Key Advances in Breeding Methodologies during Phase 1

ABS - Polycross versus Controlled cross - Heterosis

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Breeding Objectives – trade offs of centralized breeding **–** farmer needs & consumer preferences have been under estimated in the past

There is only 1 objective "the better variety" (Röbbelen) and a variety must be good overall traits

Yield, Stability & Adaptation: decentralized breeding and farmer participation to adapt for a) agro-ecological zones and consumer preferences and b) yield stability with in agro-ecological zones (yield stability ⇔ harvest index stability ⇔ storage root initiation stability (Grüneberg et al. 2004, 2005, 2009; Firon et al. 2009)

Taste & Nutrient density: moist and sweet, dry and starchy, high pro-vitamin A contents, (high iron and zinc contents)

Resistance to sweetpotato virus disease (SPVD) across regions - SP clorotic stunt virus (SPCSV) - the important component of SPVD (generally SP is very resistant to virus but …) most likely one or two recessive inherited traits – vertical resistance but also horizontal resistance

Resistance to weevil damage - all drought prone regions - Central and South America, SSA and SWCA) - storage roots deep in the soil & clearly tapering at top - latex in storage root skin / varieties like New Kawogo (Stevenson et al. 2009) from Uganda, Santo Amaro from Brazil, PZ06.120 from Peru are clearly less affected – appears to be a more complex trait as we imagined in the past

Drought tolerance – sweetpotato is quite tolerant to drought (Van Heerden & Laurie 2008) but vine survival and adequate response to rains in genotypes adapted to drought prone areas are much more important as we imagined in the past

Storage root yield as a breeding objective has highest priority (Uganda 2006)

Farmer select varieties on basis of much more trait than only yields – those who do not realize this will learn it the hard way

Polyploidy and Genetics of SP

- A) **Heterozgous genotypes occur at much larger frequencies in 6x than in 2x (see Fig. 1 a**) => heterosis much more important (study heterosis in autopolyploides is very cumbersome) – for yield and yield stability we want highly heterozygous genotypes

B) Recessive inherited traits are quite difficult to fix (See Fig. 1 b) for example resistances or quality traits

=> **The challenge in sweetpotato breeding:** achieve high level of heterozygousity for yield, yield stability, and biomass together with a high level of inbreeding for resistances and quality!!!

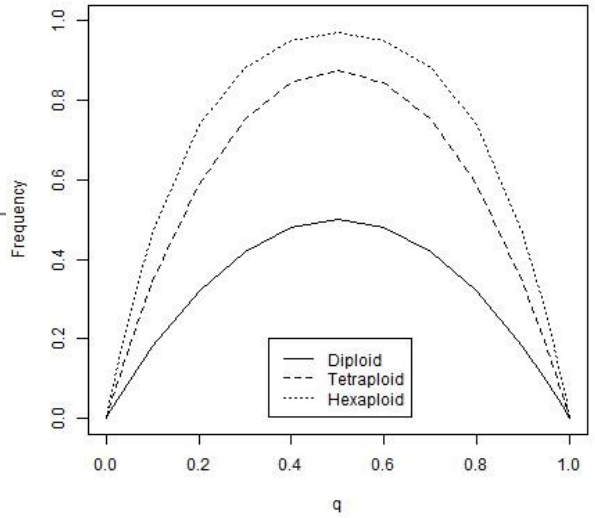


Fig 1a. Effect of ploidy level on the frequency of heterozygous genotypes (modified from Gallais 2003 by introducing to 6x curve

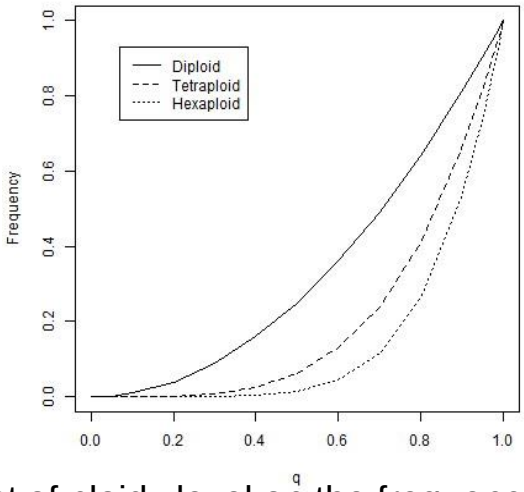


Fig 1b. Effect of ploidy level on the frequency of phenotypes expressing a one locus recessive inherited trait as a function of the allele frequency of the recessive allele.

Breeding Methods - Selection

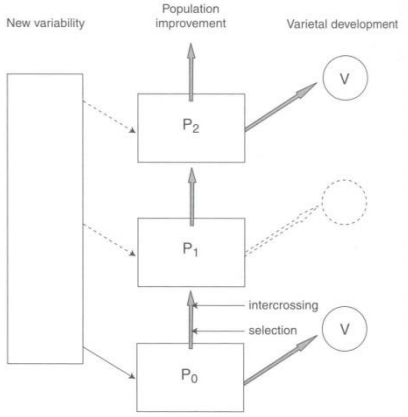
Selection for Varieties

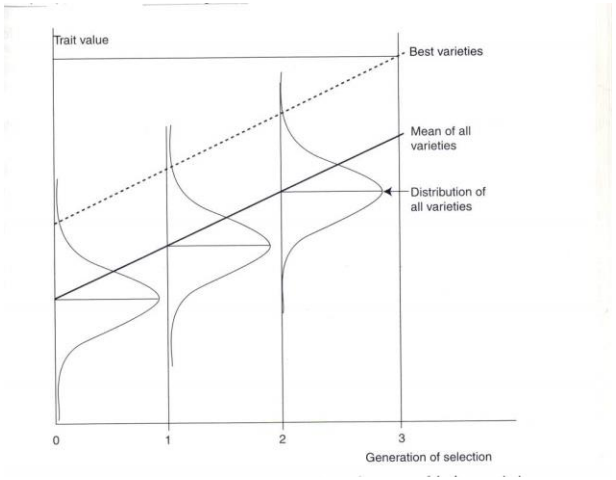
1. **Later Breeding stages** (quite well investigated: (e.g. Cochran 1951; Hanson and Brim 1963; Finney 1966; Utz 1969, 1984, Grüneberg et al. 2004; Mi et al. 2014) – note: a dozen of PhD students have been working on this by model calculations
2. **Early breeding stages clonally propagated crops – (ABS)** (Grüneberg et al. 2009) – note: extreme rapid adoption (fostered by SASHA & AGRA funds)

