resistant) were planted in 7 oz plastic drinki cups filled with silt loam soil. A small hole was punched in the bottom of each cup for drainage. Each line was replicated six times and the plants were grown in growth chambers at 30°C and 50% relative humidity with a 16-hour photoperiod. The plants were given nutrient solution once a week.

At 3 weeks of age, the leaves were analyzed for free proline using a spectrophotometric method (1). The entire experiment was replicated two times.

Field Experiment. The sorghum lines listed above were planted in a silt loam alluvial soil at the Evapotranspiration Field Research Site located 14 km south of Manhattan, Kansas on June 8, 1973. Free proline was measured as above at stages 4.2-5.2 (9), under no drought stress, on the uppermost fully expanded leaf. Each line was replicated four times

Two plots of soybeans (Glycine max (L.) cv. Calland) were grown under a rainout shelter (8). One plot was brought to field capacity (0-150 cm before planting, and recieved no more water during the growing season. The second plot was near 80% soil moisture depletion (0-150 cm) at plantin and received light waterings only as needed to prevent death of the plants Both plots were planted June 1, 1973.

Proline was analyzed as above and soil moisture was measured with a neutron attenuation meter[3/]. Leaf water potential was measured with the pressure bomb (6), and stomatal diffusive resistance was measured with a porometer (2). All measurements were replicated three times.

RESULTS AND DISCUSSION

Table 1 shows the comparative data on the 24 sorghum lines taken in both the laboratory and field. Yield data was obtained under very drought conditions and should be an indicator of relative drought resistance (4). Table 2 shows comparative data on the 3 lines of corn.

Rank correlation between the different experiments and yield in sorghum is very poor (-.181 to +.091). Poor correlation between experiments and relative drought resistance in corn is also evident which indicates that proline concentration under non-drought stress conditions

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[3/] Troxler Model 2601.

Table 1. Comparative free proline (μmoles/g F.W.) from lab and field experiments with 24 sorghum lines under no drought stress, and yield under droughty conditions.

Line	Yield[1/] Kg/ha	Yield[1/] Rank	LAB EXPERIMENT 1 Proline	LAB EXPERIMENT 1 Rank	LAB EXPERIMENT 2 Proline	LAB EXPERIMENT 2 Rank	FIELD EXPERIMENT Proline	FIELD EXPERIMENT Rank
298001	2554	19	.673	2	.397	3	.241	23
2	3988	8	.433	6	.271	22	.254	22
3	4402	4	.796	1	.252	23	.363	16
4	3318	14	.598	3	.357	8	.611	1
5	3960	9	.582	4	.381	5	.256	21
6	3296	15	.323	11	.301	18	.366	15
7	2856	16	.339	10	.349	10	.463	7
8	4006	7	.309	14	.294	19	.369	14
9	2412	21	.283	24	.313	15	.477	6
10	3391	13	.257	23	.370	6	.392	12
11	4578	2	.349	9	.351	9	.350	18
12	2731	17	.286	16	.441	1	.323	19
13	1929	23	.285	17	.294	20	.383	13
14	2107	22	.308	15	.382	4	.362	17
15	2500	20	.276	20	.242	24	.302	20
16	4070	6	.318	12	.334	11	.240	24
17	2667	18	.527	5	.407	2	.504	5
18	182	24	.384	7	.328	13	.397	10
19	4350	5	.382	8	.319	14	.524	3
20	5142	1	.278	19	.287	21	.393	11
21	4525	3	.280	18	.313	16	.435	8
22	3775	11	.264	21	.367	7	.514	4
23	3884	10	.264	22	.302	17	.414	9
24	3672	12	.311	13	.331	12	.585	2

[1/] Data from M. A. Faris, Penta Costa, Fortaleza, Brazil.

Table 2. Proline concentration (μmoles/g F.W.) in corn lines WC7 (drought susceptible), W1736 (intermediate), and W3670 (resistant) under no drought stress.

Line	EXPERIMENT 1	EXPERIMENT 2
WC7	.455	.217
W1736	.179	.171
W3670	.538	.218

is not a reliable indicator of drought resistance as it is under stressed conditions (5,7).

Table 3 shows soil moisture depletion (SMD), proline concentration, leaf water potential (Ψ_{leaf}), and stomatal resistance (R_s) in soybean. Since the soybeans in the dry plots were always growing under conditions of low soil moisture they were able to adapt (harden) themselves to the droughty conditions, and generally had lower levels of proline, stomatal resistance, and leaf water potential than the plants in the plot which began with adequate supplies of soil moisture and then ran short (hardenir Proline concentration in the hardened plants was lower than the hardening plants which is opposite the relationship between proline and drought resistance (7). This indicates that the metabolic processes that cause drought hardiness may not be the same as those that cause drought resistance. It is clear that much additional work will be required before we attain a complete understanding of the relationship between proline, hardening, and drought resistance.

