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Review Paper

Coffee improvement by interspecific hybridization: A review

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Abstract. Considerable studies on creation of the interspecific hybrids using induced tetraploid *Coffea canephora* P. and *Coffea arabica* L. varieties for improvement of resistance to Coffee Berry Disease (CBD) and Coffee Leaf Rust (CLR), growth habits, fertility, yield performance, genetic diversity, biochemical attributes and beverage quality as well as similar information on their backcrosses to Arabica coffee genotypes are reviewed in this paper. Whereas the F_1 hybrids obtained by crossing induced tetraploid Robusta and Arabica coffee exhibited low fertility, backcrossing the hybrids to *C. arabica* parents restored the fertility to near normal levels. The yields and beverage quality of backcrosses improved to the levels of the best parents and in some cases they were even better. Of major significance was the resistance to CBD and CLR that the tetraploid Robusta was able to impart into the backcross progenies. These progenies were therefore potential substitutes for the susceptible Arabica coffee varieties in addition to taking the place of Robusta which has inferior beverage quality.

Keywords: Coffee, interspecific hybrids, Backcrosses, induced tetraploid Robusta.

INTRODUCTION

Coffee belongs to the genus *Coffea* L in the family Rubiaceae. The genus is classified into two subgenera: *Coffea* and *Paracoffea* (Kumar et al., 2008). The subgenus *Coffea,* consists of approximately 105 taxa (Kumar et al., 2008). The two agronomically important species; *C. arabica* and *C. canephora,* are derived from this subgenus (Anthony et al., 2002). However, other *Coffea* species constitute a valuable gene reservoir which can be used for different breeding purposes (Lashermes et al., 1993). The relative ease of interspecific hybridization within the genus *Coffea* together with the evidence from cytogenetic studies indicate that all coffee species, probably evolved from an original species with a basic chromosome number x = 11 (Owour and Van der Vossen, 1981). *C. Arabica* is tetraploid (2n = 4x = 44) and is self-fertile while other *Coffea* species are diploid (2n = 2x = 22) and generally self-incompatible (Masumbuko et al., 2003; Gichuru et al., 2008).

The first coffee breeding work in Kenya was done by Doughty, from 1924 to 1940 (Kushalapa and Eskes, 1989). Some of the varietal crosses made at that time were distributed to other coffee growing countries but several inter-specific crosses involving *C. arabica* x *C. canephora* were lost during World War II (Kushalapa and Eskes, 1989). Artificial crosses between *C. arabica* and *C. canephora* to create triploids hybrids were made in Java in 1923, in Kenya and Brazil in 1930s (Kushalapa and Eskes, 1989). In 1971, Kenya embarked on an intensive breeding programme for Arabica coffee which was triggered by the outbreak of coffee leaf rust *(Hemileia vastatrix Berk. & Br.*) and Coffee Berry Disease (*Colletotrichum kahawae* Waller and Bridge) in the late 1960s. The main objective of this breeding programme was to develop cultivars that combine high yield and superior quality with resistance to Coffee Berry Disease (CBD) and Coffee Leaf Rust (CLR) (Van Der Vossen et al., 1976).

Interspecific hybrids between *C. arabica* and various diploid species have been successfully produced (Lashermes et al., 2011, Gimase et al., 2014a). The approach of introgressing resistance genes to Arabica coffee via interspecific hybrids was first developed by the

Table 1. Induced tetraploid Robusta genotypes and Arabica coffee parents of the interspecific F_1 hybrids.

Genotype	Species	Status	Source
UT3	Induced Tetraploid *C. canephora*	Conserved genotype	Kawanda, Uganda
UT6	Induced Tetraploid *C. canephora*	Conserved genotype	Kawanda, Uganda
UT8	Induced Tetraploid *C. canephora*	Conserved genotype	Kawanda, Uganda
UT10	Induced Tetraploid *C. canephora*	Conserved genotype	Kawanda, Uganda
SL28	*C. arabica*	Commercial variety	Kenya
SL34	*C. arabica*	Commercial variety	Kenya
N39	*C. arabica*	Commercial variety	Lyamungu, Tanzania
Caturra	*C. arabica*	Commercial variety	Brazil