Selection of Parents for new Populations

(here we have the importance of index selection – Pesek Baker 1969, not new but …)

1. **Poly-Cross versus Controlled Cross Breeding**
2. **Selection of parents on off-spring performance – Heterosis (HEBS)**





Figures: From Gallais (2003) part III: 'Population improvement and varietal development' to illustrate the relevance of variety development and populations improvement

Later Breeding stages – Variance Components Yields – we have some information but we can use more i.e. about 6 to 10 series of trials across locations and years

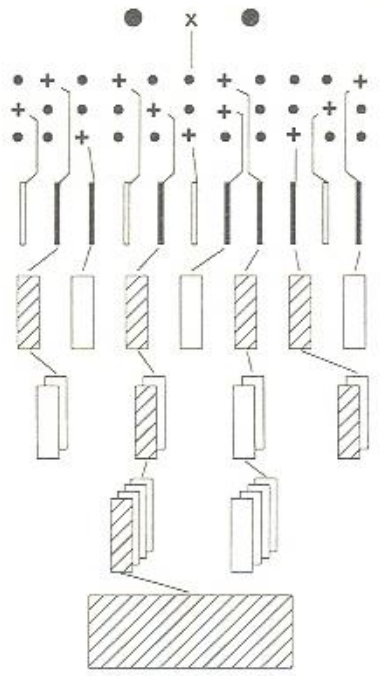
Table. Variance component ratios for storage root yield

Vg	Vgxe	Verror	Country	Method	Referance
1	1.27	1.93	Cameron	Anova	Ngeve and Boukamp (1993)
1	0.69	0.55	Peru	Anova	Manrique and Herman (2002)
1	0.78	0.21	Peru	Anova	Grüneberg et al. (2005)
1	6.12	10.62	Uganda	Anova	Tumwegamire (2011)
1	5.85	2.44	Peru	REML	Grüneberg et al. (2004)

Vg	Vgxl	Vgxs	Vgxlxs	Verror			
1	0.32	0.06	0.50	1.33	Cameron	Anova	Ngeve (1993)
1	-0.38	-0.21	1.97	3.34	Cameron	Anova	Ngeve (1993)
1	1.46	0.96	1.83	2.62	Uganda/Kenya	REML	Grüneberg et al. (2004)
1	2.21	-0.87	4.39	10.05	Uganda	Anova	Tumwegamire (2011)

Important is to note that storage root Vgxs is always or most often the smallest Vgxe this means that a breeder can replace temporal variation of test environments with variation of test environments **in other words this means a breeder can test in less years and compensate the loss of precision by using more locations**

ABS – Accelerated Breeding Scheme for clonally propagated crops – traditional breeding methods are too slow to achieve “good” progress, to make breeder happy & attract young scientists, **and donors**



This figure “The general breeding scheme of clonally propagated crops” is from Becker (1992)

Similar scheme can found in many other textbooks – unfortunately !!!

Two approaches to make things faster

1. **Accelerated breeding (ABS) by less years and more locations on basis of variance component estimations incl. early breeding stages**
2. Genomic selection – heavy use of SNP markers and prediction models

Note: approach 1 (ABS) adapted rapidly within few years (from 2005 to 2010) and resulted already in many accelerated variety releases for sweetpotato in SSA within the period 2009 to 2012 (see our last slide) – of course approach 2 promised to be better

How to explain ABS in one slide?

Planting the ABS at San Ramon in 2005 (one of 3 locations) – with 1 year in controlled crossings / with 2 years in polycrosses you select the material for later breeding stages

Plot size: 1m row plot in early breeding stages not more not less and no plot replications !!!

Three levels to investigate the efficiency of ABS (see APA paper)

=> **Estimate heritabilities** when you apply ABS in early breeding stages (4 times in applied breeding material at CIP Lima all with consistent results – when study do not apply perivous selection !!

$h^2 = \sigma^2_G / [\sigma^2_G + \sigma^2_{GL}/L]$

=> **Estimate heritabilities** when you apply ABS in early breeding stages with a check clone and **plant the selected fraction again with the check** for one further breeding stage to estimate the observed response to selection [3 studies in SSA (Ghana, Uganda and Mozambique) in process]

$h^2 = \sigma^2_G / [\sigma^2_G + \sigma^2_{GL}/L]$

R_{obs} = mean across sel fraction rel. to check in year 2 – mean all clones rel to check in year 1

=> **Estimate heritabilities when you apply ABS** in early breeding stages with a plot replication (2 plots per location) and **replant all clones in year 2 without selection** at same locations and same replication numbers and estimate the observed R and predicted R with models / testing different breeding senarios [study at CIP Lima in the frame of the poly versus controlled cross breeding study]

$h^2 = \sigma^2_G / [\sigma^2_G + \sigma^2_{GY} + \sigma^2_{GL}/L + \sigma^2_{GLY}/L + \sigma^2_e/L]$

R_{obs} = mean across sel fraction rel to check in year 2 – mean all clones rel to check in year 1

R_{pre} = standard models of selection theory

Variance components estimations in early breeding stages were leading to the ABS (accelerated breeding scheme)

Table Variance components and operative heritability for observed traits† in early breeding stages of the population 'Jewel 2005' planted at three locations (Loc) in Peru (San Ramon, La Molina and Cañete) without replications in 1-m row plots.