Table 3. Soil moisture depletion (SMD), free proline, stomatal resistance (R_s), and leaf water potential (Ψ_{leaf}) of soybean grown on plots irrigated to field capacity at planting (WET) and maintained at low soil water levels (DRY).

	Plant age (days)	SMD (%)	Proline µmole/ g.F.W.	R_s (sec/cm)	Ψ_{leaf} (-bars)
WET	34	58.2	.562	5.3	4.1
DRY	34	85.5	.598	4.6	6.8
WET	51	85.2	.888	14.1	10.8
DRY	51	89.0	.407	14.1	11.1
WET	58	83.1	.502	11.2	7.0
DRY	58	99.7	.442	14.6	11.1
WET	72	98.8	.625	17.1	11.5
DRY	72	100.0	.616	17.5	13.3

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APPENDICES

APPENDIX A

Complete data from the laboratory study of proline accumulation under drought stress.

SORGHUM			SOYBEAN		
Ψ_{leaf} (-bars)	R_s (sec/cm)	Proline (μmole/g F.W.)	Ψ_{leaf} (-bars)	R_s (sec/cm)	Proline (μmole/g F.W.)
10.4	3.18	0.205	4.1	4.34	0.286
10.4	5.92	0.254	5.2	2.87	0.388
10.4	5.30	0.872	5.5	3.78	0.422
11.7	5.37	0.273	5.5	11.06	0.667
12.4	6.11	0.220	5.5	13.19	2.157
12.4	6.63	0.186	5.5	15.86	0.605
12.4	13.70	0.124	6.2	1.59	0.341
12.4	19.21	0.807	6.2	13.13	0.270
12.4	29.70	0.403	6.2	37.09	1.055
13.8	8.98	0.189	7.6	19.98	0.385
13.8	19.41	0.130	12.4	24.41	0.341
13.8	19.81	0.208	13.8	49.68	7.448
15.2	26.05	0.196	18.6	66.08	16.758
20.7	72.91	1.664	20.7	76.25	13.906
24.2	∞	2.110	22.8	119.81	21.600
24.2	∞	2.421	24.2	∞	22.965
27.6	∞	9.310	24.2	∞	23.099
27.6	∞	11.917			
28.3	∞	19.365			
31.1	∞	21.103			
32.4	∞	18.372			
33.8	∞	44.687			
40.0	∞	44.689			

∞ stomates completely closed

APPENDIX B

Complete Data From 1972 Field Study

Plots Descriptions

Soybean. Plots used for this study were part of a separate, larger study of irrigation scheduling conducted by Dr. S. M. Goltz of the Evapotranspiration Laboratory, Kansas State University. Plots were maintained at 20, 40, and 60% available soil moisture depletion (SMD) by irrigation. The first irrigation was applied at different times during the growing season: vegetative stage (V), flowering (F), and podding (P). In addition, some plots were not irrigated as controls (C).

Plots for proline sampling were selected solely on the basis of available SMD (high, intermediate, and low) in an effort to obtain differences in drought stress. Rows were 91.4 cm apart with 22 plants/m.

Sorghum. Plots used for this study were part of a separate study on competition between weeds and sorghum. Plot 7 was completely free from weeds and plots 11 and 12 had volunteer wheat growing in competition with the sorghum. As with the soybean plots, they were selected solely on the basis of available SMD with plot 7 being low and plots 11 and 12 being high. Rows were 45.7 cm apart with 11 plants/m.

Table B-1. Available soil moisture depletion (SMD), free proline concentration, mean stomatal resistance (R_s), leaf water potential (Ψ_{leaf}), and NO_3 reductase activity of soybean grown under changing weather conditions.

