Optimizing Biological Nitrogen Fixation Inexpensively as Part of a Sustainable Agriculture Kit (SAK) Strategy to Assist Subsistence Farmers

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Funded by the Canadian International Food Security Research Fund (CIFSRF)
University of Guelph

• 1hr drive from Toronto, Canada

• Canada’s oldest and largest agricultural university

• Ranked #9 in agricultural research output (globally) and #1 in Canada for inventions per faculty

• ~20,000 students
The Raizada Lab

Hanan Shehata
PhD Student

Malinda Thilakarathna
Post-doc
Globally 75% of malnutrition is in rural areas of which amino acid deficiency is especially problematic. Nitrogen fertilizers are also expensive.

**Symbiotic Nitrogen Fixation (SNF)** - Rhizobia bacteria inside legume root nodules convert atmospheric $\text{N}_2$ gas into ammonia to **build protein**, chlorophyll and other organic molecules which can be released into soil during decomposition **as organic fertilizer**
How can SNF be improved to help farmers, especially smallholder farmers?

**Problem 1:** Sub-optimal rhizobia in soil  
**Solution:** Coat seeds or spray soil with compatible/improved rhizobia bacteria (technology called “rhizobia inoculant”)

**Problem 2:** Poor crop variety  
**Solution:** Breed/select legumes with improved SNF (e.g. more active nodules, or resistance to drought stress)

**Problem 3:** Low micronutrients in soil (or P fertilizer)  
**Solution:** Add fertilizers to the soil (Mo, B)

For all of these, one needs to diagnose the problem and test different possible solutions (e.g. test many rhizobia strains)
Current methods for assessing SNF and their limitations

- Dry matter yield method (DM)
- Total N difference method
- Nodule observations
- Acetylene reduction assay (ARA)
- Hydrogen evolution
- Xylem-solute technique
- $^{15}$N isotope (%Ndfa)***

Limitations
- Vary in reliability
- Time consuming
- Expensive ($10-20 per sample)
- Can analyze only few samples at a time
- Difficult to examine nodule to nodule variation

We need an efficient, low cost method to measure SNF in developing nations
1. Optimizing symbiotic nitrogen fixation (SNF) in legumes
   1.1. Introduction to SNF and the *GlnLux* biosensor
   1.2. Detection of SNF in colonies of rhizobia *in vitro*
   1.3. Detection of SNF in legumes *in planta*

2. Helping farmers to overcome barriers to maximize legume production

3. The Sustainable Agriculture Kit (SAK) strategy
Legumes – a portion of fixed nitrogen is transferred to leaves as amino acids such as glutamine (Gln)
The GlnLux biosensor is an *E. coli* auxotroph that detects the amino acid glutamine, grows and releases measurable photons (Tessaro and Raizada, 2012).
GlnLux Agar Assay for High-Throughput Screening Bacterial Colonies for N-fixation (1-2 h protocol)

Illustration by Lisa Smith

Shehata, Tessaro, Annan, Dong and Raizada (2016) In preparation
The GlnLux biosensor can detect nitrogen fixation output in single colonies on agar.

Colonies of *Bradyrhizobium japonicum* (510, 110) and *Sinorhizobium meliloti* (1312, JO810) wild type versus mutant nif strains on GlnLux agar after incubation for 8-22 hrs. Images were taken using CCD camera using a 600 sec exposure.

Shehata, Tessaro, Annan, Dong and Raizada (2016) In preparation
**GlnLux** agar technology was used to detect nitrogen fixation in bacterial endophytes isolated from **maize seeds**

<table>
<thead>
<tr>
<th>Detection of BNF</th>
<th>Number of endophyte strains</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Total GlnLux +</td>
<td>54 (out of 96)</td>
<td></td>
</tr>
<tr>
<td>GlnLux + and ARA or DBH +</td>
<td>53</td>
<td></td>
</tr>
<tr>
<td>GlnLux + and ARA or DBH -</td>
<td>1 (possible false positive)</td>
<td></td>
</tr>
<tr>
<td>GlnLux - and ARA or DBH +</td>
<td>5 (possible false negatives)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>59</td>
<td></td>
</tr>
</tbody>
</table>

**ARA**
acetylene reduction assay

**DBH**
Dot blot hybridization with *nif* probe

Shehata, Tessaro, Annan, Dong and Raizada (2016) In preparation
GlnLux assays permit high throughput screening of *in vitro* nitrogen fixation

• Thousands of colonies can be screened in a single day inexpensively and rapidly

• However, there are capital costs

• May enable screening for:
  -- new nitrogen fixing bacteria
  -- selection of inoculants (e.g. directed evolution) for improved nitrogen fixation under stress conditions or specific niches
1. Optimizing symbiotic nitrogen fixation (SNF) in legumes

1.1. Introduction to SNF and the GlnLux biosensor

1.2. Detection of SNF in colonies of rhizobia in vitro

1.3. Detection of SNF in legumes in planta

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3. The Sustainable Agriculture Kit (SAK) strategy
Current problem is detecting extent of low activity nodules within a root system (e.g. due to stress): *GlnLux Agar Assay for Detecting SNF Output at Nodule Scale Resolution (1-2 h protocol)* for any Legume Species/Variety-Rhizobia Combination.
Methodology: Primarily indoors without added N (except starter N)
Identification of Active Sites of SNF using Split Root Systems (wild-type vs \textit{nif} mutant rhizobia)

Green peas (\textit{Pisum sativum} L.)