Traits	Vg	Ve	Vgxe	N clones	N Loc	Operational heritability
Storage root yield, t²/ha	47.7	23.2	98.0	4175	3	**0.59**
Foliage yield, t²/ha	237.0	52.1	349.0	4167	2	**0.58**
Dry matter content of roots, % FM§	13.94	8.18	6.22	2709	2	**0.82**
Carotene content of roots, ppm DM§§	33651	3453	9539	2709	2	**0.88**
Iron content of roots, ppm DM§§	7.41	5.79	7.61	2709	2	**0.66**
Zinc content of roots, ppm DM§§	3.10	4.63	2.92	2709	2	**0.68**

† Variance components: Vg = σ^2_G = genotypes, Ve = σ^2_E = environments, Vgxe = σ^2_{GxE} = genotype by environment interactions; h^2= operational broad-sense heritability.
§ FM = fresh matter; §§ DM = dry matter.

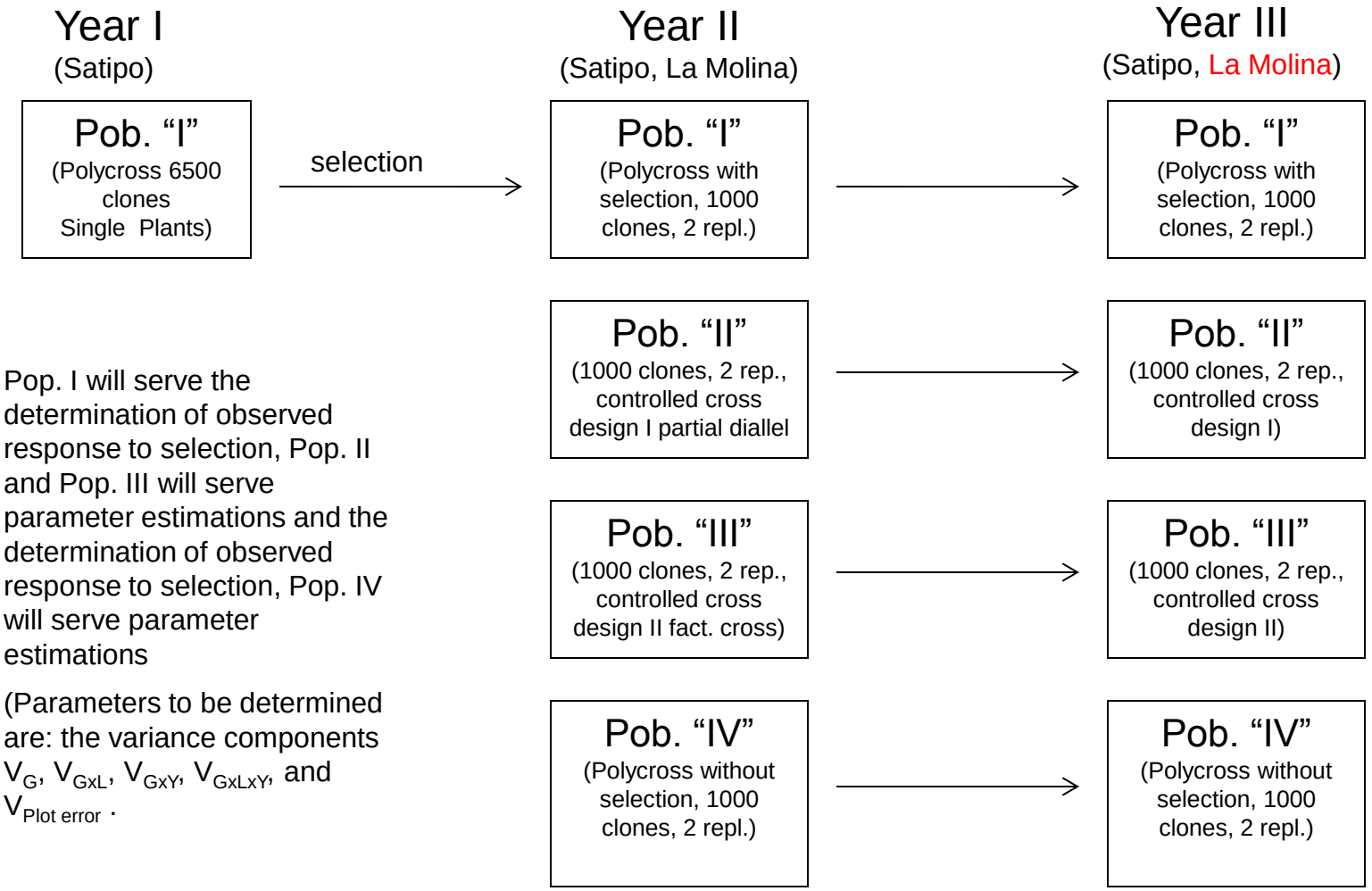
Variance components estimations in early breeding stages were leading to the ABS (accelerated breeding scheme)

Table Variance components and operative heritability for observed traits† in early breeding stages in 3 groups of the poly cross versus controlled cross breeding experiment – 2 locations (Satipo, La Molina), 2 years, 2 plot replications (1m row plots) – no selection applied.

Traits	Vg	Vgxl	Vgxy	Vgxlxy	Operational heritability in experiment	Operationalh eritability for ABS with 3 environments
Storage root yield, t²/ha	44.7	36.8	45.6	14.8	0.39	0.25
	55.9	36.2	18.0	20.2	0.48	0.33
	47.0	44.1	26.9	40.3	0.41	0.29
Harvest index, %²	129.2	43.1	11.0	1.9	0.74	0.63
	118.1	28.5	11.9	14.7	0.71	0.57
	122.7	39.4	16.2	30.9	0.67	0.53
Dry matter content of roots, % FM§	19.8	1.0	0.7	1.1	0.91	0.83
	19.3	1.9	0.6	0.4	0.90	0.84
	21.4	1.6	1.0	0.8	0.90	0.85

† Variance components: Vg, genotypes; Vgxl, genotypes by location, Vgxy; genotypes by year; Vgxlxy, genotypes by locaction by year

Polycross versus controlled cross breeding (PvC) study with 22 mega-clones as parents - Overview



Pop. I will serve the determination of observed response to selection, Pop. II and Pop. III will serve parameter estimations and the determination of observed response to selection, Pop. IV will serve parameter estimations

(Parameters to be determined are: the variance components V_G, V_{GxL}, V_{GxY}, V_{GxLxY}, and $V_{Plot\ error}$.

