GENE PYRAMIDING IN COWPEA: A REVIEW

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ABSTRACT

Cowpea is attacked by 35 major diseases, 85 insect species damage to cowpea and 55 species of nematode has been reported on cowpea. Aphids (Aphis craccivora) attack cowpea especially in the seedling stage, flower thrips (Megalurothrips sjostedti) at flowering stage, pod borer (Maruca vitrata) at flowering and pod formation stage, a complex of pod sucking bugs at podding stage, and weevil (Callosobruchus maculatus) during seed storage. Cowpea is also susceptible to a number of fungal, bacterial, and viral diseases such as Cercospora leaf spot, ashy stem blight, bacterial blight, blackeye cowpea mosaic potyvirus (BICMV), cowpea aphid-borne mosaic potyvirus (CABMV), and cowpea mosaic comovirus (CPMV). Cowpea plants are also attacked by the parasitic flowering plant Striga gesnerioides. To overcome all these problems the gene pyramiding technique is described in this article

Introduction

Cowpea (*Vigna unguiculata* L. Walp. Subsp. *Unguiculata Fabaceae*) is one of the most important food and forage legumes in the tropics. Cowpea is grown on 10.5 million ha, with an annual grain production of about 4.9 million tones (Anonymous, 2011). It is mainly cultivated for the seeds, however other important products from it include the pods (fresh or dried) and leaves (Duke, 1990). By far Nigeria is the biggest producer of cowpea, followed by Niger republic, Burkina Faso and Ghana. Cowpea is, however, devastated by many biotic and abiotic stresses. Prominent among the biotic constraints are various types of insect pest, diseases and *Striga gesnerioides*. Resistance to insect pests in cowpea has been extensively studied and most cowpea accessions have been screened for resistance to the major insect pest. High to moderate level of resistance has been reported for some of the pests (Singh *et al.*, 1997) and many different levels of these resistances have incorporated into several genotype.

Gene pyramiding

Gene pyramiding is defined as a method aimed at assembling multiple desirable genes from multiple parents into a single genotype. The end product of a gene pyramiding program is a genotype with all of the target genes. Generally speaking, the objectives of gene pyramiding include: 1) enhancing trait performance by combining two or more complementary genes, 2) remedying deficits by introgressing genes from other sources, 3) increasing the durability of disease and/or disease resistance, and 4) broadening the genetic basis of released cultivars.

Traditionally, gene pyramiding is mainly used to improve qualitative traits such as disease and insect resistance. This is associated with the fact that the presence of target trait genes must be confirmed by phenotyping mostly at the individual level and that individual phenotypic performance is a good indicator of the genotype only if genes have a major effect on phenotypic performance and the error of phenotyping is

minimal. In addition to the reliability of phenotyping at individual level other factors influencing the success of gene pyramiding are the inheritance model of the genes for the target traits, linkage and/or pleiotropism between the target trait and other traits. For instance, allelic genes cannot be combined in the same genotype. The effect conferred by a recessive gene cannot be evaluated on heterozygous individuals and progeny testing is required. If the target gene is tightly linked to genes with large negative effects on other traits, these undesirable genes may be transferred together with the target gene into the recipient line and result in reduced performance of other traits (linkage drag). Therefore, any improvement in the knowledge of the trait genetics (inheritance, genetic relationship, etc.) and techniques for inferring genotype-phenotype relationship will be useful.

To accumulate into a single genotype the genes that have been identified in multiple parents, assume we have n loci of interest and set of founding parents labeled (Pi, I = [1…n]) with Pi being homozygous for the favorable alleles at the remaining n-1 loci. It was assumed that recombinant fractions between the loci are known and we want to derive the ideal genotype (ideotype) that is homozygous for favorable allele at all n loci.

The objectives of gene pyramiding

I. Enhancing trait performance by combining two or more complementary genes,
II. Remedying deficits by introgressing genes from other sources
III. Increasing the durability of disease and/or disease resistance, and
IV. Broadening the genetic basis of released cultivars

Selection Strategies used for gene pyramiding (Ishii and Yonezawa, 2007b)

There are four strategies of marker-based selection.

1) Recurrent selection with crossing between selected plants (RSC),

In strategy RSC, the haplo-diploidization method is not used; a plant with the most promising marker genotype that has the highest potential to leave an ideotype (denoted IG in short) in its progeny is selected in each generation, being self-fertilized to give rise to a population for the next round of selection. In the absence of any promising genotype (denoted PG), two plants with the best complementary genotypes (denoted CGP) are crossed for the next round of selection. The selection is performed in *T* generations at the maximum, ending in any generation before the *T*th when an IG is found (counted as a success), or, neither IG, PG, nor CGP is found (counted as a failure). A maximum of *Ni* plants is allowed in generation G*i* (*i* = 1, 2, … , *T*). When the markers are dominant, IG and PG cannot be distinguished from each other, and plants with all kinds of PG exhibit the same promising marker phenotype (denoted PP). In this case, a plant with PP found first is selected and self-fertilized in each generation, or, in the absence of such a plant, two plants with the best complementary marker phenotypes (denoted CPP) are crossed. The selection ends at a generation (excluding the first generation G1 in Fig. 1) when all tested plants exhibit PP (counted as a success because the plants can be regarded as having been fixed to IG), or no plants with PP or CPP are found (a failure).

