Project title: **Genetic Effects on Nitrogen Use Efficiency in Winter Wheat**

**Principal investigators**


| **Nathan O. Nelson**<br>Department of Agronomy<br>nonelson@ksu.edu<br>785-532-5115 | **Allan K. Fritz**<br>Department of Agronomy<br>akf@ksu.edu<br>785-532-7245 |
|---|---|


**Introduction with project justification**

Increasing nitrogen use efficiency (NUE) is an agronomic, economic, and environmental priority for grain production. Nitrogen use efficiencies in grain production are typically less than 50% and can be as low as 30% (Raun and Johnson, 1999), indicating that 50 to 70% of applied N fertilizer is not used for grain production. This represents a substantial economic input by farmers that does not directly return a profit. Furthermore, N that is not taken up by plants can contribute to environmental problems such as greenhouse gas emissions, eutrophication of surface water, and contamination of groundwater. **Thus improving NUE will simultaneously increase profits for farmers and reduce environmental impacts of food production.**

We must understand the factors that influence NUE if we intend on increasing NUE. Nitrogen use efficiency is the grain production per unit of N available in soil and applied fertilizer. It is the product of the ability of plants to take up N (uptake efficiency, UPE) and the ability of plants to utilize the N that has been taken up for grain production (utilization efficiency, UTE). The utilization efficiency can be further impacted by other plant factors, such as the grain production per unit of total biomass (harvest index) and the ability of the plant to move N from leaves and stems into developing grain (translocation efficiency). Because NUE is the product of several variables, it can be influenced by a variety of plant, soil, and management factors.

Much of the previous work on increasing NUE has focused on fertilizer and soil management to reduce N losses prior to plant uptake, thus increasing N availability in soil (Raun and Schepers, 2008). Improved soil and fertilizer management has only increase NUE up to 50 to 70%, which still leaves room for further improvement (Raun and Schepers, 2008). As previously mentioned, NUE is influenced by plant factors, which are a function of plant genetics. For example, research has shown that 21 to 41% of fertilizer N can be lost through wheat leaves as volatilized ammonia (Harper et al., 1987; Daigger et al., 1967). Muurinen et al. (2006) found that increased nitrogen uptake efficiency resulted in improved NUE in wheat and oat varieties grown in Finland. Other researchers found that differences in NUE among durum wheat varieties were largely a result of different N utilization efficiencies (Giambalvo et al., 2010). Although several studies have documented differences in NUE among winter wheat varieties, these have been conducted in Europe with varieties and growing conditions different from those in the central Great Plains of the United States (Foulkes et al., 1998; Baric et al., 2007). **These studies illustrate the impact that genetics can have on NUE in wheat. However, additional research is needed to characterize differences in NUE for wheat varieties common to Kansas Agriculture.**

Development and selection of wheat cultivars with high nitrogen use efficiency will help farmers increase the return on fertilizer investment. Varieties with high NUE will produce more grain with less N fertilizer. Characterization of NUE and N use parameters in winter wheat varieties will help breeders as they choose varieties for further development. For example, varieties with high uptake efficiency may be crossed with varieties that have high utilization efficiency in order to maximize NUE. **However, these types of genetic gains and improvements in NUE are dependent on knowledge concerning the NUE of the current varieties.**

## Project goals and objectives

The overall goal of the project is to improve the efficiency of nitrogen fertilizer inputs in winter wheat production systems. This will be accomplished by enhancing our understanding of the variability of nitrogen use efficiency across winter wheat varieties. The specific objectives of the project are as follows

i. Determine the nitrogen use efficiency of 30 different hard-red winter wheat varieties.
ii. Characterize nitrogen uptake and utilization parameters related to NUE in hard-red winter wheat varieties
iii. Determine the relationship between yield gains and NUE in hard-red winter wheat.
iv. Select winter wheat varieties for future research on genetic effects on N response.