Soybean (\textit{Glycine max} (L.) Merr.)

Thilakarathna and Raizada (2016) Submitted
Identification of Active Sites of SNF using Split Root Systems (wild-type vs \textit{nif} mutant rhizobia)

Alfalfa (\textit{Medicago sativa} L.)

Thilakarathna and Raizada (2016) Submitted

Lentil (\textit{Lens culinaris} Medik.)
Most applicable to scientists working with smallholder farmers: 

**GlnLux 96-well Liquid Assay for *in planta* nitrogen fixation:**

Uses a single leaf punch

(3 h protocol, $1 USD per sample)

1. Punch a leaf disk
2. Freeze in liquid nitrogen
3. Grind with sand + add protease inhibitor
4. Collect supernatant (dilute 1/100)
5. M9 minimal medium + plant extracts + GlnLux bacteria
6. Incubate at 37 °C
7. Allow luminescence to accumulate
8. Compare tissue Gln level
9. Measure Lux

**Grow plants**

without N

or only starter N
Effect of different rhizobia strains on SNF of lentil: *GlnLux* leaf punch liquid assay

Rhizobia strains

**d**

![Graph showing GlnLux (RLU) vs. %Ndfa](image)

\[ R^2 = 0.997 \]

\[ p = 0.001 \]

Thilakarathna, Moroz and Raizada (2016) Submitted
Effect of different crop varieties on SNF of common bean: *GlnLux* leaf punch liquid assay

Crop varieties: R99, OAC Mist, OAC Rico, Sanilac

![Image](image_url)

**Figure b:**
- GlnLux (RLU) for R99, OAC Mist, OAC Rico, Sanilac
- Bars represent mean ± SE.
- Different letters indicate significant differences.

**Figure c:**
- Amount of N fixed (mg N plant⁻¹) for R99, OAC Mist, OAC Rico, Sanilac
- Bars represent mean ± SE.
- Different letters indicate significant differences.

**Figure d:**
- Scatter plot showing the relationship between GlnLux and amount of shoot N fixed (mg plant⁻¹)
- R² = 0.94

Thilakarathna, Moroz and Raizada (2016) Submitted
Relevance of *GlnLux* leaf punch liquid assay from the greenhouse to the field: **Common bean**

**Greenhouse data**

**Field Data (low N field)**

*Mean %Ndfa of bean cultivars under field conditions in 2011 and 2012 (Farid and Navabi 2015)*
Conclusions to Part I – GlnLux biosensor

- *GlnLux* biosensor is a **new method to measure SNF output** in non-transgenic ureide- and amide-exporting legumes.

- *Glnlux* agar permits **screening of colonies** of rhizobia for SNF activity, potentially to permit strain improvement.

- *GlnLux* 96-well liquid assay uses **single leaf punches** to measure relative SNF output in plants growing without exogenous N, making it a **rapid, low-cost, high throughput** screening method.

- *GlnLux* agar permits **visualization of active sites** of nitrogen fixation.

- *GlnLux* can be used to **pre-screen** plants inoculated with different **rhizobia candidates** prior to field testing.

- *GlnLux* may be useful for **pre-screening of crop varieties that vary in SNF** prior to field testing.

- *GlnLux* measures Gln only which limits the assay.
GlnLux may be a good tool for the early stages of legume variety and rhizobia screening, but can be used on smallholder farms to quantitatively diagnose low nitrogen fixation and improved nitrogen fixation?
GlnLux field trials with terrace farmers in Nepal
Kaski (Nepal) terrace field trial - Adjusted grain yield (g) demonstrates that rhizobia inoculant response is site-specific and hence an inexpensive diagnostic technology is needed for site-specific recommendations.

### Common bean

<table>
<thead>
<tr>
<th>Farmer (anon Number)</th>
<th>T1 Uninoculated</th>
<th>T2 B + Mo micronutrients</th>
<th>T3 Local rhizobia</th>
<th>T4 Canada/US rhizobia</th>
<th>T5 Canada/US rhizobia + B +Mo</th>
</tr>
</thead>
<tbody>
<tr>
<td>22</td>
<td>194</td>
<td>292</td>
<td>548</td>
<td>348</td>
<td>559</td>
</tr>
<tr>
<td>29</td>
<td>1076</td>
<td>447</td>
<td>466</td>
<td>294</td>
<td>518</td>
</tr>
</tbody>
</table>

### Cowpea

<table>
<thead>
<tr>
<th>Farmer (anon Number)</th>
<th>T1 Uninoculated</th>
<th>T2 B + Mo micronutrients</th>
<th>T3 Local rhizobia</th>
<th>T4 Canada/US rhizobia</th>
<th>T5 Canada/US rhizobia + B +Mo</th>
</tr>
</thead>
<tbody>
<tr>
<td>22</td>
<td>286</td>
<td>259</td>
<td>199</td>
<td>133</td>
<td>170</td>
</tr>
<tr>
<td>39</td>
<td>96</td>
<td>87</td>
<td>158</td>
<td>256</td>
<td>273</td>
</tr>
</tbody>
</table>

_GlnLux_ technology has been transferred to Nepal (NGO, LI-BIRD) and we are awaiting final Year 1 _GlnLux_ field analysis.
1. Optimizing symbiotic nitrogen fixation (SNF) in legumes

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2. Helping farmers to overcome barriers to maximize legume production

3. The Sustainable Agriculture Kit (SAK) strategy
Helping farmers to overcome barriers to maximize legume production as part of the Sustainable Agriculture Kit (SAK) Project on Nepalese terrace farms

1. Funded by a $2.3 million grant from the Canadian International Development Research Centre (IDRC) and Global Affairs Canada (Canadian PI: MN Raizada).