2) Recurrent haplo-diploidization and crossing (RHC),

Selection in RHC starts with a population of haplo-diploidized plants produced from the root genotype I0, and ends at a generation when an IG is found (success), or neither IG nor CGP is found (failure). Otherwise, two plants with the best CGP are crossed to produce a hybrid, which is haplo-diploidized for the next round of selection. In strategy RHC, selection is performed in *T* rounds at the maximum, and the type of

dominance, co dominance versus dominance, makes no difference because all tested plants are homozygous. Strategy RHC is of the same type as RDHS of Howes *et al.* (1998) and the recurrent selection strategy adopted in Charmet *et al.* (1999); in all of these strategies, selection is performed recurrently with haplo-diploidized plants. These strategies diff er in some procedural parameters, most importantly, in the number of plants selected per round; in RHC, only two plants with the best complementary marker genotypes are selected and crossed for the next round of selection, whereas, in the strategies of Howes *et al*. (1998) and Charmet *et al*. (1999), multiple plants are selected and crossed in multiple pairs. Not only haplo-diploidized plants selected but also one of the two parents used for the initiation of the population are incorporated in the crossing in the strategy of Charmet *et al.* (1999). Strategy RHC is more practicable (resource-saving) than the previously discussed ones. The idea of recurrent selection with haplo-diploidized plants traces back to the theory of Fouilloux (1980).

3) Haplo-diploidization of F_2 plants (HF_2)

In strategy HF_2 of Fig. 1, selection starts with a population (G1) produced via self-fertilization of I0. The selection ends at generation G1 when an IG (success) or none of IG, PG, and CGP (failure) is detected. Otherwise, a plant with the best PG is haplo-diploidized to raise a population for the second (final) round of selection, or in the absence of PG, two plants with the best CGP are crossed to produce a hybrid plant, which in turn is haplo-diploidized to raise plants for the final round of selection. When the markers are dominant, the selection ends at G1 when neither PP nor CPP is found (a failure). Otherwise, PP found first or a hybrid plant of the best CPP is haplo-diploidized for the final selection. Strategy HF_2 resembles RF_2 Sel of Howes *et al.* (1998) and DH of Bonnett *et al.* (2005) that was defined under item "with F_2 enrichment for all marker loci". In all of these strategies, selection starts with F_2 population. In RF_2 Sel, all F_2 plants with desirable marker genotypes (having all target markers in either homozygous or heterozygous state) are selected and randomly intercrossed to give rise to a population, from which plants with desirable genotypes are again selected and haplo-diploidized for the final round of selection. In the DH with F_2 enrichment of Bonnett *et al.* (2005), all F_2 plants with desirable marker genotypes are haplo-diploidized for the final selection. Both of these strategies will be impracticably resource-consuming because many plants are treated for crossing and/or chromosome doubling. Our HF_2 is practicable and used here as a check for examining the efficiency of combined use of F_2 enrichment and haplo-diploidization.

4) Single round of haplo-diploidization and selection (SH)

Strategy SH is the simplest one using haplo-diploidization method, in which selection is performed only once for haplo-diploidized plants raised from the root genotype. Strategy SH is the same as DH of Bonnett *et al*. (2005) that was defined under item "without selection in F_2 generation". There will be no difference between SH and RHC when only a few markers are targeted with a large population size (*Ni*) because in both strategies the selection ends in a success with only one round of selection. In any strategy employing haplo-diploidization method, much of the resources could be saved if plants are genotyped at the haploid stage, with only those with a desirable marker genotype being subject to chromosome doubling.