Note: Check clones included in each population are Tanzania, Jonathan, and Resisto.

ABS – Accelerated Breeding Scheme

Table 2. Variance component estimations on basis of 2 environments and 2 replications for observed traits for populations generated in different ways from a similar set of parents (22 parents).

	Var comp	Root yield (t^2/ha^2)	Upper biomass (t^2/ha^2)	Harvest index ($\%^2$)	Storage root dry matter ($\%^2$ fwb§)	Starch ($\%^2$ dwb§§)	b-carotene NIRS (mg^2 / $100g^2$ fwb§)	Fe (ppm^2 dwb§§
Pop – Poly-cross ws† (N=1021)	G	10.7	163.3	105.9	17.3	51.4	25.3	8.9
	GxE	48.8	50.9	48.4	1.7	7.7	0.9	1.8
	Error	189.2	572.6	226.1	8.1	43.8	5.1	8.1
Pop – Poly-cross (N=1015)	G	21.4	178.2	124.2	21.3	61.9	21.2	9.0
	GxE	31.8	48.7	57.1	2.0	4.3	1.5	2.9
	Error	98.9	498.2	149.6	9.1	14.1	3.0	7.5
Pop – Diallele (N=1041)	G	31.5	153.4	144.8	21.5	51.4	14.6	8.4
	GxE	26.1	30.7	45.0	1.4	3.2	1.6	0.3
	Error	148.4	623.6	187.4	8.8	16.0	3.7	6.9
Pop – Factorial (N=1042)	G	15.7	238.5	146.4	23.7	83.4	18.6	13.2
	GxE	38.6	107.8	20.8	2.7	5.4	3.0	2.0
	Error	119.0	761	163.6	7.8	13.1	2.7	6.3

†poly-cross with initial single plant selection (reduction of 5349 clones to 1021 clones by single plant selection)
‡ CC, color charts. §fwb, fresh weight basis. §§dwb, dry weight basis.

=> These variance component estimations are further indications that ABS works !!!

PvC – Polycross versus controlled cross breeding

Table . Genetic gains (Response) for storage root yield for polycross versus controlled cross breeding.

	Number of genotypes	Number of locations	Rep	Selected genotypes	Mean Root yield (t/ha) ‡‡	Response standardized	Response (t/ha)	Root yield (t/ha)
Pop – Poly-cross (1 step selection)	6000†	2	1	100	15.0 (14.5-15.5)	1.235	5.714	20.7
Pop – Poly-cross (2 step selection)	6000 =>1000 ††	1 =>2	1 =>1	100	15.0 (14.5-15.5)	1.350	6.243	21.2
Pop – Diallel	1000‡	2	1	100	18.4 (17.8-18.9)	0.904	5.073	23.5
Pop – Factorial (best in nutrient with the rest)	1000‡	2	1	100	14.6 (14.1-15.1)	0.715	2.834	17.4

† **Test capacity** 12000 1m row plots; †† **Test capacity** 8000 1m row plots; ‡ **Test capacity** 2000 1m row plots;
‡‡Confidence interval 95% in bracket

Polycross 1 step selection, 22 parents; Polycross 2 step selection, 22 parents; Controlled cross partial diallel (4x22); Controlled cross factorial [the best in nurtion with the rest (5 x 17)]; Mean b-carotene Pop 1 : 171 ppm; Pop 2: 171; Pop 3: 166 ppm; Pop 4: 209 ppm

Heterosis and Heterosis increments

Offspring is superior to the mid-parent performance (see figure) – heterosis increment or gain

F1 > (P1 + P2) /2 - where F1 is the family mean and P1 and P2 is the parental performance

What is the offspring in a clonally propagated crops such as sweetpotato? The family derived from a cross – note in a heterotic cross combinations / families you still can select for "the best" clone

In a experiment with so-called mega-clones (important clones across regions) **- we found positive heterosis increments in 18 out of 48 families with a heterotic yield advantage of the family** – without separation of genepools, without inbreeding, without selection of recombining ability

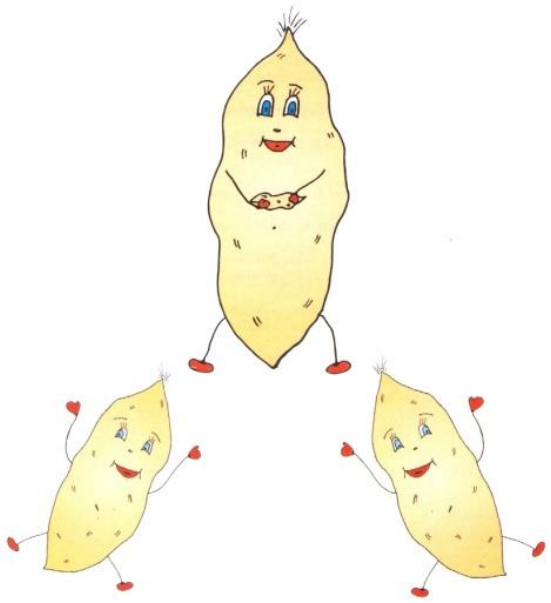


Fig. Illustration of Heterosis

Heterosis increments in sweetpotato - Family means in offsprings derived from 4x12 cross combinations

The overall aim:

Systematically exploit what we find in a single breeding population – these are heterosis increments in offsprings of specific cross combinations

Table 1. Storage root yield (t/ha) of four male and 12 female sweetpotato parents (underlined), their offspring means and heterosis increments of offspring on basis of mid-parent – mid-offspring estimates (italics) evaluated at two locations, San Ramon and La Molina, in Peru.

