AN ABSTRACT OF THE THESIS OF

_____Nu Nu Wai_____ for the _____Master of Science_____

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Title: _____Water Relations in Vetiver Grass [*Vetiveria zizanioides* (L.) Nash.]_____

Abstract Approved: _____R. Laurie Robbins_____

Water relations and drought resistance in vetiver grass [*Vetiveria zizanioides* (L.) Nash.] were studied in laboratory and greenhouse experiments. Vetiver clones used in this study were obtained from the Plant Materials Center, Galliano, Louisiana. The plants were grown in plastic pots (25 cm x 25 cm, height x diameter) with Cornell mixture. My study focused on five important water-relations parameters: xylem pressure potential (XPP), relative water content (RWC), bulk modulus of cell wall elasticity ($\in$), transpiration rate (q), and leaf diffusive resistance (LDR) to understand the plant-water relations. The measurement of XPP under different water regimes was used to estimate theoretical limits of drought resistance in the experimental plants. All the water-relations parameters were measured in the laboratory, and experiments on drought resistance were conducted in the greenhouse.

The predawn xylem pressure potential of well-watered plants was recorded as -0.09 ± 0.005 MPa (SE). It decreased to -0.93 ± 0.036 MPa at midday, but the experimental plants fully recovered from the midday stress by the next dawn. Approximately 50% of leaves on plants dried when the mean predawn XPP was -4.4 ± 0.35 MPa (SE). Vetiver grass has elastic cell walls [$\in = 0.35 \pm 0.076$ MPa, (SE)] and low osmotic potential when compared to many forage grasses. The minimum

transpiration rate of the plants was 0.242 ± 0.04 μg cm^{-2} s^{-1} (SE), and a negative

exponential relationship was found between transpiration rate and XPP. The minimum

leaf diffusive resistance (LDR) of the experimental plants was calculated as 1.47 ± 0.27

s cm^{-1} (SE) and the maximum LDR was greater than 127.3 s cm^{-1}. The LDR in vetiver

grass appears to be more influenced by atmosphere vapor pressure deficit than by leaf

water potential. Under well-watered conditions, the relative water content (RWC) of the

experimental plants ranged from 90.4 to 95.8%. The mean RWC at the point of turgor

loss, as obtained from pressure-volume analysis, was 40.1%. The RWC of the leaves

decreased linearly with its xylem pressure potential. Results showed that vetiver grass

behaves similarly to many drought-resistant C_4 grasses in water relations and possesses

most of the physiological characteristics that favor the plant's growth and survival in

semi-arid or arid environments.

WATER RELATIONS IN VETIVER GRASS [*Vetiveria zizanioides* (L.) Nash.]

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The Department of Biological Sciences

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_____

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PREFACE

This thesis follows the style of Journal of Plant Physiology.

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INTRODUCTION

Vetiver grass [*Vetiveria zizanioides* (L.) Nash] has been used for various purposes, including thatching, aromatic oil production, and its medicinal properties (Dafforn, 1996). It is commercially valuable for the aromatic oil that is extracted from its roots and used in the perfume industry (Truong, 1996b). Vetiver grass has a history of use for land stabilization in Africa and Asia. Its prominent role in soil conservation began about 50 years ago in Fiji, where it was imported by the Colonial Sugar Refinery (CRS) to control soil erosion on steep canelands (Greenfield, 1989). The vetiver has not shown any invasive potential in Fiji over the fifty-year period (Greenfield, 1989). The vetiver plants (Fig. 1) used in hedges do not possess most of the characteristics ascribed to invasive species (Dafforn, 1996). Most of the strains that have been tested were propagated clonally and showed little genetic diversity (Kresovitch et al., 1994).

Soil erosion is truly a global problem, and the need for conservation has become critical in many countries. However, enough attention has not been given to the need to reduce soil and water losses caused by excessive rainfall runoff (Grimshaw, 1993). Vietmeyer (1993) has estimated that runoff carries 20 million tons of soil a year worldwide, resulting in the loss of between 5 million and 7 million hectares of arable land. The changes brought by erosion are: lost land; reduced productivity on farms and in forests; increased flooding; silted harbors, reservoirs, canals, and irrigation works; washed-out roads and bridges; and destroyed wetlands and coral reefs. Soil erosion undermines the economic security of most countries. The problems are most severe in the warmer regions of the world. Vietmeyer (1993) pointed out that previous efforts made to control soil erosion problems were rarely successful over any extensive area because

conventional techniques are enormously expensive. Therefore, any solution to the problem of worldwide erosion must be inexpensive, easy for farmers to understand, and simple to maintain under Third World conditions.

One solution to worldwide soil erosion problems might be found in vetiver grass (National Research Council, 1993). Vetiver grass might offer the practical intervention for soil erosion control worldwide because the vetiver grass system is a simple, inexpensive, and very effective means of soil and water conservation (Grimshaw, 1993; Vietmeyer, 1993). It can also be used for sediment control, land stabilization, and land rehabilitation (Greenfield, 1989; Truong, 1996a). After a four-year assessment, the U. S. National Academy of Science panel reported that vetiver hedges are effective in their ability to slow erosion and retain moisture (Dafforn, 1996). Truong (1996b) reported that steep slope (1:1) embankment and gully erosion were stabilized by establishing vetiver on contour lines. He also suggested that vetiver plants in rows form a hedge that slows and spreads runoff water and traps sediment. As water flow is slowed, its erosive power is reduced and more water infiltrates the soil. Hence, vetiver grass hedges reduce soil erosion, conserve soil moisture, and trap sediment on a site. Although other species have been shown to effectively stabilize soil, vetiver grass, because of its extraordinary and unique morphological and physiological characteristics, is superior to other tested systems.

Vetiver is a C_4 tropical grass, native to south India. The genus *Vetiveria* belongs to the Andropogoneae tribe (Hitchcock, 1971). *V. zizanioides* is one of the 12 known species in the genus (Dafforn, 1996). Wild forms of *V. zizanioides* from Pakistan to the South China Sea produce abundant fertile seeds and form a genetically-diverse group called North India vetiver (Dafforn, 1996). These plants are not useful for erosion

control, because they do not form dense clumps, but they might also be potentially invasive. *V. zizanioides* from south India has a greater root mass, forms a dense crown, and only reproduces vegetatively. Those cultivars are called "South India" or "nonflowering" types because they rarely flower and have never been known to set fertile seed. South Indian origin cultivars do not produced seeds by natural pollination or hand pollination and they could only be maintained by vegetative propagation (Ramanujam and Kumar, 1962). Jack Harlan in Dafforn (1996) has stated that vetiver grown for essential oil is a domesticated plant; it can not survive in the wild. Truong (1996b) reported that vetiver from the south India group has unique morphological characteristics that make it an idea erosion barrier. The plants are perennial, have stiff stems, form dense clumps, and have a deep root system--3 to 4 meters. They do not have rhizomes or stolons. Studies have showed that vetiver is well adapted to pH values from < 4 to 11, temperatures from -10 °C to 45 °C, prolonged droughts, inundation, and high salinity (Truong, 1996b). The remarkable plasticity of this plant includes a strong resistance to periodic drought. Experiments with greenhouse plants have shown extreme drought tolerance. After 50 days without watering under controlled conditions, the xylem pressure potential of experimental plants was recorded at -67 bars (Nu Nu Wai, unpublished data). These experimental plants recovered when they were re-watered after the experiment. According to the U. S. National Research Council (1993), vetiver's climatic limits seem to be remarkably broad, but more research is needed to define them. Dafforn (1996) has suggested that vetiver should be considered a primary plant resource and that it should receive the same research focus and development as other major crops.

Figure 1. Vetiver grass [Vetiveria zizanioides (L.) Nash.].

Water stress is a dominant environmental factor influencing the growth and survival of tropical grasses (Ludlow, 1976). Many areas where vetiver grass is likely to be grown for soil erosion control practices are in monsoon climates, semi-arid tropics, and subtropics that have a seasonal rainfall varying from high to low. Most of these areas have a hot and dry summer after the rainy season, resulting in water shortage during the remainder of the year. Therefore, vetiver plants grown in these areas might be subjected to broad ranges in water availability throughout the year, from prolonged flooding to severe drought. Seasonal leaf water potentials as low as -10.0 MPa have been recorded in some C_4 grasses (*Panicum maximum* var. *trichoglume, Hetropogon contortus, Cenchrus ciliaris* cv. Biloela) under such environmental conditions (Ludlow, 1980). Although tropical grasses show tolerance to a broad range of water stresses, seasonal drought severely limits the growth in savannas (Baruch, 1994). Patterns by which plants respond to water stress differ from species to species (Beadle et al., 1973). Information on water relations in vetiver is very limited, and little is known about its physiological responses to water deficits.

