

Analytical Breeding

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Abstract

Ploidy manipulations consist on scaling up and down chromosome numbers of a species within a polyploid series. Chromosome sets are manipulated with haploids, $2n$ gametes, and through interspecific-interploidy crosses. Analytical breeding schemes rely mainly on ploidy manipulations to “capture” diversity from exotic (wild or non-adapted germplasm) and use $2n$ gametes to incorporate this genetic diversity through unilateral (USP; $n \times 2n$ or $2n \times n$) or bilateral (BSP; $2n \times 2n$) polyploidization. Most interesting examples of analytical breeding in crop improvement are in vegetatively propagated species such as potato, sweetpotato, cassava, among roots and tubers, and plantain/banana among fruit crops. This breeding approach appears promising in blackberry, blueberry, strawberry and other fruits. An evolutionary crop breeding method was developed for further improvement of the plantain genome. This method ensued from the genetic knowledge accumulated during the analytical breeding of this triploid ($3x$) crop. Heterozygous $3x$ landraces are crossed with diploid ($2x$) wild or cultivated accessions showing the desired characteristic(s). Primary tetraploid ($4x$) hybrids are recovered after USP, i.e., $2n$ eggs from $3x \times n$ pollen from $2x$. Secondary triploids result by crossing selected $4x$ with selected $2x$ stocks, both producing n gametes. Triploid *Musa* hybrids may also occur due to USP among $2x$, i.e., one $2x$ producing $2n$ gametes. Hybrids may result from artificial hand-pollination or through polycrosses among selected $4x$ and $2x$ parents according to their specific combining ability. Synthetic populations derived from the polycrosses may be tested in other locations to identify promising offspring for cultivar selection. Local selections may lead to dynamic conservation of genetic resources because farmers will preserve distinct locally adapted, improved genotypes across environments. Analytical breeding, coupled with this evolutionary breeding approach, offers an option for broadening the genetic base of fruit crops, enhancing their adaptation, and sustaining breeding gains in respective gene pools.

BACKGROUND

Analytical breeding (or ploidy manipulations) includes scaling up and down chromosome numbers of a species within a polyploid series (Chase, 1962; Peloquin and Ortiz, 1992). The main steps for this breeding approach are: (1) reducing polyploid to diploid level through parthenogenesis, often maternal haploids, (2) intensive breeding and selection at $2x$ level, and (3) re-synthesis and testing of polyploid forms for cultivar development. Analytical breeding capitalizes at the genome level on selecting favorable alleles within individual chromosome sets, and by combining whole chromosome sets in physiologically effective systems; which combine high yield and best quality (Chase, 1963). Ploidy manipulations rely on advantages provided by crops with euploid series and vegetative propagation but possessing the ability for sexual reproduction, having easy haploid systems through parthenogenesis, as well as a wealth of available genetic resources such as $2x$ wild species and other breeding stocks, and $2n$ gametes for sexual polyploidization (Ortiz, 2002).

Sexual polyploidization refers to the production of progeny of a higher ploidy than

expected from normal meiosis because at least one parent produces $2n$ gametes or a meiotic product with the sporophytic chromosome number (Veilleux, 1985). Sexual polyploidization with $2n$ gametes seems to be the main path for crop evolution in polyploid species such as potatoes, sweet potatoes, yams, bananas and plantains, among the most important food crops (Harlan and De Wet, 1975; Hermesen, 1984a). Veilleux (1985) provides an in-depth review of functional $2n$ gametes in crop plants.

Abnormal microsporogenesis and megasporogenesis lead to $2n$ pollen (or diploandroidy), and $2n$ eggs (or diplogynoecey), respectively. In most plant species, $2n$ pollen occurs due to pre-meiotic disturbances (endomitosis in archesporial cells, syndiploidy), meiotic disturbances (e.g. first division restitution or a FDR mechanism, and premature cytokinesis leading to a second division restitution or a SDR mechanism), abnormal cytokinesis and co-orientation of second division spindles (Hermesen, 1984b). Similar arrays for abnormal megasporogenesis account for $2n$ eggs. Genetic analysis of $2n$ gametes provides insights concerning maximum heterozygosity through FDR mechanisms and homozygosity by SDR mechanisms (Peloquin and Ortiz, 1992). Major genes affect meiosis leading to $2n$ gametes, but also minor genes and the environment influence the expressivity and penetrance of the trait. For example, heat treatment enhances $2n$ gamete production in pear branches (Lewis, 1943). Variable expressivity and incomplete penetrance keep diploidy in the population following $2x-2x$ crosses with only n gametes, and a continuous introgression from $2x$ species to $4x$ breeding pool(s) through sexual polyploidization.

