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 N_2 fixation. Two goals of the faba breeding program in Saskatchewan are to develop cultivars with high levels of stress tolerant N_2 fixation to support protein in yield, and to supply sustainable N to the next rotation through stover.

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 N_2, 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 acids at flowering pick out 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, a 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.

- Sampled 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.

- Dried, ground, and used to measure dry weight, total N content, N derived from biological fixation (Ndfa) by natural abundance ^{15}N isotopy, and total amount of actual fixed N. Amino acids measured by gas chromatography (EZfaast method, Phenomoenex, Torrance, CA).

- Rosthern 2009 and 2010 data was pooled with Saskatoon 2009. Correlations between various 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 **stem 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:

$$NALAvST = \frac{ASN + ASP + GLN + GLU + ALA}{PRO}$$

  [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.

Table 1. Model fits (R^2) of predictive models for total shoot biomass (g m^{-2}), total N content of shoot (g N m^{-2}), proportion of N derived from fixation (Ndfa), N fixed by the shoot and yield of faba grown in 2009 and 2010. All models use amino acid concentrations from the stem fraction of a faba plant, from one of three growth stages, flowering, mid pod and late pod/ physiological maturity. $R^2 < 0.60$ represents a poor fit.

Model	Growth stage	Shoot biomass (g m^{-2})	Total shoot N content (g N m^{-2})	Ndfa	N fixed by shoot (g N m^{-2})	Yield (g m^{-2})
Method 1	Flowering	0.72	0.75	0.22	0.75	0.45
	Mid pod	0.70	0.73	0.24	0.73	0.44
	Late pod	0.57	0.62	0.18	0.62	0.41
Method 2	Flowering	0.72	0.73	0.33	0.50	0.65
	Mid pod	0.73	0.75	0.42	0.55	0.43
	Late pod	0.81	0.83	0.47	0.72	0.47
Method 3	Flowering	0.69	0.71	-	-	-
	Mid pod	0.69	0.70	-	-	-
	Late pod	0.60	0.64	-	-	-

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 Saskatoon, 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 shoot total 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 the full amount 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 either the colored or white-flowered faba set, prediction method 1 at mid-pod could detect 2 (white) or 3 (colored) of the 5 top ranked genotypes.

Tables 2 and 3. Means for predicted total N and shoot biomass by Prediction Method 1, and actual measurements for colored and white-flowered faba grown at Rosthern and Saskatoon 2011. * indicates locations differ at P<0.05, the top 5 genotypes are listed and † indicates significant at P<0.05 by Fishers protected LSD.

Factor / Genotype rank	Flowering Total shoot N Prediction1 (g N m^{-2})	Mid pod Total shoot N Prediction1 (g N m^{-2})	Late pod Total shoot N Prediction1 (g N m^{-2})	Actual measurement - End of season Total shoot N (g N m^{-2})	Ndfa	N fixed by shoot (g N m^{-2})	N concentration (% shoot dw)
COLORED FLOWER FABA							
Rosthern	44.4*	55.9*	55.8*	58.4*	0.46*	21.8*	2.61*
Saskatoon	59.8	62.6	38.2	36.1	0.36	16.6	2.78
TOP 5 GENOTYPES							
1	FB61_3	Florent	FB18_20 †	186S_11	FB50_9	FB50_9	FB50_9
2	Florent	FB136_14	FB50_9	FB136_14	Taboar	FB50_43
3	228aS_24	FB9_4	FB9_4	Taboar	Taboar	FB136_14	FB9_4
4	187_8	186S_11	FB61_3	Florent	Divine	Divine	FB18_20
5	CDCSSNS_	FB50_9	FB50_9	NPZ5_768	CDCSSNS_	FB61_4	FB61_3
WHITE FLOWER FABA							
Rosthern	40.7	40.7*	52.0	37.7*	0.78*	29.7*	2.70*
Saskatoon	41.8	60.8	55.8	23.3	0.59	14.6	2.40
TOP 5 GENOTYPES							
1	NPZ97330	FB22_10 †	NPZ97207 †	219_18	Imposa	Imposa †	219_16
2	219_18	Imposa	Snowbird	Imposa	219_16	219_16	219_18
3	FB34_2	143_1	Imposa	FB22_10	FB22_10	219_18	NPZ97220
4	FB34_7	NPZ97207	NPZ4_754	219_16	224_34	224_34	221_5
5	143_1	NPZ4_754	FB128_4	224_34	NPZ97330	FB22_10	176_2

Factor / Genotype rank	Flowering Shoot biomass Prediction 1 (gm^{-2})	Mid pod Shoot biomass Prediction 1 (gm^{-2})	Late pod Shoot biomass Prediction 1 (gm^{-2})	Actual measurement - End of season Shoot Biomass (gm^{-2})	Harvest Index (small sample)	Harvest Index	Yield (gm^{-2})
COLORED FLOWER FABA							
Rosthern	1619*	1879*	1971*	2233*	0.58*	0.41	858*
Saskatoon	2108	2183	1470	1307	0.47	0.43	520
TOP 5 GENOTYPES							
1	FB61_3	Florent	FB18_20 †	Taboar †	FB18_20 †	FB50_43 †	NPZ5_768
2	228aS_24	Taboar	FB136_14	186S_11	FB61_4	FB9_4	186S_11
3	Florent	186S_11	FB9_4	Florent	FB61_3	FB18_20	FB61_4
4	187_8	FB61_3	FB61_3	NPZ5_768	FB50_9	NPZ5_768	FB50_43
5	FB9_4	FB9_4	FB50_9	186S_21	FB9_4	FB61_3	CDCFatima
WHITE FLOWER FABA							
Rosthern	1540	1496*	1747*	1391*	0.54*	0.50	672*
Saskatoon	1508	2064	1915	955	0.45	0.49	428
TOP 5 GENOTYPES							
1	219_18	FB22_10 †	NPZ97207 †	Imposa	219_18 †	138_1	FB22_10 †
2	FB34_2	Imposa	Imposa	Snowbird	219_16	FB22_10	219_18
3	FB34_7	143_1	NPZ4_754	219_18	202_23	219_18	Snowbird
4	FB80_17	NPZ4_754	138_1	FB22_10	143_1	221_5	Imposa
5	221_5	FB34_7	FB22_10	224_34	221_5	NPZ4_754	NPZ4_754

CONCLUSIONS

- We can predict shoot biomass and total N content of the shoot to about 70% accuracy (R^2 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 wide range of soil N, with and without inoculation.