Parameters that have been extensively used to study plant-water relations are: total leaf water potential, relative water content, bulk modulus of elasticity, transpiration rate, and leaf diffusive resistance (Kramer and Boyer, 1995; Levitt, 1980; Slatyer, 1967). These physiological responses represent ways that plants can resist water deficit and indicate the degree of plant resistance to water stress (Nash and Graves, 1993; Hsiao, 1973; Kramer and Boyer, 1995; Levitt, 1980). They are also used as screening criteria for drought resistance in many plant species (Matin et al., 1989). Leaf water potential has been widely accepted as a basic measure of plant water relations (Hsiao, 1973). Leaf water potential is a quantitative measure for moisture stress, becoming more negative as

water deficit develops (Slavik, 1974). Total water potential in plants can be generally defined as a sum of pressure potential and osmotic potential (Levitt, 1980; Milburn, 1979; Slatyer, 1967). The potential gradient between plants and soil is a driving force that causes water movement through the soil-plant system (Kramer and Boyer, 1995). Therefore, total water potential or the maximum leaf water potential in plants is a basic parameter in need of study to better understand plant water relations. The maximum leaf water potential should be measured on well-watered plants at predawn when stomata are closed and the plants have mostly recovered from transpiration stress (Richter, 1976). Predawn or maximum leaf water potential in plant varies with species (Table 1). Predawn and midday leaf water potential are measured to see the behavior of the plants regarding transpiration and water uptake, development of water stress, and plant recovery from midday stress (Slatyer, 1967).

Relative water content is a commonly-used indicator for plant water status because it is easy to measure on various plant materials (Slavik, 1974). The relationships between relative water content and water potential differ with species, and it might explain a part of the plant's responses to environmental conditions (Hsiao, 1973). Under stress conditions, a species with higher relative water content at given water potential is more drought resistant (Kramer and Boyer, 1995). Studies have shown that the maximum relative water content is a useful criterion to differentiate between drought-resistant and drought-susceptible cultivars (Matin et al., 1989). For reasonable comparisons on the degree of drought resistance among different studies, Levitt (1980) suggested that a species with higher relative water content at water potential of -1.5 MPa is more drought resistant.

Table 1. Maximum (predawn) and minimum water potential in various plant species.

Species	Water potential (MPa)		Reference
	Maximum	Minimum	
Zea mays	-0.45	-1.7	Sanchez-Diaz and Kramer, 1971; Ludlow, 1976
Sorghum vulgare	-0.64	-1.7	............”............
Panicum maximum	>-0.6	-1.2	Ludlow, 1976; Levitt, 1980
Astrebla lappacea		-5.0	Ludlow, 1976
Panicum virgatum	>-0.2	<-6.4	Knapp, 1984
Andropogon gerardii	>-0.2	<-6.2	Knapp, 1984
Andropogon scoparius	>-0.2	<-6.6	Knapp, 1984
Dactylis glomerata	-0.2	-1.6	Volaire et al.,1998
Lolium perenne	-0.3	-1.5	Volaire et al.,1998
Hordeum vulgare	-0.1		Matin et al., 1989
Pennisetum setaceum		-2.5 to -3.5	Williams and Black, 1994
Heteropogon contortus		-3.0 to -4.0	Williams and Black, 1994
Cotton	-0.8	-1.6	Mauer, 1977
Soybean	-0.2	-2.4	Mauer, 1977
Sunflower	-0.2	-1.8	Mauer, 1977
Tomato	>-0.7	-1.9	Slatyer, 1967; Levitt, 1980

The bulk modulus of elasticity (cell wall elasticity) and bulk parameter of water relations (osmotic potential at full and at zero turgor), and relative water content at maximum turgor and at turgor loss point, are useful tools in studying plant responses to water stresses (Cheung et al., 1975). The Pressure-Volume Curve technique can be used to calculate the elasticity of cell walls and those bulk parameters (Tyree and Hammel, 1972). The cell wall elasticity is measured to estimate the rate of changes in water content with respect to the changes of water potential (Kramer and Boyer, 1995). There is evidence that plants with an elastic cell walls (low elasticity) have a larger decrease in water content than plants with rigid cell walls (high elasticity), causing a unit change in water potential (Sanchez-Diaz and Kramer, 1971; Slatyer, 1967). A large decrease in water potential by slight dehydration gives an advantage to plants, allowing them to extract water from the soil without severe dehydration (Kramer and Boyer, 1995). Cheung et al. (1975) stated that the low osmotic potential at full turgor helps maintain turgor under water stress and the osmotic pressure at zero turgor indicates the limit of water potential where a plant still maintains turgor. Therefore, the differences in cell walls properties and bulk water relations among the plants (Table 2) might result the different plant response to water deficits.

Transpiration rate is a measure of the rate of water loss from the leaves, and leaf diffusive resistance is a counter pressure that plants exert to prevent water loss from the leaves. Transpiration is a cause of water stress in plants (Gates, 1976), but leaf diffusive resistance, mainly stomatal, is a major physiological control of water loss (Ludlow, 1976). Both are associated with stomata closure, and thus depend on leaf water potential (Hsiao, 1973). In addition to leaf water status, water vapor content of the atmosphere might be important to stomata function (Slatyer, 1967). Therefore transpiration and leaf

Table 2. Osmotic potential at full turgor ($\Psi\pi^{100}$), osmotic potential at zero turgor ($\Psi\pi^{0}$), and bulk modulus of elasticity of cell walls ($\in$) calculated from pressure-volume curves for different forage grasses (MPa).

Species	Osmotic Potential		$\in$	Reference
	$\Psi\pi^{100}$	$\Psi\pi^{0}$		
Andropogon gerardii	-1 to -2	-3.0	39.1	Barker et al., 1993; Knapp, 1984
Panicum virgatum	-1 to -2	-2.8	45.6	Barker et al., 1993; Knapp, 1984
Sorghastrum nutans	-0.9		36.1	Barker et al., 1993
Andropogon scoparius	-2.3	-3.1		Knapp, 1984
Hyparrhenia rufa	-1 to -2		4 to 8	Baruch and Fernandez, 1993
Trachypogon plumosus	-1 to -2		5 to 10	Baruch and Fernandez, 1993
Bromus inermis	-2.0		0.5 to 8	Barker et al., 1993;
				Bittman and Simpson, 1989
Agropyron cristatum			0.29	Bittman and Simpson, 1989
Leymus angustus			0.70	Bittman and Simpson, 1989
Agropyron desertorum	-2.2	-2.7		Frank et al., 1984
A. smithii	-1.8	-2.2		Frank et al., 1984
A. intermedium	-1.7	-2.1		Frank et al., 1984
Heteropogon contortus	-1.3	-1.5	25.0	Williams and Black, 1994
Pennisetum setaceum	-1.0	-1.1	19.0	Williams and Black, 1994

diffusive resistance might be influenced by atmosphere vapor pressure deficit (Slatyer, 1967; Kramer, 1971; Levitt, 1980). The relationships between leaf diffusive resistance and leaf water potential might show the nature of stomatal response to water deficit (Hsiao, 1973). The minimum and maximum leaf diffusive resistance varies with species (Table 3).

Measurements of plant responses to water deficits have been used in many studies to estimate drought resistance (Klar et al., 1978; Matin et al., 1989; Moustafa et al., 1996; Salih et al., 1999; Williams and Black, 1994). Studies have suggested that leaf water potentials could be used to differentiate between drought-resistant and drought-susceptible cultivars (Levitt, 1980; Matin et al., 1989). For possible comparisons between different studies, Levitt (1980) suggested an absolute measuring system for drought resistance: the water potential that kills 50% of plant tissues is the drought resistance value and a species that tolerates lower water potential without injury is more drought resistant. Therefore, water potential measured over a range of values is useful for predicting plant responses to drought stress (Johnson, 1978).

Studies have not been done on water relations and drought resistance in vetiver grass. The objective of this study was to analyze the plant water relations of vetiver grass by measuring leaf water potential, relative water content, bulk modulus of elasticity of cell walls and bulk water-relation parameters, transpiration rate, leaf diffusive resistance, and to estimate the theoretical limit of drought resistance in experimental plants. Results from this study might provide preliminary data to better understand plant water relations in vetiver grass and its physiological performance in response to drought under field conditions.

Table 3. Minimum (open stomata) and maximum (closed stomata) leaf diffusive resistance values in various plant groups and species.

Species	Leaf diffusive resistance(s cm^{-1})		Reference
	Minimum	Maximum	
Cultivated species	0.5 to 3	6 to 52	Mauer, 1977
Wheat	0.6 to 2.4		Kramer, 1971
Zebrina	1.5		Kramer, 1971
Sugar beet	1.6 to 1.8		Kramer, 1971
Cotton	1.0		Kramer, 1971
Zea mays	1.0 to 2.0		Sanchez-Diaz and Kramer, 1971
Sorghum vulgare	1.0 to 2.0		Sanchez-Diaz and Kramer, 1971
Hordeum vulgare	0.01 to 0.3		Matin et al., 1989
White pine	40 to 50		Kramer, 1971
Pteridium aquilinum	200 to 400		Kramer, 1971
Pinus banksiana	8	300	Mayo J., pers. comm.

MATERIALS AND METHODS

Plant Materials and Growing Conditions

Vetiver grass used in this study was obtained from the Plant Materials Center, Galliano, Louisiana. Plants were grown in plastic pots (20 cm x 20 cm, height x diameter) filled with Cornell mixture (Hanan et al., 1978) in a greenhouse for multiplication of planting materials. The plants were watered daily and given 30 grams of N: P: K fertilizer (8:8:8) once a month. Individual clones from the stock plants were grown in pots (25 cm x 25 cm, height x diameter) with Cornell mixture and were watered daily. The greenhouse day temperatures ranged from 27 to 43 $^{\circ}$C and night temperatures ranged from 18 to 27 $^{\circ}$C. Relative humidity was varied from 32% to 60%, and a 12 h photoperiod was maintained throughout the study. Bone meal (4-12-0) at a rate of 30 grams per pot was first applied 15 days after planting and every 45 days thereafter. All water-relations experiments were performed in the laboratory and dry-down experiments were conducted in the greenhouse to test the drought resistance of the species.

