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# Varietal differences in symbiotic nitrogen fixation and nitrogen redistribution in common bean

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**The objective of this study was to investigate varietal differences in symbiotic nitrogen fixation (SNF) and redistribution of N in the leaves and stems. Five Andean genotypes Sisi, Bukoba, Red Hawk, Mecost and Cardinal were evaluated for SNF and N redistribution in a field trial conducted at University of Zambia, Lusaka, Zambia. Nitrogen derived from the atmosphere (NDFA) ranged from 15.9 kg ha$^{-1}$ (Cardinal) to 48.4 kg ha$^{-1}$ (Mecosta) with an average of 30.9 kg ha$^{-1}$. Significant differences in N redistributions in the leaves and stem were observed. Both Mecosta and Red Hawk, which comparatively had higher NDFA, also had high redistribution of N from the leaves for Red Hawk and from the stem for Mecosta. This result suggest that despite Mecosta and Red Hawk being superior in SNF, both had significant dependency on the N redistributed mainly from the leaf and stem, respectively, to meet their seed N requirements and support high seed yield. This result confirms the important role of N redistribution in common bean from stems and leaves regardless of the SNF ability of a genotype. Mecosta, which had the highest NDFA, also had highest seed yield (1925 kg ha$^{-1}$), providing further evidence on the important role of SNF in common bean productivity especially in Africa where farmers do not apply N fertilizers to common bean.**

**Keywords:** Nitrogen, symbiotic nitrogen fixation, nitrogen derived from atmosphere, nitrogen redistribution.

## INTRODUCTION

Nitrogen (N) is the most limiting element of seed yield in common bean (*Phaseolus vulgaris*). Common bean meets its N growth requirements through uptake of N from the soil and/or symbiotic nitrogen fixation (SNF). Contributions of either of these two sources depend on nutritional status of the soil and the SNF ability of a genotype.

In Africa and some parts of Latin America, small-scale farmers grow common bean without N fertilizer and on soils with less optimal N fertility. In low input faming systems such as this, SNF plays an important role in meeting the N requirements for successful growth and productivity (Mafongoya et al., 2009). Adequate genetic variation for SNF exists within the primary gene pool of common bean to support development of genotypes with high SNF ability (Barbosa et al., 2018; Diaz et al., 2017; Bliss, 1993; Farid et al., 2017; Graham, 1981; Handarson et al., 1993; Kamfwa et al., 2015, 2017, 2019; Heilig et al., 2017). Identification of genotypes with high SNF ability could support breeding efforts for genetic

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enhancement of SNF.

Prior to flowering, vegetative tissues including leaves and stems act as sinks of N obtained from either the soil or SNF. From flowering to physiological maturity, the developing pod and seed become a major sink of N. Common bean like other legumes has a relatively higher N seed requirement than other crops such as cereals. This high N requirement is met primarily through N uptake from the soil, SNF or N redistributed from the vegetative organs to the seed during grain filling period. Nitrogen sourced directly from the soil or SNF is not sufficient to meet the high N requirement of the developing seed. To meet this N deficit, the plant remobilizes N from its sinks including leaves, stems and pod walls. The quantity of N remobilized depend on several factors including the amount of N stored in the sinks prior to reproductive growth stage and also the genotypic efficiency of N redistribution. In soybeans, about 50-60% of N in the mature seed is N redistributed from vegetative organs including leaves, stems and petiole while the rest is from soil uptake and SNF (Hanway and Weber, 1971; Egli and Leggett, 1973). N redistribution from vegetative organs is a way plants compensate for the shortfalls in N from the soil and SNF. The relationship between NDFA and degree of N redistribution has not been studied in common bean. Because common bean is relatively weak in SNF, and in Africa it is grown on soils with low N and without N fertilizers, redistribution of N from within the plant may be critical to meeting the high N requirement of the developing seed and for seed yield. Genotypes that can efficiently mobilize N from these sinks would provide more N to the seed, and have higher seed yield. The objective of this study was to investigate varietal differences in SNF and redistribution of N in the leaves and stems.

## MATERIALS AND METHODS

A total of five Andean genotypes were evaluated for SNF and for N redistribution from leaves and stems between flowering and physiological maturity. These genotypes included Sisi, Bukoba, Red Hawk, Mecost and Cardinal. All five varieties have determinate growth habit (Type 1). The two varieties Sisi and Bukoba are from Tanzania and have yellow seed color. The other three varieties, Red Hawk (light red kidney), Mecosta (light red kidney) and Cardinal (cranberry) are from the USA. The five genotypes were evaluated in a rain-fed field trial conducted at University of Zambia, Lusaka, Zambia in 2018. Evaluation was conducted on soil classified as fine loamy isohyperthermic paleustalf. A randomized complete block design with three replications was used. Each genotype was planted in a single row plot 4 m long and inter-row spacing of 0.50 m. A non-nodulating mutant called R99 was included in the experiment as a check for N stress and also for calculating the percentage of NDFA.

