

## RESEARCH ARTICLE

# Use of molecular markers in participatory plant breeding: assessing the genetic variability in cotton populations bred by farmers

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## Abstract

In participatory plant breeding, farmers are involved in simple selection schemes that are not suitable for assessing genetic variability in the segregating populations. We propose to use information derived from molecular marker analyses to help monitoring such populations. In this study, we used three indicators to compare genetic variability in eight genetic structures, that is three plant populations selected by farmers over five generations, three nonselected populations and two commercial varieties. The three indicators were the polymorphic locus rate, heterozygosity rate and dissimilarity index. The results highlighted that the genetic variability decreased more with farmers' selection than with environmental factors. The breeding process was not complete because genetic variability in the selected populations was midway between that of the nonselected populations and that of the commercial varieties monitored. The three proposed indicators were relevant for describing the studied populations. They could be interpreted according to a grid drawn up on the basis of the results of the present study.

## Introduction

Participatory plant breeding brings together scientists and benefiting stakeholders. Breeding operations are decentralised because selection plots are set up in farmers' fields. During the first years, involved farmers manage simple selection schemes that are usually not designed to monitor environmental or kinship parameters. Mass selection enables breeders and farmers to learn to work together on segregating lines (Sthapit *et al.*, 2000; Lançon *et al.*, 2004).

To enhance management of these selection schemes, professional breeders require indicators to assist farmer breeders in monitoring genetic variability patterns over selection cycles and in determining the genetic gain that could be expected. Statistical methods have been proposed for estimating expected genetic gain and expected degree of heterozygosity in lines (Ceccarelli *et al.*, 2000; Atlin *et al.*, 2001; Witcombe & Virk, 2001). They could not be applied to estimate reduction in genetic diversity as a result of selection.

Molecular DNA markers are used for an increasingly broad range of plant breeding applications, including marker-assisted selection, genetic diversity, linkage disequilibrium analysis, etc. In hybridisation-based breeding programmes, DNA markers are especially useful for monitoring and quantifying the genomic contributions of parents to their progeny and estimating genetic homogeneity (VanToal *et al.*, 1997; Steele *et al.*, 2004). In cotton, although DNA markers have emphasised the relatively low level of polymorphism within cultivated types of *Gossypium hirsutum* (Rungis *et al.*, 2005), we have demonstrated the possible use of the allelic variability of microsatellite markers for studying the diversity of cultivated and wild types of tetraploid *Gossypium* accessions (Lacape *et al.*, 2006).

In Benin, farmers have been managing five successive mass cotton selection cycles at three sites since 1997. The efficacy of the selection pressure they exert on the evolution of agronomic traits has been demonstrated (Lançon *et al.*, 2004). In this experiment, DNA samples from

plants derived from these selections were analysed using amplified fragment length polymorphism (AFLP) and microsatellite, or simple sequence repeat (SSR), markers. The results enabled us to develop indicators of the genetic variability of selected populations and to measure the impact of selection pressure exerted by farmers on this variability.

## Materials and methods

### Plant material

The origins and phenotypic traits of the plant material used in this study were described in Lançon *et al.* (2004). An initial hypervariable population, AGP0, was obtained in 1996 by randomly intercrossing 14 *G. hirsutum* varieties: seven selected in Africa, including three in Benin (Stam 18A, H 279-1 and H 279-A), three in Cameroon (Irma 772, Irma Z856 and Irma BLT-PF) and one in Senegal (G 440), and seven selected for more intensive cropping systems, including four in USA (Deltapine 90, DES 119, Stoneville 907ne and 1324), one in Australia (Sicala 34, from North American germplasm) and two in Argentina (Guazuncho 2 and Chaco 520). These varieties were chosen on the basis of their agronomic traits and interesting fibre qualities.

After one selfed generation, AGP0 seeds were released in 1997 to three cotton farmer breeders (FB sites) in three cotton-growing regions of Benin, that is Kandi in Alibori department in the north, Djougou in Donga department in the east and Koutago in Collines department in the south.

From 1998 to 2002, the farmers selected the best 200 plants out of 1000 sown in their plot (Lançon *et al.*, 2004). Their preference went to plants with numerous branches, short internodes and fully open bolls; that are easy to pick and regularly distributed from the bottom to the top; and with little leaves remaining at harvesting time. They were also associated to the choice of the best remaining 50 plants for technological traits (ginning outturn; seed index and length, strength, maturity or colour of the fibre). Koutago's group of farmers did the most constant and reliable work, while Djougou's group had to face coordination problems in 2002, as the initial leader became a member of the national parliament.