Xylem Pressure Potential

Predawn and midday xylem pressure potentials (XPP) of the experimental plants were measured in July 1999, three months after planting. The pressure chamber method (Scholander et al., 1965) was used to measure the XPP of the experimental plants. I randomly selected 10 plants to provide 10 replications for predawn and mid-day measurements. Measurements were performed three times on each replicate (07-18-99, 07-26-99, 08-05-99), and the mean values of XPP at predawn and at midday were recorded. I also measured the XPP of experimental plants on 07-19-99 and the data were

compared to the predawn and midday XPP measured on 07-18-99 to estimate the recovery of the plants from transpiration stress. To test the differences among XPP of the experimental plants under different growing conditions, the mean predawn XPP recorded in the August 1999 trial was compared to the XPP of the December 1999 and February 2000 trials. All measurements were made on the third leaf from the top of the plant near the center of each pot. I cut the leaf at the middle (approximately 50-70 cm from the tip) and leaf samples were placed immediately in a cooler lined with damp paper towel. The excised leaf was inserted through a rubber stopper that was fitted to the cover of the pressure chamber (PMS Instrument Co., Corvallis, OR). After placing the sample in the pressure chamber, nitrogen gas pressure was increased at a rate of 0.025 MPa s^{-1} (Slavik, 1974). Xylem pressure potential was determined as the balance pressure required to express sap from the cut surface of the leaf. Predawn XPP was measured between 05:00 and 05:30, and midday XPP was measured between 11:00 and 13:00. Average predawn and midday temperatures during the experiment were 27 $^{\circ}$C and 38 $^{\circ}$C, and relative humidity was 60% and 41%, respectively.

Relative Water Content (%)

Relative water content (RWC) was calculated using the leaf disc saturation method after Slavik (1974). The third leaf from the top of a plant was used to determine RWC. I removed five leaf discs (0.8 cm diameter) from each leaf with a punch and immediately weighed them to record the fresh weight of each disc. The discs were saturated with water in a polyfoam-lined petri dish that had individual holes for each disc. I removed the discs from the polyfoam after four hours of saturation and reweighed

them to determine their turgid weight. Finally, the discs were oven dried at 75 °C for 48 hours and a dry weight was recorded. I calculated RWC using the following equation:

$$\%RWC = [(\text{fresh wt} - \text{dry wt}) / (\text{turgid wt} - \text{dry wt})] \times 100.$$

Maximum Relative Water Content

I sampled the leaf from well-watered plants at predawn to determine the maximum RWC of the experimental plants. One leaf from each of five randomly-chosen pots was tested. I removed leaf discs and followed the procedures previously mentioned to calculate percent relative water content. The mean RWC calculated in this experiment was noted as the maximum relative water content of the experimental plants.

Xylem Pressure Potential (XPP) and Relative Water Content (RWC)

To test the relationship between XPP and RWC, I calculated RWC at different levels of XPP. The xylem pressure potential of excised leaves was measured using a pressure chamber, and RWC was determined following the leaf disc saturation procedures previously mentioned. I chose five pots of well-watered plants at random, and sampled five leaves from each pot. One leaf from each pot was placed on a bench as a group. The groups were allowed to dry for obtaining different XPP. Xylem pressure potential of the samples in a group was measured and RWC was calculated at 2-hour intervals throughout the experiment.

Bulk Modulus of Elasticity of Cell Walls and Bulk Parameters of Water Relations

Bulk modulus of elasticity (ϵ) was calculated from the Pressure-Volume Curve

(PVC) technique (Tyree and Hammel, 1972). I used a pressure chamber to generate the

PVC. Bulk parameters of water relations, i.e., the original volume of the symplast

(intercellular volume = V_0), osmotic potential at full turgor ($\Psi\pi^{100}$), osmotic potential at

zero turgor ($\Psi\pi^{0}$), and volume of the symplast at zero turgor (incipient plasmolysis = V_p)

were estimated from the PVCs. A total of 14 PVCs were tested in two groups. The first

group of ten PVCs was generated in July and August, using leaves of 3- to 4-month-old

plants. The second group of four PVCs was generated in January 2000 from the leaves

of 8- to 9-month-old plants. I sampled one leaf from each pot to generate PVCs. During

the experiment, greenhouse average midday temperature ranged from 24 to 43 °C and

average relative humidity ranged from 32% to 60%.

Pressure-Volume Curve (PVC)

Sampled leaves were rehydrated for 24 hours under saturation conditions and low

light in the laboratory. A hydrated leaf was enclosed inside a pressure chamber except

for the cut end, which protruded through an airtight seal into the open air. I used

compressed air for the gas pressure in the chamber. The air pressure of the chamber was

increased until fluid flowed out of the leaf. The fluid was collected in a weighing bottle

using a piece of highly absorbent material and the quantity expressed was recorded. The

pressure was lowered until fluid neither flowed in nor out the leaf. At this point the

pressure was noted as balancing pressure, P. The above process was repeated and each

time the volume increment and the new balancing pressure were recorded. The data were

plotted as 1/P against the total volume expressed (V_e) at each balancing pressure. Total

osmotic pressure at full turgor ($\Psi\pi^{100}$) was read where the extrapolation of the linear part

of the PVC reached $V_e = 0$. Total volume expressed at the turgor loss point or relative

water content at zero turgor was read at the point which the non-linear part of the PVC

became linear (V_e'). The osmotic pressure at V_e' was recorded as the osmotic potential at

zero turgor ($\Psi\pi^0$). The extrapolation of the linear part of the PVC to 1/P = 0 gave the

value of V_0, which is the volume of the original symplast.

Cell Wall Elasticity

To estimate the bulk modulus of elasticity of cell walls ($\in$), each V was

calculated as ($V = V_0 - V_e$) and V_p was calculated as ($V_p = V_0 - V_e'$). The difference between

osmotic pressure (π) and balancing pressure for each V was read as the volume average

turgor (VAT). The VAT values were plotted against $(V - V_p)/V_p$ on log-log graph paper.

Cell wall elasticity was read at the point where the extrapolation of the VAT to

$(V - V_p)/V_p = 1$.

Transpiration Rate and Leaf Diffusive Resistance

A transpiration rate (q) was calculated in order to calculate leaf diffusive

resistance (LDR). I calculated transpiration from the rate of water loss of the leaf, using

a simple potometer (Slavik, 1974). The potometer was assembled using a 10 ml beaker

and a rubber stopper that fit the opening of the beaker. Plastic tape was used to provide

an vaportight seal for the beaker and stopper assembly. I randomly selected twenty pots

from a total of 40 pots from the greenhouse. The third leaf from the top of each plant

was used in the measurements. Leaf tips, 20 to 25 cm long, were removed seven days

before the samples were taken for measurements. After removing the tips, the adjacent

20 to 25 cm length of the leaves was cut and immediately placed in a cooler lined with

moist paper towels to prevent moisture lost. The potometer was filled with water, each

cut leaf was added, and the whole assembly was weighed using an analytical balance

(Mettler H 54 AR, Alfie Packers, Inc., Omaha,NE). The potometer assembly was placed

in a desicator (30 cm diameter) with either water or appropriate salt solutions [Ca $(NO_3)_2$,

KCl, or KOH] for humidity control. Leaf and air temperatures were measured using fine

wire (0.1mm Cu-Constantan) thermocouples. Leaf temperature was measured before the

samples were placed in the desicator and air temperature was recorded at the same time.

A small laboratory fan was calibrated to apply 45 cm s^{-1} velocity of wind in the

desiccator. The weight of the potometer assembly was recorded again after 1-2 hrs to

determine the amount of water loss. The areas of the leaves were also measured and

water loss rate was calculated as transpiration rates (q) in $\mu g cm^{-2} s^{-1}$.

Leaf diffusive resistance (LDR) was calculated using the following equations

after Mayo and Ehret (1980):

[1] $R_L = [(C_{sat} - C_a)/q] - R_a$

Where R_L = leaf diffusive resistance (s cm^{-1})

C_{sat} = saturation absolute humidity at leaf temperature

C_a = absolute humidity at air temperature

q = transpiration rate

R_a = estimated leaf boundary layer resistance

[2] $R_a = (\propto)^a / D_j$

      Where D = the diffusivity of water vapor

      $(\propto)^a$ = boundary layer thickness estimated from Eq. 3:

[3] $(\propto)^a = 0.4 (L_{leaf} / V_{wind})^{1/2}$

      Where L = the leaf dimension in the down wind direction

      V = wind velocity (applied in determination of transpiration rate)

Minimum Leaf Diffusive Resistance

To calculate the minimum leaf diffusive resistance, I randomly chose five pots of well-watered plants for sampling. I sampled four leaves from each pot at predawn and the samples were rehydrated for two hours in the laboratory. Five leaves, one from each pot, served as five replicates for each measurement. The transpiration rate was measured under 100% relative humidity. I repeated the measurements for four times on different days to determine the mean LDR of a replicate and the overall mean LDR of the experimental plants.