Dutch in Indonesia in 1923. They obtained selections with promising yield and resistance (Kushalapa and Eskes, 1989).Viable and fertile inter-specific hybrids between the allotetraploid *C. arabica* L. (2n = 44) and *C. canephora* Pierre (2n = 22) were developed in Kenya through induced tetraploid forms of *C. canephora* (Owour and Van der Vossen, 1981). Doubling of the chromosome number in *C. canephora* was achieved in Uganda through colchicine treatment and the materials were acquired in Kenya for hybridization with *C. arabica*. Similar work was successfully carried out in Brazil in 1950 (Owour and Van der Vossen, 1981). The resultant interspecific hybrids have been used in coffee breeding programmes for introgression of CLR and CBD resistance into *C. arabica* or for improvement of the quality of Robusta coffee by direct use of their F_1 hybrids (Owour, 1985; Gimase et al., 2014a).

This paper focuses on review of considerable studies in Kenya and other countries on creation of interspecific hybrids between induced tetraploid *C. canephora* (Ex Fr.) and *C. arabica*. The induced tetraploid Robusta used in the Kenyan programme were introduced from Uganda in 1972 and crossed with four Arabica coffee varieties of SL28, SL34, N39 and Caturra (Gimase et al., 2014a). The current status and source of interspecific F1 hybrid parent are shown in Table 1. The hybridization was done to improve the tolerance of Arabica coffee to CBD and CLR while at the same time improving the quality of Robusta coffee. A major drawback was the reduced fertility of the hybrids which was successfully restored by backcrossing to Arabica.

SELECTION FOR RESISTANCE TO CBD AND CLR

Most of the cultivated *C. arabica* varieties are susceptible to CBD and CLR. Since *C canephora* is resistant to the two diseases, inter-specific crosses between *C. arabica* and tetraploid *C. canephora* were made to impart resistance to these two fungal diseases to *C. arabica*. Seven tetraploid Robusta genotypes; UT2, UT3, UT6, UT7, UT8, UT10 and UT12 were introduced from Kawanda in Uganda in 1972 and were used to cross with four Arabica coffee varieties SL28, SL34, N39 and Caturra (Table 1) to generate interspecific F_1 hybrids commonly referred to as Arabusta (CRF, 1976). Preselection tests done on hypocotyls seedlings and leaves of the F_1 hybrids for CBD and CLR respectively revealed that they were highly resistant to both diseases (CRF, 1976). Pre-selection tests for CBD resistance was carried out by inoculating the hypocotyl seedlings with a spore suspension of CBD inoculums and the symptoms were scored on a scale of 1 to 12 (Van Der Vossen et al., 1976; CRF, 1977). In general, scale 1 to 4 score is regarded as resistant, 5 to 6 medium resistant, 7 to 9 medium susceptible while 10 to 12 as susceptible (Van der Vossen et al., 1976). Interspecific F_1 hybrids derived from UT3, UT6, UT8 and UT10 were the best sources used for CBD resistance (Table 2). Of the F_1 interspecific hybrids tested, SL28 x UT6, SL34 x UT6, N39 x UT6 and SL34 x UT10 showed a higher degree of resistance to CBD with mean scores of between 2.8 to 3.8 that was better than selfed progenies of a standard resistant variety Rume Sudan, that scored 4.2 as shown in Table 2. Rume Sudan is considered as the best progenitor for CBD resistance within pure Arabica genotypes. These hybrids also showed resistance to all known strains of *Hemileia vastatrix,* the causal agent of CLR (CRF, 1977).

Pre-selection for CLR was carried out on a scale of 0 to 9 as described by Eskes in 1983. The genotypes that were scored in classes 1 to 3 were regarded as resistant; 4 to 5, medium resistant; 6 to 7, medium susceptible and 8 to 9 as susceptible. This scale can be used in resistance determination of an individual leaf or entire plants and determine a range of heterogeneous resistance types especially in interspecific hybrids (Eskes, 1983). In this scale, young leaves to adult leaves gives a better result as older leaves are more susceptible and therefore the need to observe reaction types in varying leaf ages (Eskes, 1983).

GROWTH HABITS AND FERTILITY OF THE F1 INTERSPECIFIC HYBRIDS AND THEIR BACKCROSS GENERATIONS

Gene exchange is possible due to the meiotic recombination that allows segments from the parental chromosomes to recombine into new genetic entities that are passed onto the next generation (Lashermes et al.,

Table 2. CBD pre-selection test on F_1 progenies of Arabica × tetraploid Robusta interspecific crosses (1974/75).