2. For more information, to go: www.SAKNepal.org
A holistic, farm-systems and human-centered based approach is being undertaken based on farmer-identified opportunities and complaints........
Lesson: If small spheres on legume roots are only yellow inside, they do not contain healthy microbes to make natural nitrogen fertilizer, but a pink colour inside means they are producing fertilizer.

1. Problem: legume leaves such as lentil are yellow causing low yields: might be disease or lack of fertilizer

2. To test, gently remove roots of few plants

3. Cut small spheres

4. If yellow or white inside, microbes are not working.

5. Must purchase nitrogen fertilizer.


7. Remove roots: if small sphere are pink inside, its means microbes are producing fertilizer

8. Less need to purchase nitrogen fertilizer.
Lesson: Sowing maize together with cowpea will yield more profit than maize only.
1. Intercropping Trials

A. Seasonal Intercropping Trials (row/mixed intercropping)

**Season 1**: Mid-March to Mid-July: Unit plot size: 30 m² for both test and non-test plots in split-plots

<table>
<thead>
<tr>
<th>Combinations</th>
<th>Intercrop (Test) Plot Yield (t/ha)</th>
<th>Non-test Plot Yield (t/ha)</th>
<th>% Increase or Decrease</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Non-legume</td>
<td>Legume</td>
<td>TOTAL</td>
</tr>
<tr>
<td>Maize-makaibodi (Kaski)</td>
<td>4.10</td>
<td>0.99</td>
<td>5.09</td>
</tr>
<tr>
<td>Maize-makaibodi (Dhading)</td>
<td>11.98</td>
<td>2.00</td>
<td>13.98</td>
</tr>
<tr>
<td>Maize-suryabodi (Kaski)</td>
<td>3.97</td>
<td>0.88</td>
<td>4.85</td>
</tr>
<tr>
<td>Maize-suryabodi (Kaski)</td>
<td>5.21</td>
<td>4.93</td>
<td>10.14</td>
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<tr>
<td>Maize-bean (Kaski)</td>
<td>7.96</td>
<td>0.11</td>
<td>8.06</td>
</tr>
<tr>
<td>Maize-bean (Kaski)</td>
<td>14.21</td>
<td>0.67</td>
<td>14.88</td>
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<tr>
<td>Maize-bean (Kaski)</td>
<td>6.80</td>
<td>0.10</td>
<td>6.90</td>
</tr>
<tr>
<td>Maize-bean (Kaski)</td>
<td>12.79</td>
<td>0.89</td>
<td>13.68</td>
</tr>
</tbody>
</table>
Lesson: A jab planter reduces people and livestock required to sow seeds

1. Traditional practice requires cattle and 2+ people. Difficult on steep hillside or narrow terrace.

2. New tool: jab planter (request from local vendors): Insert seed at top, then as you walk along row, press down to sow seed.

3. Single person can use

4. Helps with line sowing
A low cost seed planter (jab planter) to reduce the need for human and livestock labour especially on narrow terraces
A low cost seed planter (jab planter) to reduce the need for human and livestock labour especially on narrow terraces

### Current progress:
- 100 farmers are currently testing different models

### Preference with respect to tiredness

<table>
<thead>
<tr>
<th>Preference</th>
<th>Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jab planter</td>
<td>35</td>
</tr>
<tr>
<td>Plough</td>
<td>6</td>
</tr>
<tr>
<td>Indifferent</td>
<td>1</td>
</tr>
</tbody>
</table>

### Will you use this in future?

<table>
<thead>
<tr>
<th>Will you use this in future?</th>
<th>Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>39</td>
</tr>
<tr>
<td>No</td>
<td>3</td>
</tr>
</tbody>
</table>
Lesson: Climbing beans can be planted at the base of the terrace wall for growth up the wall to maximize usage of the vertical surface area.

1. Traditional practice: maize or other large crops at terrace edge are stunted

2. Soil erosion at terrace edge

3. Traditionally, low yield near terrace edge

4. Improved practice: climbing beans planted at base of terrace wall which grow up

5. Less erosion from terrace edge

6. Terrace wall is better utilized

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Lesson: Waterfall-type legumes can be planted at the top edge of the terrace wall and grow down the wall to maximize usage of the vertical surface area.

1. Traditional practice: maize or other large crops at terrace edge are stunted

2. Soil erosion at terrace edge

3. Traditionally, low yield near terrace edge

4. Improved practice: sow waterfall-type legumes at base of terrace wall which grow down

5. Less erosion from terrace edge

6. Terrace wall and edge are better utilized

- blackgram
- ricebean
- cowpea
- kidney bean

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### 3. Edge Crops: Yield and Income Benefits