Interspecific gene transfer in many crop species was successful through ploidy manipulations in many crop species (Singh et al., 1990). Some of the barriers to interspecific hybridization are associated to abnormal embryo development, endosperm disintegration, abnormal development of ovular tissue (or somatoplastic sterility due to prolific growth of nucellus or integuments), weak F_1 hybrids owing to genetic disability or disharmony (e.g. nuclear genome-cytoplasm incompatibility), and sterile F_1 hybrids, which may occur due to the lack of pairing arising from non-homology or structural incompatibility between donor genomes. Fertile F_1 hybrids provide a greater versatility for gene introgression or incorporation.

Ploidy manipulations overcome some of above shortcomings because it increases crossability, leads to F_1 hybrid viability by a proper handling of $2n$ gametes and the endosperm balance number (EBN). The EBN is an endosperm dosage system that influenced the speciation of many polyploid crop species (Ehlenfeldt and Ortiz, 1995), and this “effective ploidy” was defined as a unifying concept to predict endosperm function in intraspecific, interploidy and interspecific crosses. In some crop species, the EBN must be in a 2:1 maternal to paternal ratio in the endosperm for crosses to succeed. Bridging crosses are sometimes needed for manipulating the EBN when a ploidy gap occurs or for transferring specific chromosome segment(s) or gene(s)/gene block(s) therein among species (Ortiz, 1998a), which are not always amenable to direct hybridization.

The analytical breeding scheme (Fig. 1) provides a means for reversing the evolutionary pathway from diploid to polyploid level. By bringing back diploidy in the breeding population, this scheme allows intensive selective breeding at this level, and set the stage for retracing evolutionary paths from ancestral diploidy and to mold polyploidy with genome arrays that meet the human needs nowadays (Chase, 1963). The advantage of diploidy, vis-à-vis polyploidy, resides in the rapid response to selection (Stebbins, 1956), which provides a means for introducing a large array of genotypes into new target environments or for isolating specific gene arrays after multi-environment testing. For example, selection at $2x$ level at s intensity against a recessive allele with a q frequency results in losing sq^2 , which will not pass their alleles to the next breeding cycle. At the $4x$ level, this will be sq^2 , which for a $q = 0.5$, it will be 4 times smaller than at the $2x$ level. Furthermore, when the frequency of $q = 0.2$ at the $4x$ level, selection response for the dominant allele ($p = 0.8$) becomes practically nil. Of course, the lower the frequency of the gene being selected for, the larger the population size needed for successful selection,

which will be easy at the 2x level. Ortiz and Peloquin (1994) discussed in detail viz. a viz. disomic and tetrasomic inheritance and showed that the probability of recovering homozygous recessive individuals will be always higher at the former genetic system, irrespective of chromosome or chromatid segregation in the polyploid.

Why bring back polyploidy? In most commercial vegetatively propagated crops, polyploidy coupled with heterozygosity appears to confer advantages, particularly when non-additive variance (due to intra- and interlocus interactions) for the trait being selected for becomes the most important (Peloquin and Ortiz, 1992). For example, inbreeding seems to play a greater role in low yield for some vegetatively propagated crops, while allelic diversity may be associated to high yields. Intralocus (heterozygosity) and interlocus (epistasis) interactions lead to within and between locus heterosis, or the progeny outperforming the best parent (or parents' mean). In a 4x genotype four distinct alleles (tetra-allelic) may be together in the same locus, and resulting, therefore, in maximum heterozygosity, and more intralocus interactions: six first order among pairs of two distinct alleles, four second order among three distinct alleles, and one third order interaction for the four distinct alleles in the same locus. The highest within locus heterozygosity, which result in 11 distinct interactions in tetra-allelic genotypes, may lead to greatest epistasis between loci. Table 1 lists the percentage of heterozygosity transmitted through distinct gametes arising from meiosis or post-meiotic chromosome doubling.