At planting, seed was inoculated with *Rhizobium tropici* (strain CIAT 899). There was no fertilizer applied to the crop. Plant samples for evaluations for SNF and redistribution were collected at flowering and physiological maturity. At flowering, three plants were randomly selected from each genotype in each replication, and cut at the soil level. Immediately after harvest, each plant was separated into leaves and stems, which were then dried in the oven for 72 h at 65°C. After drying, leaves and stems were ground separately to a fine powder using a Thomas-Wiley Lab Mill fitted with a 1 mm screen. At physiological maturity, three plants were randomly selected and harvested similarly to the plants at flowering stage. Harvested plants were separated into leaves, stems and pods and were dried in the oven at 65°C for 72 h. After drying, pods were later separated into pod wall and seed. The leaves, stems, pod wall and seeds were ground to a fine powder using a Thomas-Wiley Lab Mill fitted with 1 mm screen. The fine powder was then packed into aluminum foil capsules and sent to the Isotope Facility at University of California, Davis, for total N and N15 measurements. The N15 natural abundance method, which is considered to be the most accurate method for estimating SNF in field trials, was used to estimate the %NDFA for each genotype using ground dry seed (Unkovich et al., 2008). Below is the equation used for estimating %NDFA using the 15N natural abundance method (Giller, 2001):

$$\%Ndfa = \left(\delta^{15}N_{reference\ \ plan\,t} - \delta^{15}N_{fixing\ \ plant}\right) / \left(\delta^{15}N_{reference\ \ plant} - B\right)$$

Where: %NDFA is percentage of N in the seed that is derived from atmosphere through SNF; $\delta^{15}N_{reference\ plant}$ is the $^{15}N$ in the non-nodulating check (R99), non-fixing plant; $\delta^{15}N_{fixing\ plant}$ is the $^{15}N$ in the fixing genotype; 'B' is the $^{15}N$ of the same N fixing genotype when grown in N-free greenhouse conditions. Each genotype had its own 'B' value derived from previous 15N estimates of the five genotypes in the greenhouse (Kamfwa et al., 2015). For the current study, redistribution was computed as the percentage reduction in N concentration in the leaves or stems between flowering and physiological maturity.

### Data analyses

Statistical analyses on all data collected from the experiment were conducted in SAS 9.1. Analysis of variance was conducted using Proc Mixed based on the following statistical model:

$$Y_{ik} = \mu + \alpha_i + \gamma_k + \varepsilon_{ik}$$

Where: $Y_{ik}$ was the response variable e.g., NDFA for genotype i, replication k; $\alpha_i$ was the fixed variable effect of the genotype i; $\gamma$ was the random variable effect of a replication; $\varepsilon$ was the residual associated with replication k in genotype i.

## RESULTS

Varietal effect was significant (P<0.001) on %NDFA, NDFA, pod wall N, and seed N (P<0.001), and seed yield. Significant varietal differences (P< 0.001) were observed in percentage reduction of N concentration in the leaf and stem between flowering and physiological maturity.

Percentage reductions in N in the leaf ranged from 51.5% (Cardinal) to 59.3% (Sisi) and an average of 55.2% for the five varieties (Table 1). The percentage reductions in N in the stem ranged from 50.3% (Bukoba) to 70.8% (Sisi) and an average of 60.4% for the five varieties. The pod wall N percentage ranged from 0.5%

**Table 1.** Means for percentage reduction of nitrogen in leaf and stem, pod wall nitrogen percentage, seed nitrogen percentage, percentage of nitrogen derived from atmosphere (%NDFA), nitrogen derived from atmosphere (NDFA) and seed yield of common bean genotypes grown in a field in 2019 growing season at University of Zambia, Lusaka, Zambia.

| Genotype | % Red Leaf N | % Red Stem N | Pod N% | Seed N% | %NDFA | NDFA (kg ha$^{-1}$) | Yield (kg ha$^{-1}$) |
|---|---|---|---|---|---|---|---|
| Bukoba | 53.7 | 50.3 | 0.8 | 2.8 | 51.9 | 21.6 | 1488 |
| Sisi | 59.3 | 70.8 | 0.6 | 3.2 | 45.3 | 23.3 | 1608 |
| Mecosta | 56.0 | 51.9 | 0.5 | 3.7 | 67.9 | 48.4 | 1925 |
| Red Hawk | 55.3 | 58.5 | 0.5 | 3.6 | 70.9 | 45.5 | 1783 |
| Cardinal | 51.5 | 70.4 | 0.5 | 3.6 | 49.6 | 15.9 | 888 |
| Avg | 55.2 | 60.4 | 0.6 | 3.4 | 57.1 | 30.9 | 1538 |
| R99 (Check) | - | - | 0.5 | 3.1 | 0 | 0 | 625 |