<table>
<thead>
<tr>
<th>Crop</th>
<th>Area (m²) covered (/plant)</th>
<th>Plant parts</th>
<th>Yield (kg/ha)</th>
<th>Price (Rs/kg)</th>
<th>Gross income (Rs.)</th>
<th>Costs (Rs.)</th>
<th>Income less cost (Rs.)</th>
<th>NET INCOME (CAD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rice bean (Kaski)</td>
<td>2.32</td>
<td>Grain</td>
<td>486</td>
<td>60</td>
<td>29,160</td>
<td>~10,000</td>
<td>19,160</td>
<td>$240</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Biomass</td>
<td>3367</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rice bean (Dhading)</td>
<td>1.98</td>
<td>Grain</td>
<td>374</td>
<td>60</td>
<td>22,440</td>
<td>~10,000</td>
<td>12,440</td>
<td>$156</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Biomass</td>
<td>535</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Horsegram (Kaski)</td>
<td>0.58</td>
<td>Grain</td>
<td>315</td>
<td>90</td>
<td>28,350</td>
<td>~10,000</td>
<td>18,350</td>
<td>$229</td>
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<td>Biomass</td>
<td>2734</td>
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<tr>
<td>Horsegram (Dhading)</td>
<td>0.48</td>
<td>Grain</td>
<td>317</td>
<td>90</td>
<td>28,530</td>
<td>~10,000</td>
<td>18,530</td>
<td>$232</td>
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<td>Biomass</td>
<td>694</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Black gram (Kaski)</td>
<td>0.28</td>
<td>Grain</td>
<td>248</td>
<td>90</td>
<td>22,320</td>
<td>~10,000</td>
<td>12,320</td>
<td>$154</td>
</tr>
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<td></td>
<td></td>
<td>Biomass</td>
<td>584</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blackgram (Dhading)</td>
<td>0.34</td>
<td>Grain</td>
<td>256</td>
<td>90</td>
<td>23,040</td>
<td>~10,000</td>
<td>13,040</td>
<td>$163</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Biomass</td>
<td>636</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cowpea (Kaski)</td>
<td>1.03</td>
<td>Grain</td>
<td>288</td>
<td>60</td>
<td>17,280</td>
<td>~10,000</td>
<td>7,280</td>
<td>$91</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Biomass</td>
<td>2308</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- **Use as fodder for livestock**
Lesson: Planting vetch in the dry season will reduce soil erosion and provide animal fodder

1. Traditional practice: nothing is sown in the dry season

2. Soil erosion when first rains arrive

3. Little animal fodder in the dry season

4. Improved practice: sow vetch prior to the beginning of the rainy season

5. Reduced erosion

6. Good animal fodder in dry season
Lesson: Kneepads can reduce pain at knees and prevent knees from becoming wet or cold such as during weeding.

1. Traditional practice causes cold, pain on knees.

2. New practice.

3. Purchase from vendor.
Lesson: Gloves reduce pain and damage to hands.

1. Traditional practice

2. New method:
   Gloves protect hands.
   Request from local vendors.
Lesson: Before sowing seeds, use a magnifying glass/sheet to help remove seeds with disease or pests.

1. Seeds for sowing may have small spots or damage due to insects or mold.

2. Purchase magnifying glass/sheet from vendor.

3. Separate unspotted, undamaged seed.

4. Sow healthy seed only.

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Lesson: Healthy seeds can be easily separated from sick seeds prior to sowing using water floatation.

1. Traditional practice: seeds with small disease spots or containing small insects may be missed, and sown in field.

2. Field will be sick, low yields.

3. Improved practice: Add seeds to water.

4. Sick seeds are light-weight and will float.

5. The light-weight seeds should not be planted as they will produce a sick field.

6. Healthy seeds are heavy and will sink.

7. The heavy seeds will produce a healthy field.

8. If seed size is large, then salt should be added to jar to better enable seed separation.
Lesson: New tools to reduce drudgery of hand removal of weeds: Long-handed, medium cost options.

1. Long handled weeders

3. Short handled weeders

2. Home-made: wood and nails

4. How to use
New weeding tool made by local Nepalese blacksmiths, creating local jobs, created using participatory testing with women farmers
Lesson: Special bags can be used to store grain which reduce oxygen inside bag which prevents insects and fungal molds from surviving, which also reduces toxins.

1. Traditional practice: stored grain is damaged by insects and mold. The mold can produce toxins in the grain.

2. New practice
   3. Dry grain completely

4. Purchase bag from vendor. Put grain in bag, remove air and tie

5. Special bag causes air to flow outside, causing death to insects and mold.

5. Put bag inside a jute bag. Elevate from ground if possible to prevent rodents.

6. High yielder if sown and less toxins in food.

7. Re-use bag many times.

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Lesson: Rather than selling harvested products in bulk, it is more profitable to package them beautifully.

1. Traditional practice: 'sell peanuts in bulk. Obtain little money at market.'

2. Improved practice: place peanuts in packages with beautiful, colourful labels.

3. Packaged peanuts obtain a higher price at the market.
Lesson: People especially pregnant women and children should eat legumes/pulses

1. Not recommended practice: pregnant women, teenage girls or children eat mostly large grains such as maize or rice or tubers such as cassava

2. Child will be stunted

3. Extended belly
4. Skin cracking
5. Hair discoloured or falling out

6. Recommended practice: add legumes, pulses, lentils, beans at each meal

7. Normal child growth

8. Normal belly
9. Normal skin
10. Normal hair

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The Challenge of Scaling Up

**Problem:** There are ~400 million smallholder farming families (<2Ha) who have little cash income. They live in remote areas, receive little knowledge extension support and have poor access to private sector inputs and markets.