## Preliminary results

In a preliminary study, the NUE of 30 winter wheat varieties was determined during the 2010/2011 growing season. The varieties were planted at the Rossville experiment field with either 0 or 80 lbs N $ac^{-1}$. Biomass and tissue samples were collected at anthesis and harvest. Nitrogen uptake at anthesis was affected by both variety and N application ($p<0.05$, data not shown). Furthermore, there was a varietal influence on fertilizer uptake efficiency at anthesis ($p<0.10$, data not shown).

Several N use parameters were significantly influenced by variety at harvest, including N content in grain and stover, N utilization efficiency, biomass production efficiency, and harvest index (Table 1). Nitrogen use efficiency ranged from 18 to 28 kg grain per kg of N and appeared to be influenced by variety, with $p< 0.10$ (Figure 1). The large range in NUE between varieties indicates potential for genetic gains, however the high variability in the data indicate that additional data is needed before firm conclusions can be stated.

**Table 1. Significance (*p*-values) for main effects and interactions from analysis of variance for nitrogen rate and variety effects on nitrogen use efficiency and related parameters.**

| | Yield | Ng | Ns | NUE | UPE | UTE | HI | BPE | NHI | NA | NRE | FUE |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Nitrogen Rate | 0.003 | <0.001 | 0.001 | <0.001 | 0.201 | <0.001 | 0.033 | <0.001 | 0.006 | 0.023 | 0.007 | - |
| Variety | 0.078 | <0.001 | 0.010 | 0.089 | 0.141 | <0.001 | <0.001 | <0.001 | <0.001 | 0.501 | 0.403 | 0.430 |
| Nitrogen Rate*Variety | 0.517 | 0.405 | 0.502 | 0.362 | 0.450 | 0.537 | 0.034 | 0.692 | 0.016 | 0.127 | 0.267 | - |

Abbreviations: Ng, nitrogen content of grain; Ns, nitrogen content of stover; NUE, nitrogen use efficiency; UPE, uptake efficiency; UTE, utilization efficiency; HI, harvest index; BPE, biomass production efficiency; NHI, nitrogen harvest index; NA, nitrogen uptake after anthesis; NRE, nitrogen remobilization efficiency; FUE, fertilizer use efficiency.