Parents		INIA100 (25.2)		Zapallo (22.0)		Wagabolige (10.9)		Tanzania (23.3)	
SR02.132	(33.5)	26.8	(-8.5%)	21.5	(-22.5%)	17.3	(-21.9%)	28.4	(-0.1%)
SR01.024	(11.7)	19.5	(5.6%)	20.8	(23.3%)	16.8	(48.9%)	22.5	(28.5%)
SR01.022	(12.7)	16.6	(-12.4%)	19.1	(9.9%)	14.2	(20.6%)	22.7	(26.0%)
LM02.082	(18.4)	19.4	(-11.2%)	23.9	(18.3%)	16.6	(13.4%)	23.3	(11.5%)
SR02.174	(22.7)	27.4	(14.7%)	28.8	(28.9%)	26.6	(58.7%)	28.2	(22.6%)
SR02.177	(41.3)	23.2	(-30.3%)	22.9	(-27.8%)	17.3	(-33.7%)	25.2	(-22.0%)
LM02.032	(23.1)	20.3	(-16.1%)	19.2	(-15.1%)	15.6	(-8.0%)	21.5	(-7.4%)
LM02.035	(13.7)	18.2	(-6.4%)	18.9	(5.8%)	15.1	(23.2%)	17.9	(-3.0%)
SR90.021	(4.6)	14.6	(-1.8%)	11.5	(-13.9%)	11.1	(43.5%)	13.1	(-6.6%)
SR01.029	(8.6)	15.0	(-11.3%)	13.8	(-10.1%)	10.9	(12.1%)	14.6	(-8.5%)
SR01.005	(11.5	15.1	(-17.7%)	12.9	(-23.0%)	8.0	(-28.7%)	12.7	(-27.0%)
SR01.002	(32.1)	24.5	(-14.5%)	19.1	(-29.6%)	18.3	(-15.1%)	20.3	(-26.7%)

Mid-parent to mid-offspring correlation r = 0.705, Pearson's correlation coefficient, N = 48.

Examples for heterosis increments we find in the crosses:

!! Wagabolige x SR02.174 (58.7%) !! or

!!! Zapallo x SR02.174 (28.9%) !!!

How to estimate heterosis increments – 1st step offspring estimates & without previous selection!

Systematic selection for parents which are generating better offsprings

<=>

HEBS (Heterosis exploiting breeding schemes)

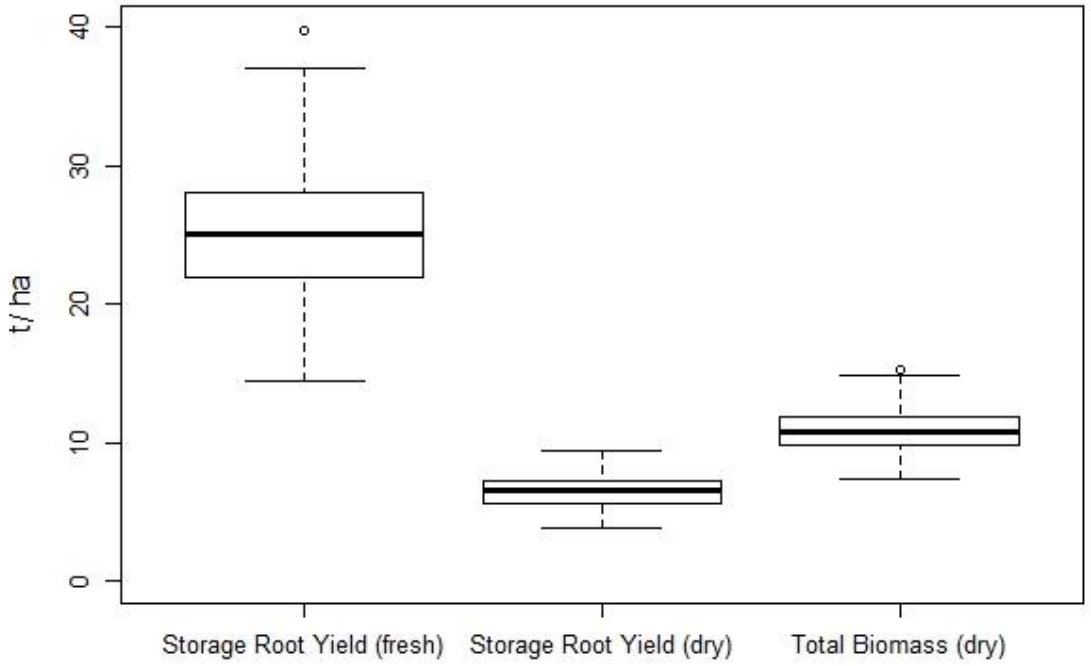


Figure: Mid offspring performance in **231 families (means**) for fresh storage root yield, dry matter storage root yield, and dry matter biomass yield – Note each boxplot shows the distribution of 231 family means - In total 6898 offspring clones tracing back to 31 PZ and 49 PJ parents recombined in 231 cross combinations / families tested at two locations and two plot replications

Heterosis increments in a hybrid population derived by crossing two mutually heterotic genepools

Systematic Heterosis exploitation with two mutually heterotic genepools so far without selection on combining ability

