Adoption and Diffusion of Potato Variety Cooperation 88 (C88) in China

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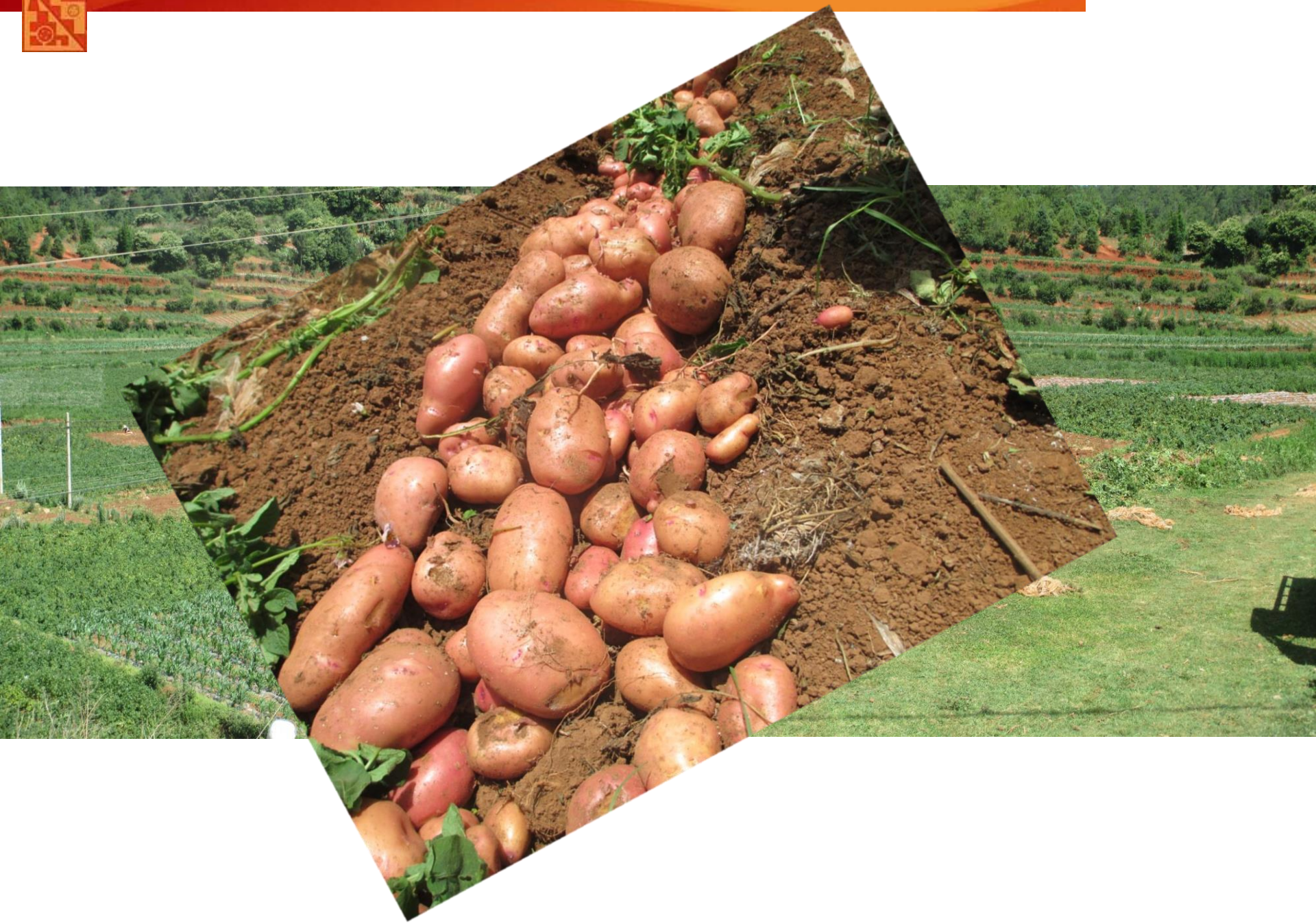
Validation of SIAC 2.1 data for Yunnan

	Crop Area (Ha)	C88	C88 Area (Ha)
EARLY SPRING	86,667	26.9%	23,333
LATE SPRING	396,667	16.7%	66,243
AUTUMN	43,333	7.7%	3,333
WINTER	60,000	55.6%	33,333
	586,667		126,242

Source: SIAC 2.1 expert elicitation workshop, March 2015



- **DNA fingerprinting** **to confirm genetic identity of putative C88 plants**
- Protocol developed for dried leaf samples, then extended to tuber samples
- Conducted at the labs of the Yunnan Normal University (Kunming)
- CIP genebank leaders provided supervision of methods and confirmed interpretation of results

