DNA fingerprinting based identification of farmers lentil varieties in Bangladesh

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Sample Seed Collection Procedures

1. Reference: Breeder seeds for each of the varieties released in the country were collected from Pulses Research Center (PRC), Bangladesh and Bangladesh Institute of Nuclear Agriculture (BINA) and serve as the reference samples.

2. Farmers: 1000 Household - Some with multiple plots.

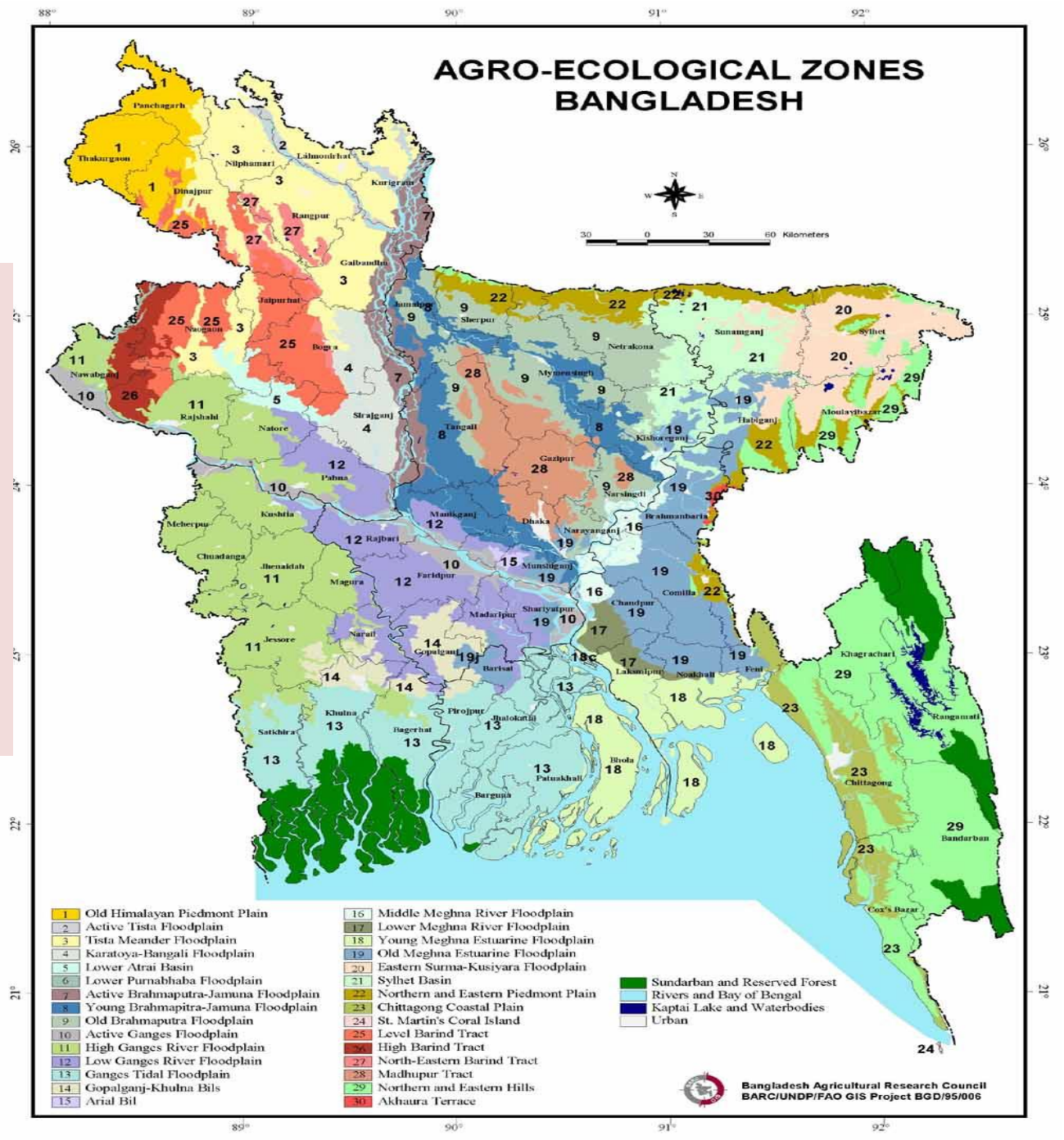
- A handful of (100-200 grains) of lentils from seed/grain storage containers.
- The seed/grain sample were placed into the plastic bag and sealed using the zip locker

Agro-ecological zone

10: Active Ganges Floodplain;

11: High Ganges River Floodplain, and

12: Low gangs river floodplain



3. Seed companies:

We were did to get seeds form seed companies. They were unwilling to provide information. For the case of the Bangladesh Agricultural Development Corporation (BADC) we received seeds from their distributors.