In parallel, samples of seed from the same AGP0 population were sown at three research stations of the National Agricultural Research Institute that were within the vicinity of the FB sites, that is Angaradébou (near Kandi in Alibori), Moné (near Djougou in Donga) and Savalou (near Koutago in Collines). At all three sites, the AGP0 population was managed with no intentional selection (NS sites) on the same plots during the period in which farmers were conducting selection trials in their own fields. Indirect selection because of the crop environment,

sowing date or rainfall pattern could affect, for example the germination vigour, the seed weight, and the ginning outturn, or the earliness, and the plant morphology. In 2000, the rainfall was very erratic at Kandi, causing a severe selection among the plants of the breeding plot.

After five selection cycles (1998–2002), we obtained six batches of seed derived from the AGP0 population:

- Three populations obtained with no selection at Angaradébou (NS1), Moné (NS2) and Savalou (NS3).
- Three populations selected by farmers at Kandi (FB1), Djougou (FB2) and Savalou (FB3).

In 2004, these six seed batches were sown at the Okpara research station, along with the two commercial varieties, Stam 18A and H 279-1. These two varieties were bred by CIRAD in partnership with the public research institutes of Ivory Coast, Togo and Benin. They descend from a single plant and can be considered as pure lines, although they remain with some genetic variability because of outcrossing during the selection phase.

At each experimental site, plants were grown under natural pollination. In West Africa, including Benin, Cameroon or Ivory Coast (Lançon, 1995), under usual cotton-growing conditions, the outcrossing rate at plant level varies between 10% and 30%. The rate decreases quickly with the distance between the plant and the other pollen source. After 10 m, it is only 3–5%. In our situation, the major pollen flow came from the plant or the population itself, and we could consider external outcrossing as negligible.

### Molecular analysis

Twenty to 30 plants were randomly chosen from each population (NS, FB and the two commercial varieties). Young just opened leaf fragments were sampled from each plant and sent to CIRAD in Montpellier (France) for DNA extraction and molecular analysis. The final number of genotyped plants depended on the quality of the sampled DNA: 132 DNA samples were analysed, including the 14 parental samples and 118 DNAs derived from eight compared treatments. These latter DNAs were distributed between the two cropped controls, that is Stam 18A and H 279-1 (three and four DNAs), the three nonselected populations, that is NS1 (21 DNAs), NS2 (20 DNAs) and NS3 (23 DNAs), and the three selected populations, that is FB1 (19 DNAs), FB2 (11 DNAs) and FB3 (17 DNAs).

Two types of molecular marker were used: AFLP markers and microsatellite (SSR) markers.

DNA extraction and molecular marker analyses were undertaken as described in Lacape *et al.* (2003).

AFLP primer pairs were selected from 64 *EcoRI/MseI* combinations using a commercial AFLP analysis kit (Gibco BRL, Gathersburg, MD, USA). Overall, 22 primer pairs

were selected on the basis of the polymorphism observed between the 14 parents. These 22 primer pairs were then used to analyse all samples, and a total of 96 bands were ultimately encoded for the presence or absence of alleles.

Sixty-six microsatellite markers from two libraries (BNL, from Brookhaven National Laboratory, and CIR, from CIRAD) were screened over the 14 parents and half of them (33) exhibited polymorphism. Details on markers and primers are available at [www.cmd.org](http://www.cmd.org). Twenty-eight SSR markers were ultimately codominantly (biallelic) encoded, with two (24 SSRs) or three (four SSRs) alleles per SSR.

The germplasm was characterised for 124 polymorphic markers overall. A list of the markers used is available from the corresponding author on request.

### Genetic variability markers

We combined molecular information derived from the two datasets (binary presence/absence encoding for AFLP markers and biallelic encoding for SSRs) to analyse the impact of selection on the genetic variability of cotton populations selected by farmers. Heterogeneity and heterozygosity traits were assessed. Heterogeneity was measured on a population scale according to the polymorphic locus rate and mean dissimilarity index, while heterozygosity was measured on a plant scale. We also calculated the frequency of specific alleles from parents of different geographical origins.

#### Polymorphic locus rate

The polymorphic locus rate in a population was expressed relative to 124 markers that were polymorphic in the parental lines.

#### Heterozygosity rate

The heterozygosity rate of a set of genotypes was estimated according to the percentage of heterozygotic loci in the population for 28 codominant SSR markers.