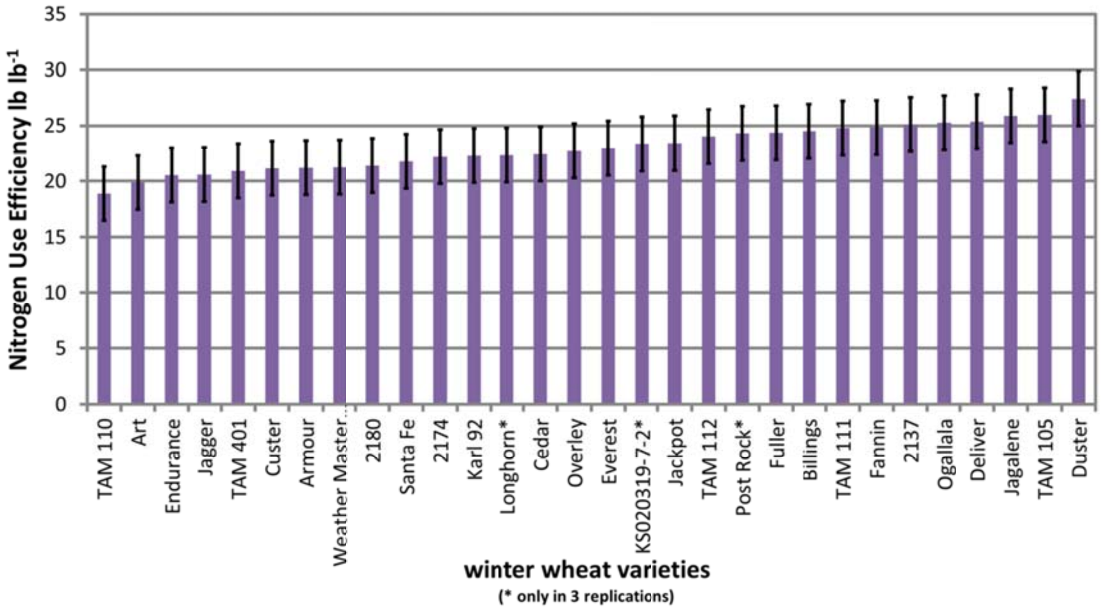


**Figure 1. Nitrogen use efficiency of 30 different wheat varieties common to the central Great Plains.**

The nitrogen utilization efficiency was strongly influenced by variety (Table 1, Figure 2). However, N uptake efficiency was not influenced by variety (Tables 1 and 2). This indicates that differences in NUE may be due to differences in the ability of wheat varieties to utilize absorbed N to produce grain rather than their abilities to uptake, or absorb, N from the soil.

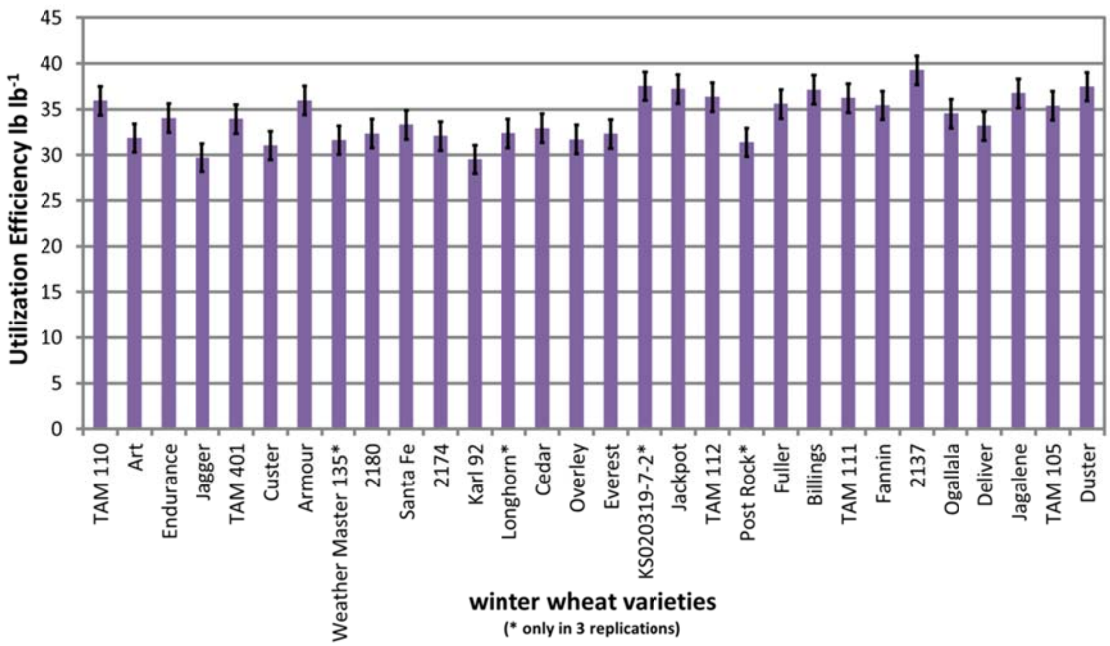


**Figure 2. Nitrogen utilization efficiency of 30 different wheat varieties common to the central Great Plains.**

**Table 2. Nitrogen use efficiency related parameters for winter wheat varieties grown in Rossville, 2010/2011 growing season.**

