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Identification of soybean cultivars tolerance to waterlogging through analyses of leaf nitrogen concentration

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IDENTIFICATION OF SOYBEAN CULTIVARS TOLERANCE TO WATERLOGGING THROUGH ANALYSES OF LEAF NITROGEN CONCENTRATION

A Thesis

Submitted to the Graduate Faculty of the
Louisiana State University and
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by
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Geaux Tigers!

2003 National Champions!

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ABSTRACT

Irrigation to soybean can cause unintended water to pond on the soil surface for more than a desired amount of time. Most soybean acreage in Louisiana is planted on poorly drained clay soils and waterlogging of soybean can cause substantial yield reductions. Although cultural practices are available for ameliorating the adverse effects of waterlogging, little is known about genotypic tolerance, therefore our objectives were to determine if percent leaf nitrogen concentration could be used as a criterion for screening for cultivar tolerance to waterlogging and to identify waterlogging tolerance among 48 commercially available soybean cultivars.

Forty-eight soybean cultivars were planted in May in 2002 and 2003 in an open- ended outdoor greenhouse at Ben Hur Research Farm near Baton Rouge, Louisiana on a Mhoon clay soil. Flooding treatment commenced the day the plants reached V4 and continued for seven consecutive days. Drained and waterlogged treatments were administered in the two halves of the greenhouse. One site received a 1-week waterlogging stress at the V4 growth stage and the other treatment received normal irrigation as necessary to avoid job stress. Each site was randomized complete block design with four replications and one factor (cultivars). Data obtained from the two year study were percent leaf nitrogen, leaf dry weight, and leaf nitrogen uptake. Analysis of variance was done by the combined analysis method of McIntosh (1983) with treatments and cultivars being fixed factors. Mean separation was accomplished by LSD ($P<0.05$) using appropriate LSD values to compare specific cultivars between drained and waterlogging treatments or to compare cultivars within or across drained and waterlogged treatments.

Results suggested that percent leaf nitrogen concentration can be an effective parameter for screening for waterlogging tolerance. Both cultivar and drainage significantly ($P<0.0001$)

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affected yield without significant interactions with other factors. The low C.V. (7.6%) shown by

percent leaf nitrogen also supports its use as a screening criterion. Cultivars showing greatest

percent nitrogen were not consistent across treatments. The decline in percent leaf nitrogen

between treatments was not consistent. No correlation occurred for cultivar percent leaf nitrogen

between treatments.

INTRODUCTION

Waterlogging is defined as ponding of water over an area of crop land (Scott and

Batchelor, 1979). It can be induced by an abundance of rainfall or supraoptimal irrigation,

especially in areas where land is poorly leveled. Since ancient times, soybean [(*Glycine max)*

(L.) merr.] has been used as a food crop. It originated in northeast China and has been grown for

thousands of years in that country. Soybean was introduced to the US from China in the 1880's.

Until World War II, soybean was grown predominately in the Midwest as a forage crop. After

World War II soybean developed into an important oil and feed crop because of its high oil and

protein content. The soybean seed has an oil content of 18 to 20% and a protein content of 38 to

40%. This made it a good crop to use as a livestock feed because of the high well-balanced

protein content; and also as an oil crop for cooking oil, margarines, and other edible products. In

the 1940's and 1950's, soybean production shifted from into seed to forage uses. By 1955, 94%

of the soybean crop in the US was grown for seed. As the crop grew in importance, grower

organizations such as the American Soybean Association were formed. Such organizations

fostered exports of US soybean to Japan, Europe, and other parts of the world. Exports grew to

where they comprised 35 to 40% of total US production (Morrison and McCormick 1996).

Although originally grown in the Midwest, soybean production started in the southeastern

US in the 1960's and grew rapidly during the 1970's. By the early 1980's, soybean acreage in

Louisiana had grown to approximately 1.2 million hectares. However, hectarage has been

steadily declining from this peak to where less than 400,000 hectares are currently grown. This

decline was caused by low prices and low yields. Lower prices resulted from increased foreign

competition, mainly from Brazil and Argentina. Brazilian and Argentine production was further

stimulated by the grain embargo placed on the USSR by President Carter in 1979 because of the Soviet invasion of Afghanistan.

According to the National Agricultural Statistics Service (NASS), Midwestern states including Illinois, Iowa, Minnesota and Wisconsin have average soybean yields of 3024 kg ha^{-1}. For 2002 Louisiana reported an average of 2150 kg ha^{-1}. The result of this 874 kg ha^{-1} decrease is attributed to several things. Low yields for soybean in Louisiana and other southeastern states compared to the midwest result from a variety of biotic and abiotic stresses. The most important of these are greater weed, insect and disease pressure. These biotic stresses are greater in the southeastern USA because of the mild winters and hot humid summer growing conditions in this region compared to the midwest. Other stresses explaining lower yield in the southeastern USA include drought, acid soils and extensive hard pans that restrict rooting to the upper zone of the soil. Thus, soybean farmers in the southeast have greater input costs and lower yields compared to farmers in the Midwest (Morrison and McCormick 1996).

