A Breakthrough: Hybrid Population Breeding Validated in Peru, Uganda, and Mozambique

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What was the problem?
Sweetpotato is a highly heterozygous hybrid propagated by cloning. Breeding progress in sweetpotato has been 0.8 to 2.5% annually for storage root yield across 4 breeding platforms over the past decade with the lowest gains in West Africa and the highest in the Amazon basin. For root β-carotene content, genetic gains were much higher with 6.3% and 6.5% annually in West- and East Africa, respectively. These genetic gains for yield correspond to genetic gains in maize when this crop was still being improved by open pollination at the beginning of the last century. We sweetpotato breeders have also improved our crop by open pollination in polycross seed nurseries, followed by selection of the best clones. However, genetic gains for quantitative traits such a yield over the medium- to long-term is determined by improving the means among prioritized traits of a breeding population, whereas the variation in a population “only” determines genetic gain in a short term. That is, when short-term genetic gains have been exploited and breeding population means are not showing further improvement, no crop enhancement takes place. Hybrid breeding has revolutionized maize breeding with tremendous genetic gains since the 1930s. Like maize, sweetpotato is a highly heterozygous hybrid, but unlike diploid maize, sweetpotato is propagated by cloning – a clone hybrid and is hexaploid1. Owing to hexaploidy, the phenomenon of heterosis – superiority of offspring over parental means (defined on the homozygous basis) might be much more important than in maize. This is a hypothesis, which will require significant time to prove, because there is no homozygous basis in sweetpotato2. Fortunately, a homozygous basis is not needed to start systematically exploiting heterosis and to conduct hybrid breeding in sweetpotato. Unfortunately, the level of knowledge among scientists about the vast range of hybrid breeding methods available is low; thus, many assume that a hybrid breeding scheme for sweetpotato is not possible. Our hypothesis was that a population hybrid breeding concept for sweetpotato can lead to genetic gains far above 2% annually for those traits found to display pronounced heterosis. A hybrid breeding approach also enables the intensification of inbreeding by intra-gene pool recombination for those traits which require inbreeding, such as the difficult to tackle sweet potato virus disease (SPVD) resistance. In this brief, we want to provide evidence of validation of the hybrid breeding concept in sweetpotato, based on results from applied breeding populations in Peru and Uganda, as well as an experimental study under abiotic stress conditions in Mozambique.

What objectives did we set?
The demonstration of genetic gains and responses to selection, respectively, are absolutely vital to justify funding public breeding. To a certain extent, this can be done by model calculations, especially with respect to selection of best clones for release from a given population. However, realized genetic gains and gains in farmer fields require surveys and/or experimental studies. Moreover, in case of recombination (crossings and recombination of parents) in a hexaploid crop like sweetpotato, model calculations to predict selection responses are of limited utility, since they cannot describe the true situation across recombination cycles.
Our objectives were to:

- Determine the response to selection for yield and quality in three applied breeding populations for a complete reciprocal recurrent selection cycle for (i) OFSP wide adaptation and earliness, (ii) OFSP for high iron, and (iii) OFSP for low sweetness after cooking.

- Apply the concept of elite crossings in sweetpotato for the first time. These are recombinations of a small number of “super” parents, based on results of large-scale trials, to fully exploit the within family variance of these crosses.

- Estimate the superiority of hybrid population means in hybrid population H0 (hybrid population established from two parental gene pools) in one applied breeding population with emphasize on yield and SPVD resistance, as well demonstrate the genetic gains to be achieved in yield and SPVD resistance by established elite crossings.

- Demonstrate for the first time in sweetpotato that hybrid breeding results in breeding populations which show pronounced heterotic increments under regular water supply and drought stress conditions. If so, the case can be made that a hybrid breeding approach leads to higher yield stability in sweetpotato bred for drought prone areas.

