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Mendelian segregation in an interspecific hybrid population of tetraploid x diploid *Coffea* species-part 1

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Abstract: Mating of two parental varieties always leads to the production of genotypic admixture of both the parental traits in F_1 and on selfing the progeny exhibits the phenotypic segregation in a definite proportion in F_2. Mendel described it as "Law of Independent Assortment". It is general belief that coffee varieties do not follow the Mendel's ratios of segregation. Keeping in view the above findings and beliefs, a study was undertaken during 2008-2011 to observe the segregation pattern in the 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, India in the year 2002. The results of the study revealed that coffee cultivars of commercial importance possessed two types of genetic traits known as dependent and independent characters. The dependent characters showed assortment of characters along with closely related characters and expressed varying degrees of expression. Therefore, the frequency of the occurrence of such phenotypic traits did not considerably match with the expected frequency of the same traits at high probability level and it matched at low probability. The genetic behavior of independent traits exhibited genetic segregation in accordance with the Mendel's law of independent assortment showing goodness of fit to the dihybrid ratio of 9:3:3:1 with high level of statistical 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 number of primary branches and reduced length of primary branches were dominant over tall type bush, thick main stem and primary shoot as well as higher number and length of primary shoots.

Keywords: Genetic Segregation, Variability, Interspecific Hybrids, Dominant Traits, Dihybrid Ratio

1. Introduction

There are various techniques that are effectively utilized to analyze the genetic constitution of an individual or population. Among these, Mendel's laws of inheritance are well explained and accepted by the scientific community. Based on one central idea, it was understood that the blending of characteristics contributed by the two parents produce the offspring with intermediate types between the parents. However, the correct explanation came from the published work of Gregor Mendel in 1886 where, he proposed the concepts of hereditary units that inherited equally from each parent and determined the observable phenotypes of the hybrids.

In coffee, cytological examinations confirmed that tetraploid *Coffea* species such as *C. arabica* possessed 2n=44 chromosomes while other diploid species had 2n=22. Studies revealed that one form of *C. arabica* known as 'bullata' had two types of plants containing 66 and 88 chromosomes respectively [6] and *C. arabica* 'monosperma' was a diploid or dihaploid material with 2n=22. Besides these sterile forms regenerated due to abnormal meiosis, commercially cultivated varieties of arabica coffee have 2n=44 chromosomes [7]. Krug *et al.* (1950) reported that the evolution of these sterile forms of arabica coffee was either because of fusion of unreduced gametes or doubling of chromosome number [8]. In India, these 'bullata' and 'monosperma' types have been explained by many research workers [3], [4] and [5]. In the present study, the hybrids produced by crossing genetically different coffee cultivars namely 'Cauvery', a tetraploid (*Coffea arabica*) and CxR (*C. congensis* x *C. canephora* var. robusta) a diploid were examined for the variability in morphological traits and identification of dominant genes controlling the major phenotypic characters generally utilized in breeding for commercial exploitation. Further, the association of imperative traits and their segregation pattern in F_2 population were also studied taking into account monohybrid and dihybrid patterns of inheritance.

2. Materials and Methods

The F_2 progenies were developed by crossing two coffee species viz; *C. arabica* cultivar 'Cauvery', a tetraploid (2n=44) and CxR, a diploid hybrid (2n=22). CxR is a hybrid cultivar of *Coffea congensis* x *C. canephora* var. robusta with tall bush stature, while, 'Cauvery' is a dwarf arabica cultivar. Both the varieties were established at Coffee Research Sub Station Chettalli, Kodagu District, Karnataka, India during 2002. Previous work on interspecific hybridization of tetraploid x diploid species also indicated regeneration and use of triploid (3n=33) F_1 hybrid of infertile genetic behavior [2] in crossing with tetraploid arabica variety that generated fertile progeny [1].

The plant population of 305 numbers was used for recording morphological characteristics. The basis of genetic variation in F_1 and segregation pattern in F_2 was formulated by taking phenotypic characters such as bush spread, main stem girth, and number of primary shoots, thickness of the primary shoots and number of internodes per primary as well as their internodal length.

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

Sl. No.	Characteristic features	Cauvery/ Catimor	CxR
1.	Bush type	Dwarf	Tall
2.	Stem girth	Thin	Thick
3.	Primary thickness	Thin	Thick
4.	Number of primary shoots	High	Low
5.	Primary length	Short	Long
6.	Number of internodes	High	Low
7.	Length between internodes	Short	Long
8.	Number of secondary shoots/primary	Low	High
9.	Leaf length	Low	High
10.	Leaf breadth	Medium	High
11.	Leaf area	Low	High

Table: 2. *Allelic symbols for the expression of genetic traits in F_2 population of Cauvery x (CxR)*

Sl. No.	Genetic traits	Allelic symbols	
		Dominant	Recessive
1	Plant type- dwarf/tall	D D	d d
2	Main stem girth- thin/thick	SgSg	sgsg
3	Thickness of primaries- thin/thick	TpTp	Tptp
4	Number of primaries- low/high	NpNp	npnp
5	Length of primaries- short/long	LpLp	Lplp
6	Number of internodes- low/high	In In	in in
7	Internodal length- short/long	Li Li	li li
8	Number of secondaries- low/high	S S	s s
9	Leaf length- short/long	LsLs	Lsls
10	Leaf breadth- narrow/broad	LbLb	Lblb
11	Leaf area- small/large	La La	la la

The classification of plants was made 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 behavior considering two pairs of allelic combinations in the inter-specific hybrid. Test of significance was done applying Chi-square test (χ^2).

