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Monohybrid Mendelian segregation in an interspecific hybrid population of tetraploid x diploid *Coffea* species- part 2

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Abstract: Coffee possesses normal sexual propagation behaviour and exhibits various characters in a large population. The work on the extent of variation is very limited and not well understood. In this context a study was undertaken during 2008-2011 to study the monohybrid segregation pattern in F_2 population of *C. arabica* cv. 'Cauvery'x (*C. congensis* x *C. canephora* var. robusta) established at Coffee Research Sub Station, Chettalli, Kodagu District, Karnataka in the year 2002. The results revealed that coffee varieties grown under Indian conditions possessed dependent and independent characters. The dependent characters followed the independent assortment along with closely associated characters and expressed the phenotypes to varying degrees. Therefore, the frequency of occurrence of such phenotypes did not match with the expected frequency of these characters at high probability confidence level. The genetic behavior of independent traits exhibited the genetic segregation in accordance with the Mendel's law of segregation and fit in to the monohybrid ratio of 3:1 and 1:2:1 with high level of hypothetical confidence ($P \geq 0.50$ up to 0.95). It was observed that the genes regulating the dwarfing effect for coffee bush, thin stem and primary girth, low primary numbers and short primary length characters were found to be dominant over tall type bush, thick main stem and primary shoot as well as more number and length of primary shoots.

Keywords: Genetic Segregation, Variability, Inter-Specific Hybrids, Dominant Traits, Monohybrid Ratio

1. Introduction

Coffee possesses high sensibility to the environmental changes and the ability to withstand in adverse circumstances. Beside the prominent attributes of the genus *Coffea* for huge range of morphological variation between the species, there is wide adaptability to the various environmental situations [4]. The basic investigations on coffee genetics have been carried out at Institute of Agronomy, Campinas, Sao Paulo, Brazil since 1933 by Krug, Carvalho and Mendes considering 25 varieties and four forms of genus *Coffea.* Their cytological examination confirmed that *C. arabica* contained 2n=44 chromosomes while other species had 2n=22. The crosses of var. arabica with 'murta' and 'nana' mutants revealed that the F_1 was normal and F_2 developed by selfing F_1 plants exhibited different types of plants grouped as arabica, bourbon, 'nana', 'murta' and a new type 'murta' plants with broader leaves. Assuming the dominant genes 'AA' carried by var. arabica besides 'NaNa' genes and 'aa' (nana) double recessive genes inherited by nana type plants, it was expected to get the plants with Aa Nana genotype in F_1 and in F_2 the plants with genotype AA nana (normal plants with larger leaves), Aa nana ('murta' type with large leaves) and aa nana (dwarf types) in the ratio of 1:2:1. Based on the above findings, Krug and Carvalho (1951) differentiated the var. typica and bourbon and confirmed var. typica with dominant alleles 'AA' and bourbon with double recessive 'aa'.

Caturra (*Ct*) is a mutant and appears to be originated from var. bourbon in the State of Minas Gerais in Brazil (Krug and Carvalho, 1951) and characterized by dwarf bush having shorter internodes and comparatively larger and dark green foliage than the var. bourbon. It has complete dominance over tall type bush and governed by 'Ct' gene. The studies on segregating behavior of S.795 revealed that this genotype followed the Mendelian ratio of 3:1(resistant: susceptible

plants). Presence of SH3 gene that was responsible for the resistance in S.795 material was thought to have been derived from tree coffee (*Coffea liberica* Bull ex Hiern). The available information suggested that the recombination of homologous chromosomes of *C. liberica* with *C. arabica* along with intra-genic recombination could be the cause for transmission of SH3 gene in Indian Selections [5].

Van der Vossen (1985) reported that dwarf effect coupled with short internodes in arabica coffee variety 'Caturra' is controlled by a single dominant gene 'Ct' that reduced about 50% intermodal length. This genetic factor was found helpful in improving production increasing plant density, early yield, and easy cultural operations besides trouble-free harvesting process. He experienced that F_1 hybrids produced with the combination of parents carrying 'CtCt' genotype would exhibit compact bush habit like parents. The scarcity of the information on segregation behaviour in coffee prompted to undertake this study on monohybrid segregation of the characters in F_2 population of an interspecific parental cross combination.