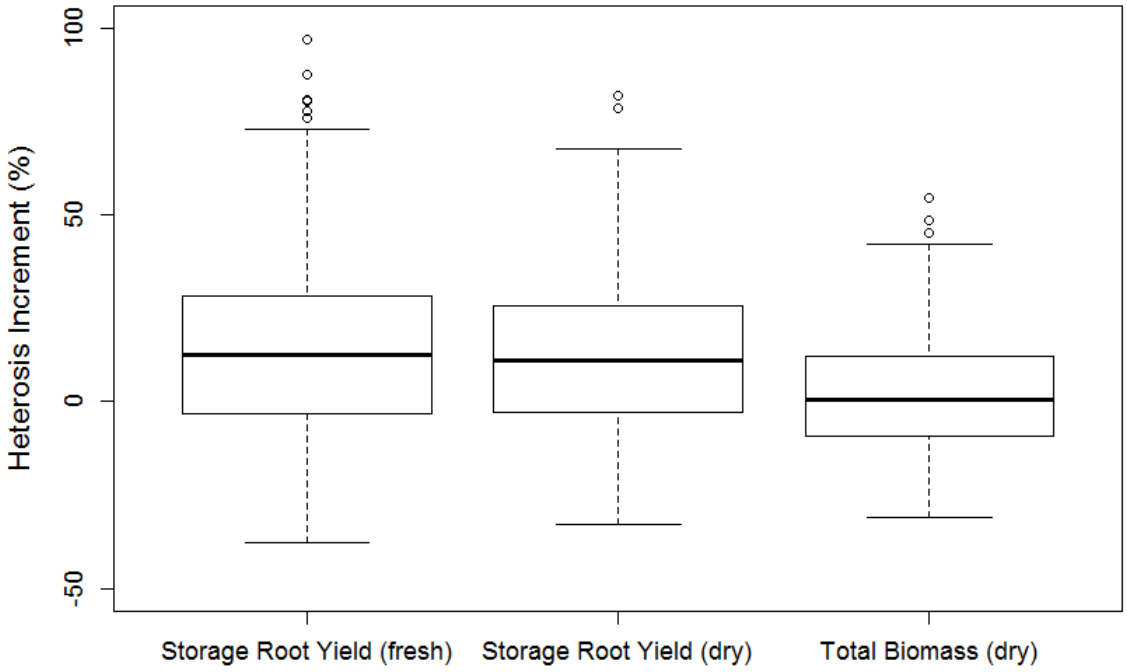
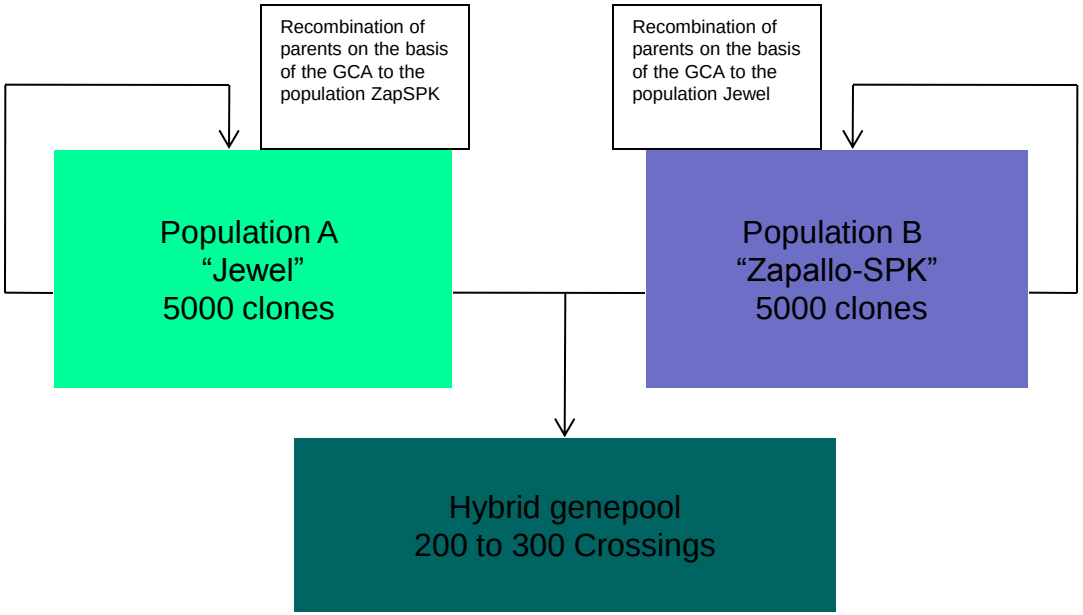


Figure: Mid parent – mid offspring heterosis increments in 231 families (means) for fresh storage root yield, dry matter storage root yield, and dry matter biomass yield – Note each boxplot shows the distribution of 231 family means - In total 6898 offspring clones tracing back to 31 PZ and 49 PJ parents recombined in 231 cross combinations / families tested at two locations and two plot replications

Selection of Parents and Heterosis Exploitation

Selection for new crosses / parents – best family makes



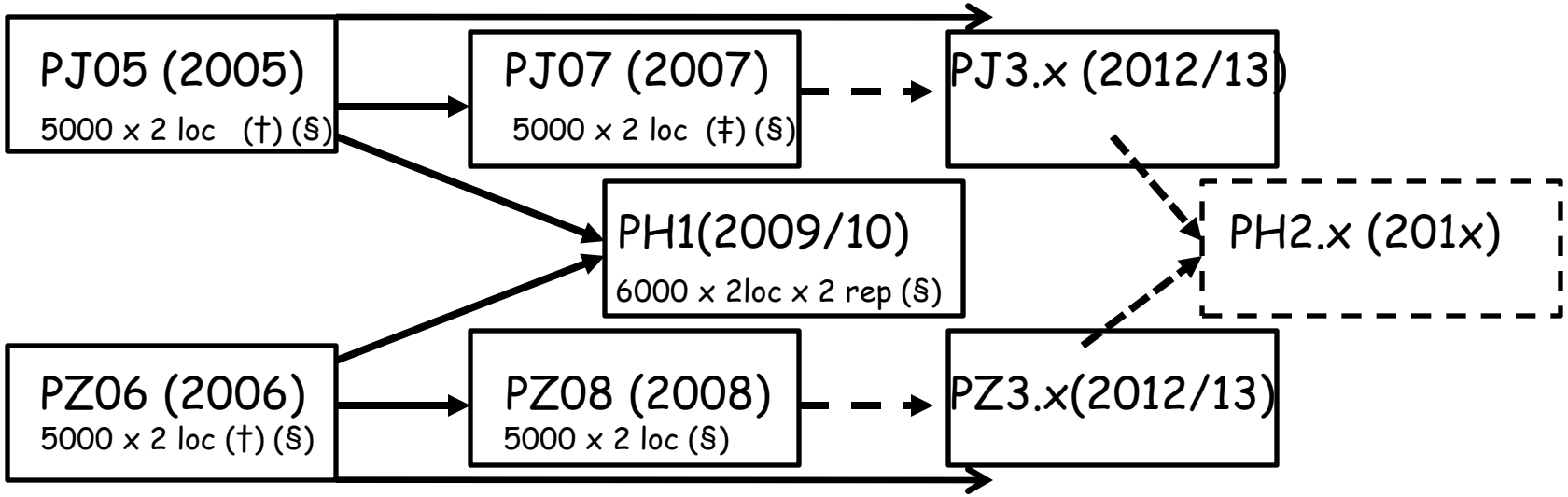
A potential heterosis exploiting breeding scheme (HEBS) - ! inbreeding by selfings not required !.