POTATO AS THE MODEL CROP SPECIES FOR PLOIDY MANIPULATIONS

Chase (1962, 1963) coined the term “analytical breeding” after considering the potato breeding approach of R.W. Hougas and S.J. Peloquin, University of Wisconsin in the early 1960s. Ploidy manipulations with haploids, wild species and 2n gametes was incorporated worldwide as a tool for enhancing the potato genome since the 1970s by Peloquin and his former students (Peloquin et al., 1989). Ortiz (1998a) provided recently a overview of both ploidy manipulations and chromosome engineering in potato.

Chromosome sets or segments to incorporate or introgress wild species germplasm into the cultigen pool of potato are easily manipulated with haploids, 2n gametes and EBN (Fig. 2). Maternal haploids or sporophytes with the gametic chromosome number are easily obtained through induced parthenogenesis in tetraploid landraces and cultivars. The haploids (2n = 2x, 2EBN) are then included in crosses with 2x, 2EBN wild species to obtain new 2x, 2EBN breeding stocks, which show chromosome pairing and recombination during meiosis because of minimal chromosome differentiation among most tuber-bearing *Solanum* taxa. Diploid wild species and other 2x potato landraces provide the source of genetic diversity, while the haploids from the 4x cultivars ‘capture’ this genetic diversity in crosses with the 2x germplasm. The diversity from the haploid-species hybrids will be transmitted to the adapted tetraploid breeding pool via 2n gametes. Important traits such as pest and disease resistance, tolerance to abiotic stresses and tuber quality were transferred using this method (Ortiz, 1998a). The allelic diversity brought by the diploid stocks may result in high tuber yield because non-additive and inter-locus interactions are most important factors for enhancing this trait in the potato crop.

GENETIC ENHANCEMENT OF BANANA AND PLANTAIN GENOMES THROUGH EVOLUTIONARY CROP BREEDING

As shown in potato, analytical breeding offers a means for developing 2x and 4x breeding populations, from which could ensue new 4x cultivars. In *Musa* 2n gametes opened the path for the genetic amelioration of both bananas (Rowe and Rosales, 1996) and plantains (Vuylsteke et al., 1997). The new plantain cultivars developed elsewhere (Ortiz et al., 1997) challenged the old concept of the intractability of plantain for genetic improvement by cross breeding methods. Interspecific hybridization, 2n gametes, embryo culture, micro-propagation, field testing and multi-environment selections resulted in the identification and release of black sigatoka-resistant hybrids (Vuylsteke et al., 1993, Ortiz and Vuylsteke 1996), which today are included in delivery programs to farmers in West

Africa, particularly in Ghana and Nigeria. Furthermore, the advantages of ploidy manipulations in *Musa* for a sustainable management of black sigatoka resistance in this crop were recently indicated (Craenen and Ortiz, in press), particularly for incorporating in farmers' fields a genetic system that slows disease development in the host plant using gene pyramiding or cultivar mixtures.

As early pointed out by Chase (1963), insights into polyploid crop genetics become available throughout the breeding cycles at distinct ploidy levels, e.g. research on dosage effects, ploidy influence on trait physiology, intra- and interlocus interactions, heterotic patterns arising from multi-allelism, genetics of pest resistance. Such knowledge could lead to evolutionary breeding proposals (Fig. 3) as suggested by the genetic enhancement of banana and plantain at the International Institute of Tropical Agriculture (IITA, Nigeria and Uganda) (Ortiz, 1997). Heterozygous 3x landraces are the source of adapted allelic diversity, which becomes available after these landraces are crossed with 2x stocks showing a specific attribute, e.g. disease resistance. Promising high-yielding 4x hybrids are further included in crosses with selected 2x stocks (after progeny testing to determine specific combining ability) to obtain improved secondary 3x hybrids (Ortiz et al., 1998). These 3x hybrids could result from hand-pollination or from open pollination in polycrosses (Ortiz and Crouch, 1997), which include selected 4x and 2x parents as per their combining ability determined in previous 4x-2x crosses. Synthetic populations from the polycross may be sent for testing to other locations that will allow selecting locally adapted offspring for further cultivar development. Such approach appears important in the *Musa* crop, because of the significant genotype-by-environment interaction affecting bunch weight (Ortiz, 1998b).

Fig. 4 shows the alternative on-going breeding *Musa* schemes at IITA that rely on ploidy manipulations. Such approaches show the unlimited prospects for the genetic enhancement of the plantain and banana genomes, which, as suggested by Vuylsteke (2000), "will at once initiate a new phase of *Musa* evolution."