#### Dissimilarity index

The DARwin 5.0 (Perrier *et al.*, 2003) software package was used to calculate the dissimilarities between individuals, that is Sokal and Michener indices for binary AFLP data (presence/absence), and the Dice simple matching index for SSR allelic data.

With AFLP markers, the dissimilarity between two individuals  $i$  and  $j$  may be expressed as:

$$\text{disAFLP}_{ij} = \frac{u}{m + u}$$

where  $u$  is the number of dissimilar variables and  $m$  is the number of similar variables.

With SSR markers, the allelic dissimilarity between two individuals  $i$  and  $j$  becomes:

$$\text{disSSR}_{ij} = 1 - \frac{1}{L} \sum_{l=1}^L \frac{m_l}{\pi}$$

where  $\pi$  is the ploidy level,  $L$  is the number of loci and  $m_l$  is the number of similar alleles at each locus.

According to the dendrograms and trees plotted by DARwin 5.0, the topologies obtained separately on the basis of AFLP and SSR dissimilarities were compared (data not shown), and a new dissimilarity rate was calculated from the two AFLP and SSR dissimilarity rates. For this calculation, a weighting coefficient of 2 was used for SSR markers. AFLP markers are not as informative as microsatellite markers (at equivalent marker numbers) because they only provide information on the presence or absence of a band (detection of only the dominant allele), whereas microsatellite markers can differentiate the homozygotic from the heterozygotic state (detection of two alleles per locus). The dissimilarity rates reported here and displayed in the tables are based on these calculated overall dissimilarity values:

$$\frac{(2 \times n \times \text{disSSR}) + (N \times \text{disAFLP})}{(2 \times n + N)}$$

with  $n = 60$  SSR alleles,  $N = 96$  AFLP alleles,  $\text{disSSR}$  is SSR dissimilarity and  $\text{disAFLP}$  is AFLP dissimilarity.

#### Specific alleles

To simplify display of the results, the 14 varieties were divided into two equal-sized continental (Africa and the Americas) groups. In each group and for both types of markers, we identified specific alleles, that is alleles present in at least one variety of the group and absent in all varieties of the other group. The allele frequency was used to calculate the following indicators in each population:

- The frequency of alleles specific to the African or to the American group.
- The percentage of specific alleles still present.

## Results

### Genetic variability in parental lines

In 22 AFLP primer pairs, the mean polymorphism between the 14 parents was 9.2% per pair, with a mean of six polymorphic bands per primer pair and a marker-dependent variation of 3% (E-AAG + M-CTA) to 17% (E-AGC + M-CTG). This moderate level of molecular polymorphism between *G. hirsutum* varieties confirms results obtained previously with isozyme markers (Bourdon, 1986), microsatellite (Rungis *et al.*, 2005; Lacape *et al.*,

2006), restriction fragment length polymorphism (RFLP) markers (Brubaker & Wendel, 2001) and AFLP markers (B.M. Bojinov, personal communication).

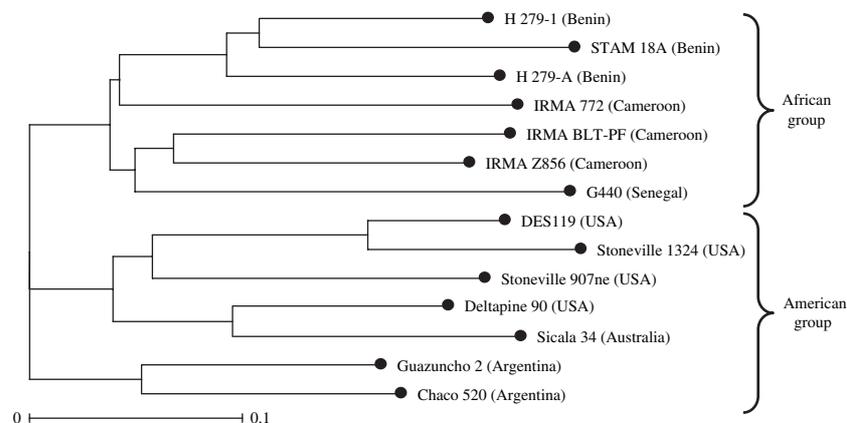
The dendrogram shown in Fig. 1 was based on the calculated overall dissimilarity values. Two continental groups (African and American) consisting of seven varieties each are clearly shown. The African group includes a subcluster of varieties from Benin, along with a subcluster of varieties from Cameroon and Senegal. In the American group, there is a four-variety US cluster associated with the Australian variety (Sicala 34, a descendant of the American cv. Deltapine 90), along with another cluster formed by the two Argentinian varieties.