PROTOCOL FOR DRIED LEAVES SAMPLES, PRESS DRY

1. Samples ideally are taken from the youngest leaves from the upper third part of a representative plant and contain 3-4 leaves. Tubers can also be collected for DNA extraction

2. Wash leaf samples / potato tubers with tap water and dry with tissue paper in a place protected from rain.

3. Leaves should not be bent when collected or when wrapped in newspaper or placed in a book during the day (secure them in order to not mix samples), while enumerators are in the field. In case of tubers, those can be stored in labeled paper bags

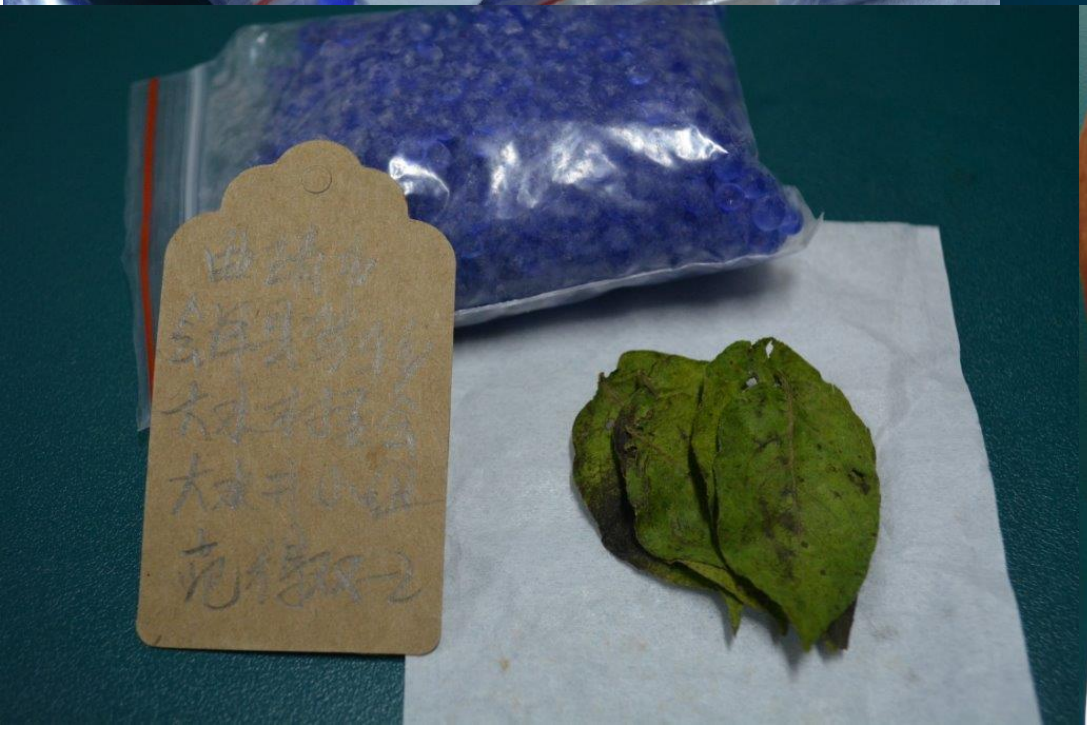
4. Label the page where the leaf sample is placed or the paper bag where the tuber is placed. Label with information of the household and plot it belongs to