Date	Plot	Available SMD (0-1.5m)	Free Leaf Proline (μmole/g F.W.)				R_s	Ψ_{leaf}	NO_3 Reductase Activity (μmole NO_2/hr/g F.W.)		Weather Conditions
			A.M.		P.M.						
		(%)	$\overline{x}$	s	$\overline{x}$	s	(sec/cm)	(-bars)	A.M.	P.M.	
8-10	9V	26.3			.406	.083	1.8	2.03	5.60		sunny, hot
	19C	79.7			.478	.064	2.2	5.67	4.51		
	40F	31.4			.441	.081	2.0	2.53	5.35		
8-14	7P	27.1	.292	.035	.417	.046	2.2	6.18	1.18	1.66	sunny, hot
	19C	83.5	.245	.030	.375	.156	2.4	6.89	1.04	0.98	
	41V	47.8	.353	.055	.414	.069	2.6	8.31	1.49	2.27	
8-17	18V		.451	.040	.411	.064			0.84	0.99	sunny, hot
	19C	88.0	.490	.119	.409	.032	8.4	19.65	0.61	0.66	
8-18	18V				.440	.035				1.18	sunny, hot
	19C				.409	.062	7.9	17.12		0.45	
8-19	18V				.573	.077				1.15	sunny, hot
	19C				.663	.283				0.89	
8-21	3C	78.2			3.273	1.024	14.8	22.49			sunny, hot
	18V	60.6	.460	.086	.799	.304			0.42	0.99	0.05 cm rain
	19C	87.6	.400	.128	.563	.068	9.1	19.75	0.76	0.94	
8-23	3C	79.9			3.551	3.719	14.1	22.79			cloudy, cool
	18V				.522	.072					
8-24	3C		1.738	1.601					0.37		cloudy, cool
	18V		.431	.020					0.48		0.15 cm rain

Table B-2. Available soil moisture depletion (SMD), free proline concentration, and stomatal resistance (R_s) of sorghum grown under changing weather conditions.

Date	Plot	Available SMD (0-1.5m)	Free Leaf Proline (µmole/g F.W.)				R_s	Weather Conditions
			A.M.		P.M.			
		(%)	$\overline{x}$	s	$\overline{x}$	s	(sec/cm)	
8-15	7	39.5						
	11	61.4						
8-17	7		.579	.338	.559	.161		sunny, hot
	11		3.663	2.053	3.247	1.407		
8-18	7				.416	.154	2.1	sunny, hot
	11				2.635	1.214	19.9	
8-19	11	65.8			4.871	4.186	22.4	sunny, hot
	12	73.6			1.024	1.004	15.1	
8-21	11		5.339	2.024	6.450	.289	61.8	sunny, hot
	12		.732	.530	.553	.303	9.9	0.05 cm rain
8-23	11				3.694	3.614		cloudy, cool
	12				.625	.369		
8-24	11	85.0	.289	.217				cloudy, cool
	12	81.2	.176	.015				0.15 cm rain

Table B-3. Free proline concentration in the uppermost, fully expanded leaf in soybean.

Date	Plot	Time (CDT)	Proline (μmole/g F.W.)				$\bar{x}$
8-10	9V	2:30	.415	.317	.377	.515	.406
	19C		.402	.465	.490	.556	.478
	40F		.468	.385	.367	.543	.441
8-14	7P	10:00	.264	.259	.317	.327	.292
	19C		.277	.214	.224	.264	.245
	41V		.405	.380	.279	.347	.353
	7P	1:40	.352	.453	.415	.447	.417
	19C		.608	.302	.287	.302	.375
	41V		.339	.480	.463	.372	.414
8-17	19C	10:10	.566	.553	.314	.538	.490
	18V		.402	.463	.498	.440	.451
	19C	1:35	.402	.377	.453	.402	.409
	18V		.324	.402	.453	.463	.411
8-18	19C	1:00	.365	.488	.354	.427	.409
	18V		.405	.488	.442	.425	.440
8-19	19C	1:00	.430	1.006	.784	.430	.663
	18V		.651	.478	.616	.548	.573
8-21	19C	10:15	.400	.587	.282	.339	.400
	18V		.581	.380	.425	.455	.460
	19C	1:20	.515	.611			.563
	18V		.742	.628	.581	1.224	.799
	3C		3.997	2.549			3.273
8-23	3C	12:35	8.847	1.282	.679	3.394	3.551
	18V		.603	.556	.440	.490	.552
8-24	3C	10:00	1.536	4.052	.509	.854	1.738
	18V		.440	.422	.407	.453	.431

Table B-4. Free proline concentration in the uppermost, fully expanded leaf in sorghum.

Date	Plot	Time (CDT)	Proline (μmole/g F.W.)				$\bar{x}$
8-17	11	10:10	6.596	3.401	2.061	2.473	3.633
	7		.402	1.082	.361	.471	.579
	11	1:35	1.446	3.891	2.757	4.792	3.247
	7		.464	.502	.471	.799	.559
8-18	11	1:00	.902	3.169	2.680	3.787	2.635
	7		.338	.258	.613	.453	.416
8-19	11	1:00	2.105	10.512	5.565	1.391	4.871
	12		2.525	.567	.590	.415	1.024
8-21	11	10:15	3.865	5.539	3.937	8.193	5.339
	12		.528	1.520	.515	.366	.732
	11	1:30	6.029	6.647	6.632	6.493	6.450
	12		.402	.593	.958	.258	.553
8-23	11	12:45	9.044	1.123	2.525	2.085	3.694
	12		.165	1.043	.747	.544	.625
8-24	11	10:00	.193	.613	.149	.199	.289
	12		.180	.157	.193	.175	.176

Table B-5. Proline profile data taken on August 9, 1972. Soybean sampled 12:30 P.M., sorghum 1:30 P.M. Top: fully expanded uppermost leaf. Mid: 8-9 nodes from top in soybean, 6 from top in sorghum. Bottom: lowest node with green leaf.