| Variety | Yield | Ng | Ns | UPE | HI | BPE | NA | NRE | FUE |
|---|---|---|---|---|---|---|---|---|---|
| | bu $ac^{-1}$ | % | % | lb $lb^{-1}$ | lb $lb^{-1}$ | lb $lb^{-1}$ | lb $ac^{-1}$ | - | - |
| 2137 | 39.9 | 4.4 | 0.85 | 0.62 | 0.38 | 102.2 | 21.5 | 0.58 | 0.43 |
| 2174 | 36.3 | 4.9 | 1.06 | 0.69 | 0.36 | 88.2 | 30.3 | 0.42 | 0.55 |
| 2180 | 37.2 | 4.4 | 1.01 | 0.67 | 0.38 | 86.2 | 31.5 | 0.55 | 0.78 |
| Armour | 36.1 | 5.1 | 0.97 | 0.60 | 0.41 | 87.9 | 28.8 | 0.53 | 0.69 |
| Art | 31.8 | 4.7 | 1.01 | 0.61 | 0.38 | 83.8 | 24.8 | 0.58 | 0.41 |
| Billings | 40.6 | 4.7 | 0.87 | 0.66 | 0.39 | 96.1 | 19.7 | 0.61 | 0.55 |
| Cedar | 38.2 | 4.4 | 1.04 | 0.68 | 0.39 | 84.4 | 32.0 | 0.53 | 0.67 |
| Custer | 34.9 | 4.4 | 1.03 | 0.67 | 0.35 | 89.8 | 24.7 | 0.51 | 0.49 |
| Deliver | 41.8 | 4.7 | 1.10 | 0.76 | 0.38 | 86.8 | 30.3 | 0.48 | 0.67 |
| Duster | 43.4 | 4.7 | 0.80 | 0.72 | 0.38 | 97.3 | 20.6 | 0.60 | 0.38 |
| Endurance | 35.8 | 4.6 | 0.94 | 0.61 | 0.36 | 92.9 | 28.5 | 0.53 | 0.70 |
| Everest | 38.4 | 5.1 | 1.04 | 0.70 | 0.40 | 80.9 | 34.8 | 0.48 | 0.62 |
| Fannin | 41.2 | 4.5 | 0.83 | 0.70 | 0.40 | 88.6 | 30.3 | 0.59 | 0.64 |
| Fuller | 38.5 | 4.6 | 0.77 | 0.67 | 0.37 | 95.6 | 22.6 | 0.61 | 0.37 |
| Jackpot | 39.9 | 4.9 | 0.80 | 0.64 | 0.42 | 88.8 | 30.1 | 0.60 | 0.63 |
| Jagalene | 41.6 | 4.8 | 0.91 | 0.69 | 0.39 | 95.4 | 24.2 | 0.55 | 0.42 |
| Jagger | 36.0 | 4.3 | 0.98 | 0.71 | 0.35 | 85.6 | 39.0 | 0.45 | 0.83 |
| Karl 92 | 37.6 | 4.4 | 1.01 | 0.77 | 0.34 | 85.3 | 43.0 | 0.41 | 0.78 |
| KS020319-7-2* | 36.9 | 4.4 | 0.83 | 0.61 | 0.40 | 93.8 | 20.8 | 0.59 | 0.39 |
| Longhorn* | 36.3 | 4.7 | 0.95 | 0.69 | 0.34 | 95.9 | 24.3 | 0.51 | 0.59 |
| Ogallala | 40.7 | 4.5 | 0.95 | 0.72 | 0.41 | 85.3 | 36.4 | 0.55 | 0.55 |
| Overley | 38.4 | 4.2 | 1.02 | 0.72 | 0.39 | 80.6 | 41.6 | 0.41 | 0.65 |
| Post Rock* | 40.4 | 4.4 | 0.98 | 0.76 | 0.36 | 88.0 | 32.5 | 0.49 | 0.52 |
| Santa Fe | 37.0 | 4.5 | 0.90 | 0.67 | 0.37 | 88.4 | 28.5 | 0.54 | 0.71 |
| TAM 105 | 41.5 | 3.9 | 0.88 | 0.73 | 0.38 | 92.3 | 33.4 | 0.53 | 0.52 |
| TAM 110 | 31.2 | 4.6 | 0.96 | 0.51 | 0.42 | 85.5 | 18.7 | 0.62 | 0.45 |
| TAM 111 | 40.0 | 4.4 | 0.81 | 0.68 | 0.38 | 96.2 | 23.1 | 0.61 | 0.59 |
| TAM 112 | 39.6 | 4.1 | 0.86 | 0.66 | 0.39 | 91.5 | 21.8 | 0.65 | 0.54 |
| TAM 401 | 34.5 | 4.4 | 0.96 | 0.61 | 0.41 | 83.0 | 27.5 | 0.56 | 0.50 |
| Weather Master 135* | 35.5 | 4.1 | 1.06 | 0.67 | 0.33 | 96.0 | 24.4 | 0.47 | 0.69 |
| L.S.D. | N.S. | 0.4 | 0.01 | N.S. | 0.02 | 9.1 | N.S. | N.S. | N.S. |