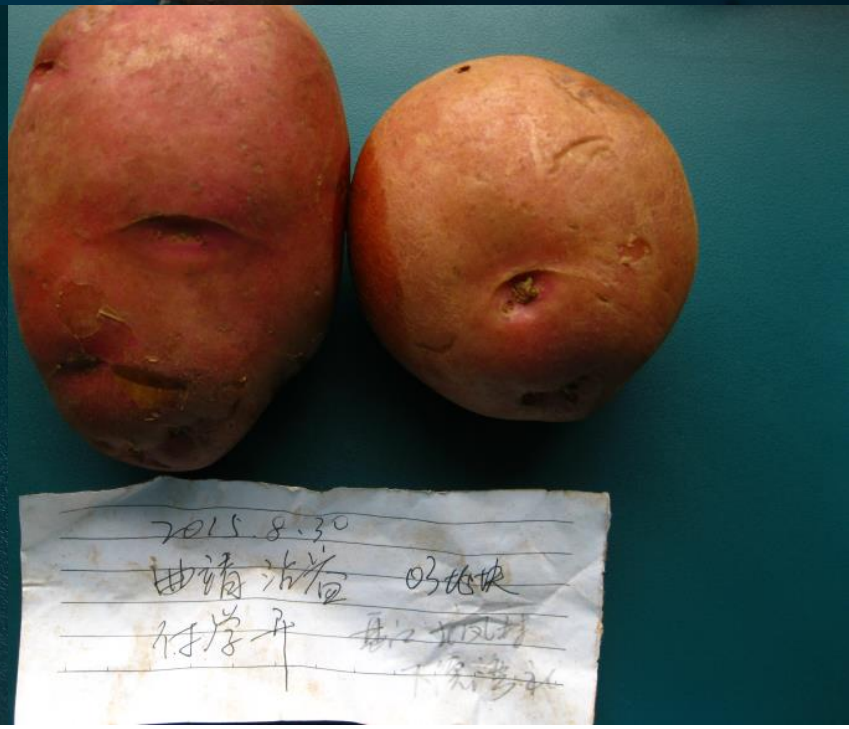
5. Move the sample from book to a drying press with the sample inside newspaper, keeping track of the label to identify samples. Maximum 10-12 samples per drying press., 3 days in the press.

6. Layers: wood, cardboard, newspaper, blotting paper, newspaper with leaves, blotting paper, newspaper,etc. Then repeat your layers etc. replace paper towel underneath and on top of each collected sample to help pull out the moisture in the samples each night

7. Keep dry samples protected from humidity and rain in their respective newspapers in plastic bags with silica.

Silica gel used to conserve leaves





Distribution of sub-sample for DNA fingerprinting

Level	Total surveyed	With leaf sampling	With tuber sampling	With leaf/tuber sampling
Household level	616	88	53	141
Village level	41	7	7	14
County level	22	5	7	12
Prefecture level	10	4	4	7

Procedure used by YNU to get DNA fingerprinting varietal confirmation of C88

1. Declared C88 tuber/leaf sample collection from household survey
2. Visual identification of putative C-88 tuber samples
3. Identification of putative C-88 samples based on cytoplasm genome diversity
4. Identification of putative C-88 samples based on SSR marker analysis of nuclear genome