Additional, waterlogging is a common production problem for soybeans in Louisiana and other southeastern states (Scott et al., 1989). This is largely due to the heavy rainfall and poor drainage encountered in many areas where soybeans are grown on clayly soils. Waterlogging of a field for two or more days reduces crop growth rate and can potentially reduce yield. Waterlogging reduces crop growth rate by replacing the soil's air with water, therefore depriving the plant's roots of oxygen. Since oxygen diffuses much more slowly through water than air, the roots soon become deprived of oxygen and are unable to maintain normal respiration. Although several abnormalities result from this rapid oxygen deprivation by soil microbe, yield loss appears linked most closely to reduced nitrogen uptake (Bacanamwo and Purcell, 1999, Bennett and Albrecht, 1994 and Kozlowski, 1984). Although cultural practices are known for alleviating

or preventing waterlogging stress (e.g. planting on raised beds, sloping the field, and precision land grading), little information is available to farmers concerning cultivar tolerance to waterlogging. Therefore, the objectives of this research were to:

1. Determine if leaf nitrogen concentration could be an effective cultivar screening method.

2. Use the appropriate screening method to identify soybean cultivars tolerant to waterlogging.

LITERATURE REVIEW

General Effects of Waterlogging

Plants become affected in a number of ways when a soil becomes flooded. Air within the soil pore spaces has an oxygen content similar to that of the atmosphere (about 20%) (Pezeshki, 1994). When a soil becomes flooded, the ordinary air in the pore spaces of the soil is replaced with water, greatly restricting the flow of oxygen through the soil. The small amount of oxygen left in the soil is quickly depleted by root and soil microorganisms' respiration. Soil oxygen depletion is accelerated under warmer soil temperatures, because oxygen becomes less soluble in water as temperature rises. Pezeshki (1994) states that once the soil becomes anaerobic, adverse effects on plants occur such as chlorosis, reduced growth rate, disruption of cell membranes, adverse effects on mineral uptake, altered growth regulator relationships, stomatal closure, leaf wilting and epinasty, reduced photosynthesis and respiration, altered carbohydrate partitioning, and potentially death. Some plant species, such as rice, are able to morphologically and/or physiologically adapt to these changes in such a way that injury is avoided. A plant such as rice has a well-developed aerenchyma tissue. Aerenchyma is tissue having large intercellular spaces resulting from parenchyma cells that have swollen and then split open (Esau 1965). This allows oxygen to diffuse from the shoot into the roots, thus maintaining normal growth. Formation of aerenchyma tissue is caused by cortex breakdown (lysigenous) or by cell separation (schizogenous). Both of these processes produce gas-filled lacunae, which helps the plant survive (Kawase 1981). For example in corn (*Zea maize*), cell lysis occurs primarily in the cortex and not in surrounding cells (Drew et al., 1979 and Campbell and Drew, 1983). In contrast, soybean is considered a flood-sensitive plant and undergoes severe injury when waterlogged. Although soybeans develop aerenchyma tissue when waterlogged stress occurs,

yield will be reduced (Pezeshki, 1994). Other flood sensitive crops in addition to soybean

include wheat (*Triticum aestivum*) (Sojka et al., 1975), cotton (*Gossypium hirsutum*) (Sojka and

Stolzy, 1980) and numerous other species (Kozlowski, 1984). For all crops listed above flood-

induced stomatal closure has been documented and supported by Oosterhuis et al. (1990).

Linkemer et al. (1998) identified some vegetative stages sensitive to waterlogging as well as

some reproductive stages being more sensitive than other stages of reproductive growth. A 93%

yield reduction was found when soybeans were waterlogged for seven days at the R3 growth

stage. The two other vulnerable stages of growth occurred at R1 or R5 and at V2 when soybeans

were waterlogged for seven days. Yield losses were reported to be 67% for R1 and R5 while

waterlogging at V2 reported a yield loss of 30%, which was a significant loss in comparison to

the rest of the growth stages (Linkemer et al., 1998).

Effects of Waterlogging on Soils

Air displacement of the soil by water in combination with warm soil temperature often

leads to lower levels of dissolved oxygen in the rooting zone (Meek et al., 1983 and Smith et al.,

1983). When a soil becomes waterlogged, there is an inhibition of gas diffusion from the

atmosphere that results in hypoxia and subsequent anoxia surrounding the plant's root zone

(Daugherty and Musgrave, 1994). Yoshida and Tadano (1978) state that when a soil becomes

absent of molecular oxygen (redox potential values are relatively insensitive to changes in

dissolved oxygen between 0.21 and 0.0021 atm (Patrick, 1980)), there are a number of other

compounds utilized by soil microorganisms as alternative electron acceptors for respiration. The

first and most important is the compound nitrate, which is reduced to nitrite, followed by nitrous

oxide and finally molecular nitrogen. Other electron acceptors are the manganese oxides.

Exchangeable and water-soluble Mn^{+2} can increase to toxic levels a couple of days when

conditions such as acid soils high in organic matter and manganese oxides are present. After several days of waterlogging, Fe^{+3} becomes reduced. Sulphate is reduced to H_2S in waterlogged soils, which decrease the solubility of zinc, copper, iron and cadmium by the formation of sulfides (Ponnamperuma, 1972; Jackson, 1983; Nathanson et al., 1984; Horst, 1986; Mortvedt et al., 1991).

When a soil becomes flooded, three types of aerobic-anaerobic zones of the soil exist: A top layer of oxygenated water and the anaerobic soil matrix, which consists of the very top portion of the soil, the soil rhyzosphere (area of plant rooting) and finally the subsoil that is oxidized (area below the roots) (Ponnamperuma, 1985).