Abbreviations: Ng, nitrogen content of grain; Ns, nitrogen content of stover; UPE, uptake efficiency; HI, harvest index; BPE, biomass production efficiency; NHI, nitrogen harvest index; NA, nitrogen uptake after anthesis; NRE, nitrogen remobilization efficiency; FUE, fertilizer use efficiency.

These preliminary data illustrate that variety selection will influence N content in stover and grain, N utilization efficiency, harvest index, and biomass production efficiency, all of which influence NUE. Furthermore, varietal impacts on NUE cannot be conclusively ruled out because of the low *p*-value found for this study ($p < 0.10$). Further research in conditions with less variability may show that there are significant differences in NUE at $p<0.05$. These data can help breeders as they focus on trait selection for development of future varieties with high NUE. These data also identify traits that may need to be enhanced through inclusion of new genetic material through wild varieties or biotechnology (i.e., uptake efficiency). **However, these are results from only one year and one location. Results from multiple years and locations are necessary before solid conclusions can be based on this data. Therefore, we are proposing to expand this research to include multiple years and an additional location.**

**Procedures**

This study will be conducted in the field at Rossville and Ashland Bottoms Experiment fields for the 2012/2013 and 2013/2014 growing seasons. The experimental design will be a split strip-plot study with variety as the whole plot factor and N rate as the sub-plot factor replicated four times (stripped across varieties, Figure 3). Sub-plot size will be 30 ft long and 5.5 ft wide. Thirty winter-wheat varieties will be evaluated for their N use efficiency at two levels of N. The winter wheat varieties have been selected to represent a range of common varieties released from the mid 1990s up until the current date and some standard check varieties (listed in Table 2). The majority of these varieties are part of larger experiments that include genotyping, therefore, these data can be related to genotypic markers in the future.

Nitrogen treatments will be a zero-rate control used to determine the N supply without N and a moderate rate of N used to represent a conservative the N recommendation typical of KSU fertilizer recommendations (80 to 100 lbs of N/ac for these soils and yield potentials). Nitrogen will be applied as ammonium nitrate with 30 lb N/ac applied at planting and the remainder top-dressed in early spring. Phosphorus and potassium will be uniformly applied based on soil tests. Appropriate herbicides and fungicides will be applied to control weeds and disease.