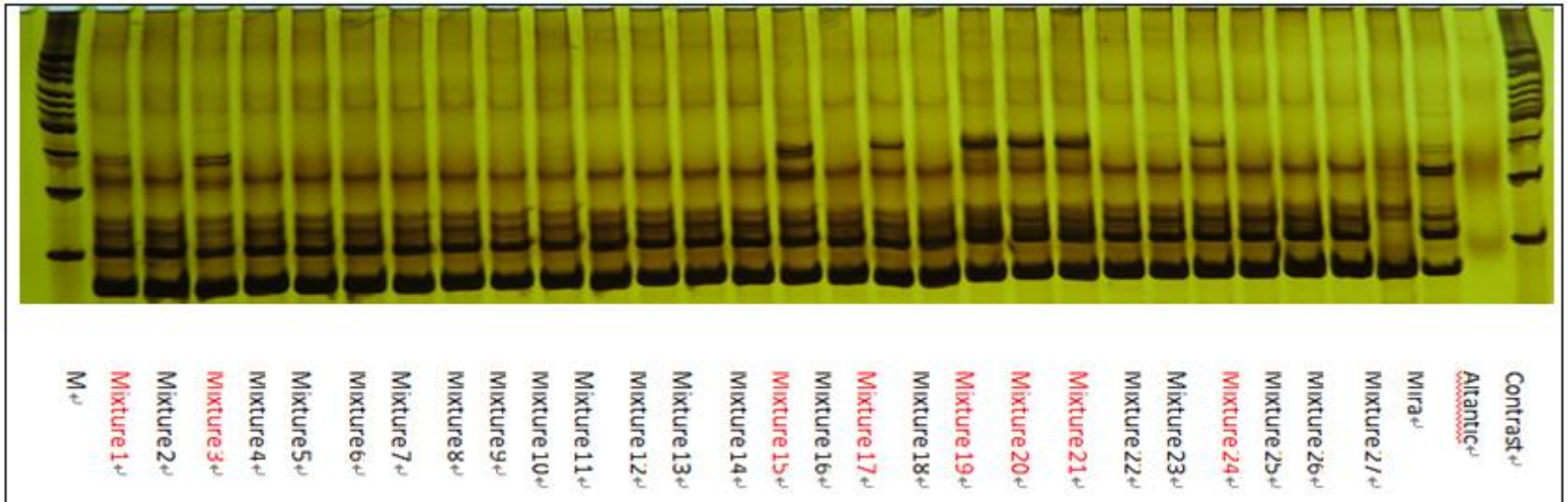


Figure 11 The result of STM0037 amplification detection （M=100bp DNA ladder）

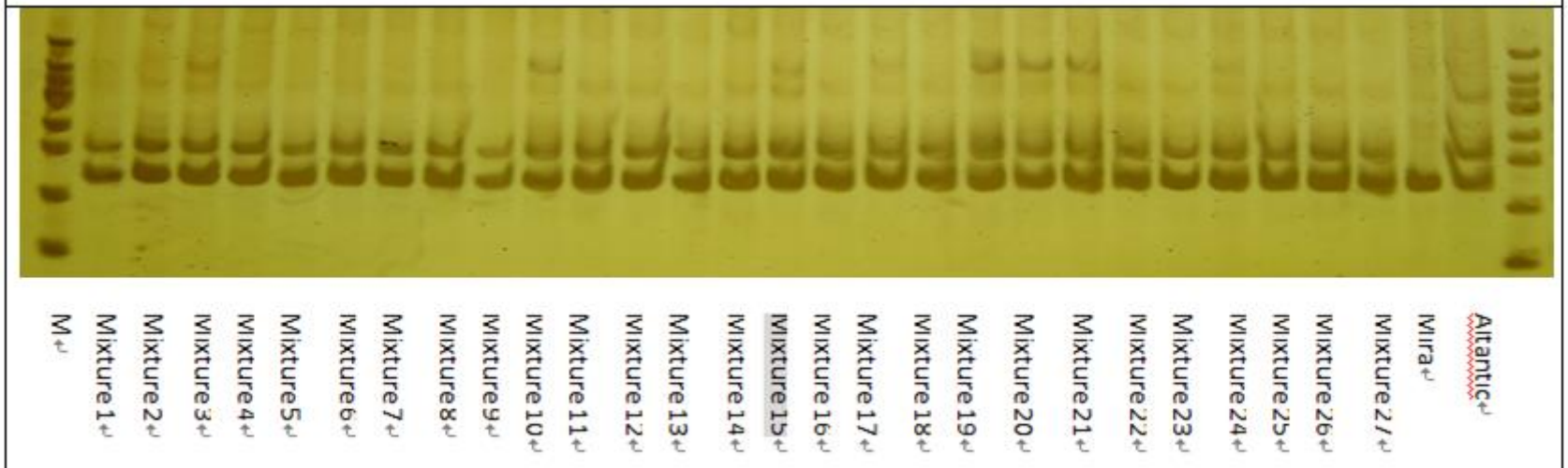


Figure 12 The result of STM3012 amplification detection （M=50bp DNA ladder）