The characteristic features of both the cultivars used in hybridization program are given in Table 1 and allelic symbols of the characters in Table 2.

3. Results and Discussion

3.1. Dihybrid Inheritance

To test the course of two or more independently segregating pairs of alleles in the F_2 population of inter-specific hybrids, the dihybrid ratio has statistically been proved using χ^2 (Chi square test) analysis. The results of the analysis confirmed that the phenotypic traits like bush spread and stem diameter followed the Mendelian law of character assortment in 9:3:3:1 proportion. The genotype D_Sg_ (Dwarf bush with thin stem diameter), D_sgsg (Dwarf bush with thick stem), ddSgsg (Tall bush with thin stem) and ddsgsg (Tall bush with thick stem) were observed to maintain 9:3:3:1 ratio. Such segregation pattern of genetic traits emphasized that tall and thick stem characters were recessive while, dwarf bush and thin stem characters transferred from the parent 'Cauvery' through 'Ct' (Caturra mutant gene) were dominant. The phenotypic ratio of 9:3:3:1 was found to be well within the acceptability level ($P \geq 0.50$) (Tables 3a, 3b, and 3c). This trend of character assortment indicated that the female parent 'Cauvery' had homozygous dominant alleles for dwarfism and thin stem character whereas CxR used as male parent possessed homozygous recessive alleles for tall bush and thick stem phenotype. This dihybrid cross involving two vegetative traits viz., plant type and main stem thickness demonstrated that dwarf plant type and thin stem were controlled by dominant genetic factors.

In another dihybrid cross with two pairs of genetic factors, plant type and primary branch thickness exhibited that D_Tp_ genotypic combination had prevailed in 65.90% and D_tptp genotype in 5.25% of the total F_2 population. Similarly, ddTp_ genotype was observed in 16.40% plants besides the expression of recessive homozygous combination ddtptp in 12.46% of the population. χ^2 test indicated that the observed frequencies of the phenotypic traits followed 9:3:3:1 ratio with low acceptance level ($P \geq 0.02$). The frequency of dwarf plants with thick primary diameter was very low (5.25%) and that did not follow the expected frequency. This kind of phenotypic combination showed that the genes governing the thickness in the primaries rarely get associated with genes controlling the dwarfing effect on bush character.

Table: 3a. *Character segregation behavior in F_2 population of Cauvery x (C x R) cross*

Plant type with stem girth	**Allelic symbol**	**% plants**	**Observed value**	**Expected value**	**Ratio**
Dwarf with small stem girth=	D _ Sg _	62.62	191	172	9
Dwarf with large stem girth=	D _ sgsg	8.52	26	57	3
Tall with small stem girth=	d dSg _	12.79	39	57	3
Tall with large stem girth=	d dsgsg	16.07	49	19	1
Total		100.00	305	305	16
	Accepted	P≥0.50	χ^2 =	1.70	
Plant type with Primary thickness					
Dwarf with thin primaries=	D _ Tp _	65.90	201	172	9
Dwarf with thick primaries=	D _ tptp	5.25	16	57	3
Tall with thin primaries=	d dTp _	16.39	50	57	3
Tall with thick primaries=	d dtptp	12.46	38	19	1
Total		100.00	305	305	16
	Accepted	P≥0.02	χ^2=	9.20	
Plant type with no. of primaries/plant					
Dwarf with less primaries=	D _ Np _	51.48	157	172	9
Dwarf with more primaries=	D _ npnp	19.67	60	57	3
Tall with less primaries=	d dNp _	22.30	68	57	3
Tall with more primaries=	d dnpnp	6.56	20	19	1
Total		100.00	305	305	16
	Accepted	P≥0.95	χ^2 =	0.32535	
Plant type with primary length					
Dwarf with short length of primaries=	D _ Lp _	46.89	143	172	9
Dwarf with long length of primaries=	D _ lplp	24.26	74	57	3
Tall with less length of primaries=	d dLp _	1.64	5	57	3
Tall with more length of primaries=	d dlplp	27.21	83	19	1
Total		100.00	305	305	16
	Accepted	P≥0.50	χ^2=	1.40	
Plant type with no. of internodes/primary					
Dwarf with less internodes/primaries=	D _ In _	60.98	186	172	9
Dwarf with more internodes/ primaries=	D _ in in	10.16	31	57	3
Tall with less internodes/ primaries=	d d In _	20.66	63	57	3
Tall with more internodes/ primaries=	d d in in	8.20	25	19	1
Total		100.00	305	305	16
	Accepted	P≥0.95	χ^2 =	0.00134	
Plant type with primary's internode length					
Dwarf with short internodes	D _ Li _	60.66	185	172	9
Dwarf with long internodes	D _li li	10.49	32	57	3
Tall with short internodes	d d Li _	15.41	47	57	3
Tall with long internodes	d d li li	13.44	41	19	1
Total		100.00	305	305	16