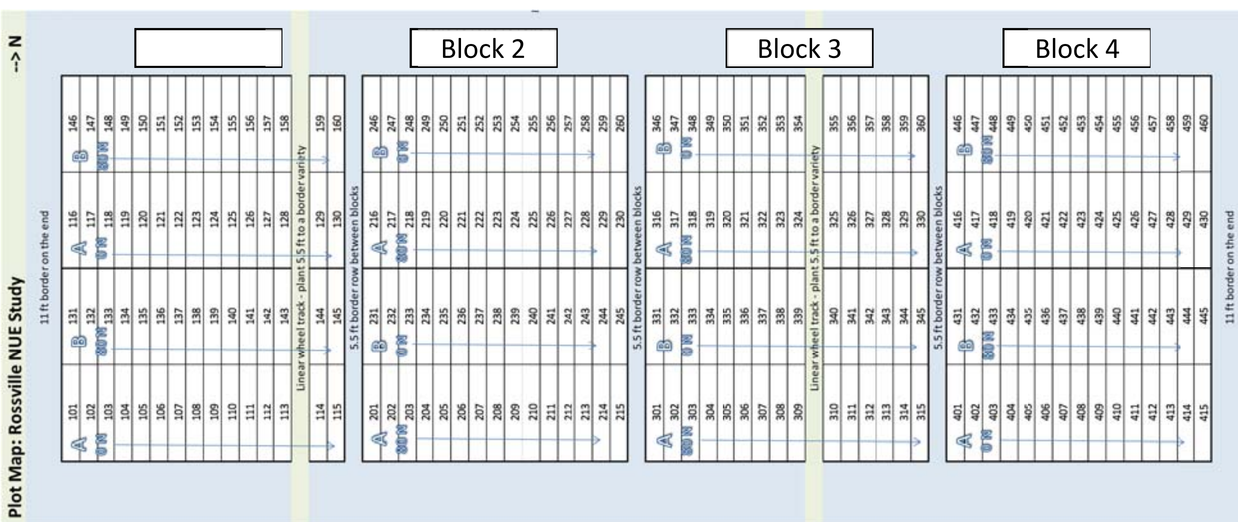


**Figure 3. Example plot map for Rossville experiment. Nitrogen treatments (A and B) are stripped across varieties. Plots 101 and 131 are planted to the same variety as are plots 102 & 132, 103 & 133, etc.**

Nitrogen use efficiency and related parameters will be determined according to conventions of Moll et al. (1982) and as described in Table 3. This will be accomplished by collecting biomass samples from 2 ft of the 4 center rows in each plot at anthesis (Feekes stage 10.51). The biomass will be dried, weighed, and analyzed for total N by combustion in the KSU Soil and Plant Analysis Laboratory. This will be used to determine N uptake at anthesis. At maturity (Feekes 11.4), whole plants will be harvested from 2 feet of the center two rows of each plot. The samples will be dried and threshed to determine harvest index. Sub-samples of grain and tissue will be analyzed for total N as previously described. The remaining plot area will be harvested with a plot combine to determine yield. The harvest index (HI) will be used to determine total biomass and stover yields based on the plot yield from the combine (Equations 1 and 2). Stover yield and grain yield will be multiplied by their respective N concentrations to determine N uptake at harvest.

Stover Yield = (Grain Yield)*((1/HI)-1) Equation 1

Biomass Yield = (Grain Yield) / HI Equation 2

**Table 3. Definitions and methods of computation for the N use efficiency related parameters that will be determined for 30 winter wheat varieties.**

| Measurement | Definition | Computation |
|---|---|---|
| Nitrogen use efficiency (NUE) | weight of grain yield per unit of nitrogen available in the soil | NUE = grain weight / nitrogen supply or UPE * UTE |
| Nitrogen uptake efficiency (UPE) | efficiency of nitrogen absorption from the soil | UPE = total nitrogen at maturity / nitrogen supply in the soil |
| Nitrogen utilization efficiency (UTE) | efficiency in which nitrogen absorbed from the soil is utilized to produce grain | UTE = grain weight / total nitrogen at maturity or HI * BPE |
| Harvest index (HI) | weight of grain yield as a fraction of total plant weight at maturity | HI = grain yield / above ground biomass |
| Biomass production efficiency (BPE) | total plant weight compared to total nitrogen at maturity | BPE = aboveground biomass / total nitrogen at maturity |
| Nitrogen harvest index (NHI) | amount of nitrogen found in the grain compared to total nitrogen at maturity | NHI = nitrogen in the grain / total nitrogen at maturity |
| Nitrogen uptake after anthesis (NA) | difference in total nitrogen from anthesis to maturity | NA = total nitrogen at maturity - total nitrogen at anthesis |
| Nitrogen remobilization efficiency (NRE) | fraction of nitrogen taken up at anthesis that was remobilized to the grain | NRE = (nitrogen in the grain - nitrogen uptake after anthesis) / total nitrogen at anthesis |
| Fertilizer use efficiency (FUE) | fraction of applied nitrogen that was absorbed | FUE = (nitrogen uptake with nitrogen applied - nitrogen uptake with no nitrogen applied) / nitrogen applied |