Farmer pilot projects by NGOs and governments are typically not scaled up to impact the ~2 billion people who need them.
Challenge of Scaling Up is Enormous in the Remote, Mountainous Regions of Nepal
Step 1. Survey local farmers for their needs and innovations with partnership with a grassroots NGO
**Example:** Nepal SAK Survey Question: Corn kernels were being removed from cob by labour-intensive methods for women

<table>
<thead>
<tr>
<th>Variable</th>
<th>Kaski</th>
<th>Dhading</th>
</tr>
</thead>
<tbody>
<tr>
<td>Finger Millet</td>
<td>Stamping with feet: 30 Beating with sticks: 12</td>
<td>Feet: 10 Beating with sticks: 15</td>
</tr>
<tr>
<td>Maize</td>
<td>Hand: 29 Cobs placed in sack and beaten with sticks: 29</td>
<td>Hand: 15 Cobs placed in sack and beaten with sticks: 8</td>
</tr>
<tr>
<td>Cowpea</td>
<td>Beating by sticks: Hand: 21</td>
<td>Beating by sticks: 1 Hand: 5</td>
</tr>
<tr>
<td>Ricebean</td>
<td>Beating by sticks: Hand: 4</td>
<td>Beating by sticks: 2</td>
</tr>
<tr>
<td>Bean</td>
<td>Beating by sticks: Hand: 13</td>
<td>Beating by sticks: 2 Hand: 4</td>
</tr>
<tr>
<td>Horsegram</td>
<td>Beating by sticks: 4</td>
<td>Beating by sticks: 2</td>
</tr>
</tbody>
</table>

Data collected by LI-BIRD, Nepal (unpublished)

**Proposed intervention:** simple kernel sheller............
Innovation: A $2 handheld tool to reduce kernels of corn from the cob (corn sheller) reduces female drudgery and prevents kernel breakage.
Sustainable Agriculture Kit (SAK) Methodology

**Step 1.** Survey local farmers for their needs and innovations with partnership with a grassroots NGO

**Step 2.** Decide menu of innovations that are low cost, purchasable, low labour, women friendly, sustainable
APPETIZERS

Chicken Wings $7.99
Fresh local chicken wings grilled and topped with buffalo, blue cheese or BBQ sauce.

Potato Skins $5.99
Baked potato filled with sour cream and bacon bits.

Onion Rings $4.99
Thick cut onions breaded and served with BBQ or Blue Cheese.

Pepper Poppers $5.99
Made with local peppers, bacon, cream cheese and jalapenos.

Chili Bean Dip $5.99
Special chili sauce served with french fries or nachos.

Grilled Sliced Sausages $7.99
Grilled to perfection served with Mustard, BBQ or Blue Cheese.

Cheese Quesadilla $5.99
Tortilla wrap with Swiss cheese and cheddar melted on the grill.

The Beach Combo $18.99
Tortilla wrap with Swiss cheese and cheddar melted on the grill.

SOUPS & SALADS

Potato Soup $6.99
Freshly baked potatoes sliced & diced with bacon bits and cheddar.

Vegetable Soup $5.99
Fresh vegetables cut and cooked in our flavorful seasoning.

Bean Soup $6.99
Tex Mex style Bean and cheese soup.

Salad Bar $7.99
Price per plate.

MAIN DISHES

Bean & Cheese Burritos $12.99
Our rich and tasty bean and cheese burritos grilled and served with sour cream and vegetables.

Grilled Pork Chops $14.99
Flame grilled with Chili, BBQ, Jack Daniels sauce.

Fajitas $17.99
Chicken, Beef or Vegetable Fajitas grilled and served with tomatoes, sour cream, grilled onions, lettuce and avocado.

Grilled Chicken Breast $13.99
Fresh chicken grilled to perfection. Add any of these sauces: Jamaican Jerk, Jack Daniels, BBQ or Chili Sauce.

Jack Daniels Ribs ½ $14.99 Full $18.99
Slow cooked to perfection and glazed. The best tasting ribs in town!

Churrasco Select $15.99
Fresh select cut of prime meat grilled to your style.

Fresh Angus Steak $24.99
Grade A. half pound Flat Meat Grilled and served with Chili, BBQ or Jack Daniels sauce.

Cowboy Steak $25.00
This special steak for only the bravest has 22oz of the best steak.

Sirloin Steak $29.99
The one and only half pound specialty steak, grilled to your taste.

The Beach Meat Lover $29.99
Har a combination of Grilled Chicken, Sausage & half a rack of ribs with Corn on the cob or baked potato.

Served with your choice of fries, baked potato or grilled vegetables.

KIDS MENU

Served with fries or fresh vegetables. Includes juice, milk, or soda.

Chicken Fingers $6.99

Mini Hamburgers $6.99

Mac & Cheese $5.99

Hot Dog Sliced $5.99

Cheese Tortilla $5.99

BURGERS

Bacon Cheese Burger $9.50
Onions, tomatoes & and cheese on a freshly toasted bun.

Doble Swiss Burger $9.50
Ground beef with Swiss cheese and served on fresh rye bread.

Ranch Jalapeno Burger $10.00
Swiss cheese, Jalapenos, Ranch sauce and served on fresh bread.

Onion Burger $10.00
Caramalized onions, Swiss cheese and served on fresh bread.

Vege Burger $9.50
Vegetarian patty with onions, tomato, and cheese on a fresh bun.

Grilled Chicken Burger $10.99
Grilled chicken with cheese, onions and tomato on fresh a bun.

Salmon Burger $15.99
Salmon fillet with onions, tomato, and cheese on a fresh bun.

