Discovering Nitrogen Use Efficiency Heritability and Potential Using ex-PVP Corn Germplasm Crop Physiology Adriano T. Mastrodomenico, Charles C. Hendrix, and Fred E. Below

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Introduction:

Genetic improvement for Nitrogen Use Efficiency (NUE) is necessary for continued sustainable corn production.

N-uptake efficiency (NUpE), N-utilization efficiency (NUtE), genetic utilization (GU), and yield response to N (RTN) are important N-use traits that can be used to characterize different corn genotypes. Genetic utilization measures the physiological efficiency of plants to produce grain utilizing the plant N accumulated without fertilizer.

Our objectives were to understand the genetic variation within in the U.S. ex-PVP

Table 1. BLUP's estimates, variance components, and broad-sense heritability (h_B²) for yield at high N (Yield +N), yield at low N (Yield –N), yield response to N (RTN), genetic utilization (GU), N uptake efficiency (NUpE), and N utilization efficiency (NUtE).

BLUP's				Variance Components					
Trait	Mean	SD	σ_F^2	σ_M^2	σ_{FxM}^2	σ_E^2	σ^2_{FxMxE}	$\sigma_{arepsilon}^2$	h_B^2
Yield +N	8931	328	1.4 x 10 ⁵	1.1 x 10 ⁵	9.1 x 10 ⁴	4.1 x 10 ⁵	6.5×10^4	7.6 x 10 ⁵	0.5
Yield -N	4611	244	1.1 x 10 ⁵	8.4 x 10 ⁴	5.7 x 10 ⁴	0.0	2.5×10^4	5.9 x 10 ⁵	0.7
RTN	4334	273	7.9 x 10 ⁴	1.5 x 10 ⁵	6.7 x 10 ⁴	2.3 x 10 ⁵	6.2×10^4	8.6 x 10 ⁵	0.4
NUpE	0.42	0.02	3.7 x 10 ⁻⁴	5.7 x 10 ⁻⁴	3.3×10^{-4}	1.2 x 10 ⁻³	0.0	1.2 x 10 ⁻²	0.3
NUtE	44	3	4.9	3.2	1.9	43.9	0.0	187.4	0.1
GU	59	2	14.9	10.7	2.9	14.8	5.2	69.7	0.6



germplasm across different corn heterotic groups (HG's) and the association of the different N-use traits with grain yield.

Materials and Methods:

Germplasm: 190 single cross hybrids from a combination of 82 ex-PVP inbreds; 31 Stiff Stalk Synthetic (SSS), 18 lodent, and 33 Lancaster. HG's were classified using principal component analysis in R with 26,679 single nucleotide polymorphisms.
 Environments: Six environments from 2 locations during 2011, 2013, and 2014.
 Design: RCBD with 3 blocks and two N fertilizer rates (0 and 252 kg N ha⁻¹) per environment.

Model: Phenotypic observations (Y_{ijklm}) were estimated as best linear unbiased predictor (BLUP's) using the linear model:

 $Y_{ijklm} = \mu + E_i + B_{j(i)} + F_k + M_l + FM_{kl} + EF_{ik} + EM_{il} + EFM_{ikl} + \varepsilon_{ijklm}$ Where μ is the overall mean and E_i , $B_{j(i)}$, F_k , M_l , and ε_{ijklm} are the random effects of environment, block, female, male, and residual error, respectively.

Analysis: BLUPS's, Pearson's pairwise correlations, and cluster analysis were calculated using the PROC MIXED, PROC SCATTER, and PROC CLUSTER procedures in SAS, respectively.