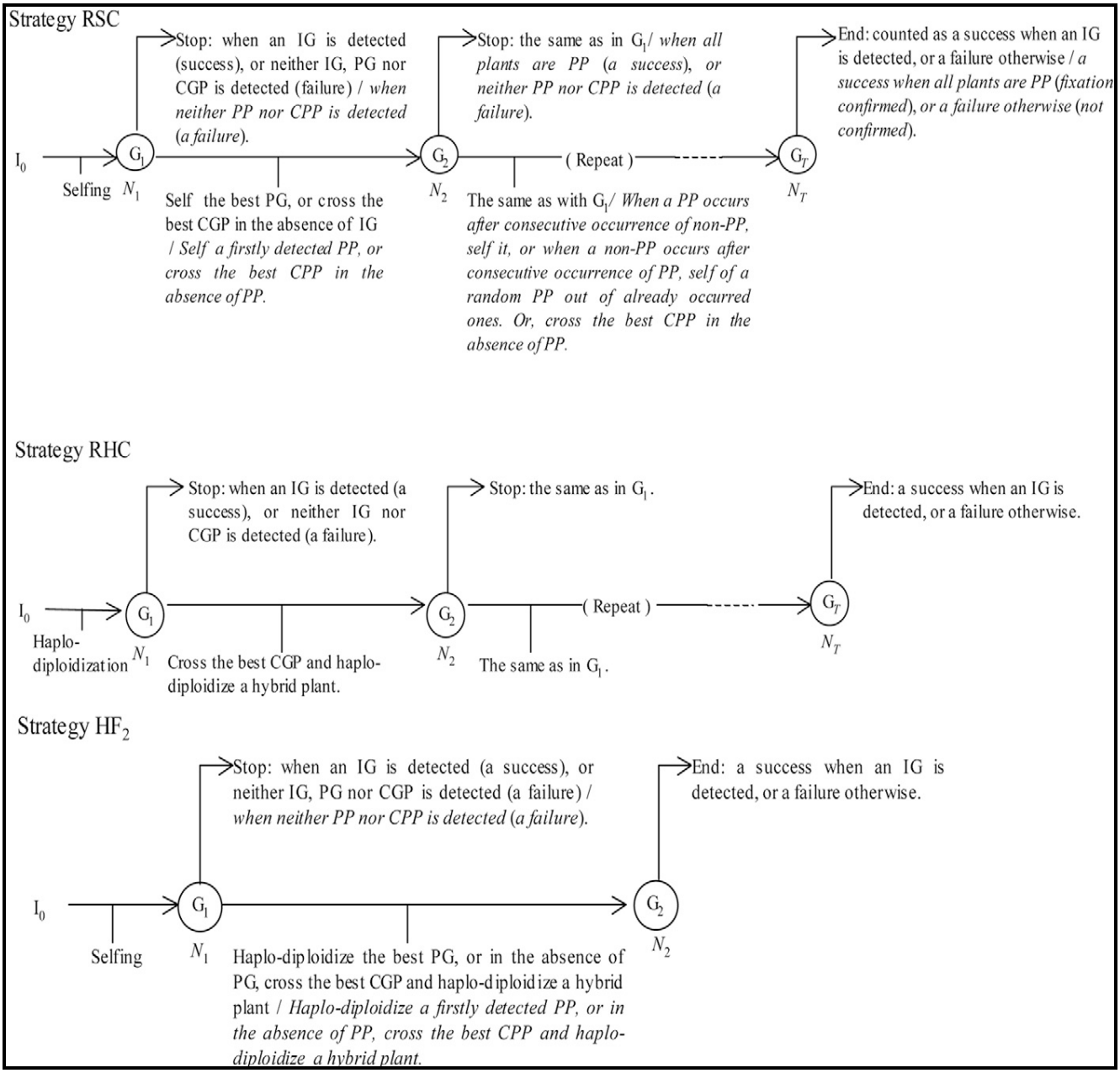


Figure 1: Selection strategies

To represents an initial heterozygous plant (root genotype) that has been produced via a schedule of crossing between multiple donor lines (Ishii and Yonezawa, 2007b). For simplicity, generations in all strategies were designated by the same symbol G*i* (*i* = 1,…, *T*). Selection procedures with codominant and dominant markers were written in roman and italic letters, respectively. No difference exists between codominance and dominance in strategy recurrent haplodiploidization and crossing (RHC). Marker genotyping may be performed for haploids, only ones with a desirable genotype being haplo-diploidized. Symbols are defined as follows: *Ni* = a maximum permissible number of plants

genotyped in generation G*i*, IG = a plant with objective homozygous marker genotype, PG = a plant with a promising genotype that has a potential to leave IG in its progeny, CGP = a pair of plants with the best complementary genotypes to leave IG, PP = a plant with a promising marker phenotype (defined when the markers are dominant), and CPP = a pair of plants with complementary marker phenotypes.

Process of designing a gene pyramiding strategy (Ye and Smith, 2008)

Bringing all the desirable alleles into a single genotype is the overall objective of a gene pyramiding program. When the number of parental lines containing the desirable genes (founding parents) is more than three, more than one crossing scheme can result in the generation of the target genotype (Fig. 2). Therefore, the gene pyramiding scheme can be divided in to twp parts. The first part is aimed at cumulating one copy of all target genes in a single genotype (called root genotype). The second part is aimed at fixing the target genes into a homozygous state, that is, to derive the target genotype from the root genotype. Sevrin *et al.* (2004) called these two parts pedigree and fixation, respectively.

