Variability of lentil *%Ndfa* as affected by reference plant position

Hossein Zakeri[1], Janet Pritchard and Rosalind Bueckert
Crop Physiology lab, Department of Plant Sciences, University of Saskatchewan
[1] hoz130@mail.usask.ca

The natural abundance $\delta^{15}N$ method has been extensively used to estimate the percentage of the nitrogen (N) derived from atmosphere (%*Ndfa*) by legume crops. In this method, soil $\delta^{15}N$, estimated by a non N-fixing (reference) plant, is compared with legume $\delta^{15}N$. Because of the high variability of soil $\delta^{15}N$, the relative position of the legume and reference plant is crucial in these experiments. A paired-plot design, where the reference plot is paired with every legume plot, is an approach for reducing the estimation errors. The paired approach becomes exceptionally costly and time consuming as the experiment expands. In addition to expensive analytical measurements, the paired plot method doubles the costs of experimental samplings. The randomized-plot approach, where one reference plot is randomly assigned within a similar set of legume plots, would be an alternative to this scenario. An example of this approach is one reference plot for a group of cultivars under same treatment in a replication of experiment. This method assumes that the estimated %*Ndfa* is not significantly affected by variability of the reference plot $\delta^{15}N$ within the similarly treated plots in each replication. However, the randomized approach has not been compared to the paired-plot method. This study aimed at investigating the effects of the reference plot position on the variability of lentil (*Lens culinaris* Medic) %*Ndfa*. First, lentil %*Ndfa* was estimated by comparing the lentil $\delta^{15}N$ to the barley (*Hordeum vulgar* L.) $\delta^{15}N$ in paired-plots (paired %*Ndfa)*. Then, estimates of lentil %*Ndfa* in each plot were repeated by using barley in 7 other positions to lentil, as reference plant (fixed position %*Ndfa)*. Finally, lentil %*Ndfa* in each row was estimated by comparing the $\delta^{15}N$ of eight lentil plots in the row to the $\delta^{15}N$ of a barley plot which was randomly selected from the row (random %*Ndfa)*

Within the experiment, eight cultivars of lentil were grown under control (0 N fertilizer - no inoculant), N fertilizer (50 kg N ha^{-1}- no inoculant) and inoculated (inoculant – no N fertilizer) treatments in four replications. The experiment was arranged in a split-plot design where eight cultivars of lentil were randomly assigned in 12 rows with varied N fertilities. A strip of barley, cv. Dolly was sown along each row of lentil; hence lentil plots were all paired with a plot of barley. The aboveground biomass of both lentil and barley were sampled at lentil flowering, full-pod and maturity stages and analyzed for isotopic N composition on a 20-20 Mass Spectrometer interfaced with an ANCA-GSL sample converter (Europa Scientific, Crewe, UK). The $\delta^{15}N$ of both lentil and barely was estimated by this equation:

$$\boldsymbol{\delta^{15}N} = \frac{\boldsymbol{atom\%^{15}N\ sample - atom\%^{15}N\ atmosphere}}{\boldsymbol{atom\%^{15}N\ atmosphere}}$$

Then, the following equation was used to estimate the lentil %*Ndfa*:

$$\boldsymbol{\%Ndfa} = \left[\frac{\boldsymbol{\delta^{15}N\ barley - \delta^{15}N\ lentil}}{\boldsymbol{\delta^{15}N\ barley - C}}\right] \times \boldsymbol{100}$$

The constant C, which is the lentil $\delta^{15}N$ in an N-free medium, was assumed to be zero. As indicated in the above equation, lentil %*Ndfa* was estimated by comparing the lentil $\delta^{15}N$ with barley $\delta^{15}N$. The %*Ndfa* was estimated for each plot of lentil in a row, using all possible positions of the barley plots in the same row. The position was considered paired, where lentil and barley plots were side-by-side in a row, otherwise positions ranked 1 to 8, depending on the distances between the barley and lentil plots. A randomly selected plot of barley in each row was also used to estimate the %*Ndfa*. The estimated values of %*Ndfa* were analyzed for the effects of N treatments, cultivars and the reference plot positions (different fixed positions and the randomly selected position in row), using the Proc Mixed procedure of SAS (SAS Institute Inc., 2009). The average %*Ndfa* of each trait, estimated by various fixed positions of barley in a row, were compared to the randomly selected barley plot by contrast comparison analysis.

A declining pattern in barley %N was observed during the growing season, while barley $\delta^{15}N$ increased by the mid season and then declined at maturity. In contrast to the mean barley $\delta^{15}N$, the coefficient of variation (%CV) of barley $\delta^{15}N$ within each row declined by mid season and then increased by maturity. Lentil %*Ndfa* remained smaller in the fertilized plots than in both inoculated and control treatments during the entire season. Analysis of variance showed that estimates of lentil %*Ndfa* due to the different fixed positions of the barley plots within a row did not significantly differ. However, the effect of barley plot position on variability of lentil %*Ndfa* was greater at early season, especially in the fertilized treatment, than other time-treatments. The randomly chosen barley plot resulted in a significantly different estimate of %*Ndfa* than those estimates made using the fixed positions. Again, the differences between fixed and random positions were clearly greater at the lentil flowering stage than the full-pod and maturity stages and in the fertilized plots compared to the control and inoculated plots.

The results of this work showed that the number of reference plots could be reduced to a minimum of one plot for each set of legume plots, when total plant N_2 fixation at maturity is measured. It also indicated that a fixed position of reference plot provided better estimates of %*Ndfa* than the randomly selected position within the row. When soil conditions (slop, fertility, weed population, etc) within rows are homogenized among the rows, the best fixed position of the reference plot in a row would have the closest possible distance from all lentil plots in the row, i.e. the 5^{th} plot of a nine plot set.