The Beach Sandwich $11.99
Imported Churrasco cut with swiss cheese, onions and mushrooms. Served with fries, baked potato, mashed potatoes or grilled vegetables.
Stage of scaling up of the SAK Menu in Nepal (growing)
Step 1. Survey local farmers for their needs and innovations with partnership with a grassroots NGO

Step 2. Decide menu of innovations that are low cost, purchasable, low labour, women friendly, sustainable

Step 3. Test candidate innovations with test farmers (2 seasons, n=20 per innovation, split plots)
Survey of test farmers on the $2 corn sheller

Preference with respect to tiredness

<table>
<thead>
<tr>
<th>Hand corn sheller</th>
<th>Traditional method</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>39</td>
<td>0</td>
</tr>
</tbody>
</table>

Results:

- One woman can remove up to 80 kg of grain in a single day
- 11,000 corn shellers have already been procured with ~15,000 more planned by 2017

Female involvement

<table>
<thead>
<tr>
<th>Gender</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>7</td>
</tr>
<tr>
<td>Female</td>
<td>23</td>
</tr>
<tr>
<td>Both</td>
<td>9</td>
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# Trials and Demonstrations: An Overview

<table>
<thead>
<tr>
<th>SN</th>
<th>Trial</th>
<th>Year 1: 2015</th>
<th>Year 2: 2016</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Total Trials</td>
<td>Test Farmers</td>
</tr>
<tr>
<td>1.</td>
<td>Intercropping trials</td>
<td>12</td>
<td>77</td>
</tr>
<tr>
<td>2.</td>
<td>Wall crops</td>
<td>3</td>
<td>68</td>
</tr>
<tr>
<td>3.</td>
<td>Edge crops</td>
<td>4</td>
<td>46</td>
</tr>
<tr>
<td>4.</td>
<td>Cropping sequence</td>
<td>5</td>
<td>89</td>
</tr>
<tr>
<td>5.</td>
<td>Inverse slope</td>
<td>1</td>
<td>7</td>
</tr>
<tr>
<td>6.</td>
<td>Rhizobium trials</td>
<td>4</td>
<td>132</td>
</tr>
<tr>
<td>7.</td>
<td>Biochar</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>8.</td>
<td>Dry season forage</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>9.</td>
<td>Seed treatment trials</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td><strong>SUB-TOTAL</strong></td>
<td><strong>33</strong></td>
<td><strong>424</strong></td>
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</table>

## Demonstrations

<table>
<thead>
<tr>
<th>SN</th>
<th>Trial</th>
<th>Year 1: 2015</th>
<th>Year 2: 2016</th>
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</thead>
<tbody>
<tr>
<td>10.</td>
<td>FYM Improvement</td>
<td>1</td>
<td>49</td>
</tr>
<tr>
<td>11.</td>
<td>Drip irrigation + poly-house</td>
<td>1</td>
<td>41</td>
</tr>
<tr>
<td>12.</td>
<td>Hybrid maize seed production</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td><strong>SUB-TOTAL</strong></td>
<td><strong>3</strong></td>
<td><strong>95</strong></td>
</tr>
</tbody>
</table>

## Products (Tools and Supplies)

<table>
<thead>
<tr>
<th>SN</th>
<th>Product</th>
<th>Year 1: 2015</th>
<th>Year 2: 2016</th>
</tr>
</thead>
<tbody>
<tr>
<td>13.</td>
<td>Tools and equipment</td>
<td>9</td>
<td>478</td>
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<tr>
<td>14.</td>
<td>Composite seeds</td>
<td>1</td>
<td>377</td>
</tr>
<tr>
<td></td>
<td><strong>SUB-TOTAL</strong></td>
<td><strong>10</strong></td>
<td><strong>660</strong></td>
</tr>
</tbody>
</table>
Step 1. Survey local farmers for their needs and innovations with partnership with a grassroots NGO

Step 2. Decide menu of innovations that are low cost, purchasable, low labour, women friendly, sustainable

Step 3. Test candidate innovations with test farmers (2 seasons, n=20 per innovation, split plots)

Step 4. Use participatory surveys with test farmers to rank best innovations for scaling up
Participatory Champion SAKs Identification Exercise @ Majthana, Nepal

Women Participants: 17  
Men Participants: 12

Place Conducted:  
Gairi Saura, Kaski

SAKs Interventions:  
26 SAKs options
## Results of Champion SAKs Identification Exercise
### By Women Farmers @ Majthana

<table>
<thead>
<tr>
<th>SAK Products/ Practices</th>
<th>Score</th>
<th>SAK Products/ Practices</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>$2 Hand Corn Sheller</strong></td>
<td>54</td>
<td><strong>Free - Terrace Wall Crop</strong> (Yam, Chayote, Pumpkin, Cowpea)</td>
<td>54</td>
</tr>
<tr>
<td><strong>Free - Yam in Sacks</strong></td>
<td>54</td>
<td><strong>Rhizobium Trials</strong></td>
<td>24</td>
</tr>
<tr>
<td><strong>$2 Composite Vegetable Kits</strong></td>
<td>54</td>
<td><strong>$200 - Mini Tiller</strong></td>
<td>26</td>
</tr>
<tr>
<td><strong>$1 Hermetic Bags</strong></td>
<td>54</td>
<td><strong>$10 - Table Corn Sheller</strong></td>
<td>26</td>
</tr>
<tr>
<td><strong>Free - Maize+Ginger+Soybean</strong></td>
<td>54</td>
<td><strong>Animal Shed+FYM Improvement</strong></td>
<td>34</td>
</tr>
<tr>
<td><strong>Free - Edge Crops (rice bean, horse gram, blackgram)</strong></td>
<td>54</td>
<td><strong>etc etc (~18 more)</strong></td>
<td></td>
</tr>
</tbody>
</table>
Sustainable Agriculture Kit (SAK) Methodology