Maximum Leaf Diffusive Resistance

Maximum leaf diffusive resistance was measured on excised leaves sampled from well-watered plants at 15:00 hr on a hot and dry day ($43^{\circ}C$ and relative humidity of 36% in the greenhouse). I sampled the leaves from nine pots, one from each pot, to provide nine replications. Samples were brought to the laboratory and the transpiration rate for each sample was immediately determined under 100% relative humidity.

Xylem pressure potential (XPP) and Leaf Diffusive Resistance (LDR)

To examine the relationship between XPP and LDR, I measured these values for excised leaves. Two treatments were used to stress the leaves for different XPP.

1. Dry-down on bench in the laboratory: I sampled five leaves, one from each of five pots, and placed them in five groups that included one leaf from each pot. Leaf samples were dried, XPP of each sample was measured, and LDR was calculated for each group at 2-hour intervals throughout the experiment.

2. Mannitol treatment: I used different concentration of mannitol solution to stress the leaves to the different levels of XPP (from -0.1 to -0.21 MPa). A total of 10 leaves, one from each pot, served as 10 replicates for the experiment, and this experiment was repeated three times.

Vapor Pressure Deficit (VPD) and Leaf Diffusive Resistance (LDR)

Leaf diffusive resistance was measured under different degrees of VPD to find the response of LDR to VPD. I used KCl, Ca $(NO_3)_2$, and KOH saturated salt solutions to control the level of relative humidity in the desiccator to 85%, 55%, and 8%, respectively. Leaf diffusive resistance measured at 100% relative humidity served as a control. I sampled the leaves and grouped them using the same procedure as in 'Minimum Leaf Diffusive Resistance' but measurements were made at a different time of the day. The duration of the rehydration period in this experiment was 24 hours.

Drought Resistance

To estimate the degree of drought tolerance, I measured xylem pressure potential (XPP) of the experimental plants as the plants were allowed to dry until 50 % of leaves on the plants died (Ludlow, 1976). I measured XPP using the pressure chamber method as previously described. Three trials of the greenhouse dry-down experiment were conducted in August (1999), December (1999), and February (2000). The August and December trials included 10 pots each and the February trial included nine pots. Pots were randomly selected from 40-60 pots of greenhouse-grown plants. Each pot represented a replication for the water stress treatment. I included five pots of well-watered plants as a control group in the experiment. Predawn XPP of these experimental plants were measured on the first day of the experiment, and the plants were then exposed to water stress by withholding water. Xylem pressure potentials were measured at nine-, seven-, five-, and three-day intervals during the dry-down period. XPP was also measured on the day when plants first showed wilting symptoms. Experimental plants were re-watered when 50% of leaves on plants died (visual estimates). XPP in the control group was also measured on the same schedule to provide a comparison to the treatment group.

Greenhouse average day/night temperatures during the experiment were approximately $41^{\circ}C/32\,^{\circ}C$ in August; however, day/night temperatures were a constant at $27\,^{\circ}C$ in December and February. Average relative humidity was approximately 41% in August and 34% in December and February. A 12 h photoperiod was maintained throughout the experiment.

Statistical Analyses

SigmaStat version 2.0 (Jandel Scientific Co.) computer software package was used for descriptive statistics and all statistical analyses. Data were tested for normality and equal variances. If a data set failed either test, non-parametric statistics were used. Repeated measures analysis of variance (RMANOVA) was used to compare the differences among XPP measured on different times and days. The differences between means were analyzed for statistical significance with Kruskal-Wallis one-way analysis of variance on ranks followed by all pairwise multiple comparison procedures in the appropriate cases for each experiment. Regression analyses were used to state the relationships between XPP and LDR, and between XPP and RWC. Means of xylem pressure potentials for control and water-stressed treatments were compared using Mann-Whitney tests. Statistical significance was assumed at $P < 0.05$. Results were reported as mean ± one standard error.

RESULTS

Under well-watered conditions, the leaf xylem pressure potential (XPP) of the experimental plants was recorded as 0.092 ± 0.005 MPa at predawn, and the XPP decreased to 0.93 ± 0.036 MPa at midday (n = 10). The predawn XPP recorded on July 18,1999 was not significantly different from the predawn XPP recorded on the next day, July the 19th (P = 0.897). However, the two values were significantly different from the midday XPP of July the 18th (P < 0.05), indicating that the experimental plants had fully recovered from midday transpiration stress at the next dawn. There was no statistical difference among predawn XPPs measured on July 18, 26, and August 5, 1999

recorded in July 1999 was higher than those recorded in December 1999 and February 2000 (P<0.05). However, no significant difference was found between the XPPs measured in the latter two trials.

Relative water content (RWC) in well-watered plants ranged from 90.4 to 95.8%, and the mean of the maximum RWC for the experimental plants was 93.24 % ± 0.52% (n = 10). At the XPP of -2.0 MPa, the RWC of 73.66% was recorded from the experimental plants. The RWC of the leaves decreased linearly with its xylem pressure potential (Fig. 2, $y = 1.4039x + 98.872$, $r^2 = 0.917$, n = 5).

Bulk modulus of elasticity of the cell walls ($\in$) and bulk parameters of water relations were estimated from pressure volume curves (PVCs). An example PVC obtained for this species is presented (Fig. 3) because all replicates were similar. The values of $\in$ independently estimated from each of 14 PVCs ranged from 0.05 to 0.9 MPa, with a mean of 0.352 ± 0.076 MPa (Fig. 4). Elasticity values measured in July and August 1999 were not significantly different from those measured in January 2000. High variability was found in estimation of symplast volumes from PVCs. Mean cell volume at full turgor or the original volume of the symplast (V_0) in the experimental plants was 270.9 ± 69.9 µg, and the mean cell volume of 110.01 ± 31.14 µg was obtained at turgor loss point or incipient plasmolysis (V_e'). The mean RWC at the turgor loss point was 40.1% (n = 14). Mean osmotic potential at full turgor ($\Psi\pi^{100}$) and at zero turgor ($\Psi\pi^{0}$) were -1.787 ± 0.069 MPa and -2.722 ± 0.053 MPa, respectively.

Transpiration rate (q) of excised leaves varied with the different degrees of vapor pressure deficit (VPD). The mean transpiration rate of 0.242 ± 0.04 µg cm^{-2} s^{-1} (n = 5) and 0.362 ± 0.01µg cm^{-2} s^{-1} (n = 5) were obtained under 100% and 8% relative humidity, respectively. There was a negative exponential relationship between transpiration rate and XPP (Fig. 5). The minimum LDR of excised leaves was 1.47 ± 0.27 s cm^{-1} (n = 5) and maximum LDR was greater than 127.3 s cm^{-1} (ranged from 127.3 to 315.68 s cm^{-1}, n = 9).

Figure 2. Relationship between the relative water content and xylem pressure potential of