Note 1: Population A and B and the hybrid genepool can be used to select clonally propagated varieties.

Such a scheme was already proposed by Hull for clonally propagated crops using sugercane as an example – (Hull, F.H. 1945 Recurrent selection for specific combining ability in corn. J. Am.Soc. Agron. 37: 134-145)

Populations PJ & PZ and Hybrid Populations PH I-III (IV)

History of the Hybrid Populations: *Population **PJ05** formed on basis of selection for orange flesh color - generated by open pollination before 2004 (phenotypically and genotypically **more similar to North American varieties** such as Jewel and Resisto). Population **PZ06** formed by factorial controlled crosses conducted in 2005 (8 male parents, namely: **Jonathan, Zapallo, Huambachero, Tanzania, Yurimaguas, Wagabolige, Xushu18, Ninshu1) x 200 OFSP** female parents, which were selected visually for agronomic performance and orange flesh color – PZ06 clones resulted in several variety releases; PJ07 and PZ08 in the pipeline for release (4 clones)*



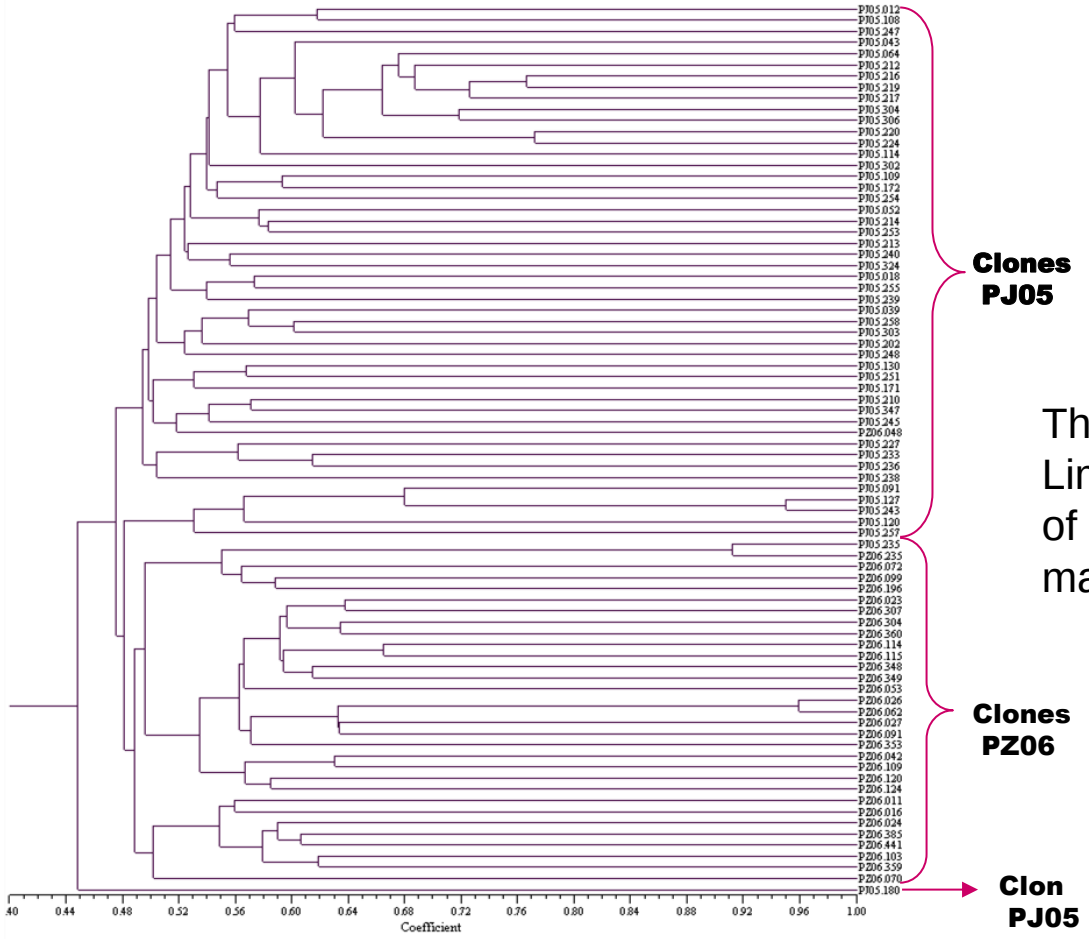
(†) *true seed of PJ05 and PZ06 (several thousands) were sent to Southern Africa and formed the **population Gurue** in Mozambique ,* (‡) *true seed of PJ07 (several thousands)send to **India***

PJ07 100g fresh storage root mean: β-carotene: 10.2 mg, iron: 0.64 mg, zinc: 0.38 mg

PZ08 100g fresh storage root mean: β-carotene: 7.9 mg, iron: 0.56 mg, zinc: 0.34 mg

Child 1 – 3 years needs per day: β-carotene: 4.8 mg, iron: 5 mg, zinc: 4 mg

The Populations PJ and PZ in Lima



PJ clones belong to the Breeding Population Jewel

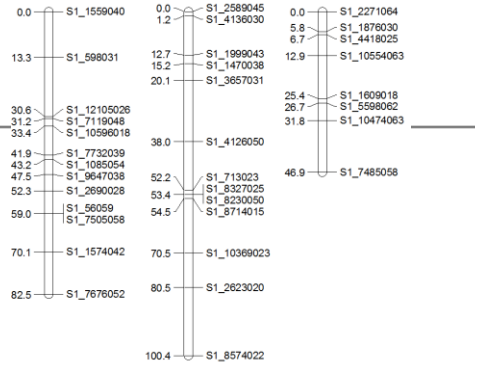
PZ clones belong to the Breeding Population Zapallo-SPKl

The heterotic breeding populations in Lima are clearly two genepools on basis of molecular characterization by SSR markers and they are mutually heterotic!!