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Step 5. Procure and sell individual items from the regional menu to consumer farmers using pre-existing snackfood/cigarette/alcohol dealers into village stalls, using the NGO-spinoff company
Little stalls in the most remote villages around the world sell snacks, cigarettes and alcohol via pre-existing distributors.
## Current SAKNepal Private Sector Vendors (2016)

<table>
<thead>
<tr>
<th>District</th>
<th>Peri-urban snackfood dealers</th>
<th>Small machinery dealers</th>
<th>Farmer cooperatives</th>
<th>Agrovet Dealers</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chitwan</td>
<td>2</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>11</td>
</tr>
<tr>
<td>Nawalparasi</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>10</td>
</tr>
<tr>
<td>Tanahu</td>
<td>2</td>
<td>3</td>
<td>3</td>
<td>2</td>
<td>10</td>
</tr>
<tr>
<td>Dhading</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>11</td>
</tr>
<tr>
<td>Gorkha</td>
<td>2</td>
<td>3</td>
<td>3</td>
<td>5</td>
<td>13</td>
</tr>
<tr>
<td>Kaski</td>
<td>2</td>
<td>2</td>
<td>4</td>
<td>4</td>
<td>12</td>
</tr>
<tr>
<td>Parbat</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>3</td>
<td>10</td>
</tr>
<tr>
<td>Baglung</td>
<td>2</td>
<td>1</td>
<td>3</td>
<td>3</td>
<td>9</td>
</tr>
<tr>
<td>Myagdi</td>
<td>2</td>
<td>1</td>
<td>3</td>
<td>2</td>
<td>8</td>
</tr>
<tr>
<td>Lamjung</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>3</td>
<td>10</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td><strong>21</strong></td>
<td><strong>21</strong></td>
<td><strong>30</strong></td>
<td><strong>32</strong></td>
<td><strong>104</strong></td>
</tr>
</tbody>
</table>
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**Step 6.** Accompany each product with instructions in picture format, and a menu of practices in picture booklets, to communicate with illiterate women farmers
Lesson: Instead of removing grains of maize by beating sacks with a stick, a hand tool can be used which is faster and less painful, and results in seeds which are healthier with fewer toxins.

1. Traditional practice

3. Damages seed and cobs which allows more disease during seed storage which can produce toxins. Also, if these seeds are sown, germination may be low

2. Painful

4. New practice

5. Purchase hand tool from vendor

6. Twist tool around cob to remove seeds

7. Fast, less pain and less breakage of seeds and cob

8. Improved germination, less disease when these seeds are sown and fewer toxins when eaten
पाठ : कोसो तथा दलहन बालीहर्को जरामा मासिना गाँठहरु हुन्छन् जसमा भएका उपयोगी जीवाणुहरुले प्राकृतिक नाइट्रोजन मल बनाउँदछ जसलेखर्द गर्दैर्य सक्नुहोस्।

1. गलत तरिका : सबै मौसममा गर्ने, गाउँ, धान, काटोको एकल बालीहरुको रूपमा लगाउने र कोसो तथा दलहन बालीहरु नलागाउने।

2. बाली कटानी

3. त्यसोबाट गाँठहरुले हिट्टैल्ने मलहरु नाइट्रोजन मल खरीद गर्नु पर्नु हुन्छ।

4. सुधारिएको तरिका : कोसो तथा दलहन बालीहरु पुसुवा बालीहरुको रूपमा लगाउने अथवा अन्य मौसममा लगाउने।

5. कोसो तथा दलहन बालीहरुको जरामा मासिना गुलाबी रेखाको गाँठहरु हुन्छन् जसमा प्राकृतिक मल बनाउँदछ आँखाले देखन नसकिने जीवाणु हुन्छ। -फोटोमा देखाएको जसले निलो तरी जिवाणुलाई आँखाले देखे सकिन्छ।

5. अन्य मौसममा कोसो तथा दलहन बालीहरुको ताउमा मर्छे, धान, गाउँ ना कोहे सोहे लगाएका पुसुवा बालीहरुको रूपमा लगाएका जस अन्य बालीले प्राकृतिक कोसेबालीको मल उपयोग गरेका फायदा लिन सक्छ।

6. कोसेबालीको रात्रि खिखाइसंगै बालीहरुको जसा र पात्रहरु कूहिन्छ। जसले पाटलाई गरिन्छ गरने काम गर्नु।

7. यसको साथै कम मात्रामा नाइट्रोजन मलको खरीद धेरै पैसाँ जीवाणु सकिन्छ।

Manish N. Raizada, Ph.D.; Lisa Smith, Illustrator
SAK Picture Book • Creative Commons
Participatory editing of the SAK Picture Book with 56 female farmers lead by Rachana Devkota in Nepal

~500 edits requested!
• African version completed
• Other versions in progress
• East/Southeast Asia
• Latin America
• North Africa/Middle East
• Users can download individual lessons and add own text translations, and create custom booklets

A Picture Book of Best Practices for Subsistence Farmers: Afro-Caribbean version

June 2016
Manish N. Raizada, Ph.D.
University of Guelph
Illustrations by Lisa Smith
University of Guelph
Step 1. Survey local farmers for their needs and innovations with partnership with a grassroots NGO