Crop	Leaf	Sun or Shade	Proline (μmole/g F.W.)				$\bar{x}$
Soybean	Top	Sun	.289	.307	.289	.352	.309
	Mid	Both	.239	.314	.338	.307	.300
	Bottom	Both	.261	.302	.188	.085	.209
Sorghum	Top	Sun	.206	.188	.226	.214	.209
	Mid	Both	.211	.188	.226	.158	.196
	Bottom	Both	.224	.163	.158	.151	.174

Table B-6. Profile data taken on August 16, 1972 showing mean ($\bar{x}$) and standard deviation (s) for irrigated and not irrigated sorghum and soybean. Original replicated data is not available.

Crop	Leaf Position	Proline (μmole/g F.W.) Irrigated		Not Irrigated	
		$\bar{x}$	s	$\bar{x}$	s
Soybean	Top Sun	1.152	.117	1.942	.196
	Mid Sun	.812	.114	.752	.106
	Mid Shade	.472	.053	.450	.045
	Lower Shade	.427	.031	.473	.039
Sorghum	Top Sun	.758	.066	.572	.065
	Mid Sun	.645	.103	.657	.120
	Mid Shade	.559	.074	.560	.083
	Lower Shade	.514	.026	.668	.036

APPENDIX C

Complete Date From 1973 Field Study

Plots Descriptions

Soybean. The soybeans were planted in and around the rainout shelter (see p.20). Plots 1-12 were the drainage lysimeters, with 1-3 being maintained around 40% available soil moisture depletion (SMD), 4-6 at 60%, and 7-9 at 80% available SMD. Plots 10-12 were given only enough water to keep the plants alive. Plot 13 was located under the shelter outside the lysimeters and was brought to field capacity before planting and no more water was applied. Plot 14 was the same as 13 except that only enough water was added to maintain growth. Plots 17 and 19 were located near the shelter and received only natural rainfall. Plot 29 was planted on July 26 to compare plants of different ages under the same conditions. All plots had rows 91.4 cm apart with 22 plants/m.

Sorghum. The sorghum plots were located within two larger sorghum fields of 1 ha each. Both plots were identical except that 26 was planted in rows 45.7 cm apart and 28 in rows 91.4 cm apart. Plant populations were 11 plants/m. Both plots received only natural rainfall. Plot 30 was planted on July 26 for comparison of different ages in rows 91.4 cm apart.

Table C-1. Available soil moisture depletion (SMD), free proline concentration, mean stomatal resistance (R_s), and leaf water potential (Ψ_{leaf}) of sorghum and soybean grown under changing weather conditions.

Date	Plot	Available SMD (0-1.5m)	Free Proline $\bar{x}$	Free Proline s	R_s	Ψ_{leaf}	Weather Conditions
		(%)	(μmole/g F.W.)		(sec/cm)	(-bars)	
7-10	13	58.2	.562	.033	5.3	4.06	sunny, hot, humid
	14	75.5	.598	.063	4.6	6.84	0.36 cm rain
	19	70.9	.456	.056	4.5	7.71	
	13		.539	.062	5.7	8.73	
	14		.484	.089	6.0	10.79	
	19		.466	.005	6.3	7.81	
7-11	26	25.1	.804	.131	9.8	10.12	sunny, hot, humid
7-17	1-3	46.0	.326	.114	7.2		sunny, hot
	4-6	51.0	.465	.066	8.9		
	7-9	53.9	.505	.011	15.4		
	10-12	98.8	1.843	2.229	28.1		
7-27	1-3	55.1	.398	.097	11.9		sunny, warm
	4-6	67.2	.389	.068	14.3		17 cm rain the
	7-9	72.7	.442	.168	13.0		previous week
	10-12	100.0	.498	.275	23.1		
	13	85.2	.888	.114	14.1		
	14	89.0	.407	.159	14.1		
	19		.594	.170	14.2		
	26		.345	.052	20.1		
	28		.404	.055	20.5		
8-3	1-3	58.1	.353	.049	20.0	7.82	sunny, warm
	4-6	55.8	.406	.067	18.5	8.42	
	7-9	81.3	.308	.028	33.4	9.87	
	10-12	100.0	.395	.031	21.3	9.89	
	13	83.1	.502	.047	11.2	6.97	
	14	99.7	.442	.030	14.6	11.09	
	19	57.3	.560	.071			
8-10	1-3	49.7	.469	.116	14.3	10.7	sunny, warm
	4-6	73.6	.407	.073	12.5	8.2	3.8 cm rain
	7-9	42.9	.515	.097	13.2	9.8	
	10-12	100.0	.407	.020	20.5	11.0	
8-14	19		.473	.068	14.7		sunny, warm
	28		.511	.054	32.1		
	29		.512	.041	11.3		
	30		.444	.034	14.3		

