# ON THE USE OF DOUBLED HAPLOIDS IN GENETICS AND BREEDING OF *COFFEA CANEPHORA* P.

**P. LASHERMES, A. CHARRIER, E. COUTURON**

**ORSTOM, Laboratoire de ressources génétiques et d'amélioration des plantes tropicales, 911 Av. Agropolis, BP 5045, F-34032 Montpellier Cedex 1, France**

## 1- Introduction

Haploid plant production followed by chromosome doubling offers the possibility of developing completely homozygous genotypes from heterozygote parents in a single generation. Doubled haploids (HD) have received considerable interest in recent years as methods of creating haploids have become more efficient. Successful applications of doubled haploids in genetic and breeding programs have been developed in a large range of crop species including cereals, rapeseed and several *solanacea*. However, utilisation of DH in woody species has been very limited due to, mainly, the difficulty for producing sufficient numbers of DH (Chen Zhenghua, 1987).

Starting in 1980, ORSTOM has carried out a large program on development and utilisation of DH in *Coffea canephora*. Preliminary outcomes were presented during the 12th ASIC conference (Berthaud et al., 1987). In this report, we intend to outline the main results obtained and will attempt to discuss aspects of application of DH in genetics and breeding of *C. canephora*.

## 2- Method of creating doubled haploids

Success in coffee haploid production by *in vitro* culture technique has been limited so far (Lanaud, 1981 ; Carneiro, 1992). Therefore, use of the spontaneous haploids (Winton and Stettler, 1974) is the only efficient way presently available to produce DH in *C. canephora*. The spontaneous development of haploid embryo in *Coffea canephora* was first reported by Dublin and Parvais in 1975. In 1982, a method of DH production was proposed (Couturon, 1982 ; Couturon and Berthaud, 1982) based on grafting of haploid embryos occurring in association with polyembryony (Figure 1). Haploid embryos are of maternal origin as deduced from inheritance of morphological and enzymatic markers (Couturon, 1982 ; Valverde, pers. com.).

Collect of immature seeds
↓
Removal and dissociation of polyembryos
↓
Grafting of the smallest embryos on four months-old diploid seedlings
↓
Identification of haploid plants
↓
Clonal propagation by cutting
↓
Chromosome doubling by colchicine treatment
↓
Grafting of DH onto heterozygous rootstock

**Figure 1.** Main steps of DH production in *C. canephora* based on the haploid embryos occurring spontaneously in association with polyembryony.

depression appeared particularly severe on general vigor and reproductive aspects including cherry yield but limited, for instance, on leaf area (-20%), hundred beans weight (-20%) and resistance to leaf rust.

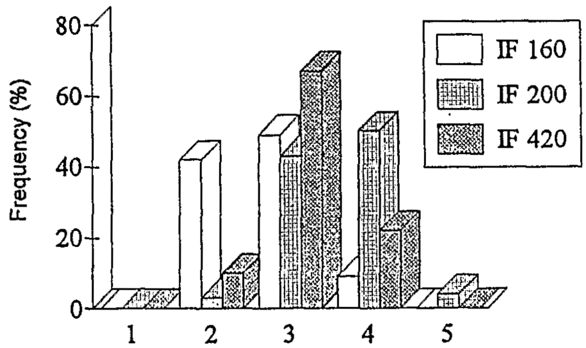


**Figure 3.** Frequency distributions of doubled haploid genotypes produced from different clones for susceptibility to leaf rust (1=resistant, 5= highly susceptible)

For all characters studied, considerable genetic variations were evidenced within and between DH families. As an example, the frequency distributions for susceptibility to leaf rust of DH's produced from different genotypes (clone) are presented in figure 3. Leaf rust (*Hemileia vastatrix*) susceptibility was scored by repeatedly observing trees on 1-5 scale. The distribution of DH's produced from the resistant clone IF 160 is skewed towards resistant levels (Berthaud and Lourd, 1982). In contrast, the distribution of DH's produced from the moderately susceptible clone IF 200 is skewed towards susceptible levels. The distribution of DH's produced from IF 420 is centred on the parental clone value (moderately resistant).

Most of DH's had a very low cherry yield and none had a production comparable to a standard heterozygous plant. The low cherry yield of DH's is for a large part due to a reduced fruit set. Independently of the plant vigor, a high proportion of flowers "fell down" and did not produce fruit. This phenomenon, usual in *C. canephora*, is notably pronounced in the DH's, and may be related to physiological deficiencies. In addition, the DH's present a high frequency of peaberries and empty berries resulting in a low fruit filling coefficient. Despite few notable exceptions, most of DH's showed a fair level of pollen viability (> 50%) as estimated by aceto-carmine staining. This result was confirmed by the high success rate of crosses using DH genotypes as male parent.