% Red =Percentage reduction; N=Nitrogen; %=percentage; %NDFA=percentage of nitrogen derived from atmosphere, NDFA=Nitrogen derived from atmosphere.
Source: Author

(Cardinal and Red Hawk) to 0.8% (Bukoba) with an average of 0.6%. Seed N percentage ranged from 2.8% (Bukoba) to 3.7% (Mecosta) with an average of 3.4% for the five varieties. The %NDFA ranged from 45.3% (Sisi) to 70.9% (Red Hawk), with an average of 57.1% for the five genotypes. For NDFA, the range was from 15.9 kg ha$^{-1}$ (Cardinal) to 48.4 kg ha$^{-1}$ (Mecosta) with an average of 30.9 kg ha$^{-1}$. The lowest yielding variety was Cardinal (888 kg ha$^{-1}$) while the highest yielding was Mecosta (1925 kg ha$^{-1}$), and the average for the five varieties was 1538 kg ha$^{-1}$ (Table 1).

## DISCUSSION

In the current study, %NDFA and NDFA significantly varies among the five genotypes evaluated. Though only a five genotypes were evaluated in the current study, the significant variability for %NDFA and NDFA observed among genotypes is consistent with previous studies that have reported significant genetic variability for SNF in common bean (Kamfwa et al., 2015). Among the five varieties evaluated, Mecosta and Red Hawk showed superior SNF ability. The %NDFA and NDFA were 71% and 45 kg ha$^{-1}$, respectively for Mecosta while for Red Hawk the %NDFA and NDFA were 67% and 48 kg ha$^{-1}$, respectively. Though all the five genotypes evaluated in the current study were determinate (bush; Type I), the %NDFA for Mecosta and Red Hawk were higher than the previously reported %NDFA averages of 40 and 60% for bush beans and climbing beans, respectively (Unkovich and Pate, 2000; Herridge et al., 2008; Peoples et al., 2009; Hardarson and Atkins, 2003; Ramaekers et al., 2013). This superior SNF ability for Mecosta and Red Hawk observed in the current study compared to other varieties shows the potential that exists within the bush type varieties for genetic improvement of their SNF. Mecosta and Red Hawk could potentially be used as parents for genetic improvement of SNF for bush type varieties. Both Mecosta and Red Hawk, which comparatively had higher %NDFA and NDFA than the other three genotypes, also had high redistribution of N from the leaves for Red Hawk and from the stem for Mecosta. This result suggest that despite being superior in N fixation, Mecosta and Red Hawk still had significant reliance on the N redistributed mainly from the leaf and stem, respectively, to meet their seed N requirements and support high seed yield. This result confirms the important role of N redistribution in common bean from stems and leaves regardless of the SNF ability of a genotype.

The variety Sisi, which had the highest redistribution of N between flowering and physiological maturity, had the lowest %NDFA providing further evidence on the important role of N redistribution in meeting the N requirements in genotypes with low SNF ability. The varieties Red Hawk and Mecosta could be considered as ideal genotypes for N nutrition because of their superiority in SNF and significant N redistribution capacity, which could ensure high seed yield even when grown on soils with low N and without N fertilizer.

In addition to leaves and stems, the pod wall is another tissue that N is remobilized from to meet the N requirements of the seed. In the current, there were significant varietal differences in N% in pod walls, which may suggest differences between varieties in their N accumulation and redistribution from the pod wall. Bukoba, which had the lowest seed N percentage (2.8%) among the five genotypes, had the highest N% in the pod walls at physiological maturity. This particular result may suggest poor N accumulation/or redistribution capacity of Bukoba in the pods or from the pods to the seed.

The two varieties Mecosta and Red Hawk, which had high SNF (%NDFA and NDFA), also had higher seed yields of 1925 and 1783 kg ha$^{-1}$, respectively than the other three varieties. This result provides further evidence on the important role of SNF in common bean productivity especially in low input agricultural systems such as the ones in Africa where farmers do not apply nitrogen fertilizers to the beans.

## Conclusion

The current study has identified significant genetic variability for SNF between five Andean genotypes. Variety Mecosta was superior to the other genotypes in SNF. Additionally, the study has demonstrated the important role of N redistribution to meeting the N requirements for seed yield in common bean. The varieties Mecosta and Redhawlk showed superior SNF ability, N redistribution and seed yields, and can be used for genetic enhancement of SNF in Andean beans, especially those with bush growth habit.

## CONFLICT OF INTERESTS

The author has not declared any conflict of interests.

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