**Where did we work?**

The concept to apply population hybrid breeding in sweetpotato started in Peru at CIP-HQ in 2009. Prior to this time, the team in Peru had established the foundation of population hybrid breeding without earmarked funding for this topic, simply by establishing two instead of one OFSP breeding populations on the basis of different genetic background, but retaining the same breeding objectives, namely high storage root yield and high number of commercial storage roots per plant in combination with medium to high β-carotene and dry matter content, as well as strong vines to facilitate planting and SPVD tolerance for levels set in Peru. The parental populations established were population “Jewel” (PJ) and “Zapallo” (PZ) with a selection of 49 PJ and 31 PZ clones to study population hybrid breeding (Fig. 2). In Peru, a hybrid population H0 was established, PJ and PZ parents were selected on basis of H0 offspring analysis, these parents were recombined by intra-gene pool crossings (PJ x PJ and PZ x PZ). Then these partially inbred intra-pool populations were evaluated in the field to select new PJ’ and PZ’ parents to establish the hybrid population H1. This was done simultaneously for three H1 populations: (i) OFSP with wide adaptation and earliness, (ii) OFSP for high iron and (iii) OFSP for low sweetness after cooking. Additionally, elite crossings were established for OFSP wide adaptation and earliness. These elite crossings (6 crosses for OFSP with root dry matter < 28%; 6 crosses for OFSP with root dry matter ≥ 28%) are now generating true seed supply for NARS breeders in Asia and the Americas.

In Uganda, the large polycross breeding nursery of originally 150 parents was divided in two gene pools only on basis of simple sequence repeat (SSR) markers, namely UG-A and UG-B. After a successful experimental study with 9 UG-A and 6 UG-B parents, which was a “go-no-go” decision point for further investments, the entire UG-A and UG-B genepool was recombined (UG-A x UG-B) to form the UG-H0 population. This UG-H0 population was evaluated for two seasons, in three environments, for storage root yield, number of commercial storage roots per plant, SPVD infection, foliage yield and Alternaria. UG-A and UG-B parents were selected on basis of H0 off-spring analysis. Twenty UG-A parents (with 8 OFSP) and 20 UG-B parents (with 6 OFSP) were selected to start intra-genepool crossings (UG-A x UG-A and UG-B x UG-B). Additionally, elite crossings were established by using three UG-A and five UG-B parents, which are treated as bi-parental isolation crosses. The latter is like “isos” established in corn breeding and uses open pollination among two parents, resulting in large amounts of true seed from best combinations without the need of laborious hand crosses and skilled...
These bi-parental isolation crosses now provide a true seed supply for NARS breeders in East Africa. In Mozambique, a study has been undertaken to test 51 UG-A x UG-B crosses and 47 UG-A x UG-A and UG-B x UG-B crosses under irrigation and not under irrigation for two seasons. The successful study, whose results determined whether further investments would be made, led to the largest crossing effort ever undertaken in sweetpotato. This crossing recombined 50 parents from Umbeluzi and 50 parents from Gurue in a 100 parent diallel to form a hybrid population designed to identify high yielding heterotic patterns in which parents are allocated to gene pools by the so-called “simulated annealing algorithm” developed at IPK in Gatersleben, Germany. Diverse aspects of the hybrid breeding approach are elsewhere described (David et al., 2018, Grüneberg et al. 2009; 2015; https://doi.org/10.13140/RG.2.2.13436.18569) and it has been reviewed by Prof. Jochen Reif from the Department of Breeding Research at IPK. Figure 3 describes CIP’s current approach to develop sweetpotato hybrid varieties.

### What did we achieve in SASHA Phase 2?

In three applied breeding populations at CIP in Peru, genetic gains for storage root yield in hybrid population 1 (H1) compared to the original base line after one five-year reciprocal recurrent selection cycle were:

- 69.7% for OFSP at 90 days harvest (H1 population mean: 18.5 t/ha);
- 117.6% for OFSP high iron a t 120 days harvest (H1 population mean: 44.9 t/ha); and
- 96.2% for OFSP low sweetness at 120 days (H1 population mean: 30.8 t/ha) (Fig. 4).