Table C-1. (continued).

Date	Plot	Available SMD (0-1.5m)	Free Proline x	s	R_s	Ψ_{leaf}	Weather Conditions
		(%)	(µmole/g F.W.)		(sec/cm)	(-bars)	
8-17	1-3	41.2	.480	.017	16.4	12.7	sunny, hot
	4-6	60.4	.455	.108	16.3	12.7	2.54 cm rain
	7-9	70.0	.513	.049	17.0	9.7	
	10-12	100.0	.807	.379	40.4	9.7	
	13	98.8	.625	.140	17.1	11.5	
	14	100.0	.616	.069	17.5	13.3	
	17	71.9	.612	.143	16.7	11.3	
	26	27.8	.359	.055	21.0	13.4	
	28	27.3	.279	.050	24.9	16.1	
	29		.459	.034	14.8	5.5	
	30		.317	.014	19.0	18.0	
8-22	1-3	38.0	.725	.343	27.2	8.8	sunny, hot, windy
	4-6	62.4	1.023	.143	22.1	10.3	
	7-9	83.9	1.375	.197	27.6	12.4	
	10-12	100.0	9.805	7.092	118.6	22.5	
	13	100.0	.616	.119	25.0	11.3	
	14	100.0	1.970	.308	51.7	19.6	
	28	31.1	.289	.019	19.5	15.4	
	17	74.9	.502	.002	17.9	11.5	
	29		.314	.004	25.8	7.3	
	30		.246	.002	18.8	15.2	
9-4	17		.574	.084			sunny, warm
	28		.525	.121			
	29		.456	.065			
	30		.433	.029			

Table C-2. Free proline concentration in the uppermost, fully expanded leaf in sorghum and soybean.

Date	Plot	Time (CDT)	Proline (µmole/g F.W.)				$\bar{x}$
7-10	13	10:00	.533	.598	.556		.562
	14		.652	.614	.529		.598
	19		.424	.424	.521		.456
	13	2:30	.514	.610	.494		.539
	14		.436	.429	.587		.484
	19		.463	.471	.463		.466
7-11	26	1:00	.639	.874	.937	.765	.804
7-17	1-3	11:00	.217	.315	.445		.326
	4-6		.489	.516	.391		.465
	7-9		.505	.494	.516		.505
	10-12		.494	.619	4.415		1.843
7-27	1-3	11:00	.287	.459	.449		.398
	4-6		.364	.446	.337		.389
	7-9		.263	.597	.466		.442
	10-12		.432	.263	.800		.498
	13		.995	.910	.769		.888
	14		.327	.304	.590		.407
	19		.476	.560	.844	.496	.594
	26		.422	.302	.329	.327	.345
	28		.331	.459	.429	.395	.404
8-3	1-3	11:30	.298	.394	.366		.353
	4-6		.329	.438	.450		.406
	7-9		.335	.279	.310		.308
	10-12		.360	.413	.413		.395
	13		.459	.552	.496		.502
	14		.413	.472	.441		.442
	19	1:00	.560	.596	.461	.622	.560
8-10	1-3	11:00	.414	.391	.602		.469
	4-6		.477	.331	.414		.407
	7-9		.407	.543	.596		.515
	10-12		.430	.400	.391		.407
8-14	19	1:00	.529	.398	.434	.532	.473
	28		.492	.471	.489	.590	.511
	29		.547	.543	.460	.496	.512
	30		.439	.487	.444	.405	.444

Table C-2. (continued)