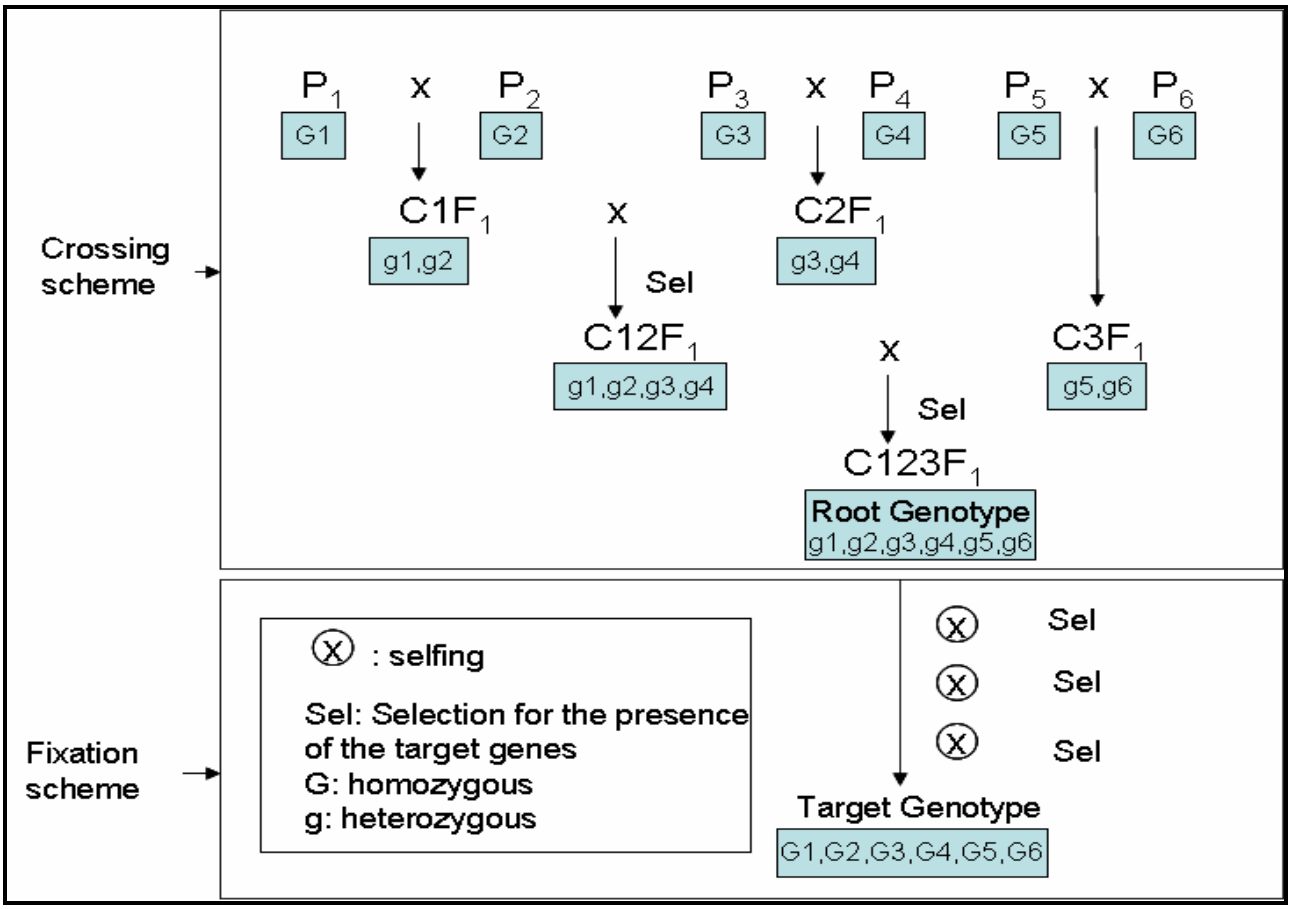


Fig. 2: Gene-pyramiding scheme cumulating six target genes from six parental lines.

Designing the Fixation Scheme

Assuming that a genotype with a copy of the desirable allele at each of the targeted loci (root genotype) is available, the design of an optimal strategy is aimed to find the minimum number of generations for genotyping and/or phenotyping required to fix all the loci for the desirable alleles within the limit of the largest possible population size applicable. The most commonly used methods for the production of homozygous individuals are the development of recombinant inbred lines (RIL), and doubled-haploid (DH) population. Therefore, it is advisable to investigate the feasibility of achieving the objective using RIL or DH. With the recombination frequencies between the target genes, the proportion of the desired genotype in the RIL or DH population can be worked out and the minimum population size required can be determined using binomial distribution. Either RIL or DH can be adopted if this size of population is practically achievable and the cost of genotyping it is affordable, although it may not be the optimal scheme.

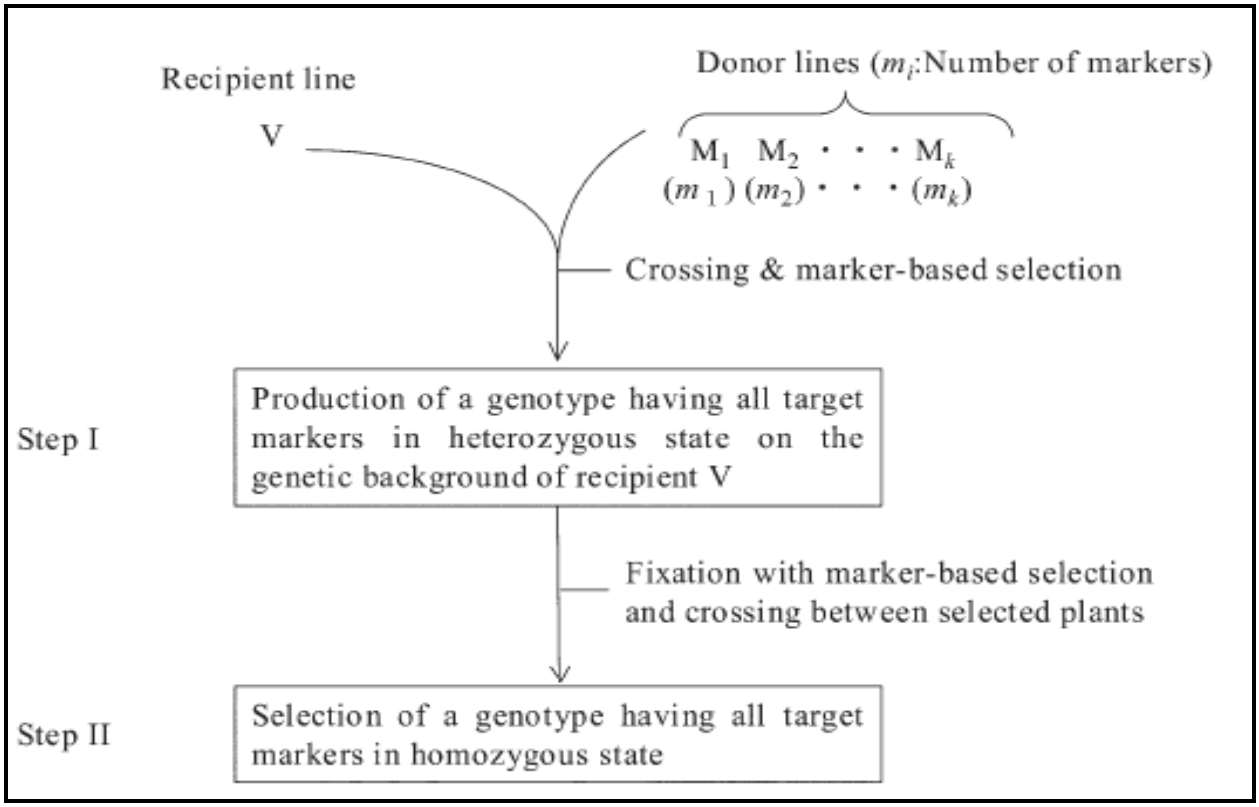
If neither RIL nor DH options are feasible, repeated selection in more than one subsequent segregating generation is required. Selection in sequential generations of individuals that have an increasing number of the desired alleles fixed at the desired loci, while heterozygous at the remaining desired loci increases the frequency of the targeted recombinant through accumulated recombination.