## 4- Combining ability of doubled haploids

With regard to coffee breeding, a crucial aspect is the performance of DH in combination. Ninety-five DH's were crossed with either heterozygous genotypes or DH to determine their combining ability. The DH's tested were produced from 9 different genotypes but in majority from the clones IF 160 and IF 200. Several agronomic trials were established, and a number of characteristics were observed (Lashermes et al., 1993b). Strong vigor was evidenced for most hybrid combinations assayed. The reduced fertility that characterised the DH's was not observed at the hybrid level. Overall frequency of peaberries which are caused by ovule abortions, is similar for both hybrid combinations and standard clones.

DH's produced appeared to be a representative sample of the parental gametes when considering the agronomic traits analysed (Figure 4). The average value of a family of DH's, in terms of combining ability, did not differ from the value of the parental clone.

The variability of the combining ability for yield observed among DH's derived from IF 200 and IF 160 is considerable. DH's showed excellent combining ability when crossing with non-related genotype. Hybrid combinations had yields comparable to standard clonal varieties (IF 126, IF 202, IF 461). The most producing combinations were intergroup hybrids between a guinean accession and different DH's produced from clones belonging or related to the congolese pool. However, high yield was also observed for hybrids involving two DH's associated to the congolese pool.

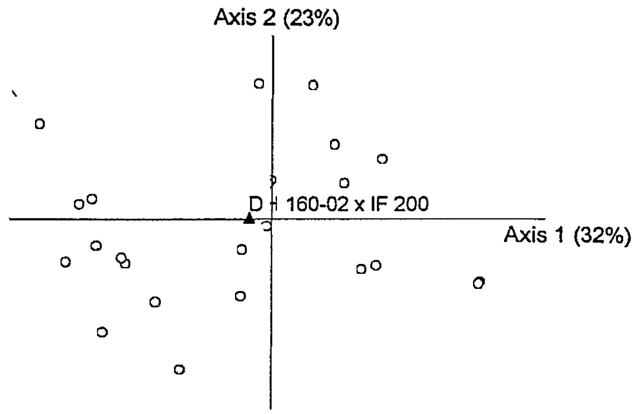


**Figure 4.** Principal component analysis for agronomic characteristics of top-crosses involving 24 DH produced from IF 200 and a common tester DH 160-02 ; position of the cross DH 160-02 x IF 200 is indicated as control.

Performances of an subset of hybrids were analysed as a factorial mating design involving 3 DH's produced from IF 160 as female parent and 4 DH's produced from IF 200 as male parent. Analyses indicated that all genetic variance was attributable to additive effects in estimates of yield as well as plant height, leaf area, leaf shape and hundred beans weight. The general combining ability variance was also predominant for stem girth and leaf rust susceptibility although effects due to interaction were detected. Importance of the general combining ability for yield (Figure 5) was confirmed by a second experiment involving 4 DH's produced from IF 160 and two genetically distant testers. Similar importance of the general combining ability has been observed by Bouharmont et al. (1986) and Leroy et al. (1993) in analysing incomplete diallel designs involving clones.

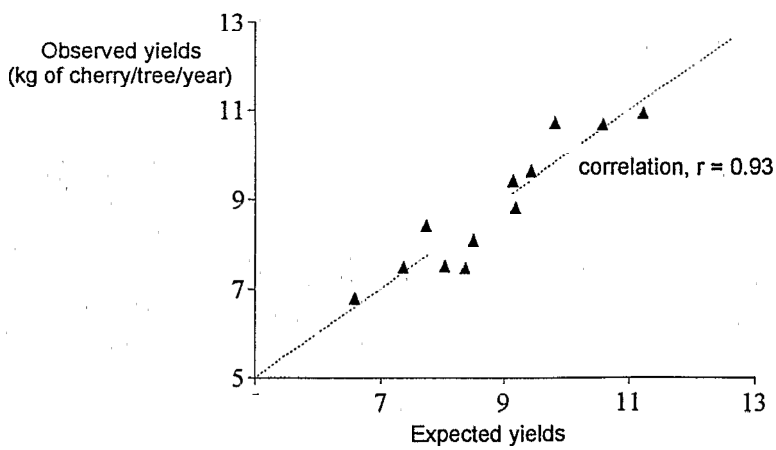


**Figure 5.** Observed yields of 12 hybrid combinations, resulting from a factorial mating design, plotted against yields predicted from parental general combining ability.

Rank correlation coefficients were calculated between characteristics of 21 DH's derived from IF 200 and their respective top-crosses using a common homozygous tester. Significant rank correlation coefficients were observed between values of DH's and their respective top-crosses for leaf shape and leaf area. On the other hand, no relationships were observed between vigor, yield of DH's, and height, girth and yield of top-crosses. These results may appear in contradiction with the importance of the general combining ability observed. If only additive effects were involved, correlation in ranking values of DH and top-crosses would be expected. A possible explanation is that evaluation of DH's could be distorted since their strict homozygosity leads to the expression of recessive deleterious alleles. These alleles, responsible for a large part of the strong inbreeding depression, would be only expressed in homozygotes. Such situation where an important general combining effect coexists with a strong inbreeding depression has been frequently reported for allogamous species (Gallais 1989) including trees species (Lanaud, 1987 ; Namkong and Kang 1990).