The upper surface layer becomes oxidized by mineral matter and plant debris from supernatant water and other soil substances that are moving upward from the anaerobic zone. This upward diffusion enriches the soil to 1 cm of the soil surface with organic matter, iron, nitrogen, phosphorus, manganese and other substances (Harrison and Alyer, 1915; DeBout et al., 1978).

With as little as a few hours of flooding, an air-dry soil becomes depleted of oxygen. Aerobic bacteria are replaced by facultative anaerobes, which ultimately will lead to strict anaerobes (Yoshida, 1978). At this time bacteria are predominate while fungi and actimomycetes are suppressed. Anaerobic bacteria prevailing in the oxygen depleted zone of flooded soils are responsible for the following changes: denitrification; reduction of iron, manganese and sulfate; methane formation and inhibition of nitrogen fixation (Yoshida, 1978; Watanabe, 1984). Nitrogen fixation is inhibited by waterlogging. Oxygen is required in plant respiration, thus when plants undergo waterlogging and anaerobic conditions exist, the glycolytic pathway produces pyruvic acid from glucose, which is converted to ethyl alcohol and carbon

dioxide. Waterlogging causes this process to be catalyzed in production of ATP from each glucose molecule. Thus, ATP synthesis and other energy-dependent processes critical for plant functioning are slowed down (Pezeshki, 1994).

In agricultural soils, there is a symbiosis of legumes and bacteria of the genera *Rhizobium,* which are responsible for fixing nitrogen. These bacteria is fast growing and capable of producing acid. The bacteria will infect the cortical cells and roots hairs of legumes and ultimately form nodules on roots. The nodule formation serves as the site of nitrogen fixation. A symbiotic relationship between the host plant (soybean) and the bacteria (*Rhizobium*) is created with the bacteria obtaining carbohydrates for energy and the soybean receiving nitrogen fixing compounds (Brady and Weil, 1996).

Altered Water Relationships During Waterlogging

An early response of waterlogged plants is stomatal closure and reduced water flux from roots to shoots. This occurs not only in soybean, but also in alfalfa, broad bean, corn, and tobacco. The mechanisms involved are complex and not fully understood. However, efflux of potassium ions from the guard cells surrounding the stomata in addition to increased levels of absisic acid due to waterlogging is a possible explanation for stomatal closure. Waterlogging reduces the uptake of potassium, and this combined with slower water flux from root to shoot, can reduce potassium levels in the guard cells resulting in stomatal closure. Reduced water flow to the leaves may result in epinasty, caused by increased levels of ethylene, in waterlogged soybeans taking on a wilted appearance. This, however, can be alleviated by stomatal closure. Wilting may last for various lengths of time depending on air temperature, the degree of stomatal closure, root resistance to water movement, and relative humidity (Pezeshki, 1994).

Alterations in Growth Regulator Relationships

Waterlogging results in an imbalance in plant growth regulators. Growth regulators made in the roots (cytokinins and gibberellins), will decline, whereas those made in the shoots (auxins, ethylene, and abscissic acid) accumulate in the leaves and stems (Reid and Bradford 1984). A common response to waterlogging is increased abscissic acid in the leaves, which may play a role in the stomatal closure described above. Watterlogging may also be related to reduced leaf growth. Leaf growth is a result of cell expansion from turgor pressure against the cell wall. Other research has also implicated increased ethylene as a factor in stomatal closure. Both ethylene and/or abscissic acid have been shown to cause changes in guard cell membranes, which interfere with water and/or ion efflux into and out of the guard cells. They are also involved in elevated carbon dioxide concentration, which also causes stomatal closure. Stomatal reopening is rapid after short periods of waterlogging, but is prolonged if stress is for an extended period of time (Pezeshki, 1994).

Another growth regulator effect of waterlogging is increased ethylene which causes production of adventitious roots. This is a morphological response that sometimes helps a plant survive waterlogging. Bacanamwo and Purcell (1999) state that ethylene also induces aerenchyma tissue development for plant survival. The beneficial effects of adventitious roots are an increased root surface exposed to air (the roots appear above the water line), increased aerobic respiration, and oxidation of the rhizosphere. Consequently, some of the adverse effects of waterlogging on root function are ameliorated (Coutts and Armstrong, 1976).

Other growth regulator changes induced by waterlogging are shoot epinasty (attenuation of leaves and stems), hypertrophy or swelling at the base of the stem, leaf chlorosis, and premature leaf senescence. Epinasty has been implicated with increased ethylene production. Ethylene

also causes swelling and lysis of cells in roots and stems in order to form aerenchyma tissues in order to help the plant survive. Auxin and gibberellins may also be involved. Hypertrophy may also be related to increased ethylene. Hypertrophy facilitates adventitious root development thus increasing aeration to the plant. Leaf chlorosis and premature senescence may be caused by decreased transport of gibberellic acid and cytokinins to the leaves, since these growth regulators are important in maintenance of membrane integrity. Reduced nitrogen transport from roots to shoot will also cause chlorosis by reducing protein and chlorophyll synthesis (Pezeshki, 1994).