2. Material and Methods

The F_2 progeny was developed by crossing two coffee species viz; *C. arabica* var. Catimor of tetraploid (2n=44) behavior also christened as 'Cauvery' in India and CxR a diploid species (2n=22). CxR is a hybrid cultivar of *C. congensis* x *C. canephora* var. robusta coffee with tall bush stature while, cultivar 'Cauvery' is of dwarf type [7,9,10]. Generally, triploids are produced by crossing a tetraploid and a diploid *Coffea* species either naturally or artificially, which exhibits infertile behavior and it is often utilized in improvement of coffee varieties [8,11]. In India, a spontaneous triploid (sterile) CxR plant was identified in a large CxR population that was characterized by large and leathery leaves and it was hybridized with 'Cauvery' a tetraploid arabica cultivar. Triploid used as female did not set the fruit while, as a pollen donor with 'Cauvery' female had more than 50% crop setting and produce normal tetraploid fertile F_1 hybrids of intermediate phenotype [7]. The plants in the F_2 progeny developed by selfing F_1 were normal tetraploid, fertile and of various phenotypes. F_2 progeny evolved was established at Coffee Research Sub Station Chettalli, Kodagu District, Karnataka, India during 2002 and studied for character segregation using morphological characters (table- 1).

The characteristic features of both cultivars used in hybridization program are as follows:

Table: 1. *Characteristic features of the parent cultivars*

Sl. No.	Characteristic features	'Cauvery'	Allelic symbol	CxR	Allelic symbol
1.	Bush type	Dwarf	D D	Tall	d d
2.	Stem girth	Thin	Sg Sg	Thick	sg sg
3.	Primary thickness	Thin	TpTp	Thick	tp tp
4.	Number of primary shoot	High	Np Np	Low	np np
5.	Primary length	Short	Lp Lp	Long	lp lp
6.	No. of internodes	High	In In	Low	in in
7.	Length between internodes	Short	Li Li	Long	li li
8.	No. of Secondary shoots/primary	Low	S S	High	s s
9.	Leaf length	Low	Ls Ls	High	ls ls
10.	Leaf breadth	Medium	Lb Lb	High	lb lb
11.	Leaf area	Low	La La	High	la la

The tetraploid F_2 plant population of 305 numbers was used for recording morphological characteristics. The basis of genetic segregation in F_2 was formulated by grouping the plants based on the observed values of morphological data recorded on bush spread, main stem diameter, and number of primary shoots, thickness of the primary shoots and number of internodes/primary as well as their internodal length. The classification of plants was based on the parental traits such as Cauvery type, robusta type and intermediate type. Beside this, some of the morphological characters like; leaf length, leaf breadth and leaf area were also used to find out the segregation patterns considering single pair of allelic combinations in the inter-specific hybrid population. Capital letters of English alphabets were used to denote the dominant and small letters used for recessive characters. The hypothesis was tested following χ^2 (chi square) test.

3. Results and Discussion

3.1. Mono Hybrid Segregation Ratio

Genetic basis for dominant traits can eventually be explained as the influence of modifier genes persist in the genetic environment of genetic constitution of the living beings that may depend on the quantum or action of the enzymes that are regulated by the genes. The 'Mendelian Law of inheritance' worked with concepts of segregation process through which alleles separate and produce haploid gametes and further assortment of different pairs of alleles. The selfed F_1 progeny of Cauvery x (CxR) produced two distinct classes

in the F_2 population, namely, dwarf type and tall type in 3:1 ratio.

The F_2 population was classified into two phenotypic categories based on the morphological traits; Cauvery type and CxR type. The single factor genetic analysis exhibited the production of plants in F_2 generation as per the 'Mendelian Law of segregation' for the traits such as bush spread, stem girth, primaries thickness, number of primary shoots, primaries length and number of nodes per primary. Similarly, the other characters like, primary internodal length and number of secondary shoots also followed the same pattern of segregation. Monohybrid segregation behavior was also noticed for leaf and fruit characters such as leaf length, leaf breadth, leaf area in addition to fruit length and fruit breadth.