POTENTIAL OF ANALYTICAL BREEDING IN FRUIT AND BERRY CROPS

Other fruit and berry crops (or their wild species) with $2n$ gametes (both $2n$ pollen and $2n$ eggs) are *Fragaria*, *Malus*, *Rubus* and *Vaccinium*. Diplandroidy was reported in *Ananas*, *Prunus* and *Pyrus*, whereas dylogoneoc occurs in *Citrus* (Veilleux, 1985). Hence, ploidy manipulation appears promising for the transfer of exotic germplasm to the cultigen pool, which will lead to broadening the genetic base of fruit and berry crops. The breeding methods discussed above for potato and *Musa*, support this analytical breeding approach.

Blackberry

Hall (1990) suggested polyploidy for blackberry breeding because fruits are small in diploid blackberry and only a genetically narrow cultigen germplasm appears to be available in this crop. Tetraploid blackberry shows larger fruit size, thicker canes, larger and thicker leaves, and greater vigor than 2x species. It appears that polyploidy offers a way for maximizing heterosis in this crop (Sanford, 1983). This hypothesis was confirmed by improvement successes when cultivars arose from crosses between distinct parents, and by the limited success of inbreeding recessive traits in cultivar development for this crop (J.N. Moore, pers. commun. as cited by Hall, 1990).

Genes from the species *Rubus ulmifolius inermis* were introgressed to *R. thyrsiger* at Long Ashton (UK). Most of the resulting offspring, from crosses of this species with an apomictic 4x clone, were sterile 3x hybrids (Crane and Darlington, 1927), but one was a 4x seedling ensuing from unilateral sexual polyploidization. This 4x hybrid, known later as 'John Innes', derived from a $2n$ gamete from *R. ulmifolius inermis* and an n gamete from *R. thyrsiger*. 'John Innes' became an ancestor of 'Merton Thornless', the donor of the recessive trait to all thornless 4x blackberry cultivars (Moore, 1984; Scott et al., 1957). Both cultivars were not isolated cases of sexual polyploidization in blackberry breeding because $2n$ gametes often participated in developing other cultivars (Lawrence,

1986; Ourecky, 1975), e.g. 6x 'Bedford Giant'. This cultivar became predominant in the UK at the end of the 1980s. The seedling originating this cultivar was grown after open pollinating the 4x cultivar 'Veitchberry'.

Blueberry

Cultigen forms of blueberry within the section *Cyanococcus* of *Vaccinium* are 4x (highbush or *V. corymbosum* and lowbush or *V. angustifolium*) and 6x (rabbiteye or *V. ashei*) (Galleta and Ballington, 1996). They originated from conventional polyploid evolution from 2x species of this section, as demonstrated by flow cytometry (Costich et al., 1993). Sexual polyploidization occurred within this section owing to 2n gametes (Ortiz et al., 1992a), which appears to be widespread among 2x species (Ortiz et al., 1992b). The high frequency of 2n pollen may facilitate the introgression of 2x germplasm into the 4x breeding pool through unilateral sexual polyploidization.

A new breeding scheme was suggested by Ortiz et al. (1992a,b) to broaden the genetic base of the blueberry crop by increasing allelic variation among 4x and 6x cultigen pools. This scheme includes (1) searching for desired traits in the 2x germplasm, (2) combining the important attributes in an improved 2x breeding population through recurrent selection, (3) and subsequent transfer of them to the 4x level by FDR 2n pollen, which occurs in blueberry (Vorsa and Ortiz, 1992).

The occurrence of 2n eggs in the 4x cultigen pool may assist in bringing 2x genetic resources to the 6x cultigen pool. Synthetic 6x hybrids may arise from 4x-2x crosses (2n × n gametes, respectively), and such hybrids may then be included in crossing schemes with the 6x cultigen pool. Hence, transmission of genetic diversity and favourable alleles from low to high ploidy levels could be accomplished effectively in the section *Cyanococcus* of *Vaccinium* with 2n gametes.

Strawberry

Modern US strawberry cultivars (*Fragaria ×ananassa*) are self-fertile 8x genotypes derived mostly from two *Fragaria* spp.: *virginiana* and *chiloensis*. *F. chiloensis* remains as the major source for resistance to root diseases, *Verticillium* wilt, Red Stele and viruses (Bringhurst and Voth, 1984). For example 'Lassen' became the most important ancestor of California cultivars because of its low chilling requirement, low temperature for growth and tolerance to high salt, which some of these traits likely inherited from *F. chiloensis*.