The objective of this step is to identify a selection scheme that leads to the production of the target genotype using the minimum number of generations and the practically allowable population sizes in each of the generations. Ye *et al.* (2007) showed how to define such a selection strategy in steps. They only considered the use of self-pollination in all subsequent generations, since it is the least expensive mating options in self-pollinated species and produces relatively more progeny. But, it may be less efficient since self-pollination breaks the already established desirable linkages between some of the favourable alleles. When crossing to another genotype can be easily and cheaply conducted, two other options may be taken as suggested by Servin *et al*. (2004). One option is to cross to a founding parent. The advantage of crossing to a founding parent is that the probability of obtaining a genotype that is homozygous for the target genes brought by the founding parent but heterozygous for the other targets is high. Hence, that target gene need not be fixed subsequently, increasing the probability of getting the target genotype. The choice of the parent to use may be subject to particular considerations depending on the value of the founding parents, the position of the loci, etc. The other option is to cross to a blank line containing none of the favourable alleles. The use of blank line increases the chance of obtaining a genotype carrying all favourable alleles in coupling and thereby increasing the frequency of target genotype in subsequent generations. If the number of generations required and/or the total population size is too large and thus genotyping is not acceptable, then the objective is deemed to be too ambitious and un- achievable and the number of genes to be pyramided has to be reduced.

Designing the Cross Scheme

A crossing scheme which leads to the production of the root genotype needs to be designed if the objective is achievable based on the above step. With the assumption that every founding parent is involved in only one cross in the gene- pyramiding scheme, Servin *et al.* (2004) described an algorithm for the building of every possible succession of pair crosses leading to the target genotype. They developed a computer program to generate all the possible schemes and associated minimal population size and the largest of the population sizes to be handled at any segregating gene- rations or steps during the

pyramiding process. The number of possible satisfactory schemes increases very fast with the number of genes. Even with the computer program, it is impossible to evaluate all the satisfactory schemes when the number of loci is more than a dozen. Ishii and Yonezawa (2007) suggested some guidelines by investigating the efficiencies of a series of crossing schemes. They also gave the procedural flow of marker-based gene pyramiding from *k donor lines.*



Guidelines for designing a gene pyramiding crossing scheme (Ishii and Yonezawa, 2007a)

Founding parents with fewer target markers enter the schedule at earlier stages

This guideline is based on the following facts: 1) Once a target gene has been incorporated into an intermediate genotype, genotyping must be done in all later stages to ensure its presence. Therefore, founding parents with moretarget genes should be used in later stage. 2) Target genes containing in a founding parent are in desired linkage phase, which may be broken down due to recombination. The more the meiosis involved the lower the probability of maintaining the desired linkage.

A cross that invokes a strong repulsion linkage should be performed as early as possible

When the target genes are linked, genes linked in repulsion at some stages of the pyramiding is unavoidable and selection for recombinants is required. As the frequency of recombinant type is always lower than that of the parental types, larger population sizes are required to recover the desired recombinant. In the genome of a plant selected at each stage, genes that were newly incorporated via the latest crossing are linked in the repulsion phase with genes that had been incorporated at

other stages before the latest. However, all genes incorporated in any stages before the latest are linked in coupling phase because it is converted to a coupling linkage after one round of MAS. On one hand, more plants and markers need to be tested in later stages since the number of target genes increases with the advancement of stages, repulsion linkage of the same strength is more disadvantageous when it occurs in at later stages. On the other hand, a repulsion linkage, once converted to the coupling phase after one round of marker selection, contributes to reduction of the number of tested plants in all subsequent stages. Similarly, the order of crossing should aim at a minimum occurrence of duplicate repulsion linkages.

More crosses should be conducted at each generation if genotyping cost is low and the practically applicable population size is large

When the maximum number of crosses is performed at each generation, the number of generations required to generate the root genotype is reduced and thus the total duration of the pyramiding program is reduced. Servin *et al.* (2004) showed that the number of generations required (h) is between ln n and n-1 (n is the number of founding parents) if every founding parent is involved in only one cross. However, the number of individuals (population size) must be large enough to ensure the recovery of the desirable genotype, which necessitates more genotyping.

One cross per generation is required if the practically applicable population size is small or genotyping cost is high

In this type of crossing design, from the second generation the desirable genotype is formed by a recombinant gamete produced by the selected genotype in the last generation and a gamete of the newly introduced parent. The probability of the desirable genotype is much higher than in schemes where the other parent is also a selected individual from the last crossing generation and thus the desirable gamete of this parent is recombinant type as well. The drawback to this crossing design is that the number of generations is large and the production of the new line is delayed.

Using backcrossing before assembling more genes

When the required population size at any stage is too large to be practicable, the use of backcrossing before assembling more genes is advisable.