## 5- Applications in coffee breeding

Current breeding programs (Charrier and Berthaud 1988) are directed to the development of varieties distributed either through seed (synthetic and hybrid varieties) or after vegetative propagation (clone). Synthetic (hybrid) varieties can be produced at much lower cost, and are easier to distribute than the clonal varieties (Duris 1985). However, the heterozygous nature of parents causes large variation in offspring and interest in synthetic and hybrid varieties is therefore limited (Capot 1977, Charmetant et al. 1990).

DH, not only provides unique information's on genetics of *C. canephora* but, could be used as homozygous parents in the production of $F_1$ hybrids (Figure 6). This new type of variety could combine several advantages: low cost, full benefit from heterosis, homogeneity. However, it may raise several difficulties. Inbreeding masks the real potential of DH and no prediction of their value as progenitors seems to be possible before trial. Number of DH that can be tested for combining ability is technically and economically limited. Results in this study indicate that the mean combining ability of a DH family can be anticipated from the value of the parental clone. So, a partial solution could be to focus the production of DH on clones showing the best combining abilities (Bouharmont et al., 1986 ; Leroy, 1993). Another difficulty in developing $F_1$ hybrid varieties is seed production. Yield of most DH is limited, and both parents of hybrid should be compatible with synchronised flowering. A preliminary selection among DH could be made on the characters directly related to the production of seeds ; the selected trees being subsequently tested for combining ability. The development of three-way crosses or double crosses could be an interesting approach to reduce seed production difficulty. In addition, three-way crosses (double crosses) varieties would be heterogeneous for the self-incompatibility genes and could avoid the necessity to mix varieties in the plantations to insure cross-pollination.

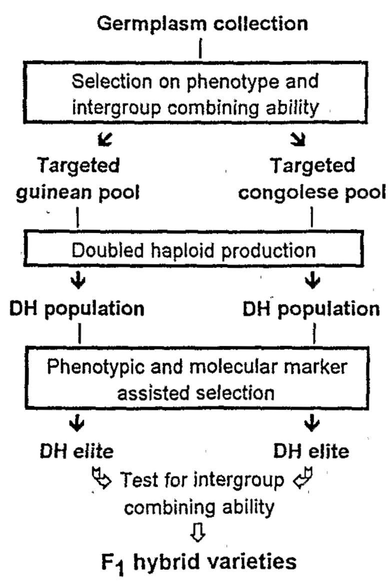


**Figure 6.** Development of hybrid varieties using doubled haploids in *C. canephora* (modified after Charrier and Berthaud, 1988).

In relation with the reciprocal recurrent selection program (Comstock et al. 1949) developed in *C. canephora* (Leroy 1993), a straightforward utilisation of DH would be to use selected DH genotypes as tester. Since the general combining ability is predominant, improvement of populations would not be affected. Absence of genetic variability attributable to the parent tester in the testing progeny should lead to a higher selection efficiency.

## 6- Conclusion and prospects

Ability to produce large number of DH from almost any genotypes in *C. canephora* affords a unique opportunity to assess the interest of doubled haploids in woody species. The method used, although time-consuming, does not require any particular facilities and production of DH based on the spontaneous occurrence of haploid plants in association with polyembryony, could be achieved in any coffee research station.

Only via haploidy can inbred lines be developed in a self-incompatible diploid crop with a long juvenile period such as *C. canephora*. Despite the inbreeding depression, DH's have a great potential in genetic and coffee breeding programmes. DH's produced from the same clone present large genetic variations and genetic analysis of important characters (e.g. incompatibility system, leaf rust resistance, caffeine content) can be undertaken. DH's are particularly well suited to detect the additive effects of quantitative trait loci (QTL) via linkage to genetic markers (Knapp et al., 1990 ; Lashermes et al., 1993c). Regarding coffee breeding, the DH's provide the possibility to develop $F_1$ hybrid varieties. This application could have a considerable impact on coffee cropping but requires additional study. Particularly, seed production technology will need further attention, and agronomic multilocated trials have to be established.

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## Summary

Doubled haploids (DH) of *Coffea canephora* were developed using haploid embryos that occur spontaneously in association with polyembryony. More than 750 DH's produced from various genotypes were brought under field conditions. Inbreeding depression was particularly severe on general vigor and reproductive aspects. Agronomic performances of hybrids involving different DH's were evaluated. Important heterosis was evidenced. Hybrid combinations had yields comparable to standard clonal varieties. Relative importance of additive and nonadditive genetic factors was estimated for different agronomic characters. Implications of such results for genetic studies and breeding of *Coffea canephora* are discussed.

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