Crosses	Mean score for CBD resistance
SL 28/ SL 34/ N 39/ Caturra x UT 3	6.0
SL 28/ SL 34/ N 39/ Caturra x UT 6	3.0
SL 28/ SL 34/ N 39/ Caturra x UT 7	10.5
SL 28/ SL 34/ N 39/ Caturra x UT 8	4.1
SL 28/ SL 34/ N 39/ Caturra x UT 10	4.6
SL 28/ SL 34/ N 39/ Caturra x UT 12	3.3

Source: CRF, 1977.

2011). However, inherent problems of interspecific hybridization such as hybrid instability, infertility, non Mendelian segregations and low levels of inter-genomic crossing-over, and features associated with polyploidy or ploidy dissimilarity limits interspecific gene transfer and crop improvement (Lashermes et al., 2011).

F_1 hybrids and their backcrosses showed normal growth under field conditions unlike their tetraploid *C. canephora* parents that were poorly adapted to the high altitudes usually required for growth of Arabica coffee. The tetraploid parents needed permanent shading to protect them from scorching heavily (Owuor and Van Der Vossen, 1981). The F_1 hybrids showed a very vigorous growth habit as they were considerably taller, with thicker stems, longer primaries, longer internodes, higher extension growth, higher percentage flowering nodes and with a higher number of flowers per node than the Arabica parents or their backcrosses (Owuor and Van Der Vossen, 1981). The vigorous growth of the F_1 hybrids, the higher percentage flowering nodes and number of flowers per node did not contribute to a higher cherry yield. This was due to the low pollen and female fertility of the interspecific hybrids (Owuor and Van Der Vossen, 1981). Based on several meiotic components including metaphase chromosome associations, distribution of chromosome over Anaphase poles and frequency of microspore formed per tetrad, the reduced fertility in interspecific F_1 hybrids is mostly an expression of their subnormal meiosis. The F_1 interspecific hybrids displayed a marked irregularity in all meiotic components except metaphase chromosome associations and had significantly more aneuploidy Anaphase I poles and higher frequency of microspore per tetrad in line with their low fertility (CRF, 1979). This study led to the conclusion that the disturbance in the F_1 interspecific hybrids and hence their low fertility arose from a poor regulation of the meiosis rather than from fundamental structural differences in the chromosomes of the genome hybridized (CRF, 1979). Increase in fertility for these inter-specific hybrids could be achieved by backcrossing to Arabica coffee genotypes (Owuor and Van Der Vossen, 1981). Considerably better results were achieved with continued backcrossing of the hybrids to Arabica which improved their fertility level to near normal levels. The low number of laterals per primary in the F_1, hybrids which is a typical characteristic of *C. canephora* completely disappeared in backcrosses (Owuor and Van Der Vossen, 1981). Similar results were reported earlier by Capot (1968). Owuor (1985) concluded that the low fertility of the inter-specific F_1 hybrids was mainly as a result of meiotic disturbances and that the dramatic response of meiosis to backcrossing might have occurred through elimination of non-homologous chromosomes without any attendant deleterious effects due to the compensation. This gives a better chance of commercial adoption for the backcross generations than the F_1 inter-specific hybrids (Owour, 1985). Normal fertility restoration between *C. arabica* and *C. canephora* inter-specific hybrids can be achieved within two successive backcrosses provided that selection for fertility is carried out for each generation (Owour and Van Der Vossen, 1981). Other morphological characters that were studied were leaf length to width ratio, stomata density, guard cell length and pollen diameter (CRF, 1979). The F_1 flower characters were intermediate between both parents but the backcrosses tended towards *C. arabica* parents. The frequency of star type of flowers in the F_1 was almost similar to SL 28 at 28 and 25%, respectively. The study further revealed that artificial doubling of chromosome number in *C. canephora was* of no consequence on self-incompatibility as no fruit set was obtained on selfing induced tetraploid *C. canephora* (CRF, 1979). The canopy differences between interspefic F1 hybrids and their parentals varied widely. The F1 hybrids was more vigorous with a denser canopy than either of the Arabica or Robusta parents as shown in Figure 1.