Vetiveria zizanioides (y = 1.4039x + 98. 872, r^2 = 0.917, n = 5). Each point

represents the mean relative water content of three leaf discs.
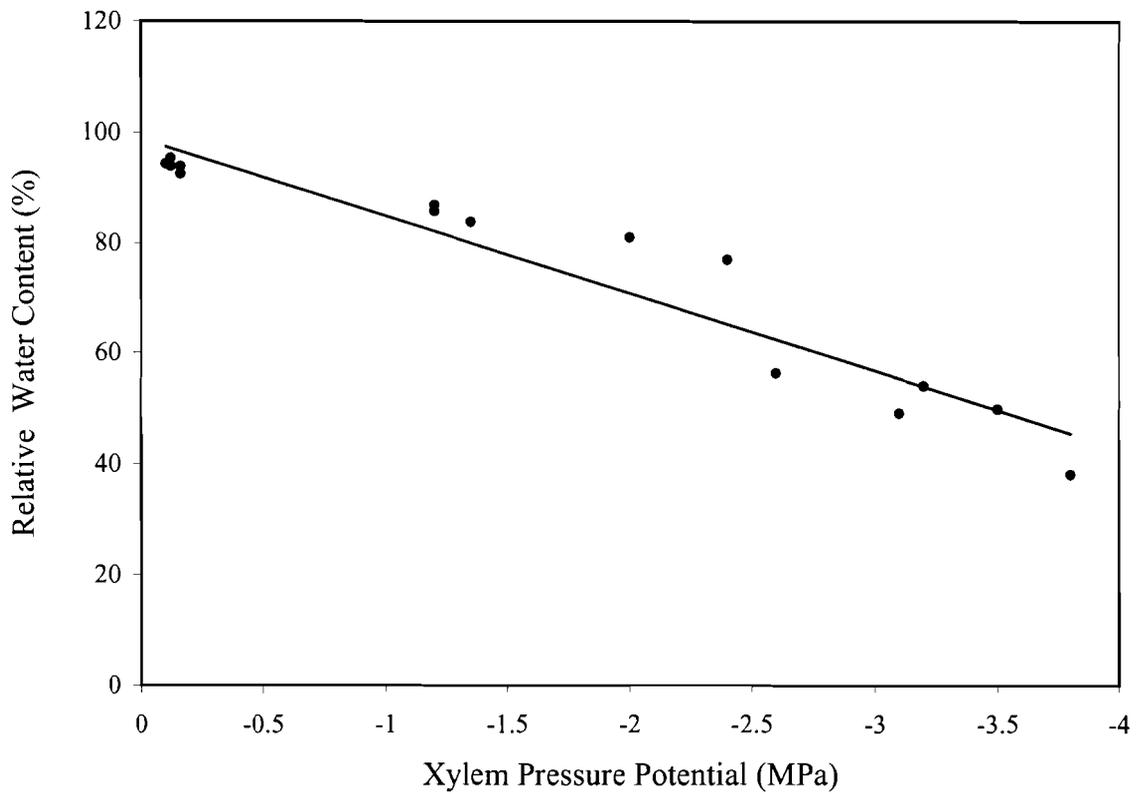
Figure 3. Pressure-volume curve obtained for *Vetiveria zizanioides* by using the pressure-chamber method. The curve is the best of fit line for the three replicates generated in July 1999 ($y = 0.9941x^{-0.924}$, $r^2 = 0.77$).
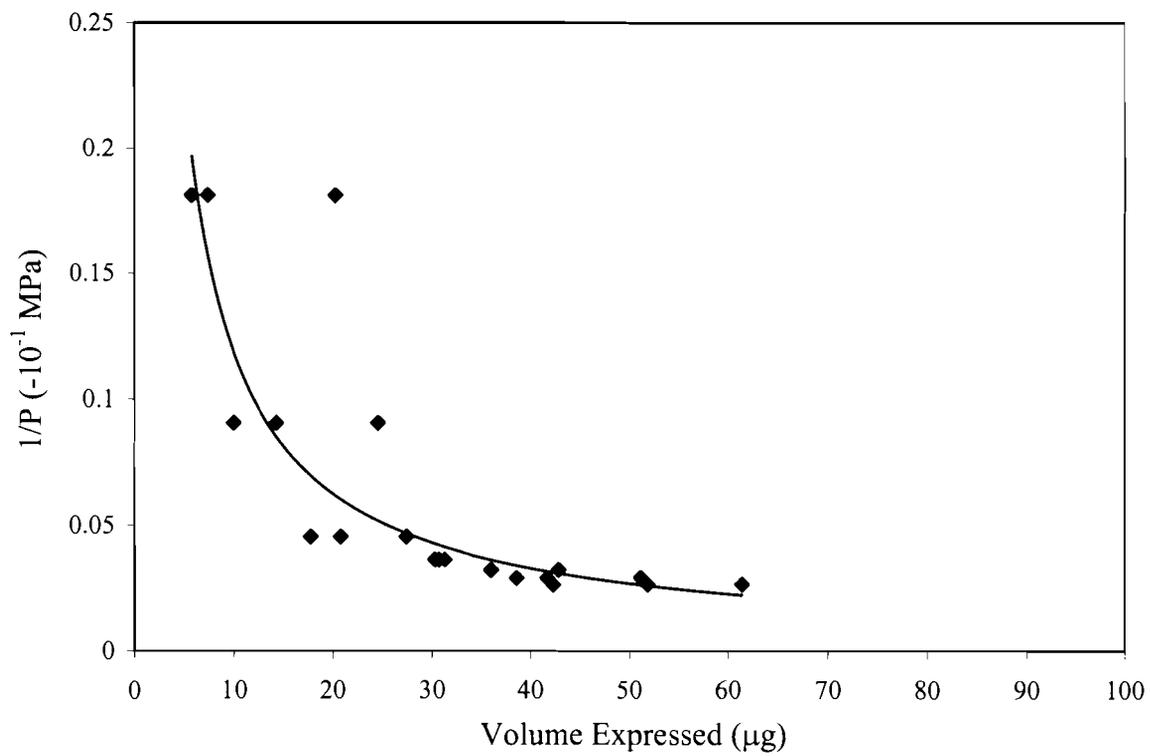
Figure 4. Cell wall elasticity ($\in$) of *Vetiveria zizanioides* estimated from the dependence

of volume averaged turgor (VAT) on the relative volume of the symplast

(V-Vp/Vp). Each point represents the mean value of the VAT and V-Vp/Vp

estimated independently from 14 pressure-volume curves.
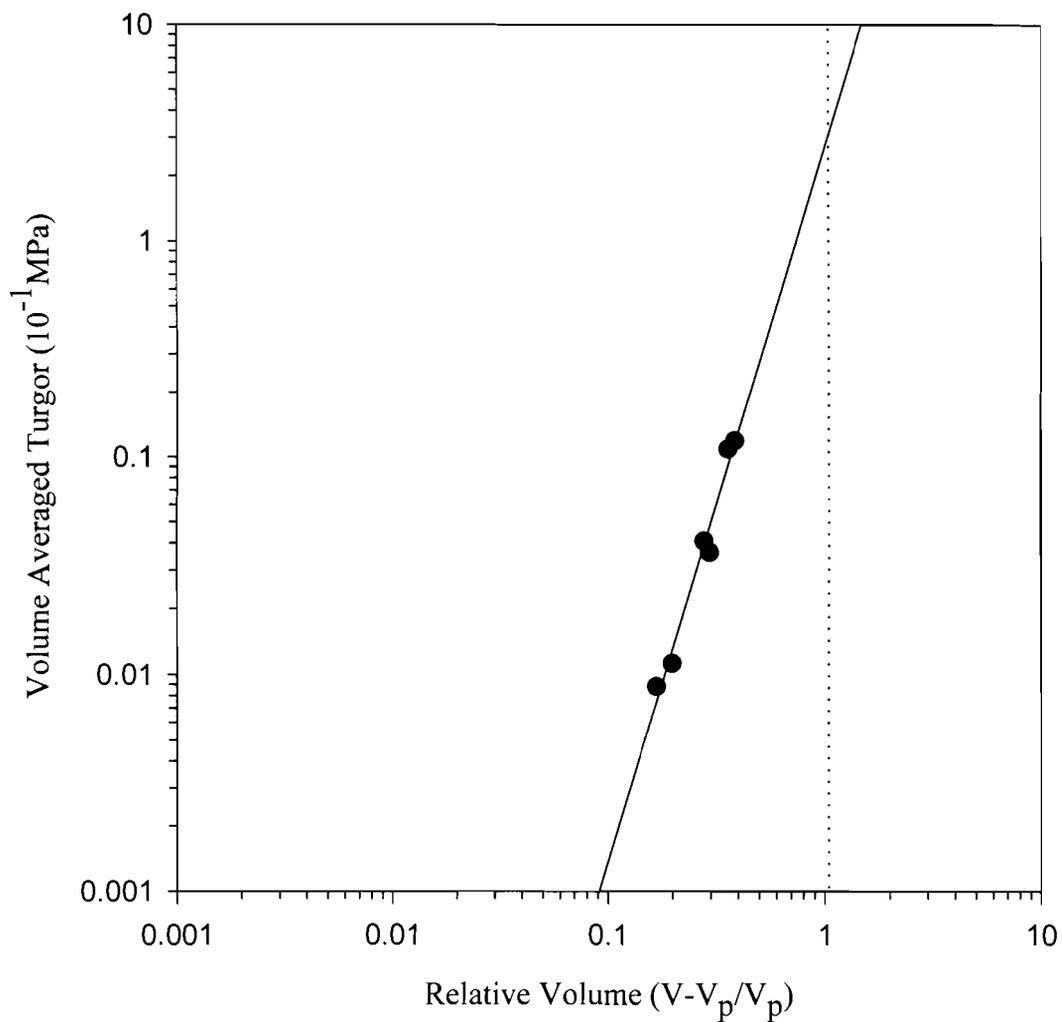
Figure 5. Relationships between transpiration rate and xylem pressure potential for