Photosynthetic Effects and Crop Growth Rate

In response to flooding, soybean shows a drastic and rapid reduction of net photosynthesis (Oosterhuis et al., 1990). Pezeshki (1994) states that in bean, net photosynthesis was reduced to near zero in response to seven days of flooding. Also, flooding for only one day reduced photosynthesis 17% as compared to controlled conditions. This reduction in photosynthetic rate is attributed partly to stomatic and non-stomatic effects. Other crop plants have similar responses. Net photosynthesis in bean was reduced to near zero in response to seven days of flooding. Relative to control conditions, beans undergoing waterlogging stress for one day reduced photosynthesis 17% and caused a reduction in plant dry weight (Pezeshki, 1994). Different results have been reported in the literature concerning the roles of stomatal closure and non-stomatic effects. In several cases, direct cause and effect relationships have been difficult to establish. For example, decreased photosynthetic activity may either be a cause for stomatal closure or an effect of stomatal closure. Decreased photosynthetic activity associated with the onset of waterlogging may also be related to reduced leaf chlorophyll content. Another possible cause of waterlogged-induced reductions in photosynthetic activity is decreased enzyme activity of ribulose carboxylase, the enzyme responsible for CO_2 fixation (Pezeshki, 1994).

Because 90% of plant dry weight results from the carbon and oxygen fixed during photosynthesis (Taiz and Zeiger, 1998), there is a strong correlation between canopy photosynthetic rate and crop growth rate. Crop growth rate is the rate of crop dry matter accumulation as measured by grams of dry matter accumulated by the crop per day per square meter of crop land ($g\ m^{-2}\ d^{-1}$). Thus, the cumulative effects of reduced canopy photosynthetic activity is suboptimal crop growth rate and reduced yield (Griffin and Saxton, 1988; Scott et al., 1989). Yield loss results from reduced pod production (Linkemer et al., 1989). Field observations have determined that crop growth rate is usually affected only when the waterlogging stress is applied for more than two days (Scott et al., 1989). Sensitivity of yield to reduced crop growth rate induced by waterlogging is apparently during the early reproductive period (flowering and pod formation stages). The early reproductive stages of soybean growth is more sensitive relative to other periods of growth (Griffin and Saxton, 1988; Scott et al., 1989).

Mineral Nutrition Effects

Mineral deficiencies commonly result when plants are waterlogged. Mineral uptake by plants is an energy-requiring process. The oxygen deprivation to the roots results in decreased root respiration, the source for Adenosine Triphosphate (ATP), the high-energy compound required for mineral uptake. Adenosine Triphosphate supplied by anaerobic respiration is not adequate to provide energy for mineral uptake. Another adverse effect of anaerobiosis on mineral uptake is decreased permeability of cell membranes in the roots which may cause nutrient leaching. Not only is root function (mineral uptake) inhibited by waterlogging, but root growth is also inhibited. This further adds to the inability of the roots to meet the mineral requirements of the shoot. The threshold oxygen concentration in the soil's air at which root

elongation is affected is believed to be around half of the concentration in normal air (i.e. 10%) (Pezeshki, 1994).

Soon after waterlogging commences, soybean demonstrates a reduced nitrogen concentration in its leaves (Bacanamwo and Purcell, 1999). Sufficiency levels for nitrogen in soybean leaves falls in the range of 4 to 5% (Jones and Benton, 2002). Waterlogging will result in a decreased nitrogen concentration below the sufficiency level ($< 4\%$). In addition, flooding results in reduced soil nitrogen through rapid volatilization and denitrification. In soybean, nitrogen deficiency is amplified by inhibition of nitrogen fixation by waterlogging. Phosphorus and potassium levels in the plant are also reduced by waterlogging (Pezeshki, 1994). Calcium and magnesium concentrations are also reduced, but not as much compared with phosphorus and potassium. Waterlogging results in increased uptake of some minerals. Anaerobic conditions result in reduced forms of manganese and iron, which are taken up by the plant more easily compared with the oxidized forms.

Morphological Mechanisms of Flood Tolerance

Some plants have developed specific anatomical structural characteristics that allow them to tolerate waterlogging. Such plants can survive and function under stressful conditions. Such features include aerenchyma tissues and adventitious root development. Adventitious roots reduce the adverse effects of waterlogging on the plant by increasing the root area exposed to the air, increasing aerobic respiration, and oxidizing the rhizosphere. Aerenchyma is parenchyma tissue with large and abundant intercellular spaces. Such tissues allow air to enter the shoot, travel down to the roots through the aerenchyma tissue, and supply oxygen so that normal root function is maintained. Such a system is what allows rice to flourish in a flooded field. Aerenchyma tissue development is a process caused by cell wall separation which results in

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breakdown of the cortex leaving empty spaces called lacunae that can carry air (Pezeshki, 1994).

Oxygen may enter through lenticels and/or leaves. Development of aerenchyma in response to

flooding is usually associated with inceased ethylene production (Pezeshki, 1994).

MATERIALS AND METHODS

Cultural Practices

Forty-eight soybean cultivars (Table 1) were planted on May 6 and May 23 of 2002 and 2003, respectively, in an open-ended outdoor greenhouse at Ben Hur Research Farm near Baton Rouge, Louisiana. Dimensions of the greenhouse were 29 m long x 9.1 m wide. The greenhouse was subdivided into 20 containment areas, each having dimensions of 2.75 m wide x 4 m long. Each containment area was surrounded by a vinyl-coated aluminum material anchored into the ground and extending 30 cm above the ground to hold water. Seed of each soybean cultivar for both years was planted at a 2.5 cm depth in an experimental unit having a row 91 cm long and 30 cm wide. Seed was heavily sown and then thinned to a population of five plants per foot of row after emergence. Based on soil test recommendations, muriate of potash (0-0-60) was applied to each containment area at the rate of 225 kg ha^{-1} and gypsum (calcium sulfate) was applied at the rate of 100 kg ha^{-1} to correct for sulfur deficiency in both years. Plants in the drained treatment and plants in the undrained treatment, when not being flooded were irrigated once a week to prevent drought stress. Flooding treatment commenced the day the plants reached V4 (fourth leaf stage). Day 1 of the flood treatment was considered to be the next day and flooding was continued for 7 consecutive days. Water level of the flood treatments were maintained at 2.5 to 5.0 cm above ground during the 7-day period through the use of timing devices that applied water to the flooded plots four times during each diurnal cycle for designated periods of time.