Although genetically controlled diploidization occurred for the four homeologous genomes of the 8x cultigen, trait transfer the 8x level appears to be easy, while gene transfer from 2x, 4x and 6x species may be unsuccessful because imbalances in the resulting hybrids (Bringhurst and Voth, 1984). Not surprisingly, recurrent selection in offspring from crosses between best cultivars or available breeding materials became the main breeding method. Selection gains arose from capitalizing on additive genetic variation, which appears to be the most important for economic traits such as yield and fruit size. To bring 2x *Fragaria* genetic resources into the cultigen pool Bringhurst and Hill (1970) suggested using 10x after chromosome doubling (CD e.g. with colchicine) of 5x hybrids resulting from 8x-2x crosses, or by intermating fertile 5x hybrids producing 2n gametes. Likewise, they indicated that 4x (arising from CD of 2x) may be crossed with 8x to obtain 6x with a high frequency of 2n gametes, which then can be backcrossed to the 4x parents to yield genomically balanced 10x. Lastly, 16x from CD of 8x may be the source for 10x after crossing with 4x.

A second approach consists of using 9x instead of 10x, and backcross them to 8x cultivars. The 9x may ensue from crosses between 5x with 2n gametes and 8x cultivars with n gametes. Both approaches (10x and 9x) were included in breeding programs to incorporate genetic resources from 2x and 4x species to the 8x cultigen pool. The third approach includes CD and redoubling of 2x forms to obtain synthetic 8x, which then can be included in crosses with the 8x cultigen pool. An alternative could be 6x-2x crosses with n gametes, and CD of the resulting 4x hybrid (Evans, 1982a,b). Hence, ploidy

manipulations appears promising for the genetic enhancement of 8x strawberry with both *Fragaria* and *Potentilla* genetic resources.

Others

Sanford (1983) and Veilleux (1985) indicated that some 3x and 4x cultivars of other fruit and berry crops may result from 2n gametes. For example seedless 3x offspring was reported after 2x-2x crosses in *Citrus* with 2n eggs (Geraci et al., 1975); up to 20% 3x hybrid genotypes were observed from such crosses, in which the interploid endosperm block appears to be partially effective (Sanford, 1983). In currant and gooseberry (*Ribes* spp.) ploidy manipulation was the tool for gene introgression (cited by Singh et al., 1990). Also most triploid cultivars arose from 2n gametes in apple (Sanford, 1983), a crop in which the interploid endosperm block seems to be absent and showing interesting polyploid heterosis associated to organ sizes.

CONCLUSION

Successful analytical breeding scheme for gene introgression (or incorporation) from exotic of wild gene pools to cultigen breeding pool(s) requires: (1) viable (and better fertile) F₁ hybrids arising from interspecific crosses, (2) “integration” of chromosome segment(s) or set(s) from a donor species into the cultigen pool, (3) ploidy manipulations with haploids and 2n gametes to accomplish the incorporation of unadapted genetic resources into cultivar development.