Table 1: Cowpea Gene index

Preferred symbol	Synonym	Character
Bgs[§]	Bg	Big seed
cd[§]		Chlorophyl deficiency
Cpi		Effective nodulation
Gc		Green cotyledon
Hbs		Heat induced browning in seed coat
Ims		Res to cowpea severe mosaic virus
Pbs		Proliferated buds
Pm-1	Pm_1	Miniature plant
Pm-2	Pm_2	Miniature plant
Pt		Nonpetiolate leaf
Pt-2		Nonpetiolate leaf-2
Rac	Ac_1	Res to *Aphis craccivora*
Rav-1		Res to *Alectra vogelii*
Rav-2		Res to *Alectra vogelii*

Rav-3		Res to *Alectra vogelii*
Rcc		Res to *Colletotricum capsici*
rcm-1		Res to *Callosobruchus macalatus-1*
rcm-2		Res to *Callosobruchus macalatus*-2
Rsg-1		Res to *Striga gesnerioides*
Rsg-2		Res to *Striga gesnerioides*
Rsg-3		Res to *Striga gesnerioides*
Rss		Res to *Shaceloma sp.*
Rsv-1		Res to *septoria vignae*-1
Rsv-2		Res to *septoria vignae*-2
sbc-1		Res to southern bean mosaic virus-1
sbc-2		Res to southern bean mosaic virus-2
Spg-1[§]	Pp-1	Stem pigmentation -1
Spg-2[§]	Pp-2	Stem pigmentation -2
Sti		Stipule colour; red dominant over green
Vv-1		*Uromyces vignae* res.-1
Vv-1		*Uromyces vignae* res.-1

Res.= resistance § = Proposed new symbol ***Singh et al. (1997)***

Singh *et al.* (1997), Singh (1998), and Singh (1999) developed several cowpea lines with resistance to Cercospora, smut, rust, Septoria, scab, Ascochyta blight, and bacterial blight (Table 2). Some of the varieties, which showed multiple resistance were IT97K-1021-15, IT97K-556-4, and IT98K-476-8. Resistance to CSMV, CABMV, and CGMV has already been incorporated in some of the released varieties like BR 10-Piaui (Santos et al. 1987), BR 12-Canindé (Cardoso *et al.*, 1988), BR 14-Mulato (Cardoso *et al.*, 1990), BR 17-Gurguéia (Freire Filho *et al.*, 1994), EPACE 10 (Barreto *et al.*, 1988), Setentão (Paiva *et al.*, 1988), IPA 206 (IPA, 1989), and BR 16-Chapeo-de-couro (Fernandes *et al.*, 1990b).

Table 2: Sources of resistance to major diseases in cowpea

Diseases	Sources of resistance
Anthracnose	TVx 3236
Cercospora	IT89KD-288, IT97K-1021-15 IT97K-463-7, IT97K-478-10 IT97K-1069-8, IT97K-556-4
Smut	IT97K-556-4, IT95K-1090-12 IT95K-1091-3, IT95K-1106-6 IAR-48, IT97K-506-6
Rust (Uromyces)	IT97K-1042-8, IT97K-569-9 IT97K-556-4, IT97K-1069-8 IT95K-238-3, IT97K-819-118 IT90K-277-2, IT97K-1021-15 IT96D-610, IT86D-719
Septoria	TVu 12349, TVu11761, IT95K-398-14 IT90K284-2, IT95K-1090-12

	IT97K-1021-15, IT98K-205-8 IT98K-476-8, IT97K-819-118, IT95K-193-12 TVu 1234, IT95K-1090-12,
Scab	IT98K-476-8, IT97K-1069-8 TVx 3236, IT95K-398-14 IT97K-1021-15, IT95K-1133-6
Ascochyta	TVu 11761
Bacterial blight	IT95K-398-14, IT95K-193-12 IT81D-1228-14, IT95K-1133-6 IT97K-556-4, IT97K-1069-8, IT90K-284-2, IT91K-93-1, IT91K-118-20

Singh et al. (1997)

Table 3: Donors for disease resistance identified from cowpea germplasm at National level

Traits	Donar line identified
YMV resistance	C 159, C 244, C 355, C422, C 566, C 685, C 722, C 1018, C 1270, C 1300, C 1308
Leaf blight resistance	C 61, C 269, C 541, C 543, C 648
Leaf crinkle resistance	C 13, C 98, C 192, C 215, C 358
Rust resistance	C 38, C 76, C 370, C 425
Web blight resistance	C 10, C 11, C 49, C 139, C 140

Acharya et al. (2006)

Table 4: Donors identified for various diseases of cow pea in Gujarat

Disease	Donor identified
Yellow mosaic virus, cowpea leaf curl virus, Cowpea aphid born virus, Root rot and leaf spot	GC-0011 GC-0012
Yellow mosaic virus and cowpea leaf curl virus	GC-9714, GC-9732, GC-5, GC-3, TC-99-1
Cowpea aphid born virus, leaf spot	GC-5
Cowpea leaf curl virus	GC-102, TC-2000-4
Root rot	GC-9040, GC-125
Cowpea aphid born virus	GC-3

Acharya et al. (2006)

