Heterosis Exploiting Breeding Schemes for Rapid Yield Enhancement

SASHA
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During SASHA Phase 1, clear evidence was generated for the first time that heterosis can be successfully exploited in sweetpotato for significant yield gains for roots and foliage. There is high potential for application to other root and tuber and banana crops, with more efficient breeding helping to solve the problem of feeding the ever growing population.

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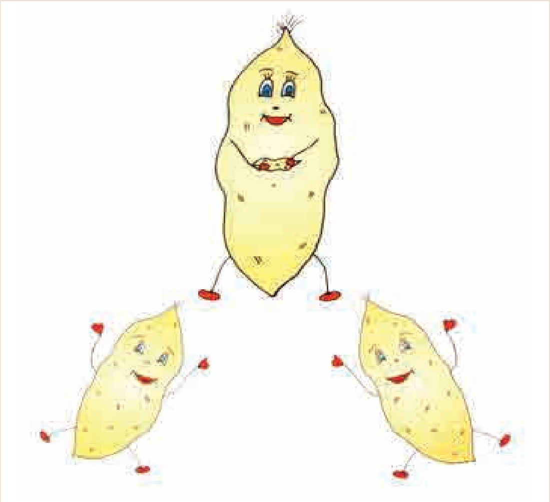

Figure 1. Two short parents producing a giant offspring (hybrid vigor in action)

There are five steps that can contribute to a solution to the problem of how to feed 9 billion people. The first is to avoid further expropriation of farm land to other uses. The second step is to grow more on farmland we are using. The third step is to use resources more efficiently such as water and nutrients. The fourth step is to shift diets. The fifth step is to reduced waste of food already produced.

Through improving breeding methods for roots, tuber, and bananas / plantains (RTB crops), which are quite efficient in using resources and so far are still untapped in their genetic potentials, we think that it is possible to contribute directly to steps 2, 3, and 4 and indirectly to step 1. Improving breeding methods for RTB crops can come through heterosis exploiting breeding schemes which have been very successfully applied to grain crops over the past decades, but so far not in RTBs.

The phenomenon heterosis (hybrid vigour) is well known in breeding as the increase in yield or size or other traits in hybrids. Gregor Mendel already reported the phenomenon in his famous pea experiments published in 1865 for the quantitative attribute "internode length". Other traits are usually those complex adaptation traits leading to abiotic stress tolerance (i.e. drought, low or high temperatures). Heterosis is everywhere around us especially in outbred species. Self-fertilization is an exception; however this strongest form of inbreeding can have certain advantages especially for generating genetic diversity. With respect to traits inherited by many genes nature prefers hybrids created by joining genetically divergent but still compatible parents. Inbreeding results in depression of performance in traits inherited by many genes, and the "right" combination of parents in highly heterozygous hybrids and heterosis, respectively. Inbreeding depression and heterosis are two sides of the same genetic phenomenon.

Heterosis exploiting breeding schemes (HEBs) for outbreeding crops ranks among the biggest impacts made by plant breeders to world food supply. Hybrid cultivars at the beginning of hybrid introduction produced yield jumps of 15% in maize, 50% in sunflower, 40% in sorghum, 30% in rice, and 20% in rye, when compared to superior open pollinated cultivars in the market (Geiger and Miedaner 1999). At the beginning these hybrids were not derived from fully inbred lines, because inbreeding depression was so strong that lines with low heterozygosity or completely homozygosity were too weak to give acceptable hybrid seed production.

There are several types of hybrid varieties:

1) population hybrids (recombining a population with a population or a F1 of two inbred lines or an inbred line),
2) double cross hybrids (recombining a F1 of two inbred lines with a F1 of two inbred lines),
3) three-way hybrids (recombining a F1 of two inbred lines with an inbred line), and
4) single cross hybrids (recombining an inbred line with an inbred) (Wricke and Weber 1986).

Thus, if you hear the argument that HEBs or hybrid breeding schemes, respective, require fully inbred lines, don't believe it. However, it is correct that the determination of the full amount of heterosis requires a homozygous basis and the total magnitude of heterosis is defined by the difference between the mean of two homozygous parents and their offspring [H = F1 – (P1 + P2)/2; where F1 denotes the offspring and P1 and P2 the homozygous parents]. This "total magnitude of heterosis" can be remarkable: yields of hybrids e.g. maize or rye may be two to three times larger than the yield of the parental inbred lines. Nevertheless, exploiting a fraction of the full amount of heterosis can be sufficiently attractive for breeders to embark on HEBs – for this fraction of the full amount of heterosis the terms 'heterosis increment' or 'heterotic gain' should be used and not heterosis. This 'heterosis increment or 'heterotic gain', respectively, can be determined without inbred lines and the parameter "mid-parent – mid-offspring heterosis increment" and provides information about the exploitable amount of heterosis at the current stage of the breeding material.