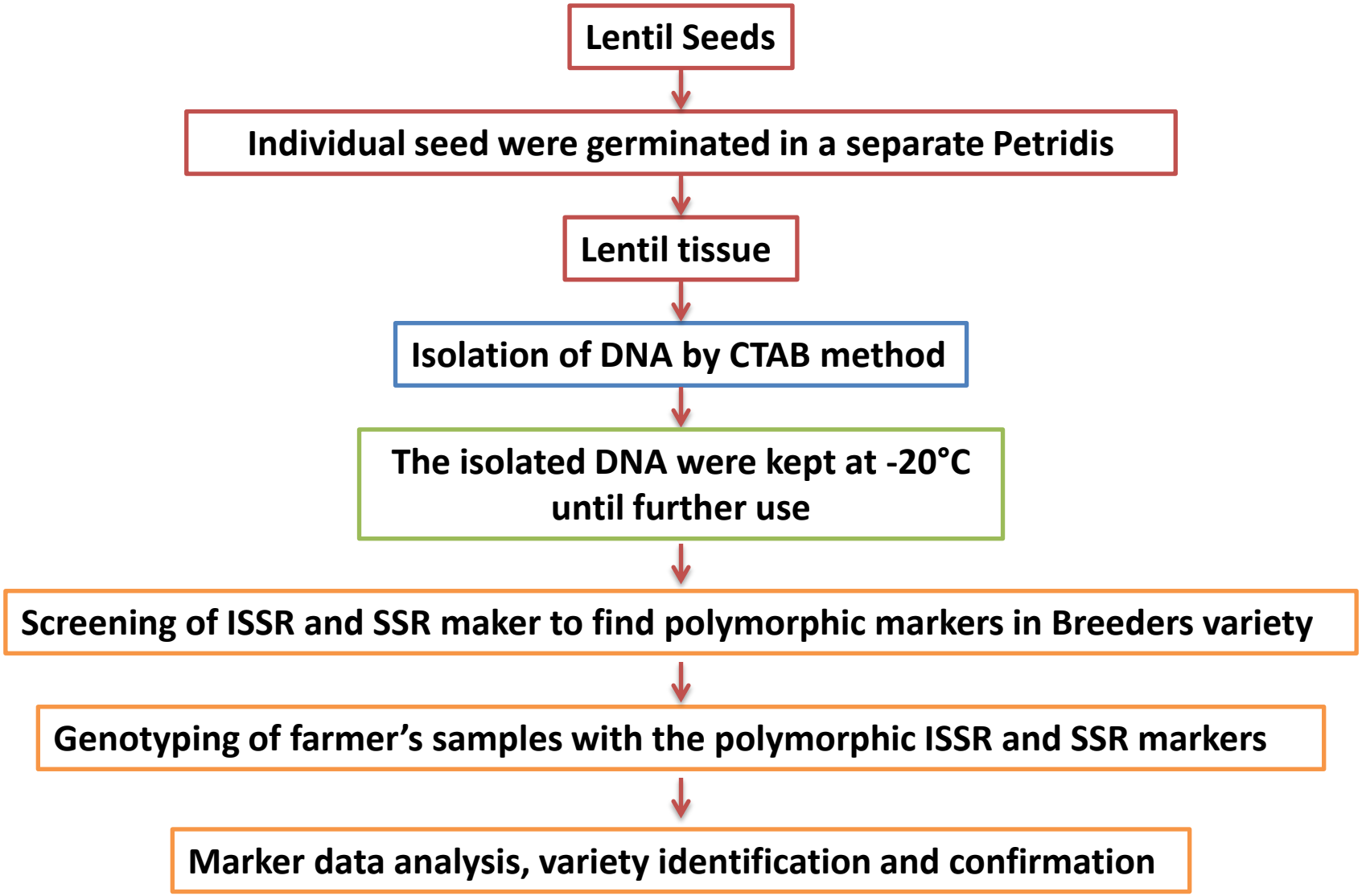
4. Local Seed dealers :

- Two samples from each of the varieties sold by a random sample of
- 2 dealers per sub district were taken using grain probes (2 X 2 X 20 X number of varieties sold)
- A total of 39 seed samples were obtained