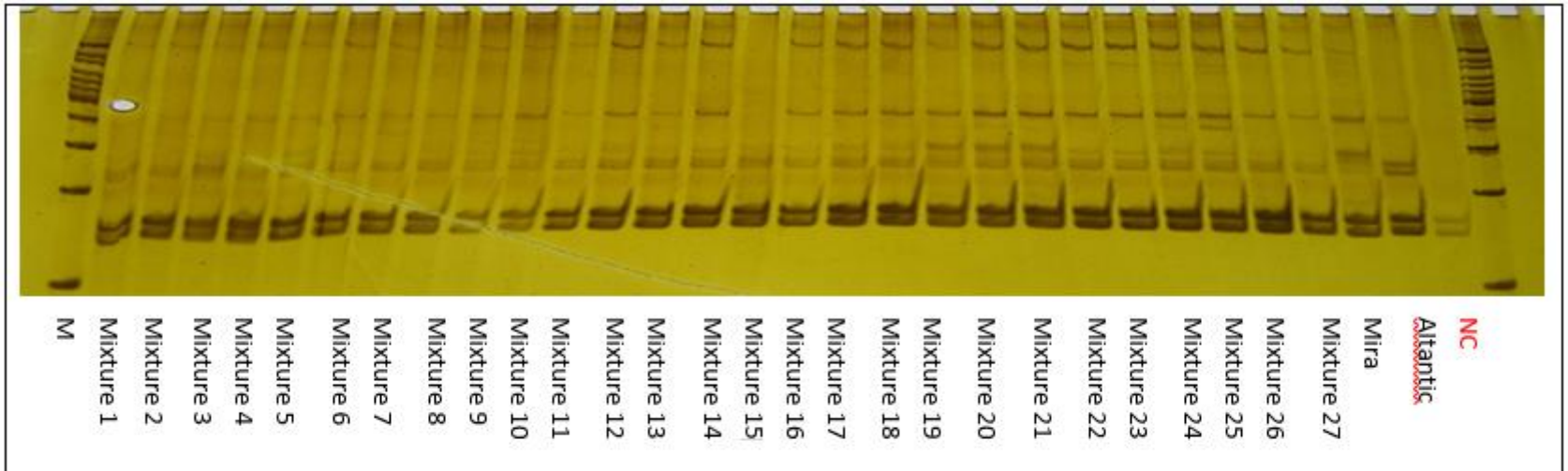


Figure 7 The result of STM1104 amplification detection （M=100bp DNA ladder）

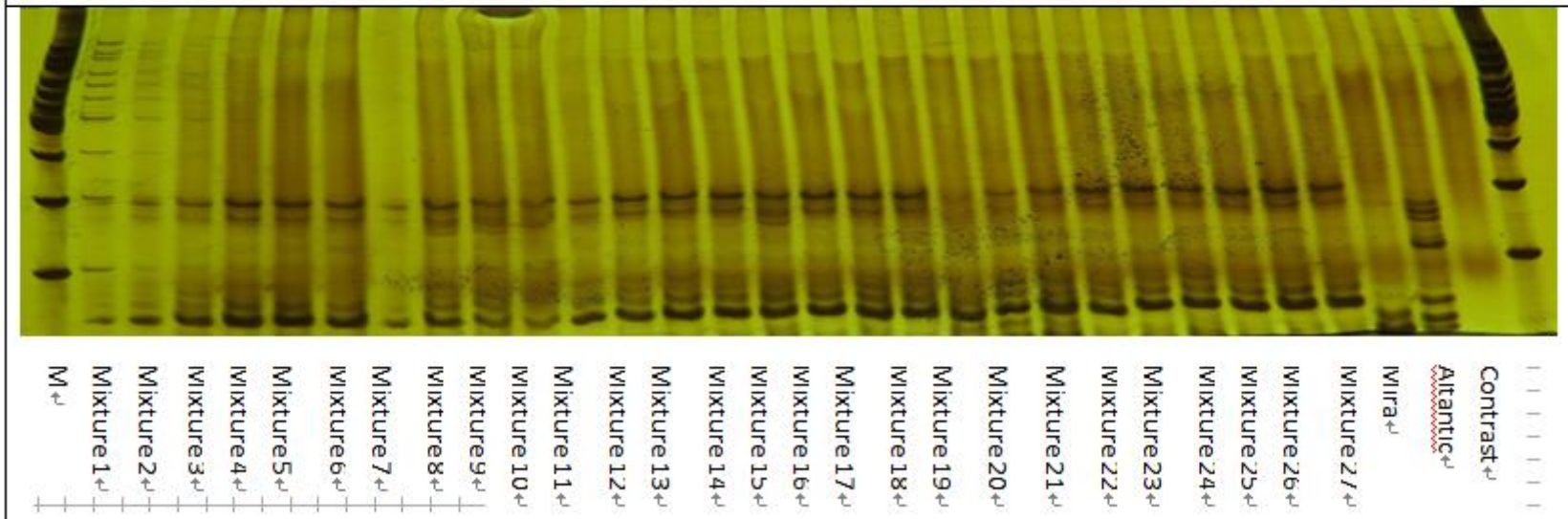


Figure 8 The result of STM1106 amplification detection （M=100bp DNA ladder）