Phenotypic traits:

Yield (kg ha⁻¹) at 0 kg N ha⁻¹ (**Yield-N**) and at 252 kg N ha⁻¹ (**Yield+N**) at 0% moisture Yield response to N fertilizer (kg ha⁻¹): **RTN** = Yield_{@ 252 kg/ha} – Yield_{@ 0 kg/ha} Nitrogen Uptake Efficiency (kg kg_{plantN} ⁻¹): **NUpE** = $\frac{\text{Plant N}_{@ 252 kg/ha} - \text{Plant N}_{@ 0 kg/ha}}{\text{N Rate}_{252 kg/ha}}$

Nitrogen Utilization Efficiency (
$$kg_{plantN} kg_N^{-1}$$
): NUtE = $\frac{11014 \text{ }\text{(}252 \text{ }\text{kg/ha}^{-1})}{\text{Plant N}_{@ 252 \text{ }\text{kg/ha}^{-1}}}$ Plant N $_{@ 0 \text{ }\text{ }\text{kg}}$

Genetic Utilization (kg kg_{plantN}⁻¹): $GU = \frac{\text{Individual Plant Yield}_{@ 0 \text{ kg/ha}}}{\text{Plant N}_{@ 0 \text{ kg/ha}}}$

Results:

HG's had similar variance for Yield+N, GU, NUpE, and NUtE (Table 1). However, SSS had greater variance than non-SSS for Yield-N, while the non-SSS had greater variance than SSS for RTN. Large environmental and error variances lead to low broad sense heritability (h_B^2) for NUpE and NUtE (Table 1). Nonetheless, Yield-N and GU exhibited high h_B^2 and appear to be more genetically controlled than Yield+N and RTN.

Yield+N was positively correlated to NUpE, NUtE, RTN, and Yield-N (Figure 1). Alternatively, Yield-N was positively correlated to GU and negatively correlated to

Stiff Stalk Synthetic O Lancaster O lodent

Figure 1. Pearson correlation coefficients, scatter matrix, and observation distributions for NUpE, NUtE, GU, RTN, Yield-N, and Yield+N. Observations are BLUP estimates from each inbred across different heterotic groups. Coefficients indicate significance at $P \le 0.001$.

Conclusions:

High genetic variance for different N use traits offers opportunity for selection (Table 1). Breeding programs targeting improved N use must consider h_B² of the different N use traits and the correlation of these traits with grain yield in order to obtain desirable genetic gain (Figure 1).
Yield at high N (Yield+N) accounted for 52% of the yield under low N conditions (Yield-N), and 48% from the yield response to N fertilizer (RTN), (Table 1). In addition, Yield-N appeared to be more genetically controlled or less influenced by the environment than Yield+N (Table 1).
High h_B² for genetic utilization (GU) and Yield-N indicate that yield improvement in modern hybrids may be achieved by selecting genotypes under low N environments and GU may be integrated in corn breeding



Figure 2. Cluster analysis for inbreds from different heterotic groups using Yield+N, Yield-N, RTN, and GU. Clusters A, B, C, and D represent groups of parental inbreds of hybrids with different N use performance.

Future research:

The integration of N-use phenotypic traits with molecular marker information from ex-PVP germplasm will be used to develop genomic selection models to accelerate NUE improvement in corn. Indirect selection for high Yield-N using GU in

RTN. Phenotypic correlations were strongly positive between RTN and Yield+N, and between GU and Yield-N, indicating that RTN and GU are good predictor traits for Yield+N and Yield-N, respectively.

Cluster analysis was performed on the hybrids for different phenotypic characteristics and divided the inbreds into 4 main clusters (Figure 2): Cluster A (R²= 0.65, n=16) high Yield+N, high RTN, low Yield-N, and low GU. Cluster B (R²= 0.60, n=24) low Yield+N, low RTN, low Yield-N, and low GU. Cluster C (R²= 0.65, n=15) low Yield+N, low RTN, high Yield-N, and high GU. Cluster D (r²= 0.70, n=28) average for Yield+N, RTN, Yield-N, and GU. programs targeting for N-use improvement.

Cluster analysis was able to differentiate inbreds based on their hybrids'

performance to multiple N use traits. Inbreds from cluster A produced hybrids that exhibited maximum yield under high N environments, but were susceptible to N deficit conditions. One breeding strategy for NUE improvement could be new inbred or hybrid combinations using the genetic pool from clusters A and C to create genotypes with high Yield+N and high Yield-N (Figure 2). genomic selection models may help to improve N utilization.

Acknowledgements:

The authors wish to thank Dr. Martin Bohn, Dr. Alex Lipka, and Dr. Pat Brown for their assistance with analysis and comments.

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