Vetiveria zizanioides (y = 0.3856e $^{0.3629x}$, r^2 = 0.88, n = 5).
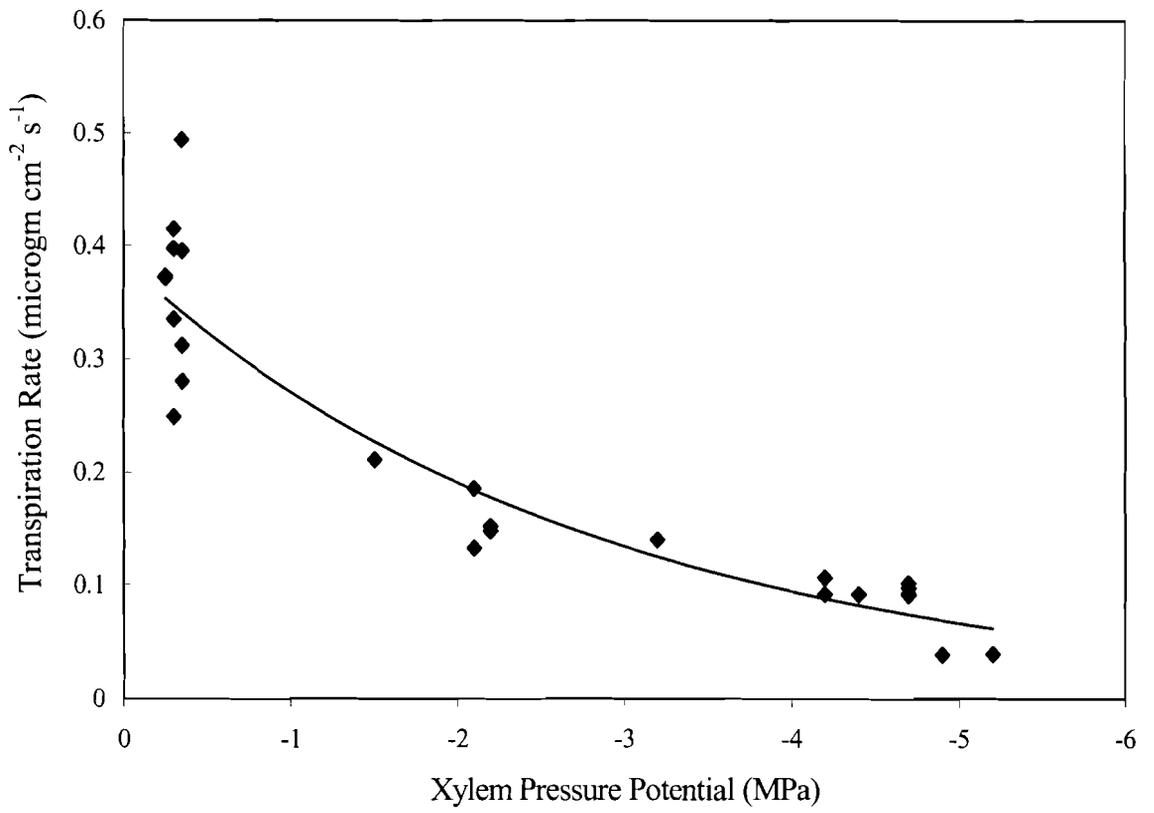
A larger variation among the data was found where transpiration rates of the leaf samples were low. Leaf diffusive resistance of the plants increased with (decrease in relative humidity) vapor pressure deficit (Fig. 6, $y = 4.394x^2 + 10.549x + 51.867$, $r^2 = 0.98$, $n = 20$) and increased as xylem pressure potential became more negative (Fig.7, $y = 9.477e^{-0.033x}$, $r^2 = 0.88$, $n = 5$). The changes in LDR in response to XPP were small over the range of XPP -0.1 to -2.0 MPa, but LDR increased rapidly where the XPP reached about -3.5 to -3.8 MPa. There was no consistent correlation between LDR and water potentials when leaf samples were subjected to osmotic stress with Mannitol solution (Fig. 8, $y = -0.036x + 12.84$, $r^2 = 9E - 05$, $n = 10$).

The mean predawn XPP of the experimental plants ranged from -0.08 MPa to -0.3 MPa under well-watered conditions and from -23.0 to <-67.0 MPa (pressure chamber limit) at the end of the stressed periods. Stress symptoms were not found in the plant for the first 7 to 9 days after withholding water (predawn XPP higher than -2.0 MPa). However, visible wilting symptoms in young leaves were found when the predawn XPP of the plants reached -2.2 MPa. About 50% of leaf death (visual estimates) occurred at the XPP of -4.48 ± 0.35 MPa (n = 29). This level of water stress was reached in the plants on the 15th, 20th and 21st days of stress-periods in the August, December, and February trials, respectively. At the end of the experiment, xylem pressure potentials in well-watered control groups were significantly different from water-stressed treatment groups in all trials (Table. 4).

Figure 6. The response of leaf diffusive resistance of *Vetiveria zizanioides* to atmosphere

vapor pressure deficits ($y = 4.394x^2 + 10.549x + 51.86$, $r^2 = 0.98$, $n = 20$).
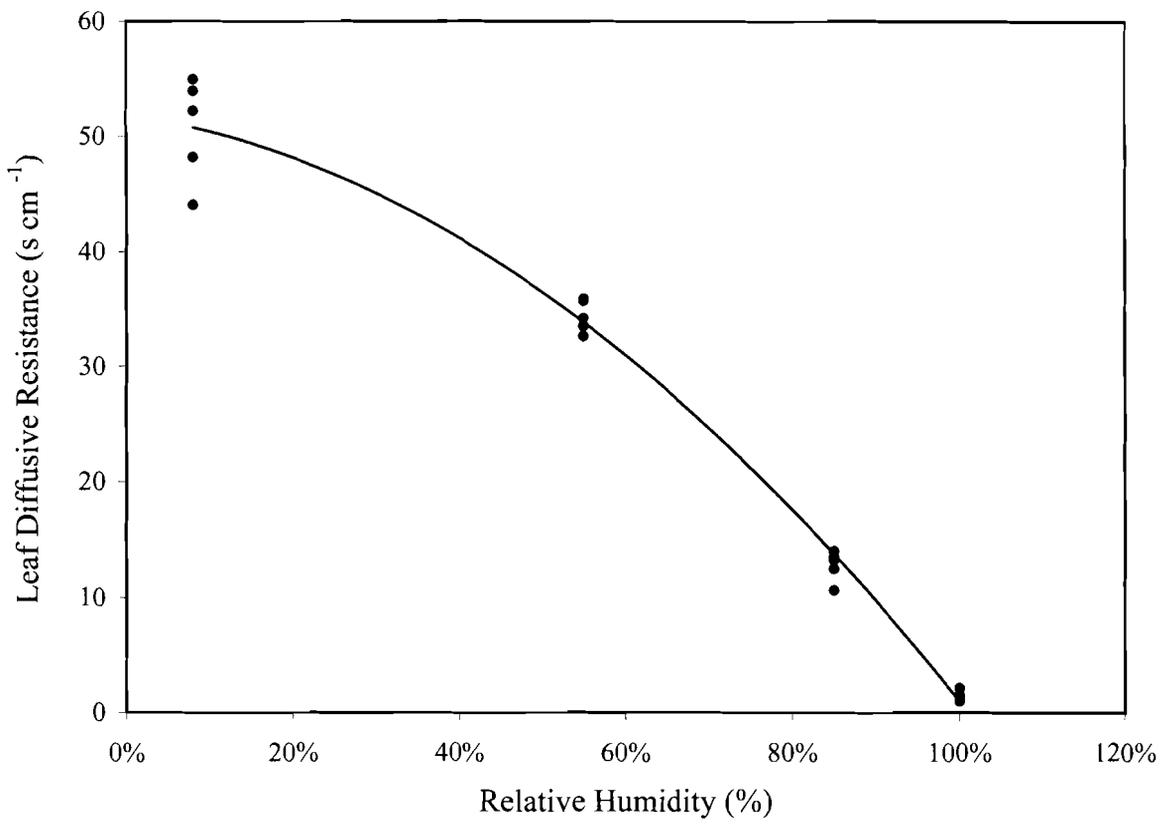
Figure 7. Relationship between leaf diffusive resistance and leaf xylem pressure

potential for *Vetiveria zizanioides* ($y = 9.477\ e^{-0.033x}$, $r^2 = 0.88$, $n = 5$).
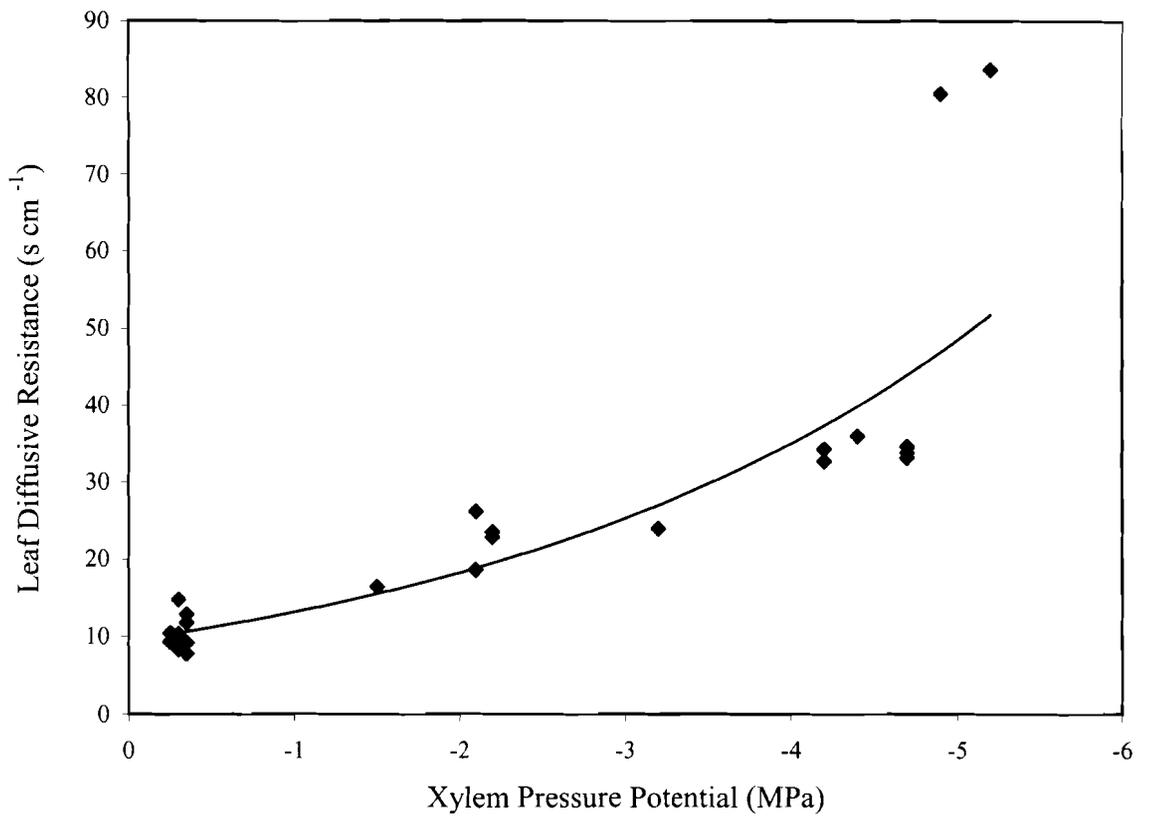
Figure 8. Relationship between leaf diffusive resistance and leaf water potential for