The monohybrid ratio expressed the production of F_2 plant population in three dwarf and one tall type classes (table- 2a). This ratio showed that 'Ct' (Caturra gene) mutant genes transmitted through 'Cauvery' as one of the parents had complete dominance over tall parent (CxR). The statistical analysis through χ^2 (chi square) test indicated that the ratio was well within the level of acceptance at 50% probability (table- 2a). Subsequently, the stem girth and primary thickness showed the inheritance of these traits in 3 thin stem: 1 thick stem (acceptable at $P \geq 0.30$) and 3 thin primaries: 1 thick primary ($P \geq 0.95$) respectively. The 3:1 ratio was also observed to be acceptable for number of nodes per primary shoot ($P \geq 0.50$) and primaries internodal length ($P \geq 0.30$) while number of secondary/primary was acceptable at higher probability level ($P \geq 0.95$) (table- 2b). The ratio indicated the dominance of less number of nodes per primary over more numbers, short length of internodes over long internodes and less number of secondary productions over large number. Length of primary character exhibited the plant population with short, medium and long length in 1:2:1 ratio the same trend of segregation for leaf length character was also observed (table-2d). Krug and Carvalho also reported 1:2:1 ratio in F_2 when the plants with 'murtha' and 'nana' mutant genes were crossed [2] The study revealed the dominant effect of major Caturra 'Ct' mutant genes that carried all the other correlated traits of mutational behavior such as thin stem girth and primaries girth, less number of primaries, less number of nodes, short internodal length and less number of secondary shoots. Twenty five percent of plant population produced low leaf length, 50 % medium and remaining 25% produced higher leaf length in 1:2:1 ratio [2] (table-2d). This kind of frequency distribution of the leaf character as well as primary length behavior indicated the occurrence of co dominance.

3.2. Identification of Dominant Traits

Based on the monohybrid and dihybrid ratio and segregation behaviors of the genetic traits in F_2 population of the inter-specific hybrid progeny, the dominant traits governed by the polymorphic genetic factors were identified and presented hereafter. The study revealed that the genes regulating the dwarfing effect on coffee bush, thin stem and primary girth, low primary numbers along with short primary length were found to be dominant over tall type bush, thick main stem and primary shoot as well as more number and length of primary shoots. Further, the monohybrid ratio observed in the experiment for character bush stature was 3:1 consisting of 217 numbers of dwarf and 88 of tall plants. Out of 305 plant population, 230 plants expressed thin stem girth and 75 plants with thick stem and the proportion of these plants was observed to be fitting in monohybrid ratio of 3:1 at 30% level of acceptability ($P \geq 0.30$).

The character primary thickness indicated the production of plants with thin and thick primaries was found to be in 3:1 ratio at greater degree of acceptability ($P \geq 0.95$). The similar trend was noticed for the numbers of primary character too where, the proportion of low numbers and higher numbers of primaries in the F_2 population matched with 3:1 ratio ($P \geq 0.30$). In case of length of primary and leaf length characters, the proportion of plants with short length, medium length and long length primary shoot and leaves were in acceptable ratio of 1:2:1 ($P \geq 0.95$). Beside this, the F_2 population had the plant frequency of low numbers of internodes and higher numbers were found in accordance with the Monohybrid ratio of 3:1($P \geq 0.50$). The internodal length character also expressed the proportion of plants with short internodes and long internodes in the ratio of 3:1 ($P \geq 0.30$). Further, the trend of secondary branches production in F_2 population was found in 3:1 ratio at higher degree of acceptability ($P \geq 0.95$). The leaf area character expressed the greater acceptability of plant frequencies with low and large leaf area as per the monohybrid ratio of 3:1 ($P \geq 0.95$). This tendency of monohybrid inheritance and character segregation in F_2 progeny of Cauvery x (CxR) inter-specific cross ascertained the presence of dominant genes segregating in the higher frequency.

The present study confirmed that the characters such as dwarf bush, thin main stem, thin primary shoots, low numbers of primary branches as well as short length of primary were dominant characters possessing homozygous condition in the parent. Nevertheless, numbers of internodes on the primary and internodal length also expressed their dominance in F_2. Beside this, the low leaf area character expressed its phenotypic appearance under the control of dominant genes. The leaf length exhibited the incomplete dominance by producing plants with less leaf length and more leaf length as well as low leaf breadth in 1:2:1.

4. Conclusion

The present study revealed that the dependent characters followed the assortment of characters in combination with closely related characters and exhibited varying degrees of expression, therefore, the observed frequency of such phenotypes could not match with the expected frequency at high probability level or sometimes it matched at the low probability. The independent characters exhibited the genetic segregation in accordance with the Mendel's law of segregation and fit in to the monohybrid ratio of 3:1and 1:2:1 with high level of hypothetical confidence ($P \geq 0.50$ up to 0.95).