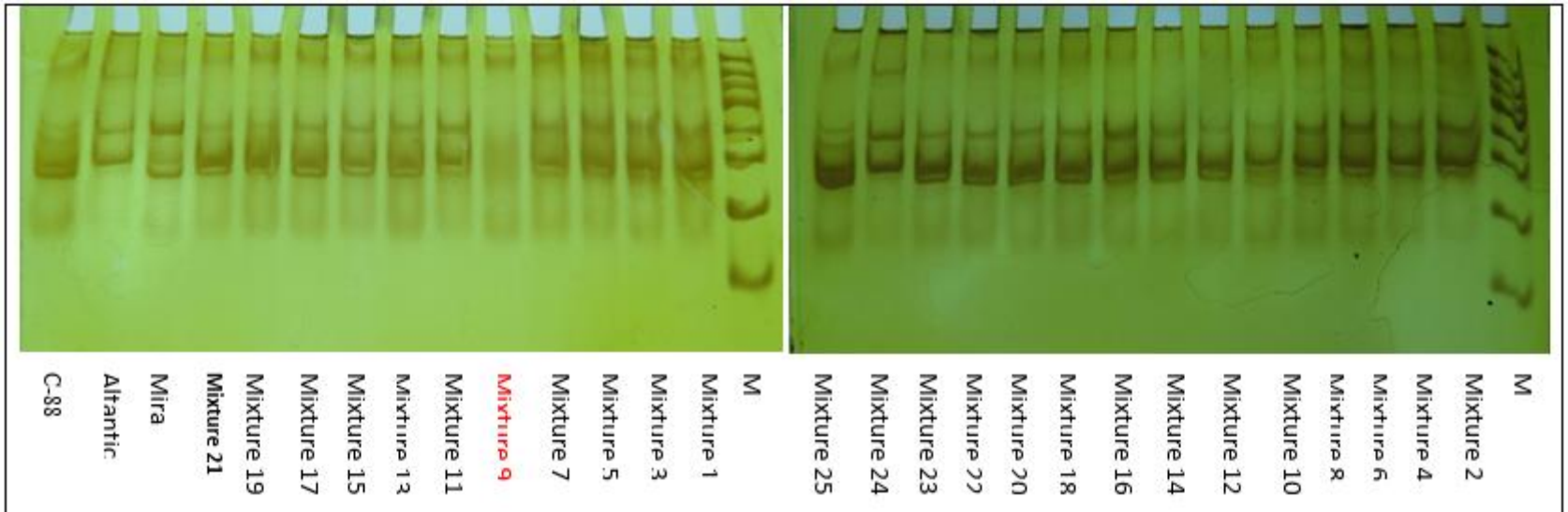


Figure 3 The result of STM2022 amplification detection （M=50bp DNA ladder）

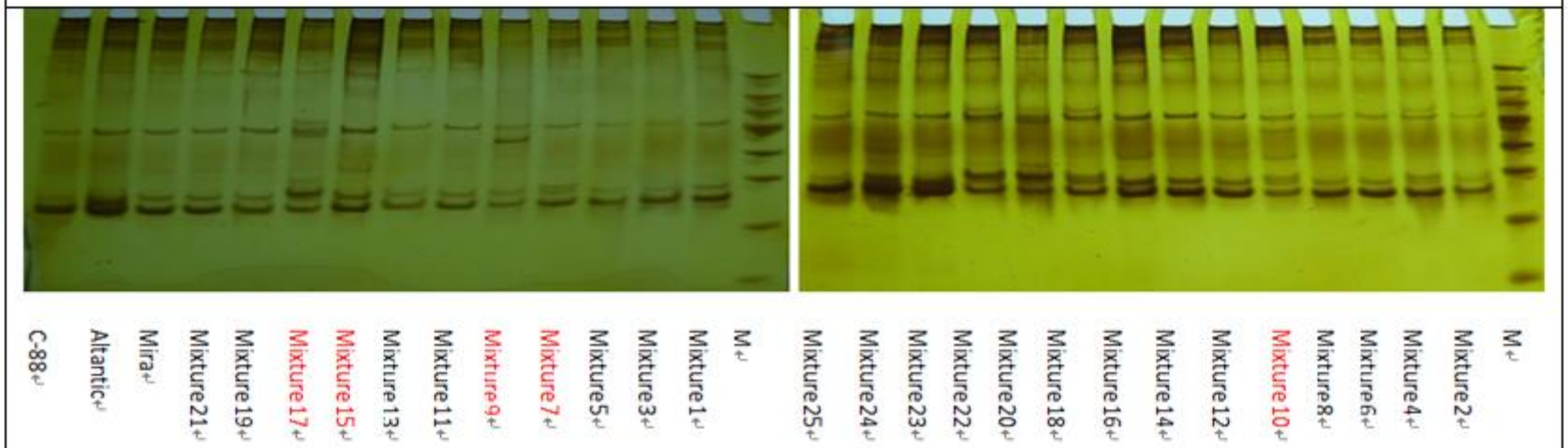
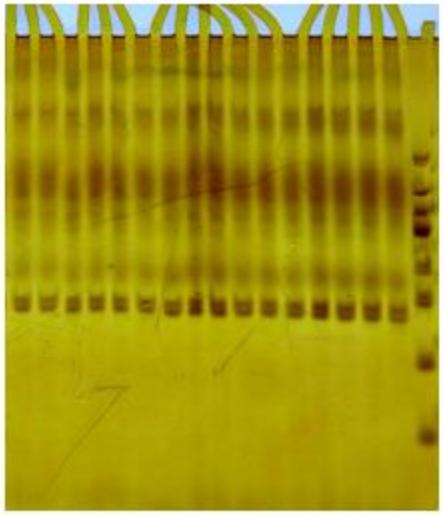
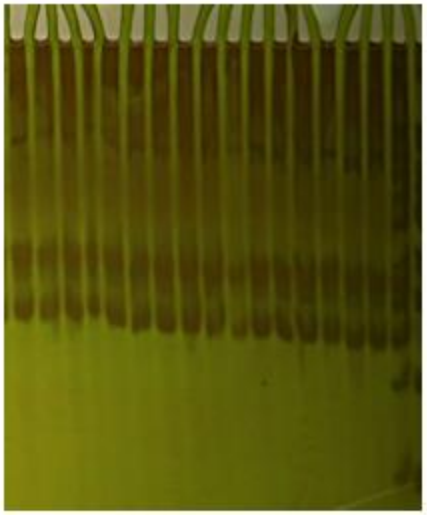