Figure: Molecular characterization of the heterotic genepools PJ and PZ by 60 SSR marker (Diaz unpublished)

Similar studies EA germplasma (Tumwegamire et al. 2011); Parental material EA breeding plat form (David 2012)

Breeding Methods – Molecular tools (will they improve yields?)



M9 x M19 (2x) linkage map

- **With exception of genepool division *I* (separation of parental material) mol. markers are still not used much in applied breeding**
- **although there are many publications on molecular marker – trait associations:**

  SPVD resistance (Mwanga 2001; Miano et al. 2008), Root-knot nematode resistance (Ukoskit et al. 1997; Mcharo et al. 2005; Cervantes 2006); Storage root dry-matter (Cervantes 2006, Solis & Grüneberg 2008); β-carotene content (Cervantes 2006; Solis & Grüneberg 2008; Mcharo & LaBonte 2010), Starch and sucrose content (Solis & Grüneberg 2008), Storage root yield (Cervantes 2006; Solis & Grüneberg 2008)
- **Two 6x mapping populations:** Beauregard x Tanzania and Beauregard x New Kawogo (NCSU, Uganda/CIP, CIP); **One 2x mapping populations** with *I. trifida:* M9 x M19 – all still not clean (perhaps end of the year one Beauregard x Tanzania population
- Developing a **SNP (single nucleotide polymorphism) platform** and GbS (genotyping by sequencing).
- To accelerate sweetpotato breeding with superior genomic tools (might need **complete SP genome sequence – preferable with 2x homozygous *I. trifida***, because the sweetpotato genome is extremly large [the haploid DNA content is 1.55–2.25 pg/C nuclei or 1515–2200 Mbp (Ozias-Okins and Jarret 1994; Kriegner 2001)]

=> Most likely there will be a new project for 4 years: Molecular tools for sweetpotato breeding

Varieties released 1994 to 2013 – restricted to SSA more information across regions in the paper for this presentation

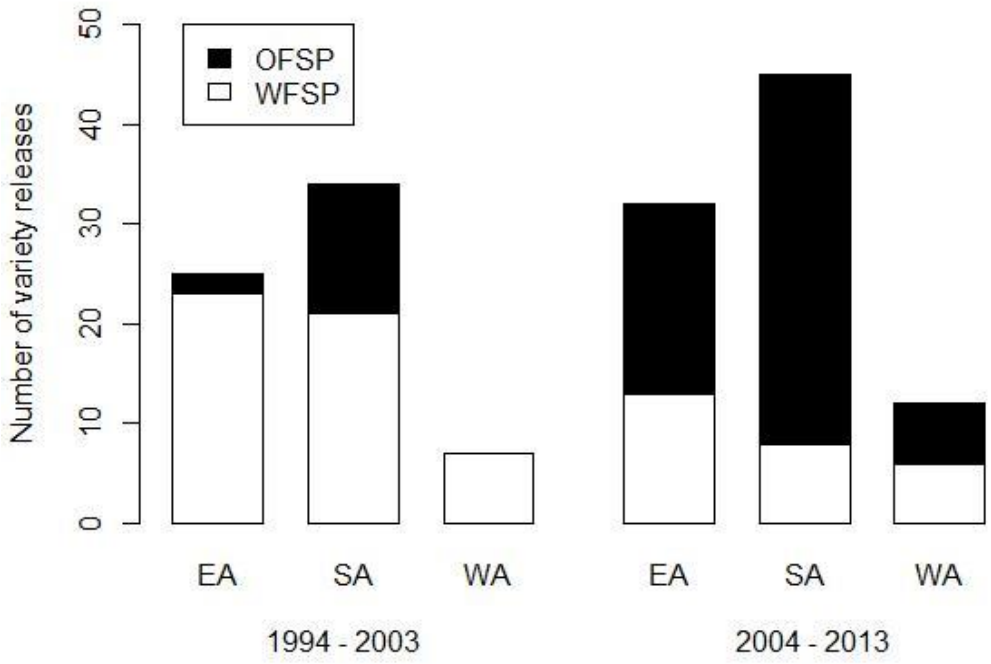
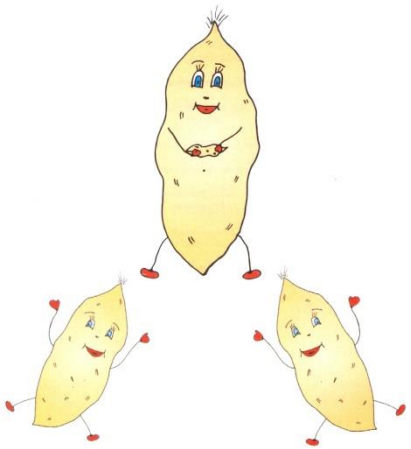


Figure. Number of variety releases in SSA during 1994–2013 by subregion and flesh color. EA, East Africa (Kenya, Rwanda, Tanzania and Uganda); SA, Southern Africa (Madagascar, Malawi, Mozambique, Republic of South Africa and Zambia); WA, West Africa (Burkina Faso, Ghana and Nigeria); OFSP, orange fleshed; WFSP, white fleshed.



Thank-you for your Attention