SCREENING FOR NITROGEN FIXATION & SUPPLY IN FABA BEAN

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RATIONALE

Faba bean is a large biomass legume crop and it can supply most of its N requirement through biological Ndfa fixation. Two goals of the faba breeding program in Saskatchewan are to develop cultivars with high levels of stress tolerant Nfix fixation to support protein in yield, and to supply sustainable N in the next rotation through forage.

OBJECTIVES

1. Assess 15 genotypes (cultivars and breeding lines) of faba in the field at two locations (SK) in 2009 and 2010 for yield and ability to take up and to fix Nfix, and to quantify amino acids in leaf, stem, pod and seed partitions before crop maturity (flowering, pod-fill, and close to physiological maturity).
2. Develop a screening tool using the amino acid concentrations to select superior N acquisition end-of-season.
3. Validate the performance of the screening tool (model) with data not used in model development. Can the prediction method based on stem amino acid concentrations at flowering predict the top performing genotypes from a group of 18 to 20?

METHODS

STEP ONE Field material

- 15 faba genotypes grown in field plots at two locations (Saskatoon, Rosthern, SK) in 2009 and 2010, representing a dry, normal and a wet year. Reference crops: pea with N fixation, barley.
- Genotypes represent germplasm diversity and cultivars including normal colored flowers (high tannin) and white flowers (low tannin), high and moderate yield, plus variation in seed protein concentration and plant height.
- Samples at 3 growth stages (flowering, mid-pod, and full-pod/physiological maturity).

STEP TWO Plant N relationships

- Plant material partitioned into leaf, stem or reproductive organs.
- Dry, ground, and used to measure dry weight, total N content, N derived from biological fixation (Ndfa) by natural abundance 15N isotope, and total amount of actual fixed N. Amino acids measured by gas chromatography (GCAst method, Phenomenex, Torrance, CA).
- Rosthern 2009 and 2010 data were pooled with Saskatoon 2009. Correlations between amino acids or ratios of amino acids were made with shoot biomass, yield, total N content, N derived from biological fixation (Ndfa), and the total of actual fixed N.
- The stem fraction data from flowering, mid-pod, and full-pod/physiological maturity were the most consistent and stem data were moved forward to model prediction.

STEP THREE Predicting shoot N from amino acids

- For each growth stage:
  End-of-season shoot biomass and total shoot N content, in addition to the proportion of N derived from fixation (Ndfa), N fixed by the shoot and yield, were each predicted by three methods based on shoot amino acid concentrations:
  1. using the best amino acid variables from correlations,
  2. using the best individual amino acid concentrations selected from stepwise multiple regression,
  3. using the best individual amino acid concentrations and ratios of several amino acids selected from stepwise multiple regression (Table 1).
- The fits of the models are listed in Table 1, with Method 1 having the simplest model based on three calculated terms:
  \( \text{NALAvST} = \text{ASN} + \text{ASP} + \text{GLN} + \text{GLU} + \text{ALA} \)
  - the sum of asparagine (ASN), aspartate (ASP), glutamine (GLN), glutamate (GLU) and alanine (ALA), then divided by proline (PRO)
- the ratio of ALA to PRO, and
- the cross-product term NALAvST x ALA/PRO.

STEP FOUR Evaluating model performance

- Test data set:
  2011 samples from various untested faba genotypes and several previously tested check cultivars in regional variety trials from three locations, Sutherland and Preston in Saskatchewan, and Rosthern.
- 18 colored genotypes
  20 white genotypes
- Plots were sampled at the 3 growth stages, organs partitioned, and measured as in STEP TWO. Shoot biomass and total shoot N content were also measured on end-of-season plants, along with yield and Ndfa.
- Associations between predicted shoot biomass and predicted total N content versus actual shoot biomass and actual total shoot N content from 2011 field measurements were tested (Tables 2 and 3).

RESULTS

Actual measurements

- Both of the two faba types, colored and white flower (low tannin), generally performed differently at Saskatoon and Rosthern (Tables 2 and 3, columns 4-6).
- Genotypic differences in shoot biomass were also detectable using actual measurements, but rarely for N variables.
- In 2011 the location, weather and soil conditions were such that the N acquisition across the range of genotypes does not have a large range of variation possible to prove that the predictive methodology is precise enough to detect elite genotypes every time.

Predicted measurements

- Predicted shoot biomass at mid pod had significant genotypic effects for white flowered faba only, and for both faba types using the late pod physiological maturity prediction equation.
- Even from one test year the protocols look promising. For N variables (Table 2), Prediction Method 1 at mid pod could predict 4 (white) or 3 (colored) of the top 5 ranked genotypes.
- When the genotypes are ranked for biomass, harvest index and yield (Table 3), within the either the colored or white-flowered faba set, prediction method 1 at mid-pod could detect 2 (white) or 3 (colored) of the top 5 ranked genotypes.

<table>
<thead>
<tr>
<th>Factor</th>
<th>Flowering</th>
<th>Mid pod</th>
<th>Late pod</th>
<th>Actual measurement - End of season</th>
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<tbody>
<tr>
<td>Genotype rank</td>
<td>Total shoot N Prediction 1</td>
<td>Total shoot N Prediction 1</td>
<td>Total shoot N Prediction 1</td>
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<td>Florent</td>
<td>FB18_20</td>
<td>1965</td>
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<td>Taboar</td>
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</table>

CONCLUSIONS

- We can predict shoot biomass and total N content of the shoot to about 70% accuracy (R² about 0.70) based on the stem data from which they were generated.
- Prediction Method 1 can detect half of the top 5 ranked genotypes out of 18 to 20.
- We plan to screen faba for shoot growth, greater N acquisition, and greater amounts of N fixed by using amino acid concentrations from the stem fraction at flowering or in early reproductive growth.

- The screening method detects total shoot N content, and greater total N fixed by inference without reference plots.
- A better test set would be a wider range of environment-years, with a full range of soil N, with and without inoculation.

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