Experimental Design

The greenhouse was divided into two equally-sized treatments: one treatment receiving a 1-week waterlogging stress (undrained treatment) at the V4 stage (fourth leaf) stage, and the other (control treatment) received normal irrigation as necessary (drained treatment). The drained treatment consisted of eight contiguous containment areas on the west side of the greenhouse and the undrained treatment had eight contiguous containment areas on the east side of the greenhouse. Each treatment was divided into four blocks (or replications) with each block consisting of two contiguous containment areas. Two contiguous containment areas in the middle of the greenhouse served as a buffer zone between treatments. Each treatment was a randomized complete block design with four replications and one factor (cultivars). There were 48 cultivars in the study. Forty-five of them were roundup-ready (RR) cultivars selected from the state-wide soybean cultivar trials. The remaining three were also roundup-ready, and were cultivars purported to have tolerance to waterlogging (supplied by J. Grover Shannon, University of Missouri). These three cultivars served as control for comparing the performance of other soybean cultivars. After the seventh day of waterlogging, five plants per plot were sampled and immediately moved to the cold storage rooms at Ben Hur Farm. Leaves were removed from the plants, dried at 60° C for two days until constant weight was reached and weight was recorded. Samples were then ground, placed into labeled glass vials and analyzed for percent nitrogen by the Plant Analysis Lab of the Department of Agronomy of Louisiana State University, Baton Rouge.

Data Analysis Methods

Data obtained from the two year study consisted of percent leaf nitrogen and average leaf dry weight obtained from all cultivars in both treatments (drained and undrained) on the day

following the end of the flooding treatment. Analysis of variance was obtained by the combined

analysis method of McIntosh (1983) with treatments and cultivars being fixed factors. Mean

separation was accomplished by LSD (P< 0.05) using appropriate LSD values to compare

specific cultivars between drained and undrained treatments and/or to compare cultivars within a

drained or undrained treatment.

RESULTS AND DISCUSSION

Screening Method

The list of the 48 soybean cultivars with their abbrieviated names is presented in Table 1. The

ANOVA calculated across 2002 and 2003 is presented in Table 2. Results indicated significant

(P<0.0001) waterlogging and cultivar effects on leaf nitrogen concentration. Results also

indicated significant year and waterlogging effects for nitrogen leaf uptake (N Lf uptake). All

other main effects and interactions had no significant effects on measured parameters. Based on

the lack of a significant year x drainage x cultivar interaction, drainage by cultivar treatment

combinations were averaged across years. According to ANOVA for the 2002 and 2003 test,

C.V.'s for percent nitrogen concentrations, leaf dry weight and nitrogen leaf uptake were 7.6%,

27.3%, and 29.1%, respectively. Because percent leaf nitrogen concentration showed significant

cultivar effects without interactions; whereas leaf dry weight and nitrogen leaf uptake did not;

leaf nitrogen concentration appears to be the best cultivar screening method. Another advantage

for using percent leaf nitrogen concentration is the C.V. is lower compared with the other

parameters. This indicates that variability is lower for percent leaf nitrogen concentration,

making it a more precise selection criterion relative to others.

Although the drainage x cultivar interaction was not significant, indicating that cultivar

rankings were generally similar between treatments, closer examination indicated that successful

screening should be done on the waterlogged treatment only. Correlation between percent leaf

nitrogen concentrations on the drained and waterlogged treatments was very low ($r^2 = 0.13$),

indicating little relationship for this parameter between treatments (Figure 1). Furthermore, an

examination of the percent decline in nitrogen between treatments indicated large differences

Table 1: 2002-2003 Soybean Varieties

Cultivar	Abbreviated Cultivar
1. Asgrow 4902	A4902
2. Hartz 4994	H4994
3. Terral 59R98	T59R98
4. Terral 58R11	T58R11
5. Terral 4890	T4890
6. Eagle Ranger	ERanger
7. Eagle Punch	EPunch
8. Eagle Marshall	EMarshall
9. Eagle Trooper	ETrooper
10. Deltapine 498	D498
11. Deltapine 5915	D5915
12. Deltapine 5644	D5644
13. Deltapine 5806	D5806
14. Deltapine 5414	D5414
15. DynaGro 3600	DG3600
16. DynaGro 3535	DG3535
17. Hornbeck 6020	HBK6020
18. AP/Garst 4512	AP4512
19. Southern States 5999	SS5999
20. AgriPro 4888	AP4888
21. AP/Garst 588	AP588
22. Asgrow 5701	A5701
23. Asgrow 6201	A6201
24. Pioneer 94B73	P94B73
25. Pioneer 9492	P9492
26. Croplan Genetics RC 4992	CGRC4992
27. Croplan Genetics RC 4995	CGRC4995
28. Asgrow 4403	A4403
29. Croplan Genetics RC 4444	CGRC4444
30. Deltapine 4344	D4344
31. Hartz 4884	H4884
32. Delta Grow 4950	Del4950
33. DynaGro 3463	DG3463
34. Deltapine 4690	D4690
35. Asgrow 4603	A4603
36. Hornbeck 4820	HBK4820
37. Delta King 4763	DK4763
38. Delta King 4868	DK4868
39. Delta King 4965	DK4965
40. Southern States RT 4902 N	SSRT4902
41. Hornbeck 4622	HBK4622
42. Asgrow 5901	A5901