Vetiver zizanioides when leaf samples were stressed by mannitol solution

(y = -0.036x + 12.847, r^2 = 9E-05, n = 10).
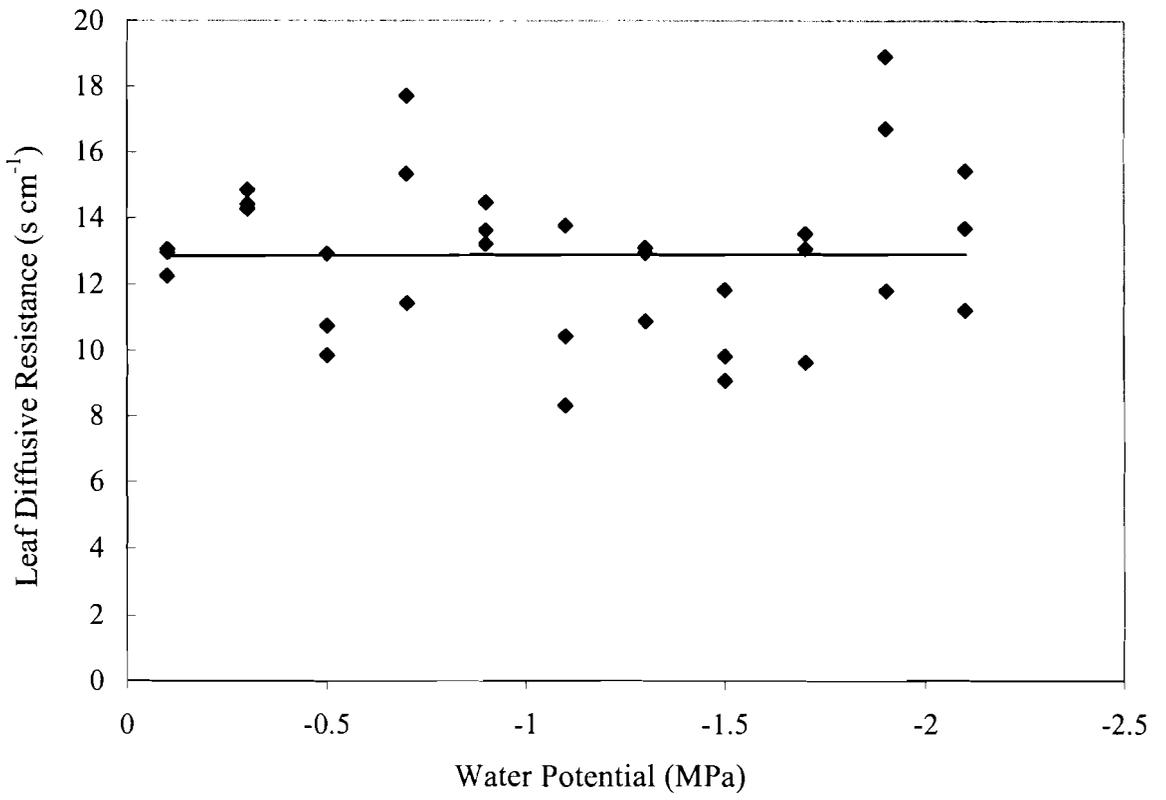
Table 4. Predawn xylem pressure potential of *V. zizanioides* under well-watered

conditions and at the end of stressed period.

| Trials | Xylem Pressure Potential (MPa) | | | |
| | Well-watered condition | | End of stressed period | |
	Control	Experimental	Control	Experimental
August (1999)	-0.102	-0.093^{ns}	-0.128	-4.210***
December (1999)	-0.200	-0.186^{ns}	-0.190	-4.200***
February (2000)	-0.230	-0.172^{ns}	-0.204	-5.100***

Experimental data represent the mean of n = 10 in the 1999 and n = 9 in the 2000 trials.

Control data are the means of n = 5

^{ns} Not significant at P = 0.05

*** Significant at P = 0.003

DISCUSSION

The water relations and physiological responses of vetiver grass from this study are discussed mostly in comparison to those reported for other grasses. It is important to note that the water relations and drought resistance of a plant species might be related to the environments to which it is adapted (Ludlow, 1976). Studies have suggested that field-grown plants generally have a lower threshold leaf water potential than the plants grown in controlled environments (Ng et al., 1975). Therefore, the comparisons among species from different studies should be interpreted with care.

Results from this study showed that predawn xylem pressure potentials (XPP) of vetiver grass, under well-watered conditions, were in the range (-0.8 to -0.18 MPa), similar to (> - 0.2 MPa) that reported for field grown switchgrass, big bluestem, and little bluestem (Knapp, 1984). Sanchez-Diaz and Kramer (1971) reported that predawn XPP of -0.4 to -0.6 MPa was found in well-watered corn and sorghum, which are genetically close relatives to vetiver grass. Midday XPP of vetiver was (-0.78 to -1.12 MPa), similar to (- 0.55 to -1.85 MPa) that has been reported for Indian grass, switchgrass, big bluestem and little bluestem (Barker et al.,1993; Knapp, 1984). Predawn XPPs of the experimental plants were consistent from day to day, indicating that the vetiver plants are able to fully recover from transpiration stress overnight. Well-watered plants in the July1999 trial had higher predawn XPP than those of December 1999 and February 2000 trials. However, a significant difference was not found between the latter two trials, where there were no differences in temperature and humidity in growing conditions. The temperature and humidity in the greenhouse (27 °C and 34%) in December and February, which were lower than the optimums for C_4 plants, might have affected the normal function of the plants.

Relative water content (RWC) is widely used to measure plant water status and its relationship to leaf water potential and has been used to estimate drought resistance (Levitt, 1980). The mean RWC in vetiver leaves whose XPPs ranged from -0.08 to -0.2 MPa was 93.24%. It was similar to the maximum RWC of corn, sorghum, barley, wheat (Beardsell and Cohen, 1974; Matin et al., 1989; Sharma and Garg, 1984), and other perennial forage grasses (Volaire et al. 1998). However, it was higher than reported for crested wheatgrass (Bittman and Simpson, 1989). Relative water content at zero turgor calculated from PVC was 40.6%. This value was much lower than the range of other C_4 and C_3 forage grasses such as switchgrass, big bluestem, Indiangrass, smooth brome, and reed canarygrass (Barker et al., 1993). Levitt (1980) stated that many grass species have little restriction of transpiration during drought and might lose 50% to 80% of plant water without suffering any damage. He also suggested that gamma grass might lose up to 98.3% of its free water without injury. Sharma and Garg (1984) reported that permanent wilting percent for wheat was 48%. Therefore, low RWC at turgor loss point in vetiver might not be a significant characteristic of the species. The relationship between its RWC and XPP was linear (Fig. 2) and the regression equation suggested that the experimental plants lost their plant water steadily at the same ratio as water potential decline. This behavior agrees with the cell wall properties of the plants.

The different behaviors of plants in response to water potential and relative water content could result from differences in cell wall elasticity ($\in$). Species with elastic cell walls have lower E values and show a larger decrease in relative water content for a unit decrease in water potential than species with rigid walls (Kramer and Boyer, 1995). Therefore, under water deficit, plants with elastic cell walls (low $\in$) maintain turgor longer than plants with rigid cell walls (high $\in$). Kramer and Boyer (1995) suggested

that species with rigid walls lower water potential by losing a small amount of water and thus generate a water potential gradient for more water uptake from the soil. Moreover, the plants lose turgor rapidly and close their stomata to prevent further water loss as water potential declines. Therefore, more rigid walls might be an important mechanism to prolong survival under drought conditions. Sanchez-Diaz and Kramer (1971) reported that sorghum lost a smaller amount of plant water content than corn for a unit decrease in water potential. The smaller reduction in the water content of sorghum is a characteristic of drought-resistant species. However, the ϵ value of vetiver grass (0.35 MPa) in this study indicated that the plant has very elastic cell walls similar to most crop species (Mauer, 1977). That value was also considerably lower than the range (8 to 61 MPa) reported for switchgrass, Indian grass, big bluestem (Knapp, 1984; Barker et al., 1993), and other perennial C_4 grasses (Pugnaire and Haase, 1996; Toft et al. 1987). An ϵ value as low as 0.35 MPa was unexpected for a drought-resistant species. However, a similar result was reported in crested wheatgrass by Bittman and Simpson (1989). According to their data, crested wheat grass--one the most drought-resistant grasses (Tadmor et al. 1970)--had a lower ϵ than that was found in smooth brome. This is evidence that a very drought-resistant species has more elastic cell walls than a less-resistant one. Bittman and Simpson (1989) concluded that greater tissue elasticity helped crested wheat grass maintain its turgor during low water potential. In addition, Knapp (1984) reported that switch grass, a more mesic grass, has more rigid cell walls than little bluestem, which is more xeric. Vetiver grass might have the same behavior as crested wheatgrass, and little bluestem in that the plant maintains turgor until a large fraction of water is lost from the plants. The ability to maintain turgor pressure as water potential declines is an important mechanism of stress tolerance in plants (Heuer and Nadler, 1998), and relatively elastic

cell walls is a characteristic of drought-resistance species (Nilsen et al., 1983). Vetiver grass characteristics correlate well with these interpretations for drought resistance.