There are 56 specific alleles in these two groups among the 156 alleles detected, 19 in the American group and 37 in the African group.

### Genetic variability in nonselected populations

At all three sites without selection (NS), specific alleles of the two continental groups were present at a mean frequency rate of 0.16 for the American group and 0.25 for the African group (Table 1). These frequencies were close to those calculated for all the parental lines (0.20 and 0.18, respectively). The frequency of African alleles was slightly higher than in the parents (+0.07), especially in the NS2 population (+0.17), while the mean allele frequency of the American group was slightly lower (−0.04).

After 5 years of cropping without intentional selection, these populations had preserved most of their initial genetic variability (Table 2), with a mean polymorphic locus rate of 94%, a heterozygosity rate of 9% and an intrapopulation dissimilarity index of 0.35. They had also preserved 94% of alleles specific to the two continental groups.



**Figure 1** Dendrogram of 14 parental varieties based on dissimilarity indices calculated for 96 AFLP markers and 28 SSR loci. Geographical origins of the varieties are shown in brackets.

**Table 1** Frequency of specific alleles<sup>a</sup> from each continental group in the nonselected and selected plant populations at sites 1 (Kandi), 2 (Djougou) and 3 (Savalou)<sup>b</sup>

	<i>n</i>	Geographical Origin	
		The Americas	Africa
NS1	21	0.27	0.26
NS2	23	0.18	0.35
NS3	11	0.14	0.13
FB1	20	0.24	0.63
FB2	19	0.14	0.26
FB3	17	0.10	0.40
Parents <sup>c</sup>		0.20	0.18
Mean NS populations		0.16	0.25
Mean FB populations		0.16	0.46
SD (means)		0.07	0.10

<sup>a</sup>Nineteen specific alleles of varieties from the Americas and 37 specific alleles of varieties from Africa.

<sup>b</sup>Differences between the NS and FB populations were not significant at the 0.05 probability level.

<sup>c</sup>Specific allele frequency in all 14 parents.

Slight differences were noted between the three NS populations (Table 2). Genetic variability was lowest for NS1, that is fewer polymorphic and heterozygotic loci, and fewer specific alleles from the two continental groups.

The mean heterozygotic locus rate of the nonselected populations was low (9%). Indeed, at panmictic equilibrium and without selection pressure, a rate of 37% could be expected on the basis of the allele frequency in the parent populations. Following one selfed generation and five generations under open pollination, this rate should range from 15% to 20% depending on the extent of outcrossing, whereas it ranged from only 5.7% with NS1 to a maximum of 11.3% with NS3.

**Table 2** Indicators of genetic variability in the nonselected populations (NS) and selected populations (FB) at sites 1 (Kandi), 2 (Djougou), 3 (Savalou) and in two commercial varieties: polymorphic locus rate, mean heterozygosity rate, mean dissimilarity index and specific allele rate<sup>a</sup>

	Polymorphic Locus Rate <sup>b</sup> (%)	Mean Heterozygosity Rate (%)	Mean Dissimilarity Index	Specific Allele Rate <sup>c</sup> (%)
NS1	90.6	5.7	0.34	89.3
NS2	96.9	10.1	0.37	98.2
NS3	95.3	11.3	0.34	94.6
FB1	71.7	5.0	0.28	80.4
FB2	92.9	5.1	0.34	91.1
FB3	81.9	3.4	0.23	80.4
Mean NS populations	94.2	9.0	0.35	94.0
Mean FB populations	82.2	4.5	0.28	84.0
Two commercial lines	37.0	1.2	0.20	n.a.
SD (means)	5.4	2.6	0.03	6.1

n.a., not applicable.

<sup>a</sup>Differences between means of the three NS populations and means of the three FB populations were, respectively, significant for the four indicators at the 0.11, 0.17, 0.10 and 0.06 probability levels. The standard deviation applied to comparisons of these two population groups.

<sup>b</sup>For a maximum number of 127 loci.

<sup>c</sup>For 56 specific alleles of the two continental groups.

### Selection effects

Selection by farmer breeders resulted, at a given site, in a greater reduction in genetic variability within the farmers-bred populations than in the nonselected populations.