Sample seeds for lentil varietal DNA identification

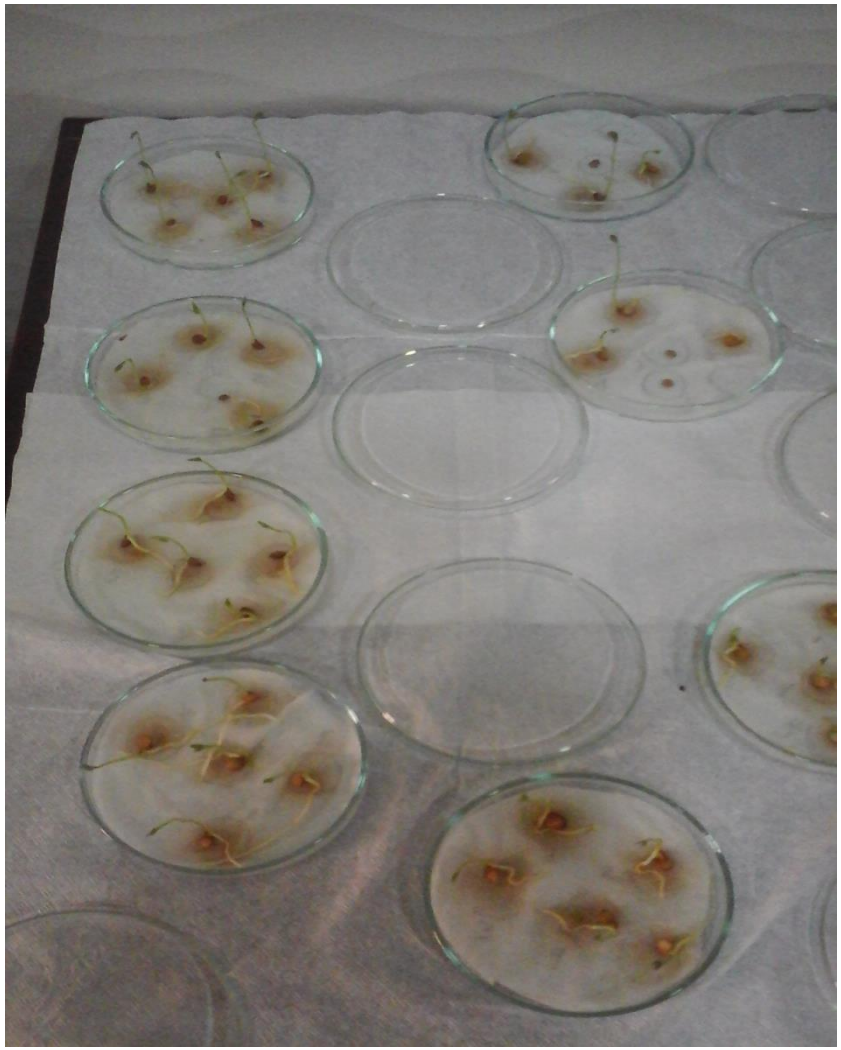
Source	Number observations for DNA identifications*
Breeders (for reference)	17
Farmers	1694
Seed dealers	39
Total	1750