**Fig 3. Diagram of CIP’s current approach to develop sweetpotato hybrid varieties**

**Fig 4. Conducting sensorial evaluation for low sugar must be done on cooked samples. This evaluation at CIP-HQ was done on 5,000 genotypes from the H1 hybrid population**
Fig 5. Genetic gain (GG) for storage root yield after one complete reciprocal recurrent selection cycle in H1 OFSP high iron population evaluated at two locations in Peru: 1) Cañete in the arid Pacific Coast and 2) Satipo in the humid tropics. Mean baseline yield (22.8 t/ha) based on 80 clones (each clone in 8 one-meter row plot replications); mean parent yield (32.6 t/ha) based on 46 clones (each clone in 8 one-meter row plot replications); mean offspring yield (44.8 t/ha) based on 3,292 H1 hybrid clones; mean baseline yield (22.8 t/ha) based on 80 clones; mean parent yield (32.6 t/ha) based on 46 clones; mean offspring yield (44.8 t/ha) based on 3,292 H1 hybrid clones; total genetic gains: 96.2%; heterosis increment in H1: 36.4%; estimated gain by one reciprocal recurrent selection cycle: 43.8%; frequency offspring clones better than check means: 44.9% (two widely adapted high yielding checks - CEMSA-74-228 and Dagga).

Fig 6. Genetic gain (GG) for root iron content by one complete reciprocal recurrent selection cycle estimated in H1 OFSP high iron evaluated at two locations in Peru: 1) Cañete in the arid Pacific Coast and 2) Satipo in the humid tropics. Mean baseline iron content (18.2 ppm) based on 80 clones (each clone in 8 one-meter row plot replications); mean parent iron content (21.67 ppm) based on 46 clones; mean offspring iron content (21.70 ppm) based on 3,292 H1 hybrid clones; mean baseline iron content (18.2 ppm) based on 80 clones; mean parent iron content (21.67 ppm) based on 46 clones; mean offspring iron content (21.70 ppm) based on 3,292 H1 hybrid clones; total GG: 19.1%; heterosis increment in H1: 0.1%; estimated gain by one reciprocal recurrent selection cycle: 19.0%; frequency offspring clones better than check means: 98.8% (two widely adapted high yielding checks - CEMSA-74-228 and Dagga)). Note: Iron contents estimated by NIRS calibrations. XRF and ICP iron estimates are usually higher than NIRS iron estimates.

Fig 7. Breeders visiting a sweetpotato phenotyping field in a SPVD hotspot in Namulonge, Uganda (Credit: T. Mendes)
All populations showed a large frequency of hybrid clones – 35.7%, 13.9%, and 44.9%, respectively – surpassing checks (two widely adapted high yielding checks - CEMSA-74-228 (cream-fleshed) and Dagga (light orange-fleshed)). For example, the very high gains in the H1 OFSP high iron population are shown in Figures 5 and 6. The H1 population for OFSP high iron has a population mean for storage root yield of 44.9 t/ha (Fig. 5) with an average root iron content of 21.9 ppm on dry weight basis (dwb) (Fig. 6), that is 98.8% of all off-spring clones were observed for iron to surpass the well-known checks CEMSA-74-228 and Dagga with an average root iron content of 13.6 ppm dwb. Note that these gains still do not include the additional gains to be achieved by selection within the breeding population nor the genetic gain jump to be achieved by using elite crosses (see branch hybrid variety selection in Fig. 2). On basis of these results we think that OFSP biofortified for iron is feasible and double biofortified varieties are on the horizon.

A H0 hybrid population from the largest parental basis in the world (150 parents original) has been evaluated for two seasons in the fields of Namulonge in Uganda, with very positive results (Fig. 7). Figures 8 and 9 display heterosis observed for storage root yield and SPVD, respectively, from the same trial. The base population was formed with 50 parents in Population Uganda A, and 80 parents in Population Uganda B, with a mean storage root yield of 6.8 t/ha. Very large heterotic effects were observed for storage root yield in families from elite crosses\(^1\), with average root yield of 13.7 t/ha compared to the family means of the entire H0 population (all progeny BxA with 8.1 t/ha) (Fig. 8). The SPVD score in elite progenies were clearly superior to the entire H0 population with about 1 score unit lower in SPVD (scores from 1 (highly resistant) to 9 (completely susceptible)) (Fig. 9). Forty-one percent of elite progenies showed SPVD scores close or below 3 after two seasons of testing across three environments – the lowest acceptable value which differentiates resistance from susceptibility to SPVD. The large frequency of clones with SPVD scores close or below 3 in elite progenies represents a breakthrough in SPVD resistance breeding. Uganda data shows that yields in elite crosses increased by more than 100% and significant reduction of SPVD susceptibility can be achieved. The breeding team in Uganda is excited and immediately started producing elite true seed from bi-parental isolation crosses for East African countries. The East African breeding platform selected parents (20 in each gene pool) for intra-gene pool recombination to move forward towards an H1 hybrid population.