Table 5: Progress in pyramiding genes for resistance in cowpea

Pest/disease factor	lfe Brown(1973)	TVx 3236 (1978)	IT82D-716 (1982)	IT84S-2246 (1984)	IT90K-59 (1990)	IT90K-76 (1990)	IT97K 499-35 (1997)	IT00K-1251
Anthracnose	S	R	R	R	R	R	R	R
Cercospora	S	R	R	MR	R	R	R	R
Brown blotch	S	R	R	MR	R	R	R	R
Bacterial pustule	S	R	R	R	R	R	R	R
Bacterial blight	MR	MR	MR	MR	MR	MR	R	R
Septoria	S	S	S	S	S	S	R	
Scab	S	MR	MR	MR	MR	R	R	R
Web blight	S	MR	MR	MR	MR	R	R	R
Yellow mosaic	S	S	R	R	R	R	R	R
Aphid born mosaic	S	S	R	R	R	R	R	R
Golden mosaic	S	R	R	R	R	R	R	R
Aphid	S	S	S	R	R	R	R	
Thrips	S	MR	MR	MR	MR	R	R	R
Bruchid	S	S	R	R	R	R	R	R
Striga	S	S	S	S	R	R	R	R
Alectra	S	S	S	S	R	R	R	R
Nematode	S	S	S	R	R	R	R	R

Acharya et al. (2009)

Table 6: Contribution of scientist in the development of multiple gene resistance in cowpea

Lima et al. (1986)	Evaluated 248 genotypes and identified four new genotypes (TVu 379, TVu 382, TVu 966, and TVu 3961) as being immune to CSMV and CABMV.
Lin *et al.* (1995)	Screened 131 cowpea varieties by artificially inoculating with *Cercospora cruenta* (*Mycosphaerella cruenta*) from which 15 varieties were identified immune and seven resistant.
Ogbuinya (1997)	Excellent sources of resistance were observed in wild species viz. *V. verxillata* and *V. oblogitelia* [hairiness—aphid—non preference mechanism]
Singh *et al.* (1997)	The conventional breeding has primarily focused on transferring desirable multiple resistance genes by using earlier variety as a parent for new variety and different gene have pyramided in different varieties
Vale *et al.* (1995)	Reported that resistance to cowpea severe mosaic comovirus (CpSMV) is controlled by single recessive gene using macaibo and pitiuba as resistance and susceptible parents respectively
Arshad *et al*. (1998)	Designated single recessive gene as bcm that controlled resistance to black eye cow pea mosaic virus (BICMV)

Lima *et al*. (1998)	Confirmed the immunity of genotypes TVu 379, TVu 382, TVu 966, and TVu 3961 to three strains of CSMV.
Wydra and Singh (1998)	screened 90 cowpea breeding lines and identified IT90K-284-2, IT91K-93-10, and IT91K-118-20 to be completely resistant to three virulent strains of bacterial blight. Eight varieties were resistant to two strains and two varieties were resistant to one strain.
Latunde-Dada *et al*. (1999)	Studied the mechanism of resistance to anthracnose in TVx 3236 cowpea. In this variety the initially injected epidermal cells underwent a hypersensitive response restricting the growth of the pathogen. The phytoalexins "kievitone" and "phaseollidin" accumulated more rapidly in the stem tissue of TVx 3236 compared to the susceptible variety.
Robert *et al*. (1996)	Screened different genotype for resistance to different population of nematodes (*Melodogyne incognita*) and *M. javanica*) and identified IT-84 S-2049 as completely resistant. They identified one dominant gene RK2 controlling resistance in this variety that was different to Rk gene identified earlier.
Singh *et al.* (1996)	Reported several improved cowpea varieties with combined resistance to aphid, thrips, and bruchid. Of these, IT90K-76, IT90K-59, and IT90K 277-2 are already popular varieties in several countries.
Singh (1999)	Screened new improved cowpea breeding lines for field resistance to major insect pests without insecticide sprays and he observed several cowpea lines with grain yield of 500 kg/ha to 856 kg/ha without any chemical protection. The local variety yielded 0 to 48 kg/ha in the same trials. The most promising varieties are IT90K-277-2, IT93K-452-1, IT94K-437-1, IT97K-569-9, IT95K-222-3, IT97K-837, and IT97K-499-38. These lines are resistant to major foliar diseases, aphid, thrips, and bruchid with pods at a wide angle and suffer less damage due to Maruca. IT94K-437-1 and IT97K-499-38 also have combined resistance to *Striga* and *Alectra*.
Van-Boxtel *et al*. (2000)	Artificially screened 14 cowpea varieties with three isolates of blackeye cowpea mosaic and 10 isolates of cowpea aphid borne mosaic virus in order to identify lines with multiple strain resistance. They observed that cowpea breeding lines IT86D-880 and IT86D-1010 were resistant to all the three isolates of blackeye cowpea mosaic and fi ve strains of cowpea aphid borne mosaic. IT82D-889, IT90K-277-2, and TVu 201 showed resistance to one or the other of the five remaining isolates and thus by using the abovementioned fi ve cowpea varieties as parental lines, it is possible to breed new cowpea varieties with combined resistance to all the 13 strains of the viruses.
Boukar *et al*. (2004)	A recently identified cowpea breeding line, IT93K-693-2, has resistance to all known races. An F2 population developed from the cross between IT93K-693-2 and the susceptible variety IAR1696 was characterized for resistance against race 3 of *S. gesnerioides* for genetic analysis and molecular mapping. IT93K-693-2 has a single dominant gene for resistance. Four Amplified Fragment Length Polymorphism (AFLP) markers, designated E-ACT/M-CTC115, E-ACT/M-CAC115 , E-ACA/M-CAG120 and E-AAG/E-CTA190, were identified and mapped, respectively 3.2, 4.8, 13.5 and 23.0 cM from resistance