Flow chart for DNA fingerprinting procedure

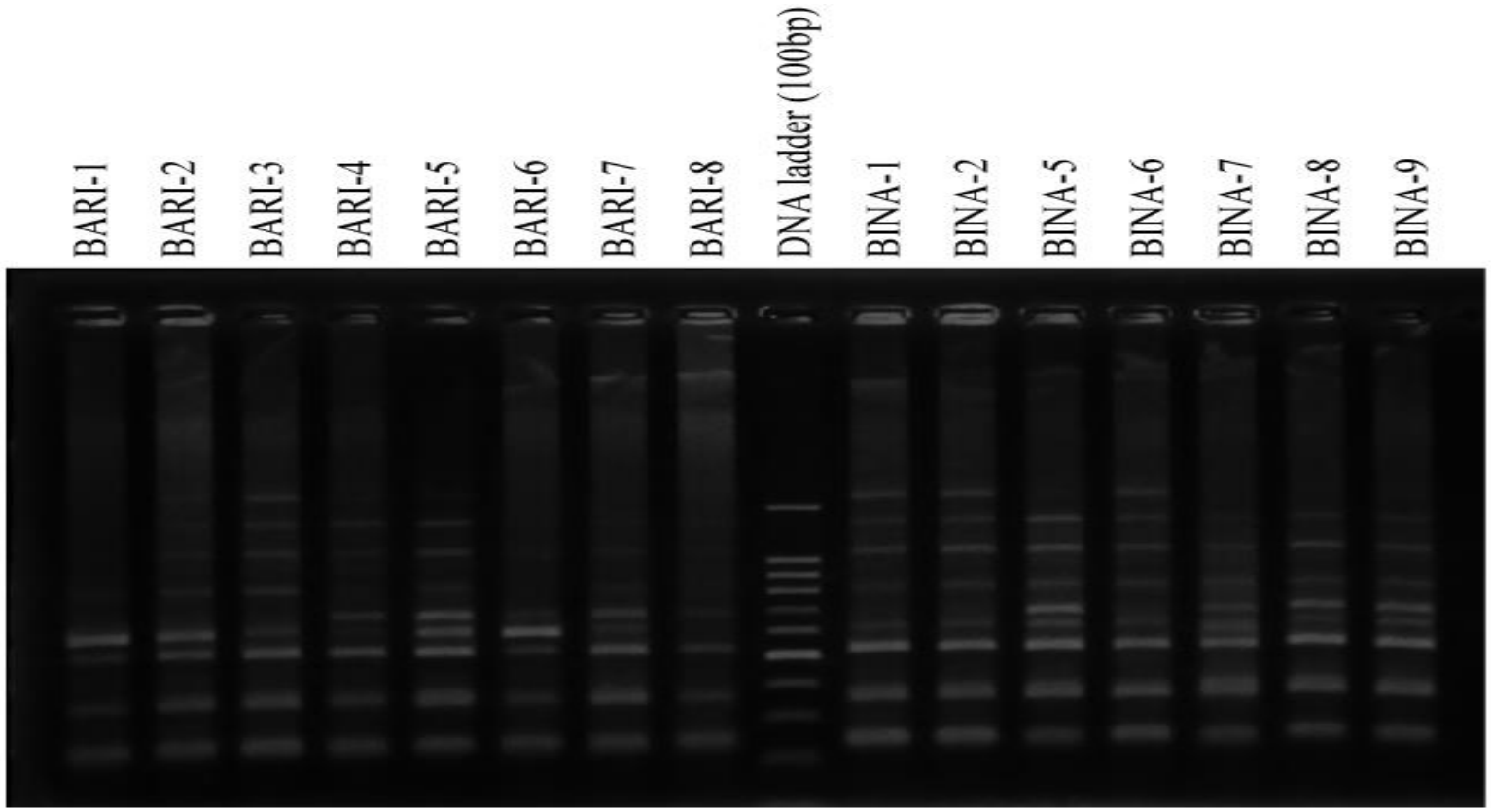


Protocol

- For each sample (4-5) uniform seeds were selected for germination,
- After germination only one germinated plant was taken for DNA extraction



DNA fingerprinting data generated by *HB011* ISSR marker across different breeder's samples



Example of comparison of DNA results of field data and breeders varieties

Sample ID	Identified variety by farmer	Matched with breeder's variety by DNA fingerprinting
Q0029	BARI-4	BARI-3
Q0360	BARI-6	Unmatched
Q0438	BARI-4	BARI-4
Q0456	BARI-3	BARI-4
Q0460	BARI-4	BARI-4
Q0789	BARI-3	BARI-3
Q0820	BARI-3	BARI-3
Q0848	Local	BARI-4
Q0007	BARI-7	BARI-7

Estimates of mismatch

- Analysis is not complete

But

So far mismatch between farmer identification and DNA identification is estimated not to exceed 5-6% of the total samples. This is work in progress and this figure may change.

Where are we in the process

1. Germination and DNA isolation completed
2. Marker screening and testing completed
3. Breeder seeds characterization completed
4. BADC/Dealer seeds characterization completed
5. Farmer seeds characterization completed 75%
6. Statistical matching and verification variety yet to completed hopefully it will done by 20 August.

Challenges of the varietal identification by DNA fingerprinting

- **Challenges of own experience**
  - Higher within-variety variations
  - Lower between-variety variations
  - Low polymorphisms between variety

- **How these challenges are addressed**
  - Large set of ISSR and SSR markers were screened to find polymorphic markers among the varieties
  - Both dominant and co-dominant marker (i.e., ISSR and SSR) were used in DNA fingerprinting
  - Clustering and comparing of DNA fingerprinting data to match farmers variety with breeder variety

Next steps

- Genotyping of remaining samples
- Data analysis and comparing data of breeder varieties with farmer identified varieties