Certainly the yield of a hybrid is a very important attribute – but two additional factors (perhaps three factors) should not be neglected in reviewing the history of hybrid breeding scheme uptakes: 1) investments into hybrid breeding schemes were attractive for breeding companies generating their incomes by selling seed, because hybrid seed varieties can only be maintained with access to the parents, and 2) the first maize hybrids in the States were – because of hybrid vigour or heterosis – clearly more drought tolerant than open pollinated cultivars grown in the corn belt in the 1930s [during the recurrent drought years of the 1930s in the corn belt this led to a break-through of maize hybrid varieties (cited from Duvick 2009)]. The third factor to be considered is that maize hybrid breeding resulted in the development of the most efficient plant breeding scheme: reciprocal recurrent selection. This breeding scheme (i) systematically exploits heterosis by recombining two genepools (mutually heterotic genepools), (ii) discards all parents within genepools from recombination which are bad hybrid family makers with respect to combination with the complementary genepool, and (iii) allows controlled and even intense inbreeding within genepools of an outbreeding crop to select for quality and biotic stress resistances without sacrificing the heterosis in the cultivar. The exploitation of heterosis in the first hybrid varieties (population, three-way, or two-way hybrids) resulted in a yield jump and then the reciprocal recurrent selection resulted in steeper slopes of regression lines for genetic gains with hybrid breeding compared to those obtained during the epoch of open pollinated variety breeding in out-breeding crops (Troyer 1991).

What has this to do with breeding RTB crops and clonally propagated crops? All clonally propagated crops are heterozygous hybrids propagated by cloning – the term "clone hybrids" should be used to reflect appropriately the genetic nature of these crops. To breed clonally propagated crops sexual recombination (sexual seed development of 20,000 to 200,000 true seeds) is required and we think that this is done better by HEBs. This should result in superior hybrid breeding populations, i.e. breeding populations with higher yield means and enhanced quality and resistances to biotic stresses. However, in contrast to "seed hybrids" such hybrid populations of "clone hybrids" are not for variety release – they are the basis for selection of the "best clone variety" in the hybrid population. This new way of breeding clonally propagated crops is particularly attractive because a large investment is not required – simply by gene-pool separation and controlled recombination (which requires skilled technicians), it should be possible to generate: (i) a yield jump for clonally propagated crops associated with enhancement of resistances to abiotic stresses by working with two mutually heterotic genepools (expected: a yield jump of 20 to 40%), (ii) steeper slopes of regression lines for genetic gains by reciprocal recurrent selection (expected: yield breeding progress of 3 to 4% per year for 3 to 4 decades), and (iii) enhanced breeding progress for quality and biotic stress resistances through controlled or more intense inbreeding within genepools (crossing relatives where clones are not self-compatible).

It should be noted that most clonally propagated crops are not diploid, except cassava (e.g. potato is usually tetraploid, although there are two attractive diploid potato species; sweetpotato is hexaploid, and bananas and plantains are triploid). In such *autopolyploids* the frequency of heterozygosity is much higher compared to diploid crops (Gallais 2003) and this indicates that the "stimulus of heterozygosity" or heterosis might be much higher in autopolyploid hybrids compared to diploid hybrids (Fig. 2). It can be hypothesized that the magnitude of quantitative trait performance in cassava is nearly similarly effected by heterosis as in diploid maize, but in tetraploid potato,

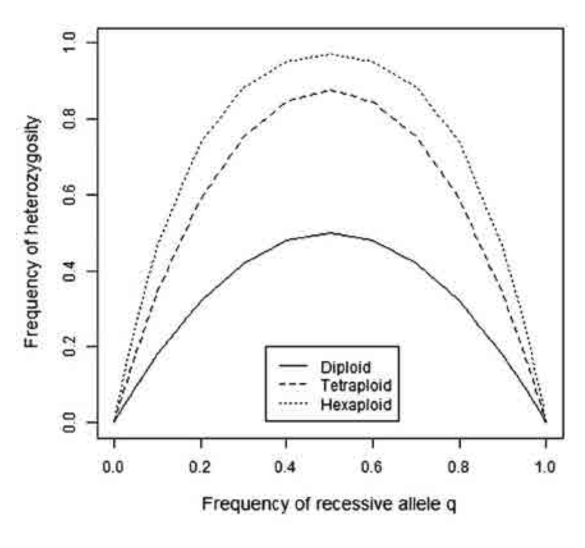

Figure 2. Effect of ploidy level on the frequency of heterozygosity in a random mating bi-allelic population at equilibrium as a function of the frequency q of the recessive allele (p + q = 1), in the absence of double reduction. From Gallais (2003), modified by inserting the hexaploid curve.