The pressure-volume relations showed that the experimental plants lost turgor when they lost above 50% of their original cell volume. However, a large variation among replications was found in estimation of the original cell volume (V_0) and the cell volume at turgor loss point (V_e'). Those variations might, in part, be explained by the physiological differences among the leaf samples and high variability associated with quantitative estimations of internal water-relation parameters from the PVC technique (Wilson et al., 1979). The osmotic potentials at full turgor ($\Psi\pi^{100}$) and at zero turgor ($\Psi\pi^0$) in vetiver grass, as obtained from PVC analysis, were lower than reported for some forage grasses and for semi-arid species (Barker et al., 1993; Baruch and Fernandez, 1993; Knapp, 1984; Williams and Black, 1994). However, the values obtained for vetiver were similar to those of crested, intermediate, and western wheatgrass (Frank et al., 1984). Barker et al. (1993) and Bittman and Simpson (1989) suggested that the low osmotic potentials in crested wheat grass, switch grass, and big bluestem result in turgor maintenance at low water potential.

The transpiration rate of vetiver grass decreased as XPP became more negative (Fig. 5) and increased with VPD. Comparable measurement techniques and experimental conditions were not found in other studies for comparisons on maximum and minimum transpiration rates. The minimum transpiration rate of excised leaves was relatively high when compared to its transpiration rates determined under (8% relative humidity) high atmosphere vapor pressure deficit (VPD). The transpiration increased rapidly as the VPD increased to a certain degree (between 100% and 55% relative humidity) but it tended to level off before it reached the threshold level of VPD for stomatal closure.

The minimum LDR (1.47 s cm^{-1}) in the experimental plants was similar to corn and sorghum (Beadle et al., 1973), and was in the range of most agricultural species (Mauer, 1977). The leaf diffusive resistance of experimental plants increased exponentially as XPP declined (Fig. 7). The response of LDR to water potential followed a usual pattern that the LDR remains almost consistent to a certain level of water stress and increases exponentially when the threshold water potential for stomatal closure is reached (Beadle et al., 1973). The maximum LDR (>127 s cm^{-1}) was high compared to cultivated species (Mauer, 1977). It is important to note that a small error in weighing and handling can severely affect the results when the transpiration rate (q) is very small. A large variation that was found in the maximum LDR data set might possibly be more associated with human error. The data for the maximum and minimum LDR suggested that the vetiver plant keeps opening its stomata until a certain level of water stress but fully closes them under extreme environments. In addition, it indicated that transpiration rate and LDR in vetiver grass appears to be more influenced by VPD than soil moisture. Similarly, the leaf diffusive resistant of the pine tree (*Pinus banksiana*) showed a high response to VPD independent of soil water potential (Mayo. J., Pers. Comm.). Sensitive stomatal response to VPD might minimize water loss and tissue dehydration under the environmental conditions where there is a high demand for evaporation (Williams and Black, 1994). When excised leaves were subjected to osmotic stress (-0.1 to -2.1 MPa) by using mannitol solution, a significant correlation was not found between LDR and leaf water potential. This is probably because the range of stress applied to the leaf samples was not large enough to show significant changes in LDR. Another possibility is that that mannitol might enter the cell and cause less osmotic stress in the leaves. However,

there was no evidence of mannitol crossing the cell membrane in other species (Kramer, 1971; Kramer and Boyer, 1995; Mayo J., Pers. Comm.).

Most crop plants reach permanent wilting stage between the water potential of -1.5 and -2.0 MPa (Salisbury and Ross, 1992; Slayter, 1967). Vetiver plants in this study showed visible wilting symptoms at an XPP of -2.2 MPa. Its threshold water potential, about 50% of leaf death, was -4.4 MPa. These values were also in the range reported for other drought-resistant C_4 grasses (Baruch, 1994; Knapp, 1984; Ludlow, 1980; Ng et al., 1975, Toft et al., 1987). It should be noted that values for vetiver grass were recorded from potted plants with a relatively large leaf area and limited area for root growth. Vetiver grass in the field has very deep and well-developed root systems as compared to many other herbaceous plants (National Research Council 1993). Jones et al. (1980) suggested that root system development is associated with drought resistance in Bermuda grass. Deep rooting systems might increase the drought hardiness of prairie grasses (Knapp 1984). Therefore, vetiver grass might have better performance in resisting drought under field conditions.

In summary, vetiver grass in this study showed that the predawn and midday XPP, and drought resistance of the plants were in the range reported for field grown switchgrass, Indian grass, big bluestem, and little bluestem. The experimental plants have a very low $\in$ value, which was unexpected for drought-resistant species. The osmotic potential of the plants was low as compared to many C_4 forage grasses. Low osmotic potential and elastic cell walls might help the vetiver plant to maintain turgor at low water potential. Relative water content in the experimental plants was similar to those of most C_4 grasses, but the plant lost turgor at lower water content. Plants lost water steadily as water stress developed, and this behavior correlated well with cell wall

properties of the plant. The transpiration rate and leaf diffusive resistance of vetiver plants in this study appear to be more influenced by atmosphere vapor pressure deficit than leaf water potential. Changes in LDR in response to XPP were not consistent when mannitol solution was used to stress the leaves. The drought resistance of vetiver grass was in the range that reported for switchgrass, Indian grass, big bluestem, and little bluestem. However, care should be taken in interpreting the results as the environment to which the plants adapted might influence their physiological responses.

CONCLUSIONS

Vetiver grass possesses most physiological characteristics of drought-resistant C_4 grasses in plant water relations and response to water stress. The drought resistance of the vetiver grass is also similar to those grasses. However, the ability of vetiver grass surviving under drought conditions might be more associated with mechanisms other than stomata sensitivity to water deficits. The most important physiological attributes for its drought resistance might include 1) the ability to maintain turgor at low water potential, 2) low osmotic potential to help maintain turgor, and 3) sensitive stomatal response to leaf-air vapor pressure deficit limiting transpiration under severe moisture stress. These characteristics might favor the growth and survival of vetiver grass in semi-arid and arid environments.

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APPENDICES

Appendix A. One Way Repeated Measures Analysis of Variance for Plant Recovery from Ttranspiration Stress (Statistical software: SigmaStat 2.0).

Normality Test: Passed (P = 0.522)

Equal Variance Test: Failed (P = 0.036)

Group	N	Missing
07/18 Dawn	10	0
07/19 Dawn	10	0
07/18 midday	10	0

Group	Mean	Std Dev	SEM
07/18 Dawn	0.910	0.173	0.0547
07/19 Dawn	0.920	0.262	0.0827
07/18 midday	11.200	2.463	0.779

Power of performed test with alpha = 0.050: 1.000

Source of Variation	DF	SS	MS	F	P
Between Subjects	9	20.347	2.261		
Between Treatments	2	705.209	352.604	180.627	<0.001
Residual	18	35.138	1.952		
Total	29	760.694			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0.001). To isolate the group or groups that differ from the others use a multiple comparison procedure.

All Pairwise Multiple Comparison Procedures (Tukey Test):

Comparisons for factor:

Comparison	Diff of Means	p	q	P<0.05
07/18 midday vs. 07/18 Dawn	10.290	3	23.290	Yes
07/18 midday vs. 07/19 Dawn	10.280	3	23.267	Yes
07/19 Dawn vs. 07/18 Dawn	0.01000	3	0.0226	No

Appendix B. One Way Analysis of Variance for Predawn Xylem Pressure Potential of
Vetiver Grass Measured in 07/ 99, 12/99, and 02/00 (Statistical software:
SigmaStat 2.0)

Normality Test: Failed (P = 0.029)

Test execution ended by user request, ANOVA on Ranks begun

Kruskal-Wallis One Way Analysis of Variance on Ranks
Data source: Data 1 in Notebook

Group N   Missing
07/99   10   0
12/99   10   0
02/99   9   0

Group	Median	25%	75%
07/99	0.850	0.800	1.000
12/99	1.600	1.500	2.000
02/00	1.500	1.150	2.125

H = 16.181 with 2 degrees of freedom. (P = <0.001)

The differences in the median values among the treatment groups are greater than would
be expected by chance; there is a statistically significant difference (P = <0.001)

To isolate the group or groups that differ from the others use a multiple comparison
procedure.

All Pairwise Multiple Comparison Procedures (Dunn's Method) :

Comparison	Diff of Ranks	p	Q	P<0.05
12/99 vs 07/99	14.200	3	3.761	Yes
12/99 vs 02/00	2.267	2	0.584	No
02/00 vs 07/99	11.933	2	3.077	Yes

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07 - 20 - 00
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Date

Water Relations in Vetiver Grass [*Vetiveria zizanioides* (L.) Nash.]
Title of Thesis Project

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Signature of Graduate Office Staff

August 1, 2000
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Date Received