At two of the three sites (FB1 and FB3), the specific allele frequency from the African group sharply increased (Table 1) relative to that of the parents (+0.22 and +0.47, respectively) and relative to that of the nonselected populations at nearby NS sites (+0.27 and +0.37).

All the variability indicators declined as a result of selection by farmer breeders (Table 2). The polymorphic locus rate decreased by 12% relative to that of the three NS populations, while the heterozygosity rate dropped by half, and the mean dissimilarity index decreased from 0.35 (three NS populations) to 0.28 (three FB populations). On average, the selected populations preserved 84% of alleles specific to the continental groups, that is 10% less than the nonselected populations.

The indicator values of the selected populations were midway between those of the commercial varieties and the nonselected populations.

Selection had a less marked effect on genetic variability with FB2 where the variability indicator values were higher than those noted for FB3 and FB1 (Table 2). The frequency of African-specific alleles increased by more than twofold at these latter two sites (Table 1).

### Discussion

Although the number of individuals per plant population was relatively low, the results of this study were in line with conclusions already put forward by a previous phenotypic study (Lançon *et al.*, 2004) – selection pressure induced by farmers has a much stronger effect than that induced by the cropping environment alone. The homozygosity and genetic homogeneity of plant populations were found to increase under such pressure.

Two factors could explain the predominance of African types in the populations selected at Kandi (FB1) and Savalou (FB3). We previously found (Lançon *et al.*, 2004) that some farmer breeders, especially at Savalou, were captivated in the field by traits of the African cotton varieties, that is tallness, high production potential and late flowering. Besides, the African parents had a better technological quality and a higher ginning yield. A preference for this African group of varieties was thus noted during the two main field and laboratory selection operations. This could indicate the presence of local adaptation quantitative trait loci (QTLs) as was found by Steele *et al.* (2004) in rice populations improved by farmers for upland adaptation.

Allelic variability was higher in germplasm derived from this participatory breeding process than in the commercial control varieties, thus indicating that the selection process was still under way. Further analyses on agronomic and technological traits could indicate whether this genetic variability would concomitantly involve phenotypic variability.

Our results suggest that breeders could use three kinds of indicators to characterise the level of homogeneity of a breeding population:

- The polymorphic locus rate that varied in our experiment from 37% for commercial cultivars to 92% for the three nonselected populations.
- The mean heterozygotic locus rate that varied from 1% (commercial cultivars) to over 10% (nonselected populations).
- The mean index of allelic dissimilarity between individuals that varied from 0.20 (commercial cultivars) to over 0.35 (nonselected populations).

The nonselected populations could be considered as highly variable, whereas the two commercial varieties represented currently acceptable levels of genetic variability on the cottonseed market. The values obtained for these two groups could thus be considered as high and low values for the three proposed indicators.

These values were determined through a single experiment in which a parent group was compared with a set of polymorphic markers. They should be assessed using different parent groups and marker sets with the aim of

drawing up a grid to assist breeders. In this study, the mean frequency of the less frequent SSR alleles was 0.27, while the rate expected from heterozygotic loci would drop if the experiment were to be carried out again with a group of parents having a lower frequency of less frequent alleles. The number of plants sampled can also bias the results, and only three to four plants were assessed when testing the commercial controls. These results should therefore now be confirmed on the basis of results obtained with other varieties.

Information resulting from analyses with neutral AFLP or SSR molecular markers is useful for breeders as a supplement to other information derived from field and/or laboratory experiments, for example homogeneity of a plant population, environmental heterogeneity, variability in highly heritable traits, field and laboratory measurements, especially in populations obtained through a participatory plant breeding scheme. The information provided by molecular markers can facilitate decision making in several areas, for instance terminating (with release of a new variety) or continuing the selection process, maintaining or modifying the selection method, introducing new genetic variability and choosing open pollination or forced inbreeding. After the study, we advised farmer breeders to use pedigree selection rather than mass selection for the purpose of intensifying the breeding process.

These results highlight the advantages of molecular DNA marker analysis techniques for describing plant material obtained through participatory plant breeding (Thro & Spillane, 2003). They demonstrated the mutual benefits of centralised plant breeding techniques and those developed for decentralised participatory plant breeding. This complementarity should, of course, be considered with local constraints. The economic and technological conditions required for mass use of molecular biology techniques may not be fulfilled in low-cost participatory plant breeding schemes, particularly in developing countries. But targeted rational use of these techniques would be possible to accelerate fixation of traits of interest (Jones *et al.*, 2002; Steele *et al.*, 2004) and to optimise management of genetic diversity in decentralised plant populations.