hexaploid sweetpotato and triploid bananas, the magnitude of quantitative trait performance should be much more than that for diploid maize – this is indeed food for thought. HEBs even could be the solution to come out of the dilemma of triploid banana breeding by working with one diploid and one tetraploid genepool (Ortiz 1997; Grüneberg et al. 2009). During the past five years a more intensive discussion has developed on heterosis-exploiting breeding schemes for clonally propagated crops (Miles 2007; Grüneberg et al. 2009). However, this discussion is not new – it was proposed earlier for breeding clonally propagated crops (e.g. Hull 1945; Melchinger and Gumber 1998), but hidden in reports concerning heterosis in traditional seed hybrid crops. Although some maize breeders say that applying HEBs in clonally propagated crops should follow the same principles as in traditional hybrid crops, there is one factor that is extremely different. That is the driving force of commerce to sell cereal hybrid seed, for example, does not exist in clonally propagated crops which are easily shared among farmers.

There are efforts underway in hybrid breeding for potatoes at the University of Wageningen (Lindhout et al. 2011) and for cassava at CIAT (Ceballos pers. comm.). To our knowledge both of these working groups are focusing on solving the compatibility problem of self-fertilization in the development of inbred lines by self-compatibility genes in potato and double diploid production in cassava. The discussion among clone breeders about the requirement of inbred lines / fully inbred lines to use HEBs is to certain extent irrelevant. Inbreeding does not require self-compatibility – certainly, it is the strongest method to achieve inbreeding progress, but inbreeding was the driving force to create the huge spectra of dog races we know today and no dog was ever selfed. Certainly, self-incompatibility of animals has not hampered animal breeders in exploiting heterosis. Moreover, for *auto-polyploids* the development of homozygous genotypes by selfing is partially illusory.

For example, in hexaploid sweetpotato (even if clones are self-compatible, which is more and more the case), it would require seven generations of selfing to reach an inbreeding coefficient of F = 0.5 (readers are referred to p. 124 of Gallais 2003), whereas F = 0.5 is reached in diploids after one generation of selfing. For this reason, at the International Potato Center (CIP) concerning sweetpotato, we focus on the most relevant parameter to reach "go" and "no-go" decisions for further heterosis investigations: that is the **mid-parent – mid-offspring heterosis increment.**

In the first study under SASHA Phase 1 among 48 sweetpotato crosses without genepool separation (recombined in a 4 x 14 factorial cross design) we observed mid-parent – mid-offspring heterosis increments of up to 60% (Grüneberg et al. in press). This "go" result (Table 1) suggested a study on mid-parent – mid-offspring heterosis increments on the basis of separate genepools. Two applied sweetpotato breeding populations supposed to be mutually heterotic were used for this study, namely "PJ" and PZ". Both populations have different genetic backgrounds (different origin of parental material used to develop the populations) and no PJ clone was used to improve the PZ population and vice versa since both populations were established. In total 6,898 offspring clones were developed tracing back to 231 families / offspring derived from PJ x PZ crosses (Fig. 3). The offspring were evaluated with their parents (49 PJ05 and 31 PZ06 clones) at 2 locations (Huaral in the Central Coast and San Ramon in the humid tropical highlands in Peru) with 2 plot replications. The mean across all families in the hybrid population was 25.0 t/ha fresh storage root yield. The hybrid population exhibited on average a mid-parent – mid-offspring heterosis increment of 13% for storage root yield (dry weight basis). With respect to storage root yield (dry), heterosis increments larger than zero existed in more than 70% of all offspring, about 25% of all offspring exhibited a heterosis increment of 20% and more, and for two offspring we observed heterosis increments of close to 80% (Fig. 4). Hence these two populations PJ and PZ are mutually heterotic. We think also this demonstrates that yield jumps of around 20% can be achieved for sweetpotato by heterosis exploitation with genepool separation, even without applying reciprocal recurrent selection. Applying the first reciprocal recurrent selection should considerably enhance the yield jump.

Note that after obtaining these results the parents were tested by Simple Sequence Repeat (SSR) markers and results indicated that PJ and PZ form separate clusters. This indicates that molecular markers, especially SSR markers are helpful in the identification or creation of mutually heterotic genepools in sweetpotato. This is similar to findings for many other crops (Lèfort-Buson and de Vienne 1985; Melchinger 1999). These positive results led to the establishment for heterotic genepools in parental breeding material at the sweetpotato breeding platform in Uganda by SSR markers.

Simultaneously, we started into the first reciprocal recurrent selection cycle in a HEBS on the basis of selection using offspring performance of parents with the PJ and the PZ population. All parents were discarded which developed offspring below the median line in the box plots presented in Fig. 4, and the remaining parents were entered into intra-genepool recombination. The resulting "inbred populations" are currently undergoing field evaluations.