Growth and development of the wheat varieties will be monitored throughout the growing season with an active remote sensor (GreenSeeker). Remote sensing measurements NDVI (an indicator of color and biomass) will be collected at least once in the fall and four times in the spring for each plot.

Data will be analyzed with SAS proc mixed to determine the effects of variety, N rate, environment (location and year), and their interactions on N use efficiency and related parameters listed in Table 3. Multiple regression analysis will be used to determine the percent of yield variability that can be explained by differences in NUE over the time of variety release.

This will be used to determine if increases in NUE can explain the increase in yield over time in the breeding program.

**Duration of the project**

This project will continue for two years (2012/2013 and 2013/2014 growing seasons)

**Expected outputs and outcomes**

We expect that varieties will differ with respect to the N content in grain and stover, N uptake, N utilization efficiency, and N use efficiency. We expect that the differences in N utilization efficiency will be the primary factor leading to differences in N use efficiency. We expect that increased N use efficiency will be highly correlated to yield increases observed in wheat varieties over time. These results will be used to focus future research for more efficient breeding and greater yield gains with reduced N inputs.

The primary outcome from this research will be improved knowledge with respect to the N use efficiencies of modern wheat varieties. This knowledge will be used to change behaviors of wheat breeders when selecting parent lines for future wheat varieties. This knowledge will also be used to direct future research investigating physiological differences in N use efficiency and differences in N responsiveness for different wheat varieties.

**Technology Transfer**

Results of this research will be included in the Annual Fertilizer Research Report, which is available on the internet. The results will also be summarized and presented at the annual winter research station stakeholder meeting at the Rossville/Silverlake experiment field. At the conclusion of the research project, the results will be presented at professional meetings (Great Plains Soil Fertility Conference and the American Society of Agronomy Annual meeting) and research station field days.

**Project budget**

The total project budget is $77,280. We are requesting $59,990 from the fertilizer research fund. The remaining $17,280 is being requested from the International Plant Nutrition Institute (IPNI) to cover plant tissue analysis. If funds are unavailable from IPNI, then the KSU wheat breeding project will cover the plant tissue analysis expenses. An itemized budget is listed in Table 4.

**Table 4. Itemized budget for funding requested from the Fertilizer Research Fund (FRF) and from other sources (Other).**

| | **Year 1** | | **Year 2** | | **Total** | |
|---|---|---|---|---|---|---|
| **Category** | **FRF** | **Other** | **FRF** | **Other** | **FRF** | **Other** |
| Salaries | | | | | | |
| GRA (M.S.) | $22,371 | | $23,042 | | $45,413 | |
| Hourly Labor | $3,000 | | $3,000 | | $6,000 | |
| Fringe Benefits | | | | | | |
| GRA (6.5%) | $1,253 | | $1,290 | | $2,543 | |
| Hourly Labor (1.3%) | $39 | | $39 | | $78 | |
| Supplies | | | | | | |
| Lab Supplies | $800 | | $500 | | $1,300 | |
| Field Supplies | $800 | | $500 | | $1,300 | |
| Travel | | | | | | |
| Rossville | $480 | | $480 | | $960 | |
| Ashland Bottoms | $138 | | $138 | | $276 | |
| Out-of-State Travel | $700 | | $700 | | $1,400 | |
| Other Direct Costs | | | | | | |
| tissue analysis | $0 | $8,640 | $0 | $8640 | $0 | $17,280 |
| soil analysis | $360 | | $360 | | $720 | |
| **Total** | **$29,941** | **$8,640** | **$30,049** | **$8,640** | **$59,990** | **$17,280** |

**Budget Justification**

*Salaries* – One M.S. graduate student paid according to graduate stipend guidelines for the Agronomy department, including a 3% annual salary increase and 5.6% fringe benefits. Part-time student work to assist with harvesting and sample processing, 300 hours per year paid at $10 per hour with 1.3% fringe benefit rate.