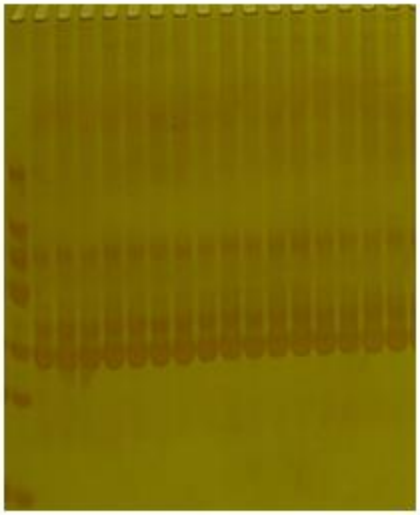
Figure 4 The result of STM3032a amplification detection （M=50bp DNA ladder）



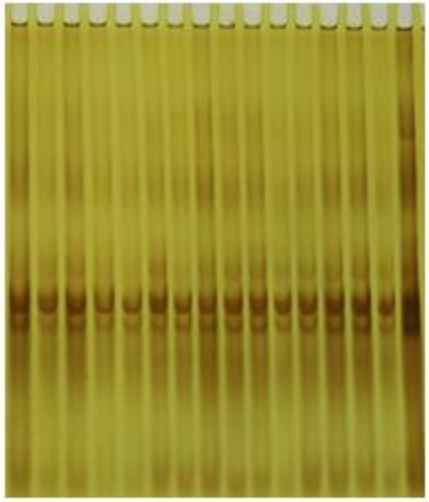
STM1049



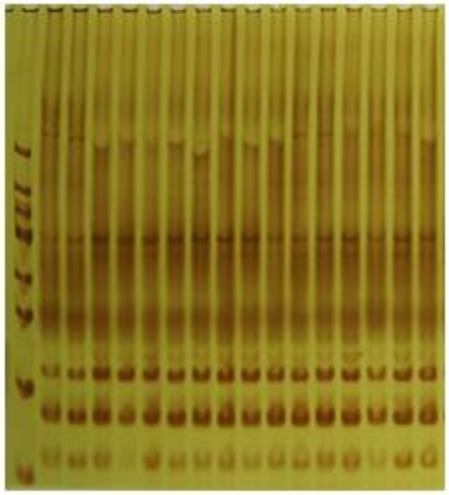
STM1053



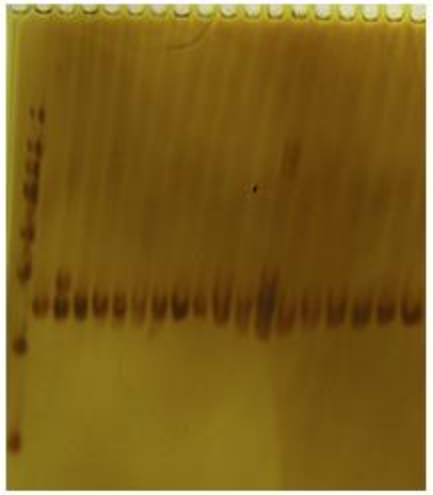
StpoAC58



STM0019a



STM0030



STM 3023a (C-88, Mira,128-143)

Figure 16： the results of 128-143 SSR amplification

Additional analysis: cytoplasmic type detection of samples with Atlantic, Mira and C-88 as check cultivars