(table con'd)

43. Pioneer 95B53   P95B53
44. Terral 52R42   T52R42
45. Terral 59R85   T59R85
46. Macon (check)   Macon
47. 8115 (check)   8115
48. Defiance (check)   Defiance

Table 2: ANOVA for percent leaf nitrogen (% Lf N), leaf dry weight (Lfdw) and nitrogen uptake (N Lf uptake) for 48 soybean cultivars grown near Baton Rouge, LA in drained and waterlogged conditions, 2002 to 2003.

ANOVA Source	Summary (df)	% Lf N	Lfdw	N Lf uptake
Year	1	1.57	30.70*	51.39*
Drainage	1	1497.67*	3.16	346.16*
Year*Drainage	1	0.40	7.66	22.79
Cultivar	47	2.76*	1.72	1.66
Year*Cultivar	47	1.13	1.48	1.45
Drainage*Cultivar	47	1.31	0.94	1.26
Year*Drainage*Cultivar	47	1.02	0.65	0.86
C.V. %		7.6%	27.3%	29.1%

* Mean square is significant at the 0.0001 probability level.

% N in Drained vs. Undrained

$y = 0.3181x + 1.2014$
$R^2 = 0.1271*$

% Leaf N in Undrained Site (y-axis)
% Leaf N in Drained Site (x-axis)

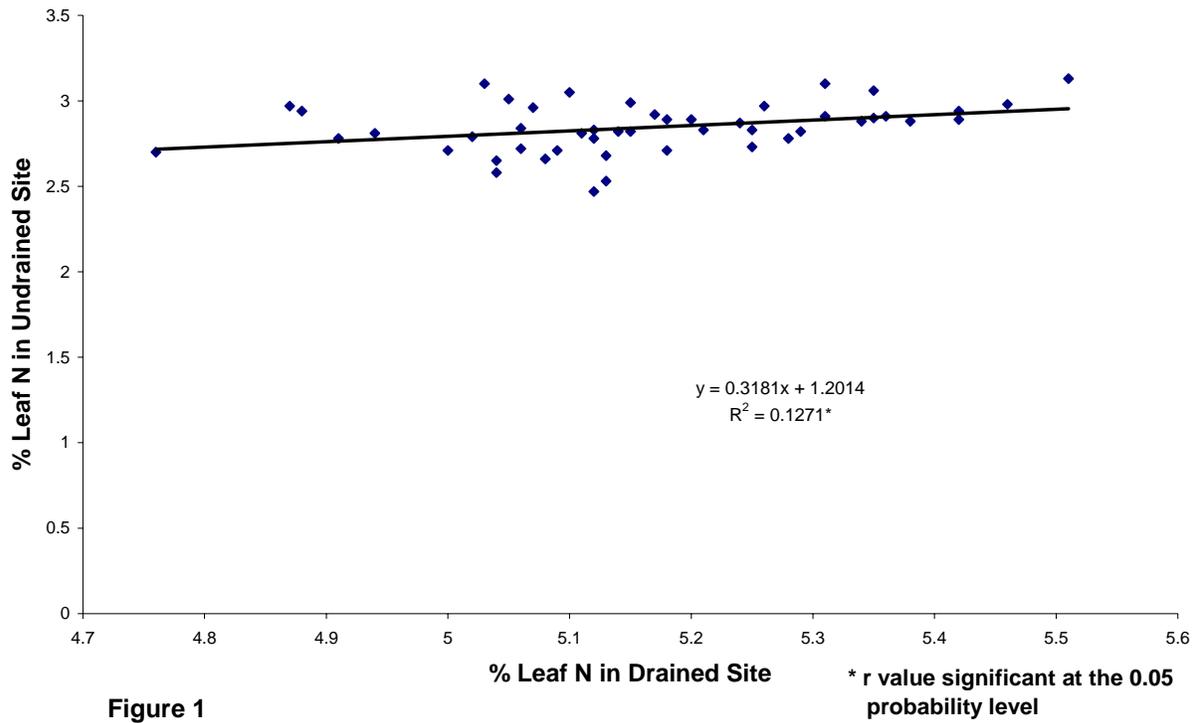**Figure 1**

* r value significant at the 0.05 probability level

between cultivars (38% to 52%) despite an insignificant cultivar effect (Tables 3 and 4). Based on these findings, we concluded that the best procedure was to screen for waterlogging tolerance on the waterlogged treatment alone. Further evidence supporting screening on the waterlogged treatment can be seen in the actual cultivar rankings between treatments (Tables 5 and 6). Cultivars showing the greatest percent leaf nitrogen concentration (levels statistically similar to the top cultivar according to LSD (0.05)) were not common to the two treatments. Out of the eight cultivars that were top-ranking in the waterlogged treatment, only Delta King 4965 was also top-ranking in the drained treatment.

Results also indicate that the seven day waterlogging treatment was sufficient for decreasing percent leaf nitrogen concentration below the critical 4% level and thus provided sufficient stress for making cultivar distinctions.