The study also revealed the genetic composition hybrid progenies and indicated that the genes controlling the morphological characters such as leaf length and breadth and shoots length either of primaries or secondaries were more responsive to environmental conditions in spite of their dominant genetic behavior.

Table: 2a. *Mono hybrid ratio in F_2 population of Cauvery x (CxR) crosses considering 3:1 ratio*

Bush spread D D x d d → F_1 D d Dwarf				**Stem girth Sg Sg x sg sg → F_1 Sg sg Thin stem**				**Primary's thickness Pt Pt x pt pt→ F_1 Pt pt Thin primaries**			
Character	*Alleles*	*Observed*	*Expected*	*Character*	*Alleles*	*Observed*	*Expected*	*Character*	*Alleles*	*Observed*	*Expected*
Dwarf type (<195.00cm)	*D _*	217	228.75	Thin stem (<5.80cm)	*Sg _*	230	228.75	Thin primary (<2.15cm)	*Pt _*	251	228.75
Tall type (>195.00cm)	*dd*	88	76.25	Thick stem (>5.80cm)	*sg sg*	75	76.25	Thick primary (>2.15cm)	*pt pt*	54	76.25
Total		305	305	Total		305	305	Total		305	305
χ^2 test			0.1202				0.8687				0.0033
Significance			NS				NS				NS
Probability			P≥0. 50				P≥0.30				P≥0.95

Table: 2b. *Mono hybrid ratio in F_2 population of Cauvery x (CxR) crosses considering 3:1 ratio*

Number of primaries *Np Np x np np*→ F_1 *Np np* Low numbers				**Number of internodes/primaries *In In x in in* → F_1 *In in* Low numbers**				**Primary's internodal length *Li Li x li li* → F_1 *Li li***			
Character	*Alleles*	*Observed*	*Expected*	*Character*	*Alleles*	*Observed*	*Expected*	*Character*	*Alleles*	*Observed*	*Expected*
Low (<12.0)	*Np _*	225	228.75	Low (<20.0)	*In _*	249	228.75	Short (<6.5cm)	*Li _*	232	228.75
High (>12.0)	*np np*	80	76.25	High (>20.0)	*in in*	56	76.25	Long (>6.5cm)	*li li*	73	76.25
Total		305	305	Total		305	305	Total		305	305
χ^2 test			0.6200				0.0074				0.6674
Significance			NS				NS				NS
Probability			P≥0.30				P≥0. 50				P≥0.30

Table: 2c. *Mono hybrid ratio in F_2 population of Cauvery x (CxR) crosses considering 3:1 ratio*

Number of secondaries S S x s s → F_1 S s Low numbers				**Leaf breadth Lb Lb x lb lb → F_1 Lb lb Medium leaves**				**Leaf area (cm^2) La La x la la → F_1 La la Smaller leaf area**			
Character	*Alleles*	*Observed*	*Expected*	*Character*	*Alleles*	*Observed*	*Expected*	*Character*	*Alleles*	*Observed*	*Expected*
Low (<10.0)	S _	207	228.75	Narrow (<9.50cm)	Lb _	187	152.5	Small (<112.0cm^2)	La _	203	228.75
High (>10.0)	s s	98	76.25	Broad (>9.50cm)	lb lb	118	152.5	Large (>112.0cm^2)	la la	102	76.25
Total		305	305	Total		305	305	Total		305	305
χ^2 test			0.0040				7.78E-05				0.0007
Significance			NS				S				NS
Probability			P≥0.95				P≥0.01				P≥0. 95

Table: 2d. *Mono hybrid ratio in F_2 population of Cauvery x (CxR) crosses considering 1:2:1 ratio*

Primary's length Lp Lp x lp lp→ F_1 Lp lp Medium length					**Leaf length Ls ls x ls ls → F_1 Ls ls Medium length**				
Character	Alleles	Observed	Expected	Ratio	Character	Alleles	Observed	Expected	Ratio
Short (<80.00cm)	Lp Lp	59	76.25	1	Short (<16.00cm)	Ls Ls	53	76.25	1
Medium (>80.00 to 120.00cm)	Lp lp	189	152.5	2	Medium (>16.00 to 20.00cm)	Ls ls	182	152.5	2
Long (>120.00cm)	lp lp	57	76.25	1	Long (>20.00cm)	ls ls	70	76.25	1
Total		305	305		Total		305	305	
χ2 test			0.000159					0.001289	
Significance			NS	P≥0. 95				NS	P≥0. 95
Probability									

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