Date	Plot	Time (CDT)	Proline (μmole/g F.W.)				$\bar{x}$
8-17	1-3	12:30	.483	.462	.496		.480
	4-6		.360	.572	.432		.455
	7-9		.462	.559	.517		.513
	10-12		.695	.496	1.229		.807
	13		.428	.725	.725	.623	.625
	14		.682	.521	.644	.614	.616
	17		.742	.678	.411	.615	.612
	26		.394	.415	.331	.297	.359
	28		.233	.237	.326	.318	.279
	29		.441	.506	.428	.462	.459
	30		.335	.301	.314	.318	.317
8-22	1-3	1:00	.398	.695	1.082		.725
	4-6		.869	1.151	1.050		1.023
	7-9		1.151	1.520	1.455		1.375
	10-12		1.723	14.987	12.706		9.805
	13		.731	.507	.610		.616
	14		2.281	1.665	1.965		1.970
	17		.504	.501	.501		.502
	28		.310	.273	.284		.289
	29		.310	.314	.318		.314
	30		.243	.247	.247		.246
9-4	17	11:00	.607	.519	.494	.677	.574
	28		.695	.415	.472	.517	.525
	29		.501	.428	.377	.516	.456
	30		.472	.428	.402	.430	.433

Table C-3. Diurnal changes in free proline concentration in the uppermost, fully expanded leaf of sorghum and soybean on August 9. (Plots 19 and 28).

Plot	Time	Proline					Sky Conditions
	(CDT)	(µmole/g F.W.)				$\bar{x}$	
Sorghum	7:00	.416	.520	.488	.539	.491	sunrise
Soybean		.455	.422	.487	.442	.452	
Sorghum	9:00	.465	.422	.461	.443	.448	clear
Soybean		.507	.457	.546	.461	.493	
Sorghum	11:00	.341	.524	.368	.304	.384	cloudy
Soybean		.467	.455	.429	.429	.445	
Sorghum	13:00	.543	.534	.505	.464	.512	part cloudy
Soybean		.344	.366	.442	.512	.416	
Sorghum	15:00	.443	.497	.469	.523	.483	part cloudy
Soybean		.574	.494	.654	.634	.589	
Sorghum	17:00	.412	.418	.415	.354	.400	clear
Soybean		.415	.508	.646	.673	.561	
Sorghum	19:00	.568	.434	.468	.458	.482	clear
Soybean		.542	.542	.665	.702	.613	

Table C-4. Free proline concentration in the canopy profile of sorghum and soybean on July 5 (canopy open), and July 31 (canopy closed). (Plots 19, 26, and 28).

Date	Plot	Leaf	Proline (μmole/g F.W.)			$\bar{x}$
7-5	Soybean	Expanding	.596	.500	.435	.510
		Upper sun	.387	.334	,407	.376
		Mid sun	.471	.479	.439	.463
		Lower shade	.330	.394	.394	.373
	Sorghum 91.4 cm row	Expanding	.399	.477	.371	.416
		Upper sun	.504	.330	.427	.420
		Mid sun	.386	,427	.435	.416
		Lower shade	.423	.471	.403	.432
	Sorghum 54.7 cm row	Expanding	.431	.443	.423	.432
		Upper sun	.504	.330	.435	.423
		Mid sun	.439	.443	.407	.430
		Lower shade	.367	.367	.367	.367
7-31	Soybean	Top sun	.450	.450	.454	.451
		Mid sun	.627	.460	.444	.510
		Mid shade	.261	.290	.409	.320
		Lower shade	.351	.428	.412	.397
	Sorghum 91.4 cm row	Flag sun	.434	.312	.467	.404
		No. 13 sun	.480	.502	.476	.486
		No. 11 sun-shade	.512	.283	.415	.403
		No. 9 shade	.348	.341	.216	.302
		No. 7 shade	.235	.235	.296	.255
		No. 5 shade	.238	.225	.248	.237

APPENDIX D

Drought Resistance Data

Table D-1. Data from lab experiment conducted in May 1973 on sorghum.

Line	Proline (μmole/g F.W.)						$\bar{x}$
298001	.673	.468	.669	1.003	.459	.564	.639
2	.291	.413	.632	.324	.421	.519	.433
3	1.381	.480	.451	1.095	.759	.611	.796
4	.708	.569	.683	.535	.535	.556	.598
5	.679	.464	.969	.618	.308	.451	.582
6	.484	.240	.223	.276	.279	.433	.323
7	.307	.431	.256	.348	.350	.341	.339
8	.363	.315	.256	.299	.303	.316	.309
9	.299	.299	.231	.192	.200	.205	.238
10	.249	.260	.256	.213	.231	.332	.257
11	.388	.332	.469	.336	.285	.285	.349
12	.281	.277	.289	.332	.281	.256	.286
13	.291	.317	.291	.256	.296	.256	.285
14	.428	.468	.256	.212	.239	.243	.308
15	.225	.256	.323	.300	.300	.252	.276
16	.397	.309	.296	.222	.309	.375	.318
17	.684	.529	.405	.411	.253	.882	.527
18	.529	.300	.441	.291	.309	.432	.384
19	.320	.421	.384	.305	.543	.320	.382
20	.264	.223	.348	.352	.279	.199	.278
21	.208	.241	.223	.251	.287	.472	.280
22	.264	.255	.292	.273	.232	.269	.264
23	.255	.255	.213	.264	.311	.287	.264
24	.208	.371	.287	.269	.464	.269	.311
25	.290	.244	.269	.410	.343	.259	.304
26	.269	.273	.236	.232	.244	.265	.253