Identification of Tolerant Cultivars

Greatest leaf nitrogen concentration for the drained treatment was shown for Delta King 4965 (Table 5). Cultivars including Croplan Genetics 4444, 8115, Delta King 4868, Hartz 4884 and Hornbeck 4820 all showed leaf nitrogen concentrations statistically similar (P<0.05) to the top cultivar. Leaf nitrogen concentration for the drained treatment ranged from 4.76% (Eagle Punch) to 5.51% (Delta King 4965). Mean leaf nitrogen concentration was 5.16%. Thus, all cultivars within the drained treatment had leaf nitrogen concentration within the sufficient range of 4 to 6% (Jones and Benton, 2002). Three cultivars consisting of 8115, Macon and Defiance were included as checks with known water tolerance obtained from the University of Missouri. Cultivar 8115 was significantly similar to the top cultivar Delta King 4965 in the drained treatment for percent leaf nitrogen concentration. Cultivar 8115 reported a percent leaf

Table 3: Percent leaf nitrogen concentration drop for the drained and waterlogged site for 48 soybean cultivars and averaged across two years at Baton Rouge, LA, 2002 to 2003.

Cultivar	% Lf N Decrease Between Treatments
H4994	52
T59R98	51
T4890	49
A5701	48
CGRC4992	48
D5806	48
DG3463	48
8115	47
D498	47
EMarshal	47
H4884	47
P9492	47
T59R85	47
A4902	46
A6201	46
AP588	46
D5915	46
DK4763	46
DK4868	46
HBK4820	46
Macon	46
SSRT4902	46
AP4512	45
AP4888	45
CGRC4444	45
D5644	45
ETrooper	45
SS5999	45
T52R42	45
A5901	44
CGRC4995	44
D4690	44
ERanger	44
HBK4622	44
HBK6020	44
A4403	43
D5414	43

(table con'd)

DG3600	43
DK4965	43
EPunch	43
A4603	42
Defiance	42
T58R11	42
D4344	40
P94B73	40
P95B53	40
DG3535	39
Del4950	38

Mean = 44.9

Table 4: ANOVA for percent leaf nitrogen drop (% Lf N drop) for 48 soybean cultivars grown near Baton Rouge, LA in drained and waterlogged conditions, 2002 to 2003.

ANOVA Source	Summary (df)	% Lf N decrease[1]
Year	1	0.26
Cultivar	47	1.29
Year*Cultivar	47	1.01
C.V. %		17.3%

[1] = Not significant

Table 5: Percent leaf nitrogen concentration (% Lf N) for 48 soybean cultivars grown under normal drained conditions and averaged across two years at Baton Rouge, LA, 2002 to 2003.

Cultivar	% Lf N in Drained Treatment
DK4965	5.51*
CGRC4444	5.46*
8115	5.42*
DK4868	5.42*
H4884	5.38*
HBK4820	5.36*
A4403	5.35
Macon	5.35
DK4763	5.34
AP4512	5.31
Defiance	5.31
D498	5.29
P9492	5.28
HBK6020	5.26
D5915	5.25
DG3463	5.25
T52R42	5.24
A4902	5.21
HBK4622	5.20
A5901	5.18
CGRC4992	5.18
CGRC4995	5.17
D5644	5.15
T58R11	5.15
SS5999	5.14
D5806	5.13
T59R98	5.13
ETrooper	5.12
H4994	5.12
SSRT4902	5.12
AP4888	5.11
P94B73	5.10
T59R85	5.09
A5701	5.08
A4603	5.07
AP588	5.06
ERanger	5.06
D4344	5.05
EMarshal	5.04

(table con'd)

T4890	5.04
Del4950	5.03
D4690	5.02
A6201	5.00
D5414	4.94
DG3600	4.91
P95B53	4.88
DG3535	4.87
EPunch	4.76

* Cultivars are statistically similar to the top cultivar at the 0.05 probability level according to LSD. LSD = 0.16%.

Table 6: Percent leaf nitrogen concentration (% Lf N) for 48 soybean cultivars grown under waterlogged conditions and averaged across two years at Baton Rouge, LA, 2002 to 2003.

Cultivar	% Lf N in Waterlogged Treatment
DK4965	3.13*
Defiance	3.10*
Del4950	3.10*
A4403	3.06*
P94B73	3.05*
D4344	3.01*
T58R11	2.99*
CGRC4444	2.98*
HBK6020	2.97
DG3535	2.97
A4603	2.96
DK4868	2.94
P95B53	2.94
CGRC4995	2.92
HBK4820	2.91
AP4512	2.91
Macon	2.90
8115	2.89
HBK4622	2.89
A5901	2.89
H4884	2.88
DK4763	2.88
T52R42	2.87
ERanger	2.84
D5915	2.83
A4902	2.83
ETrooper	2.83
D498	2.82
D5644	2.82
SS5999	2.82
AP4888	2.81
D5414	2.81
D4690	2.79
P9492	2.78
SSRT4902	2.78
DG3600	2.78
DG3463	2.73
AP588	2.72

(table con'd)

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CGRC4992	2.71
T59R85	2.71
A6201	2.71
EPunch	2.70
D5806	2.68
A5701	2.66
EMarshal	2.65
T4890	2.58
T59R98	2.53
H4994	2.47

* Cultivars are statistically similar to the top cultivar at the 0.05 probability level according to LSD. LSD = 0.16%.

nitrogen concentration of 5.42% statistically similar to Delta King 4965 with a reported reading of 5.51%.