Table 1 summarizes results from Mozambique. Here we tested hybrid seed introductions from the Ugandan breeding program for SPVD resistance under drought and irrigated conditions at Umbeluzi and compared with regular breeding lines and intra-pool crossings. Despite their relative lack of adaptation, hybrid clones performed very well, particularly under abiotic stress conditions, which is consistent with hybrids showing higher levels of yield stability under stress conditions. This is an advantage in the context of climatic change. Based on such encouraging results, CIP in Mozambique is recombining local parental material from two gene pools since February 2019 (100 parent diallel) to establish a H0 population and elite cross populations (Fig. 10). This is a huge recombination task, requiring 20 persons to conduct controlled hand crossings from 7 to 10 am six days a week for the next six months. This H0 hybrid population will be evaluated in 2020 and hopefully will result in the establishment of elite crosses and bi-parental isolation crosses at the end of 2020, together with the selection of about 12 to 16 parents in each gene pool.

\(^1\) Elite crosses were made from 3 SPVD resistant parents from Pop A crossed with 5 SPVD resistant parents from Pop B. The parents were chosen on basis of offspring SPVD resistance observations.

What is next?

Certainly, the evidence shows that it is worthwhile to invest in moving hybrid breeding in sweetpotato from proof-of-concept to full implementation in population development programs. Within the next three years, CIP and its partners are targeting to release the first hybrid variety of sweetpotato. In Peru, hybrid clones are in the second stage of a three season, multi-stage selection process and the Uganda hybrid program is in the first stage of a three season, multi-stage selection process. We are aware of the level of complexity that developing hybrids brings to sweetpotato breeding, and also the increased time required on a population improvement cycle basis (until genomic selection models are developed.
Table 1: Predictions for inter-pool (9 A x 6 B clones) and intra-pool (9x9 A & 6 x 6 B clones) crosses and differences by drought stress treatment for Ugandan true seed introductions tested in Mozambique in 2015 and 2016; total number of genotypes: 1,010.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Inter-pool AxB crosses (N=51)</th>
<th>Intra-pool AxA &amp; BxB crosses (N=47)</th>
<th>AxB Cross superiority in %</th>
<th>P-Value</th>
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<tr>
<td>Root yield (t/ha)</td>
<td></td>
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<td></td>
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<tr>
<td>Irrigation</td>
<td>13.0</td>
<td>12.2</td>
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<td>0.013</td>
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<td>No irrigation</td>
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<td>Foliage (t/ha)</td>
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<td></td>
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<tr>
<td>Irrigation</td>
<td>21.1</td>
<td>19.5</td>
<td>8.2</td>
<td>&lt;0.001</td>
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<tr>
<td>No irrigation</td>
<td>21.0</td>
<td>17.4</td>
<td>20.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Biomass (t/ha)</td>
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<tr>
<td>Irrigation</td>
<td>33.4</td>
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<tr>
<td>No irrigation</td>
<td>28.8</td>
<td>24.4</td>
<td>18.4</td>
<td>&lt;0.001</td>
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</table>

and validated). However, we think that such extra experimental complexity and effort is more than offset in terms of the pivotal key performance indicator, genetic gain per year. It has been calculated that the genetic gain for storage root yield in the H1 OFSP wide adaptation and earliness corresponds to 36 years of breeding work by polycross breeding. OFSP hybrid breeding for double biofortified sweetpotato (i.e. for iron and beta-carotene) is a major breeding objective of CIP given that iron deficiency is a worldwide problem.

An additional goal is to attract the private sector into sweetpotato breeding and to combine elite crosses with in-vitro germination and later with genomic selection. Here, we propose to use the purple-fleshed sweetpotato, in high demand in Asia, as a model.

**Fig 10.** The massive 100 parent diallel crossing block established in February 2019 in Mozambique (Credit: J. Low)

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