YIELD PERFORMANCE OF INTER-SPECIFIC HYBRIDS AND THEIR BACKCROSSES TO ARABICA COFFEE

In Arabica coffee, the economic value of a variety is determined both by the yield potential and the bean and cup quality (Agwanda et al., 2003; Gichimu et al., 2013). Yield performance for inter-specific hybrids and their backcrosses to *C. arabica* was low with a large variation in high altitudes suitable for Arabica coffee (CRF, 1985). Selection for evaluation in lower altitude zones (suitable for Robusta coffee) was carried out (CRF, 1985). In a

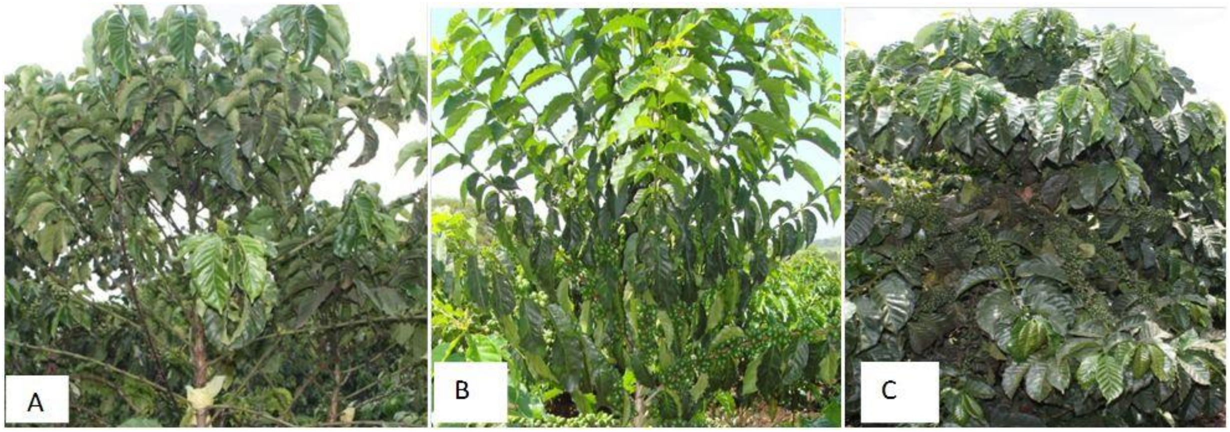

Figure 1. Representative canopies of F_1 interspecific hybrids and their parents; (A) induced tetraploid Robusta, (B) SL 28, (C) F1 interspecific hybrid.

comparative trial carried out by Omondi and Owour (1992) to evaluate the performance of the inter-specific hybrids and their backcross to *C. arabica* in the lower regions of western Kenya, better results were obtained as compared to the high altitude zones. Robusta coffee is adapted to the low altitude coffee growing areas (Omondi and Owour, 1992). In one trial site (Busia), one clone of the inter-specific hybrid recorded a higher mean yield over a 4 years period, with an increase of 21.4% than the standard Robusta (Omondi and Owour, 1992) that is best suited for the region. Omondi and Owour (1992) attributed this increase to the vigorous growth of the interspecific F_1 hybrids resulting to increased bearing surfaces. Although backcrosses to Arabica coffee recorded low yield in low altitude areas, they showed better adaptability to prolonged dry periods and the low yield could be compensated by high density planting especially on backcrosses to Caturra, which exhibited compact growth (Omondi and Owour, 1992).

GENETIC DIVERSITY OF THE INTER-SPECIFIC HYBRIDS IN RELATION TO THEIR PARENTS

Diversity in genetic resources is the basis for genetic improvement. Genetic resources will have little value unless it is efficiently conserved and properly utilized (Kathurima et al., 2012). Efficient utilization as well as conservation of genetic resources depends on the availability of reliable genetic diversity information. As new coffee varieties are continuously being developed through hybridization, there is a need to determine the level and sources of genetic variation within and between new and existing coffee varieties because genetic consistency within varieties is also essential to quality assurance for any agricultural product (Gichimu and Omondi, 2010).