The intention is to select new PJ and PZ parents and to recombine these for the establishment of a new hybrid population. Through comparison of the clones in the new hybrid population with the original 49 PJ05 and 31 PZ06 clones (held in trust in CIP's genebank), it shall be possible to determine the yield jump achievable by genepool separation and reciprocal recurrent selection (expected yield jump for sweetpotato 30 to 40%). In addition to the HEBS yield jump, expect clearly larger genetic gains in breeding for recessive inherited traits such as resistance to sweetpotato virus disease (SPVD) and non-sweet sweetpotatoes through selection among moderate inbred clones in intra-genepool recombination. HEBS could become a game changing tool for breeding sweetpotatoes.

Figure 3. Harvest of the first hybrid breeding population systematically developed by inter-genepool crossings at San Ramon / Peru - in total 6,898 offspring clones tracing back to 31 PZ and 49 PJ parents recombined in 231 cross combinations / families planted in 1m row plots per clone comprising three plants.

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.

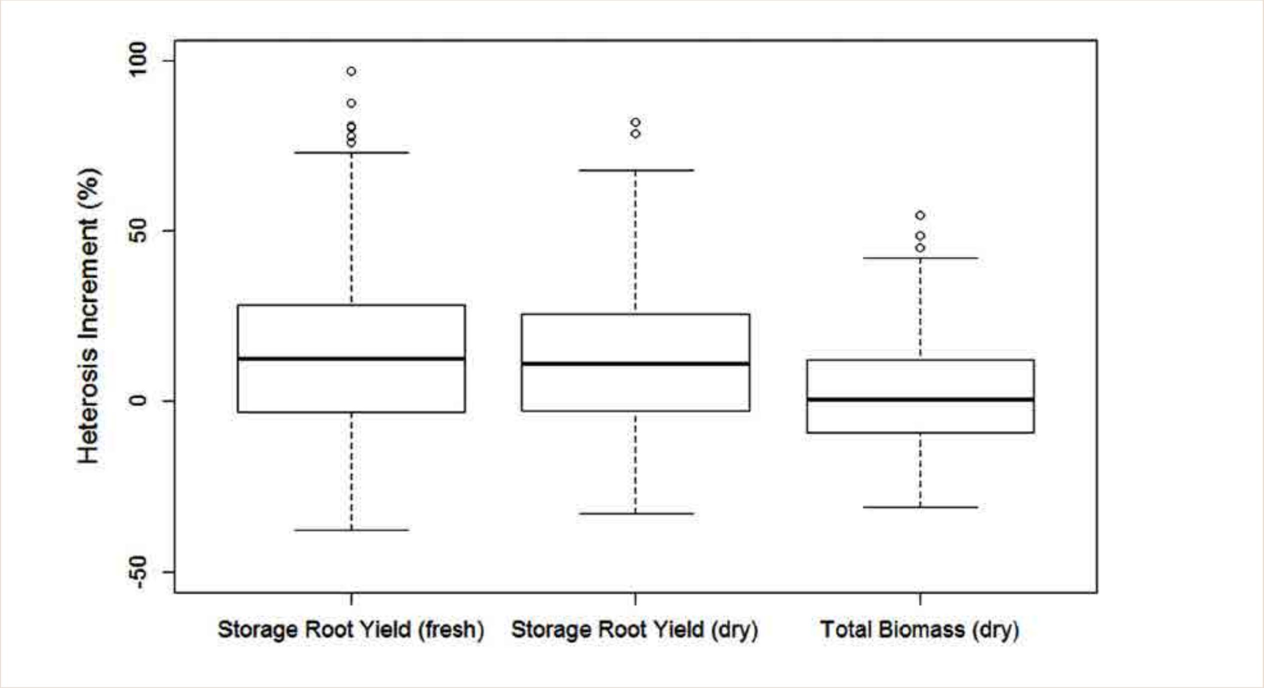

Figure 4. Heterosis Increments for Storage Root and Biomass Yields in Sweetpotato with Genepool Separation (so far without Reciprocal Recurrent Selection). Mid parent – mid offspring heterosis increments in 231 families (means) for storage root yield (fresh and dry weight basis) and biomass yield (dry weight basis) – each boxplot shows the distribution of 231 family means - in total 6,898 offspring clones tracing back to 31 PZ and 49 PJ parents recombined in 231 cross combinations / families were tested at two locations and two plot replications.

It all begins with crossing between two mutually heterotic genepools (credit G. Ssemakula)

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CONTACT

Wolfgang Grüneberg (CIP)
W.Gruneberg@cgiar.org