Table D-2. Data from lab experiment conducted in June 1973 on sorghum.

Line	Proline (μmole/g F.W.)						$\overline{x}$
298001	.391	.442	.556	.348	.325	.321	.397
2	.254	.231	.211	.293	.337	.297	.271
3	.250	.305	.227	.227	.250	.254	.252
4	.325	.325	.325	.450	.372	.344	.357
5	.446	.532	.337	.337	.337	.297	.381
6	.297	.290	.286	.297	.293	.344	.301
7	.193	.488	.346	.346	.435	.290	.349
8	.314	.330	.278	.363	.242	.242	.294
9	.359	.274	.346	.342	.242	.314	.313
10	.403	.270	.326	.439	.463	.318	.370
11	.334	.314	.314	.415	.395	.334	.351
12	.427	.403	.391	.463	.479	.483	.441
13	.302	.286	.290	.294	.298	.294	.294
14	.308	.390	.318	.308	.504	.475	.382
15	.270	.258	.230	.238	.226	.230	.242
16	.334	.294	.274	.306	.483	.314	.334
17	.375	.383	.320	.323	.527	.518	.407
18	.310	.419	.387	.274	.262	.314	.328
19	.308	.435	.272	.304	.280	.316	.319
20	.288	.268	.256	.232	.340	.336	.287
21	.332	.332	.371	.300	.300	.240	.313
22	.292	.359	.292	.367	.427	.463	.367
23	.435	.256	.268	.260	.240	.351	.302
24	.431	.276	.260	.280	.328	.411	.331
25	.232	.220	.201	.205	.348	.259	.244
26	.259	.247	.251	.239	.351	.239	.264

Table D-3. Data from lab experiment conducted in May and June 1973 on corn.

Line	Proline (μmole/g F.W.)						x̄
			May 1973				
W1736	.190	.199	.153	.157	.165	.207	.179
W3670	.360	.662	.463	.376	.931	.438	.538
WC7	.281	.525	.463	.269	.749	.381	.445
			June 1973				
W1736	.178	.181	.158	.162	.174	.170	.171
W3670	.216	.178	.282	.201	.224	.209	.218
WC7	.282	.259	.220	.170	.178	.193	.217

Table D-4. Field data on the corn plots of Dr. Wassom taken on August 9, 1973. (K41 x H28 drought resistant, K731 x K724 drought susceptible).

Line	Age	Proline (μmole/g F.W.)				x̄
K731xK724	5.0	.513	.299	.305	.331	.362
Zaplote c.	6.0	.351	.351	.448	.516	.417
H28xK724	5.0	.315	.354	.487	.442	.400
3149	5.5	.399	.552	.328	.477	.439
K41xH28	5.0	.315	.292	.309	.279	.299
Oh7BxP8	5.8	.286	.344	.377	.338	.336
3368P	6.0	.510	.510	.383	.383	.447
Oh7B	4.5	.715	.486	.400	.601	.551
K731	3.5	.945	1.174	.695	.968	.957
K21	3.9	.358	.246	.304	.277	.296
K62	5.0	.422	.253	.364	.337	.344
H28	4.0	.715	.935	.381	.678	.677
K724	4.0	.321	.321	.280	.219	.285
P8	5.0	.236	.236	.395	.321	.297
K41	4.5	.459	.381	.482	.472	.449
K695	4.5	.327	.300	.300	.219	.287

Table D-5. Field data on sorghum taken July 10-12, 1973 at physiological age 3.0.