Genetic diversity study on inter-specific F_1 hybrids using SSR and RAPD molecular markers were carried out by Gimase et al. (2014a). The SSR results indicated a high diversity with 50% allele heterozygosity for inter-specific F_1 hybrids as compared to a low diversity with 6.9% allele heterozygosity for Arabica coffee parental genotypes. The result was similar for RAPD primers as it indicated a higher genetic diversity in the inter-specific F_1 hybrids but low diversity in Arabica parental genotypes. The higher genetic diversity in the interspecific F_1 hybrids resulted from Robusta gene introgression and was also as a result of the high polymorphic nature in the *C. canephora* species (Teressaet al., 2010). The low molecular polymorphism in Arabica cultivars is attributed to the allotetraploid origin and moderate speciation of ***C.*** *arabica* due to restricted genetic base of the original population from which the varieties evolved (Agwanda et al., 1997). The study suggested that the inter-specific F_1 hybrids were a good source of genes for further genetic improvement of the Arabica coffee genotypes (Gimase et al., 2014a).

BIOCHEMICAL COMPONENTS, BEVERAGE QUALITY AND GREEN BEAN PHYSICAL CHARACTERISTICS OF THE INTER-SPECIFIC HYBRIDS AND THEIR BACKCROSSES TO ARABICA COFFEE

Sensory characteristics are used to determine the market potential, particularly in the food or drink based products (Lazim and Suriani, 2009). Beverage quality in coffee, determines the desirability of coffee for consumption purposes and acts as a yardstick for price determination (Agwanda et al., 2003). Different levels of biochemical components contribute variously to the final quality of the cup (Buffo and Freire, 2004). A study on sensory diversity on inter-specific hybrids revealed a highly significant ($p < 0.05$) variation in the sensory attributes of fragrance, flavor, after taste, acidity, body, balance and overall perception with significant (0.01) positive correlations among these cup quality traits (Gimase et al., 2014b).

The inter-specific F_1 hybrids recorded a total score of 80 points or above based on a Specialty Quality as per the Specialty Coffee Association of America (SCAA). The varieties SL28 and SL34 (Gimase et al., 2014b), the standard commercial varieties, were used as a control for comparisons. In the study, biochemical attributes of caffeine, oil and sucrose were significantly ($p < 0.05$) different among the interspecific hybrids while chlorogenic acids (CGA) and trigonelline were not. Also Sucrose had significant ($P < 0.05$) positive correlations with sensory variables of fragrance, flavour, after-taste, acidity and overall perception but not for body and balance while oil had a significant ($P < 0.05$) negative correlation with caffeine (Gimase et al., 2014b).

Green coffee beans are categorized into seven grades based on size, shape and density (Kathurima et al., 2010). E – Elephant beans which are the largest coffee beans and are retained on screen 21. AA – Flat beans that passes through screen No. 21, and are retained on 18 (7.2 mm), AB passes through screen No. 18 and are retained on screen No. 16 (size 6.35 mm). C grade describes flat beans that passes through screen No. 16, and retained on screen No. 10, size (3.96mm). TT are light beans extracted from AA and AB by use of pneumatic separator; Pea Beans (PB) – are retained by a piano wire screen on 12, size 4.76 mm (4.43 mm); T – Very small beans and broken bits (CRF, 2011). Grade AB is the majority in the products. Most of the inter-specific F_1 hybrids (over 60%) were usually categorized as the PB grade (CRF, 1981). However, this grade was significantly reduced in their backcross progenies (BC_1) to Arabica coffee, whereby the PB grade accounted for about 40% (CRF, 1985). In the sixth backcross (BC_6), the percentage of PB grades had been sharply reduced to lower than 27% while AB grade increased to 28% (CRF, 1993). This result suggested that the poor genetic features from the wild genotypes may not have significant effect on the beverage quality in the hybrids as several studies have reported that there are no correlations between cup quality and coffee bean physical characteristics (Kathurima et al., 2009; Carvalho et al., 1988; Roche, 1995).

CONCLUSION

The principal objective of coffee breeding and selection is to develop high yielding, superior bean quality and disease resistant cultivars which are adapted to specific growing conditions. The interspecific crosses exhibit desirable traits for improvement of resistance to CBD and CLR in Arabica coffee and cup quality in Robusta coffee. Interspecific crosses and their backcrosses to Arabica coffee exhibit a better performance in lower-latitude regions. A further study for selection and commercialization on these backcrosses in lower regions is therefore recommended.

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