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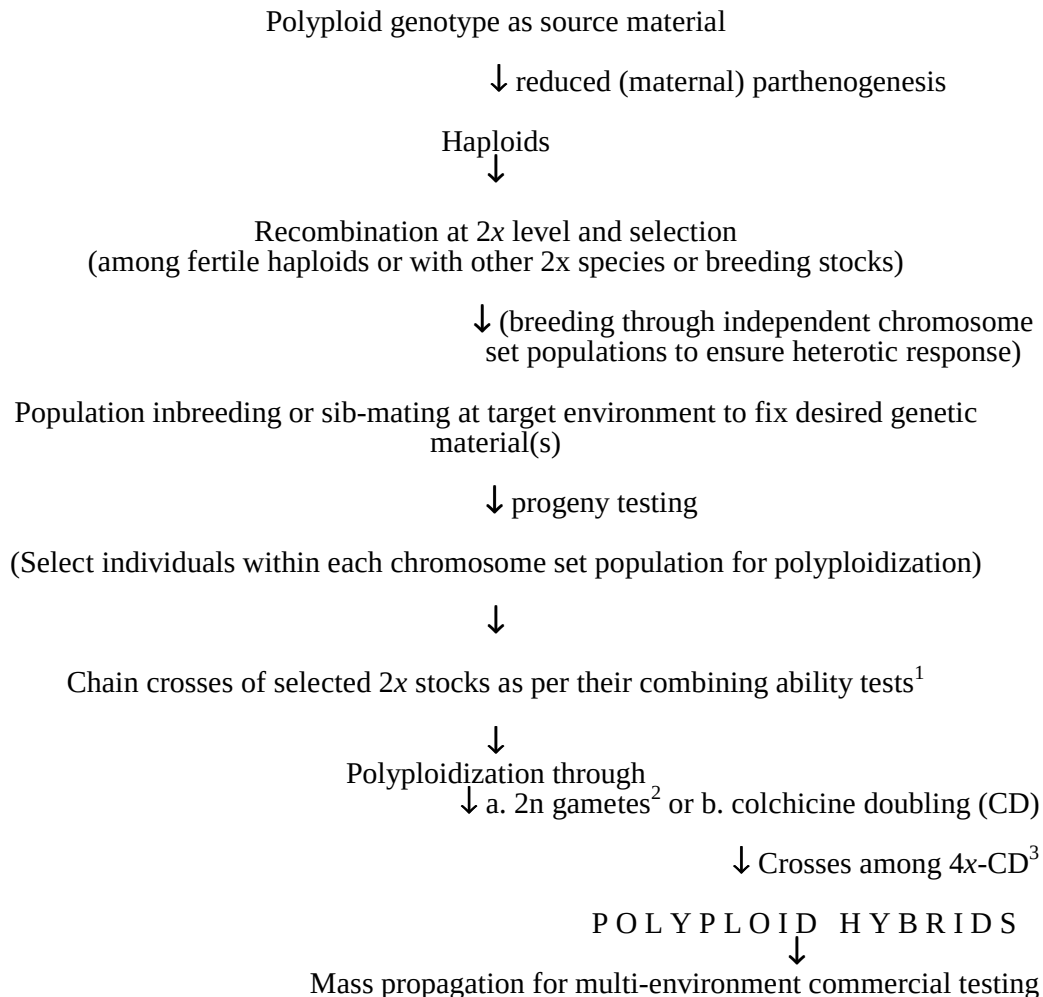
Tables

Table 1. Heterozygosity transmitted by distinct meiotic mechanisms assuming a 2x as original source (After Sanford, 1983).

Mechanism	Parental heterozygosity to offspring (%)
Apospory	100
Asynapsis plus first division restitution	100
First division restitution	75–83 ¹
Somatic doubling (CD) followed by normal meiosis	67
Second division restitution	33–50 ¹
Post-meiotic doubling	0
Normal reductional meiosis	0

¹Depends on chromosome morphology and crossing over pattern

Figures



¹Among those with weakest heterotic interactions to maximize this interaction in polyploid breeding (as per in double-hybrid scheme for maize)

²Synaptic mutants avoiding crossing over may allow intact transfer of chromosomes from parents to offspring, thereby maximizing heterotic potential

³Not recommended for traits negatively affected by inbreeding because F₁ hybrids from duplex genotypes are partially inbreds (coefficient of inbreeding or F = 0.166)

Fig. 1. Analytical breeding scheme (after Chase, 1963).

Sexual Depolyploidization and Polyploidization

$4x_1$, 4 EBN cultivar $\times$ Haploid inducer

$\downarrow$ parthenogenesis

Maternal haploid ($2x$, 2 EBN) $\times$ $2x$, 2 EBN species
 n gamete $\downarrow$ n gamete n gamete

$4x_2$, 4 EBN cultivar $\times$ $2x$, 2 EBN haploid-species hybrid₁ (HS₁)

n gamete $\downarrow$ $2n$ gamete
 n egg $\downarrow$ $2n$ pollen

or HS₁ $\times$ $2x$, 2 EBN haploid-species hybrid

$\downarrow$ $2n$ gamete

$4x$, 4 EBN cultivar

Chromosome Engineering

$4x_1$, 4 EBN cultivar $\times$ $4x_1$, 2 EBN species
 n egg $\downarrow$ $2n$ pollen

$6x$, 4 EBN hybrid. $\times$ $4x_2$, 4 EBN cultivar
 n gamete $\downarrow$ n gamete

$5x$, 4 EBN hybrid. $\times$ $4x_3$, 4 EBN cultivar
 $\downarrow$ n gamete

4 EBN addition lines

$2x$, 1 EBN species $\times$ $2x$, 2 EBN species
 $2n$ eggs $\downarrow$ n pollen

$4x_1$, 4 EBN cultivar $\times$ $3x$, 2 EBN hybrid

Haploid inducer $\times$ $5x$, 4 EBN hybrid. $\times$ $4x_3$, 4 EBN cultivar
 pathenogenesis $\downarrow$ n gamete $\downarrow$ n gamete
 $2x$, 2 EBN species $\times$ 2 EBN haploids
 n gamete $\downarrow$ n gamete
2 EBN addition lines

Fig. 2. Ploidy manipulations and chromosome engineering in potato (After Ortiz, 2000).