Line	Proline (μmole/g F.W.)				$\bar{x}$
298001	.406	.406	.371	.324	.377
2	.473	.567	.460	.473	.493
3	.581	.460	.453	.453	.487
4	.541	.669	.629	.798	.659
5	.392	.378	.366	.473	.402
6	.439	.534	.473	.473	.480
7	.473	.439	.446	.460	.455
8	.460	.460	.460	.460	.460
9	.676	.756	.473	.574	.620
10	.584	.499	.594	.623	.575
11	.508	.709	.709	.639	.641
12	.522	.585	.445	.492	.511
13	.655	.655	.749	.530	.647
14	.623	.616	.492	.623	.589
15	.515	.772	.725	.702	.679
16	.632	.632	.546	.546	.589
17	.741	.569	.686	.632	.657
18	.473	.655	.589	.515	.558
19	.779	.796	.796	.762	.783
20	.415	.429	.497	.497	.460
21	.659	.559	.617	.590	.606
22	.542	.521	.563	.566	.548
23	.439	.521	.552	.381	.473
24	.230	.333	.257	.274	.273
25	.854	.868	.583	.532	.709
26	.713	.566	.394	.394	.517

Table D-6. Field data on sorghum taken July 26, 1973.

Line	Age	Proline (µmole/g F.W.)				$\bar{x}$
298001	4.2	.242	.278	.224	.221	.241
2	4.5	.251	.260	.260	.245	.254
3	4.8	.363	.334	.405	.348	.363
4	5.0	.550	.725	.659	.510	.611
5	4.8	.242	.317	.242	.221	.256
6	4.5	.387	.387	.311	.378	.366
7	5.0	.477	.508	.445	.423	.463
8	4.5	.423	.393	.332	.326	.369
9	5.1	.505	.553	.426	.422	.477
10	4.4	.324	.354	.415	.476	.392
11	4.6	.384	.256	.329	.329	.350
12	4.6	.334	.294	.291	.373	.323
13	5.2	.391	.371	.348	.420	.383
14	4.5	.400	.373	.359	.317	.362
15	4.7	.356	.268	.314	.271	.302
16	4.5	.269	.266	.210	.216	.240
17	4.2	.510	.510	.516	.480	.504
18	4.8	.499	.392	.345	.350	.397
19	4.4	.559	.535	.489	.412	.524
20	4.3	.379	.390	.418	.386	.393
21	4.9	.426	.389	.521	.403	.435
22	4.5	.556	.546	.532	.421	.514
23	4.5	.396	.389	.403	.469	.414
24	4.6	.626	.626	.617	.470	.585
25	4.2	.414	.511	.445	.371	.435
26	4.2	.598	.389	.365	.372	.431

EVALUATION OF FREE LEAF PROLINE CONCENTRATION AS A PRACTICAL METHOD FOR MEASURING DROUGHT STRESS IN PLANTS

by

RICHARD PAUL WALDREN

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AN ABSTRACT OF A MASTER'S THESIS

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A simple, rapid colorimetric determination of free proline in plant leaves is described. This technique uses 3% aqueous sulfosalicylic acid as the extracting medium during homogenization of the leaf material. This solution precipitates out chlorophyll and proteins. The extract is then reacted with acid-ninhydrin and glacial acetic acid for one hour at 100°C. After cooling, the reaction mixture is mixed well with toluene which extracts the chromophore. The absorbance of the toluene-chromophore mixture is determined in a spectrophotometer at 520 nm.

In the laboratory, free proline accumulation was measured in leaves of intact sorghum (*Sorghum bicolor* (L.) Moench cv. Pioneer 846) and soybean (*Glycine max* L. cv. Calland) grown in growth chambers and subjected to "normal" drought stress. Free proline was also measured in the leaves of sorghum and soybean grown in the field under conditions of drought stress and adequate soil moisture. Stomatal diffusive resistance and leaf water potential were used to determine the degree of stress at the time of proline analysis.

Free leaf proline levels in non-stressed plants were found to change during the day and during the growing season, although the changes were not great. High light intensity increases free proline accumulation and high variation was found in leaves located in the middle of the plant which are both shaded and receive light.

In drought-stressed plants, free proline accumulation did not rise significantly until the plants were under severe stress with visible signs of wilting present. Variation was high in both stressed and nonstressed plants. However, factors that affect transpirational demand can also affect free proline accumulation which probably accounts for most of the variation.

Free proline was not found to be a good indicator of drought stress in the field for irrigation scheduling because it did not increase until after the critical time for water application needed to maximize yield.

Free proline concentration was measured in sorghum and corn (*Zea mays* L.) lines having a wide range of drought resistance under drought stress. It was found that proline concentration under non-stressed conditions was not as good an indicator of drought resistance as it has been shown to be under drought stress.

Soybean was grown under conditions of low soil moisture that induced drought hardiness. When compared to hardening plants under the same conditions of drought stress, the already hardened plants had lower proline concentrations. Since drought resistant plants have been shown to have higher proline levels than less resistant plants, the metabolic processes that induce drought hardiness may not be the same as those that cause drought resistance.