Within the waterlogged treatment, Delta King 4965 was also the cultivar having the greatest leaf nitrogen concentration (Table 6). Cultivars showing leaf nitrogen concentrations statistically similar ($P<0.05$) to Delta King 4965 were Defiance, Delta Grow 4950 , Asgrow 4403, Pioneer 94B73, Deltapine 4344, Terral 58R11 and Croplan Genetics 4444. Leaf nitrogen concentration for the waterlogged treatment ranged from 2.47% (Hartz 4994) TO 3.13% (Delta King 4965), thus all cultivars suffered leaf nitrogen stress. Average leaf nitrogen concentration among all 48 cultivars was 2.84%. Thus, percent leaf nitrogen concentrations in this study fell below 4% for all 48 cultivars in the waterlogging treatment. Under these conditions, yield losses are likely to arise. Physiological disruptions likely to occur are reduced root growth, reduced nodulation, lower nitrogen fixation, decreased photosynthesis, biomass accumulation, and decreased stomatal conductance (Schmitthenner, 1985; Sallam and Scott, 1987a, b; Oosterhuis et al., 1990; Scott et al., 1990). Among check cultivar performance, only Defiance was significantly similar to the top cultivar Delta King 4965 with a reported reading of 3.13%.

Soybean producers in Louisiana might consider planting cultivars demonstrating the greatest leaf nitrogen under waterlogged conditions. Cultivars found to have the highest levels of percent leaf nitrogen under waterlogged conditions include Delta King 4965, Defiance, Delta Grow 4950, Asgrow 4403, Pioneer 94B73, Deltapine 4344, Terral 58R11, and Croplan Genetics 4444.

Although the current method shows promise as a screening technique for identifying soybean cultivars tolerant to waterlogging, other simpler methods could be developed. Perhaps a

spad meter or leaf color chart would be a more economically feasible way to screen for waterlogging tolerance among soybean cultivars due to the time and cost of the analysis of percent leaf nitrogen concentration encountered with the nitrogen laboratory. Problems associated with spad meter reading include non-precise and non-accurate measurements. As do weed scientists with herbicide injury ratings to weeds over rates and times, perhaps agronomists could develop color rating charts for soybeans in attempt to quickly identify potential cultivar tolerance when exposed to different flood durations at different growth stages. This technique would save lots of time and money in trying to identify soybean cultivar's tolerance to waterlogging.

According to the 2002 and 2003 test, farmers in Louisiana should note that when making cultivar decisions one may consider cultivars with higher levels of percent leaf nitrogen regarding water tolerance. When subjected to seven days of waterlogging, all soybean cultivars tested in this test responded statistically similar, although some cultivars were found to be numerically different regarding percent leaf nitrogen concentration. New cultivars are breed each year and perhaps in the future, soybean breeders will find the genes to enable soybeans to withstand waterlogging to a better degree.

CONCLUSIONS

This research suggests that cultivar screening for waterlogging tolerance should be done on a waterlogged treatment and that a seven day flooding period was sufficient to distinguish cultivar differences. Cultivar rankings between drained and waterlogged treatments were not similar enough to allow for screening on the drained treatment alone. Using percent leaf nitrogen concentration as a screening criterion was justified by significant cultivar effects, lack of significant year x drainage or cultivar x drainage interactions and a low C.V.

For the waterlogged treatment, cultivars Delta King 4965, Defiance, Delta Grow 4950, Asgrow 4403, Pioneer 94B73, Deltapine 4344, Terral 58R11, and Croplan Genetics 4444 all showed significant similarities to the top cultivar Delta King 4965 in percent leaf nitrogen concentrations. Numerically, these cultivars had the closest percent leaf concentrations to 4%, implying potential to have greater waterlogging tolerance relative to the other 40 cultivars in the study.

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VITA

Curt Jude Riche' was born on December 15, 1976, in Alexandria, Louisiana. He has two wonderful sisters Callie and Hannah whom he is very close to and visits with regularly. His home town is a beautiful place called Cottonport located in Avoyelles Parish. Curt is the son of Jon Craig Riche' and Deborah Brochard Riche'. Curt attended St. Mary's Assumption Catholic School from kindergarden to eighth grade. From there Curt went on to Bunkie High School and graduated with honors in the top 10 percent of his class in 1995. He then attended Louisiana State University at Alexandria for two and one half years before attending Louisiana State University in Baton Rouge for an additional two and one half years where he earned a B.S. in Plant and Soil Science.

In June of 2000, Curt accepted a job as an extension associate in plant science working with soybeans and corn. He has experience in weed science, entomology, plant pathology and agricultural engineering. Dr. Walter Morrison, his boss at the time had Curt highly involved with county agents and farm producers across the state. Curt took part in the Louisiana Soybean Research Verification Program along with state-wide field crop demonstrations. Curt is currently stationed at the Dean Lee Research Station in Alexandria, Louisiana with the same job responsibilities under a new boss, Dr. David Y. Lanclos. Dr. Lanclos and Curt work with crops including soybeans, corn and grain sorghum in addition to extension weed science responsibilities. Curt has been employed with the LSU AgCenter for nearly four years now and plans to continue working there as a research associate.

Curt is currently living in Mansura, Louisiana, and enjoys outdoor activities such as hunting, fishing, exercising, cooking, gardening, maintaining his yard, and playing with his pets.

He will be married June 5, 2004 to his lovely fiancée, Brooke Leigh Gauthier, R.N., and plans to

have several children.