Wild 2x *Musa* spp.

↓ mutation, recombination, selection

2x *Musa* cvs.

↓ parthenocarpy and polyploidization

3x or other poly-x *Musa* cvs.

↓ human selection¹

Ancestral 3x *Musa* landraces in centres of diversity

↓ mutation², migration, farmers' clonal selection

MODERN CULTIVARS³

(grown world wide by farmers commercially or as own food)

↓ × 2x wild or other *Musa* stocks

↓ breeders select by specific combining ability (SCA)

Polycross with 2x stocks⁴ ← 4x *Musa* hybrids⁵ × 2x breeding stocks

↓
Synthetic *Musa* population(s)

↓
3x *Musa* hybrid

↓ decentralized, participatory breeding with farmers ↓ multi-environment test

Locally adapted cultivars To farmers after national release(s)

¹Instant domestication of good strains (linkat) that narrowed population genetic base

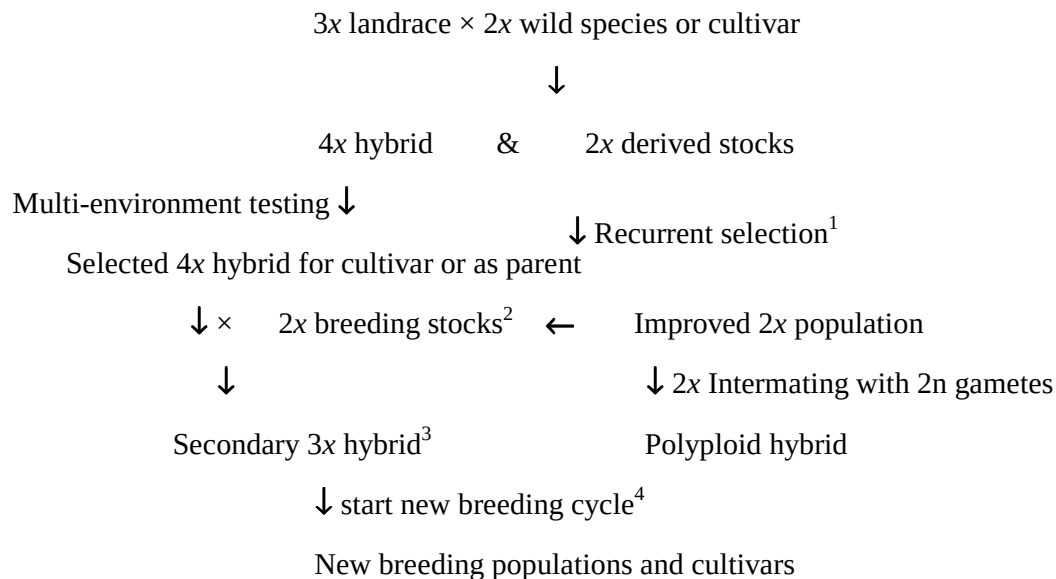
²Including somaclonal variation arising after micropropagation

³Genetic bottleneck occurs, e.g. only about 120 plantain cultivars are available world wide nowadays

⁴Selected to be together in isolated fields for open pollination as per their SCA tests

⁵Broadening genetic base of the crop

Fig. 3. Evolution and ensuing breeding approaches for the genetic enhancement of plantain and banana genomes (After Ortiz, 1997).



¹Genetic research and DNA marker-aided selection may be undertaken

²After progeny testing to select for combining ability

³Arising from hand-pollination or through open pollination from polycrosses. A secondary 3x may be a potential new cultivar after selecting for yield, quality and other desired attributes, e.g. pest resistance

⁴Following above schemes from top of this diagram

Fig. 4. Ploidy manipulation for the genetic enhancement of the *Musa* genome at IITA (After Tenkouano, 2001).