## References

- Atlin G.N., Cooper M., Bjornstad Å. (2001) A comparison of formal and participatory breeding approaches using selection theory. Special issue: breeding for low-input conditions and consequences for participatory plant breeding. *Euphytica*, **122**, 463–475.
- Bourdon C. (1986) Polymorphisme enzymatique et organisation génétique des deux espèces cultivées tétraploïdes de cotonnier, *G. hirsutum* et *G. barbadense*. I. Evolution et domestication des deux espèces. *Coton et Fibres Tropicales*, **41**, 191–210.
- Brubaker C.L., Wendel J.F. (2001) RFLP diversity in cotton. In *Genetic Improvement of Cotton, Emerging Technologies*, pp. 81–101. Eds J.N. Jenkins and S. Saha. Enfield, NH, USA: Science Publisher Inc.
- Ceccarelli S., Grando S., Tutwiller R., Baha J., Martini A.M., Salahieh H., Goodchild A., Michael M. (2000) A methodological study on participatory barley breeding. I. Selection phase. *Euphytica*, **111**, 91–104.
- Jones E.S., Breese W.A., Liu C.J., Singh S.D., Shaw D.S., Witcombe J.R. (2002) Mapping quantitative trait loci for resistance to downy mildew in pearl millet: field and glass-house screens detect the same QTL. *Crop Science*, **42**, 1316–1323.
- Lacape J.-M., Nguyen T.B., Thibivilliers S., Courtois B., Bojinov B.M., Cantrell R.G., Burr B., Hau B. (2003) A combined RFLP-SSR-AFLP map of tetraploid cotton based on a *Gossypium hirsutum* × *Gossypium barbadense* backcross population. *Genome*, **46**, 612–626.
- Lacape J.-M., Dessauw D., Rajab M., Noyer J.-L., Hau B. (2006) Microsatellite diversity in tetraploid *Gossypium* gene pool: assembling a highly informative genotyping set of cotton SSRs. *Molecular Breeding*, **19**, 45–58.
- Lançon J. (1995) L'amélioration du cotonnier au CIRAD-CA. In *Traitements Statistiques Des Essais de Sélection*, pp. 275–293. Ed. C. Cilas. Montpellier, France: Série Colloques du CIRAD.
- Lançon J., Djaboutou M., Lewicki S., Sêkloka E. (2004) Decentralised and participatory cotton breeding in Benin: farmer-breeders' results are promising. *Experimental Agriculture*, **40**, 419–431.
- Perrier X., Flori A., Bonnot F. (2003) Data analysis methods. In *Genetic Diversity of Cultivated Tropical Plants*, pp. 43–76. Eds P. Hamon, M. Séguin, X. Perrier and J.-C. Glaszmann. Montpellier, France, Enfield, USA: Science Publishers.
- Rungis D., Llewellyn D., Dennis E.S., Lyon B.R. (2005) Simple sequence repeat (SSR) markers reveal low levels of polymorphism between cotton (*Gossypium hirsutum* L.) cultivars. *Australian Journal of Agricultural Research*, **56**, 301–307.
- Steele K.A., Edwards G., Zhu J., Witcombe J.R. (2004) Marker evaluated selection in rice: shifts in allele frequency among bulks selected in contrasting agricultural environments identify genomic regions of importance to rice adaptation and breeding. *Theoretical and Applied Genetics*, **109**, 1247–1260.
- Staphit B., Shrestha P., Subedi M., Castillo-Gonzales F. (2000) Mass selection: a low cost, widely applicable method for local improvement in Nepal and Mexico. In *Participatory Approaches to the Conservation and Use of*

- Genetic Resources*, pp. 111–116. Eds E. Friis-Hansen and B. Sthapit. Rome: IPGRI.
- Thro A.-M., Spillane C. (2003) *Biotechnology-Assisted Participatory Plant Breeding: Complement or Contradiction?* PRGA, PPB monograph No. 3, 153 pp. Cali, Colombia: CGIAR Systemwide Program on Participatory Research and Gender Analysis (PRGA Program).
- VanToal T.T., Peng J., St Martin S.K. (1997) Using AFLP markers to determine the genomic contribution of parents to populations. *Crop Science*, **37**, 1370–1373.
- Witcombe J.R., Virk D.S. (2001) Number of crosses and population size for participatory and classical plant breeding. *Euphytica*, **122**, 451–462.