	gene Rsg1-3. The AFLP fragment from marker combination E-ACT/M-CAC which is linked in coupling with Rsg1-3 was cloned, sequenced, and converted into a Sequence Characterized Amplified Region (SCAR) codominant marker to enhance its usefulness in breeding programs. There is an urgent need to develop additional DNA markers in cowpea to enhance pyramiding of resistance genes.
Aliyu and Ishiyaku (2013)	The reaction of IT84S-2246-4, a hitherto aphid resistant genotype, which supported higher levels of survival of the larvae relative to other known susceptible genotype IAR-48, may be an indication of the presence of a new biotype of *Aphis craccivora* endemic to Zaria environs, or that of the ability of insects to overcome hindrances to their survival including various forms of resistance.
Tignegre *et al*., 2013	Evaluated cowpea genotypes in fields infested with *S. gesnerioides* at three striga hot spots in Burkina Faso and in pots under artificial infestation with striga races SR 1, SR 5 and SR Kp to identify new, adapted and striga-resistant sources. Cowpea genotypes showed differential reactions for striga resistance over sites and for striga races in pot experiments, indicating differences in the races involved, and SR Kp was reported as a new race. Resistant sources conferring site-specific or multiple striga-race resistance were identified. Genotypes 58-57, Sanga 2, IT84S-2049, IT98K-205-8, IT93K-693-2, KVx771-10, KVx775-33-2, KVx61-1, Gorom local, Mouride and Melakh conferred resistance to all three striga races. These genotypes are potential donor parents for breeding new, adapted and striga-resistant genotypes. Cowpea landraces including Moussa local and Niaogo local with farmers' preferred traits were susceptible and need improvement for striga resistance.

Factors affecting gene pyramiding (Ye and Smith, 2008)

Characteristics of the target traits/genes

When the genes to be pyramided are functionally well characterized and markers used for selection are equal to the gene itself (perfect markers), gene pyramiding will be more successful. For qualitative traits controlled by one or a few genes, the identification of the genes and tightly linked markers is easier provided phenotyping is carefully conducted one or two markers per gene can be used for tracing the presence/absence of the target genes. Bulk segregant analysis (BSA) is the preferred method for the identification of markers tightly linked to a major gene (Michelmore *et al.* 1991). For BSA plants from a segregating population are grouped according to phenotypic expression of the trait into two bulks. The bulks are screened with a large number of markers to id entify those that distinguish the bulks and, by inference, must be genetically linked to the trait locus. When the target genes are QTL with moderate or small effects, pyramiding may be less successful due to the following reasons. Firstly, the identified QTL may be more likely to be a false positive. Secondly, inaccurate QTL localizations result in the need to select for mo re marker loci covering large genomic segments to be certain that target QTL alleles are retained in selected progeny. Thirdly, QTL effects may be specific to a particular genetic background. Moreover, markers identified for a QTL can be ineffective in monitoring the QTL since the marker-QTL association might be different from population to population. Fourthly, more QTL need to be pyramided to

achieve a significant improvement.

Reproductive characteristics

The propagation capability of a crop is determined by the number of seeds produced by a single plant. This capacity determines the population size applicable if seed has to be collected from only a single plant. In a gene pyramiding program, in most generations this is the case, since the chance of selecting two or more individuals of exactly the same genotype in previous generation is very low. For example, although a fairly large F_2 population can be obtained by collecting seed from many F_1 plants of the cross between two homozygo us parents, from the F_3 generation seed can only be collected from a single plant. The fact that F_1 plants of the cross between two homozygous parents are genetically the same can also be used to increase the size of a progeny population of the F_1 plants of two crosses (double cross) or of the F_1 plants of one cross and an inbred line (Three-way cross or testcross). The efficiency of hybridization may be an important constraint for some crop species. When wild relatives are used as the donor of desirable genes, many more reproduction related constraints may exist including cross incompatibility between the wild species and cultivated crop. F_1 hybrid sterility, infertility o f the segregating generations reduced recombination between the chromosomes of the two species. Appropriate techniques that may include chemical treatment and immature embryo culture for overcoming these problems must be established

A breeder's capability to identify the 'desired' genotypes

It is obvious that the desirable genes must be present in all generations leading to the target genotype. To ensure the presence of the target genes individuals of desired genotype (which may change with generation advance) must be identified among all individuals in each generation. Breeder's capability to identify the desired genotypes has been greatly enhanced by the use of tightly linked or diagnostic markers It might be appropriate to consider the importance of marker and trait gene linkage here.

Operating capital

All breeding programmes are operated within the limits of available operating capital. Therefore, reducing the overall cost is always an important consideration when choosing a strategy. In addition to the use of the most economic mating and testing approaches, other factors affecting the cost also need to be considered. In the context of gene pyramiding cost affects both what can be achieved and how to achieve it. Increasing the number of generations (duration) will reduce the pressure on population size required in each generation and may result in the reduction of the total cost However, increasing the duration delays the release of the new cultivar and consequently reduced market share. The well-known trade-off between duration and cost in breeding has no exception in gene pyramiding. To find an optimum balance between duration and cost is desired but very difficult to achieve. In practice, the best strategy may be de fined as the one that enabled the breeder to achieve the objectives with the shortest duration and within a fixed expected investment.

Conclusion

- Pyramiding of gene for resistance to aphid, bruchid, thrips and striga as well as field resistance to Maruca pod borer and pod bugs, should be pursued so as to minimize or eliminate the need for insecticidal protection.
- Cowpea breeder should also seek to increase the genetic potential of plant for higher grain and fodder yield, to enhance the role of cowpea in sustainable (crop/livestock) farming system in tropics.

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