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Step 7. Use mobile phones to obtain feedback from consumer farmers on efficacy and improvements needed
The cell phone penetration rate in Nepal is 83%
SAKNepal Project Goal: To reach 100,000 people (25,000 households) by early 2018 with private and public sector partners
Conclusion

1. Optimizing symbiotic nitrogen fixation (SNF) in legumes
   1.1. Introduction to SNF and the GlnLux biosensor
   1.2. Detection of SNF in colonies of rhizobia in vitro
   1.3. Detection of SNF in legumes in planta

2. Helping farmers to overcome barriers to maximize legume production

3. The Sustainable Agriculture Kit (SAK) strategy
SAKNepal Acknowledgements

• Funded by the Canadian International Food Security Research Fund (CIFSRF) (IDRC and Global Affairs Canada)
• Dr. Kevin Tiessen, IDRC
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• Roshan Pusasaini, SAKNepal coordinator, Nepal
• Bhawana Ghimire, LI-BIRD, Nepal and LI-BIRD Staff
• Dr. Ram Rana, LI-BIRD, Nepal
• Lisa Smith, SAK Picture Book graphic designer
• Anamolbiu staff (Nepal)
• Canadian private sector collaborators: PlantProducts (Ontario), XiteBio (Manitoba), Agriculex (Guelph)
• Post doctoral fellow: Dr. Malinda Thilakarathna
• Graduate students: Hanan Shehata, Rachana Devkota, Kamal Khadka, Finlay Small, Eamonn McGuinty, Michael Tessaro
• Gryphon Therault-Loubier, SAKNepal website coordinator
• Undergraduate research assistants: Jaclyn Clark (now MSc), Austin Bruch, Sara Wyngaarden, Caleb Niemeyer, Nick Moroz, Sophia Watts, Myla Manser
• Faculty advisors: Prof. Cate Dewey, Prof. Ralph Martin, Prof. Helen Hambly, Prof. Alastair Summerlee, Prof. Ali Navabi and other graduate committee members
• 400 Guelph undergraduate students: Canadian Youth Agrifood Food Trade Ambassadors (CYAFTA)
• Prof. Manish N. Raizada: raizada@uoguelph.ca
• Picture books available starting Jul2016: www.SAKBook.org
• Visit SAKNepal website: www.SAKNepal.org
• Sign up for Twitter: @SAK_nepal (>7500 followers)
Extra slides
Ureide exporters
(amino acids as minor fraction)

Amide exporters
(amino acids as major fraction)

* Soybean
* Common bean
* Kidney bean
* Cowpea
* Pigeon pea
* Black gram

* Lentils
* Pea
* Groundnuts
* Chickpea
* Clover
* Alfalfa
GlnLux 96-well Assay to Measure Relative SNF Output from Rhizobia Liquid Cultures (3 h protocol, 10-20 cents per sample)

Illustration by Lisa Smith
GlnLux agar can detect N-fixation activity from rhizobia colonies plates on GlnLux agar (*Sinorhizobium meliloti*).

*GlnLux* cells co-incubated with rhizobia in 96-well liquid culture plates can measure how N fixation responds to the environment.

---

**C**

<table>
<thead>
<tr>
<th>Rhizobial strain</th>
<th>Rm1021 (WT)</th>
<th>Rm1312 (nifD-)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trial 1</td>
<td><img src="image1.png" alt="Graph" /></td>
<td><img src="image2.png" alt="Graph" /></td>
</tr>
<tr>
<td>Trial 2</td>
<td><img src="image3.png" alt="Graph" /></td>
<td><img src="image4.png" alt="Graph" /></td>
</tr>
</tbody>
</table>

---

**D**

<table>
<thead>
<tr>
<th>Oxygen treatment</th>
<th>Rm1021 (WT)</th>
<th>Rm1312 (nifD-)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anaerobic</td>
<td><img src="image5.png" alt="Graph" /></td>
<td><img src="image6.png" alt="Graph" /></td>
</tr>
<tr>
<td>Aerobic</td>
<td><img src="image7.png" alt="Graph" /></td>
<td><img src="image8.png" alt="Graph" /></td>
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</tbody>
</table>

---

**E**

<table>
<thead>
<tr>
<th>Nitrogen treatment</th>
<th>Rm1021 (WT)</th>
<th>Rm1312 (nifD-)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Without N</td>
<td><img src="image9.png" alt="Graph" /></td>
<td><img src="image10.png" alt="Graph" /></td>
</tr>
<tr>
<td>With N</td>
<td><img src="image11.png" alt="Graph" /></td>
<td><img src="image12.png" alt="Graph" /></td>
</tr>
</tbody>
</table>

Shehata, Tessaro, Annan, Dong and Raizada (2016) In preparation
Effect of different rhizobia strains on SNF of soybean: *GlnLux* leaf punch and agar assays

---

**a**  
![Image](image1.png)  
GlnLux (RLU)  

**b**  
![Image](image2.png)  
Shoot total N% (dry weight basis)  

**c**  
![Image](image3.png)  
% Nitra  

---

Thilakarathna, Moroz and Raizada (2016) *Submitted*
Effect of different rhizobia strains on SNF of soybean: GlnLux leaf punch and agar assays

Thilakarathna, Moroz and Raizada (2016) Submitted
Effect of different rhizobia strains on SNF of lentil: \textit{GlnLux} imaging of roots

Thilakarathna, Moroz and Raizada (2016) Submitted
Effect of different crop varieties on SNF of common bean: *GlnLux* imaging of roots

Thilakarathna, Moroz and Raizada (2016) Submitted