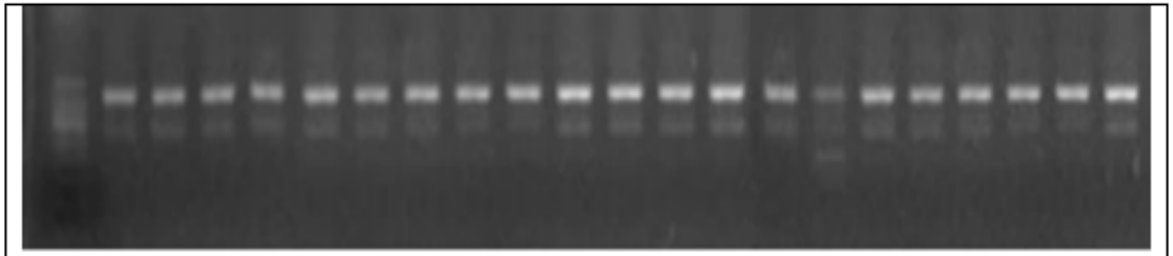


Fig. 1 1-105 混合样 T 引物扩增检测结果

加样顺序(从左至右)：泳道 1：Marker 为 50bp；泳道 2：1-5；泳道 3：6-10；泳道 4：11-15；泳道 5：16-20；泳道 6：21-25；泳道 7：26-30；泳道 8：31-35；泳道 9：36-40；泳道 10：41-45；泳道 11：46-50；泳道 12：51-55；泳道 13：56-60；泳道 14：61-65；泳道 15：66-60；泳道 16：71-75；泳道 17：76-80；泳道 18：81-85；泳道 19：86-90；泳道 20：91-95；泳道 21：96-100；泳道 22：101-105； 其中 Marker（共 9 条带，分子量由上至下分别为：500，400，350，300，250，200，150，100，50bp，其中 300 bp 为最亮带）

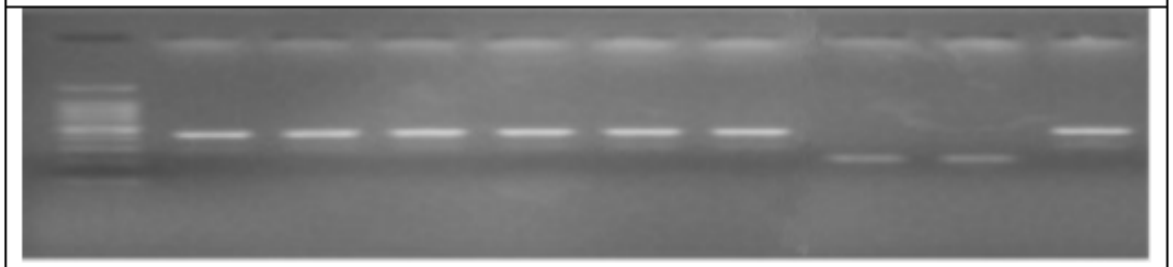


Fig. 2 106-143 样品 T 引物扩增检测结果

加样顺序(从左至右)：泳道 1：Marker 为 100bp；泳道 2：106-110；泳道 3：111-115；泳道 4：116-120；泳道 5：121-127；泳道 6：128-135；泳道 7：136-143；泳道 8：Mira；泳道 9：Atlantic；泳道 10：C-88；其中 Marker（共 11 条带，分子量由上至下分别为：1500，1000，900，800，700，600，500，400，300，200，100bp 其中 500 bp 为最亮带）

YNU report highlights:

- 615 households surveyed, 141 mentioned planting C88.
- Based on visual observation, only one tuber sample of the C-88 collected from Zhanyi county of Qujing city was found to be mixed with another red-skin cultivar.
- According to the results of cytoplasmic type detection, one leaf sample collected from Ninglang county of Lijiang city had different cytoplasmic type (T/β type).
- Additionally, the SSR marker-based fingerprinting further clarified three samples showed different SSR genotypes at two loci (STM1049 and STM3032a) in comparison with the other samples and the reference C-88.
- **Therefore, it was confirmed that over 97% (137/141) of the fresh samples (leaves and tubers) were C-88**

Some lessons learned

- DNA fingerprinting effective in confirming genetic identity of C88 => additional confidence on adoption estimates.
- Visual identification enough?
- Cost of DNA fingerprinting extraction
- Coordination with local partners challenging:
  - Protocols different but compatible: ask reference literature from partner.
- Logistics:
  - Coordination of HH surveys with sample extraction: additional constraints on window of opportunity for field work
  - Larger team in the field and therefore higher supervision and transportation costs
  - Accessibility to HH can be compromised
  - Matching samples with HH surveys
  - Trade-offs need to be assessed, detailed planning becomes critical.

Thank you