*Supplies* – Field and laboratory supplies include paper bags, plastic bags and sample containers for collection, processing, and storage of plant tissue samples (1440 samples per year). Expendable safety supplies, including respirators, safety glasses, and safety gloves. Purchase of fertilizer, herbicide, and fungicide for field plot maintenance.

*Travel* – Travel to and from plots in Rossville (80 mile round trip) and Ashland Bottoms (24 mile round trip). Twelve trips to each site per year at $0.50 per mile. Out-of-state travel for one individual to attend a regional conference to present research results (such as the North Central Extension-Industry Soil Fertility conference in Des Moines, IA or the Great Plains Soil Fertility Conference in Denver, CO).

*Other Direct Costs* – Laboratory analysis of soil and plant tissue samples. Twenty-four split profile soil samples per year (3 per block per site) at a cost of $15 per sample (pH, P, K, organic matter, NH4-N, NO3-N in the surface and NH4-N and NO3-N in the sub-surface).

Nitrogen analysis of 1440 plant tissue samples per year (240 plots per site, 3 tissue samples per plot) at a cost of $6 per sample. The expense of tissue analysis will be covered from other funding sources. We are seeking funding from the International Plant Nutrition Institute (IPNI) for the tissue analysis. If we are unsuccessful in garnering funds from IPNI, then the KSU wheat project will cover the expense of tissue analysis.

**References**

Baric, M., H. Sarcevic, S. Keresa, I.H. Jercic, and I. Rukavina. 2007. Genotypic differences for nitrogen use efficiency in winter wheat (triticum aestivum L.). Cereal Res. Commun. 35:213-216.

Daigger, L.A., D.H. Sander, and G.A. Peterson. 1976. Nitrogen content of winter wheat during growth and maturation. Agron. J. 68:815-818.

Foulkes, M.J., R. Sylvester-Bradley, and R.K. Scott. 1998. Evidence for differences between winter wheat cultivars in acquisition of soil mineral nitrogen and uptake and utilization of applied fertilizer nitrogen. J. Agric. Sci. 130:29-44.

Giambalvo, D., P. Ruisi, G. Di Miceli, A.S. Frenda, and G. Amato. 2010. Nitrogen use efficiency and nitrogen fertilizer recovery of durum wheat genotypes as affected by interspecific competition. Agron. J. 102:707-715.

Harper, L.A., R.R. Sharpe, G.W. Langdale, and J.E. Giddens. 1987. Nitrogen cycling in a wheat crop: Soil, plant, and aerial nitrogen transport. Agron. J. 79:965-973.

Moll R., Kamprath E. and Jackson W. 1982. Analysis and interpretation of factors which contribute to efficiency of nitrogen-utilization. Agron. J. 74:562-564.

Muurinen, S., G.A. Slafer, and P. Peltonen-Sainio. 2006. Breeding effects on nitrogen use efficiency of spring cereals under northern conditions. Crop Sci. 46:561-568.

Raun, W.R., and J.S. Schepers. 2008. Nitrogen management for improved use efficiency. p. 675-693. In J.S. Schepers and W.R. Raun (eds.) Nitrogen in agricultural systems. ASA-CSSA-SSSA, Madison, WI.

Raun, W.R., and G.V. Johnson. 1999. Improving nitrogen use efficiency for cereal production. Agron. J. 91:357-363.