Plant type with stem girth	**Allelic symbol**	**% plants**	**Observed value**	**Expected value**	**Ratio**
	Accepted	P≥0.50	χ^2 =	1.70	
Plant type with no. of secondaries/primary					
Dwarf with less no. of secondaries=	D _ S _	51.48	157	172	9
Dwarf with more no. of secondaries=	D _ s s	19.67	60	57	3
Tall with less no. of secondaries=	d d S _	16.39	50	57	3
Tall with more no. of secondaries=	d d s s	12.46	38	19	1
Total		100.00	305	305	16
	Accepted	P≥0.95	χ^2 =	0.0001	
Plant type with leaf length					
Dwarf with less leaf length	D _ Ls _	39.02	119	172	9
Dwarf with more leaf length	D _ lsls	32.13	98	57	3
Tall with less leaf length	d dLs _	13.77	42	57	3
Tall with more leaf length	d dlsls	15.08	46	19	1
Total		100.00	305	305	16
	Accepted	P≥0.02	χ^2 =	8.30	
Plant type with leaf breadth					
Dwarf with less leaf breadth	D _ Lb_	44.59	136	172	9
Dwarf with more leaf breadth	D _ lblb	26.56	81	57	3
Tall with less leaf breadth	d dLb_	16.72	51	57	3
Tall with more leaf breadth	d dlblb	12.13	37	19	1
Total		100.00	305	305	16
	Accepted	P≥0.50	χ^2 =	1.40	

Assuming the dihybrid cross combination of plant type with the characters namely number of primary shoots per plant, number of internodes per primary, number of secondary shoots per primary and leaf area, it was noticed that there was a strong association of bush size with these traits that resulted in the 9:3:3:1 Mendelian ratio of independent assortment (Table 3a). Further, the observed frequency had acceptance with expected ratio of 9:3:3:1 at higher degree of probability (P≥0.95).

Beside the association of bush spread with stem girth, the other phenotypes such as primary shoot length, internodal length and leaf breadth also showed their close association with bush spread (Table 3a). The Dihybrid character segregation of the above mentioned characters followed the segregation pattern in 9:3:3:1 ratio that was highly acceptable (P≥0. 50) on χ^2 test (Table 3b). The diallelic cross combination of bush character with leaf length exhibited the segregation frequency in the dihybrid ratio of 9:3:3:1 acceptable at lower level of probability (P≥0.02).

Table: 3b. *Dihybrid ratio in F_2 population of Cauvery x (CxR) crosses*

Plant type with leaf area	**Allelic symbol**	**% plants**	**Observed value**	**Expected value**	**Ratio**
Dwarf with less leaf area	D _ La _	47.21	144	172	9
Dwarf with more leaf area	D _ la la	23.93	73	57	3
Tall with less leaf area	d d La _	19.34	59	57	3
Tall with more leaf area	d d la la	9.51	29	19	1
Total		100.00	305	305	16
	Accepted	P≥0.95	χ^2 =	0.00285	
Stem girth with primary thickness					
Thin stem girth with thin primary girth	Sg _ Tp _	69.84	213	172	9
Thin stem girth with thick primary girth	Sg _ tptp	5.57	17	57	3

Plant type with leaf area	Allelic symbol	% plants	Observed value	Expected value	Ratio
Thick stem girth with thin primary girth	sg _ Tp _	12.46	38	57	3
Thick stem girth with thick primary girth	sgsgtptp	12.13	37	19	1
Total		100.00	305	305	16
	Accepted	P≥0.30	χ^2 =	2.80	
Stem girth with number of primaries/plant					
Thin stem girth with less primaries	Sg _ Np _	54.75	167	172	9
Thin stem girth with more primaries	Sg _ npnp	20.66	63	57	3
Thick stem girth with less primaries	sgsgNp _	19.02	58	57	3
Thick stem girth with more primaries	sgsgnpnp	5.57	17	19	1
Total		100.00	305	305	16
	Accepted	P≥0.50	χ^2 =	0.81412	
Stem girth with primary length					
Thin stem girth with low primary’s length	Sg _ Lp _	47.54	145	172	9
Thin stem girth with more primary’s length	Sg _ lplp	27.87	85	57	3
Thick stem girth with low primary’s length	sgsgLp _	1.31	4	57	3
Thick stem girth with more primary’s length	sgsglplp	23.28	71	19	1
Total		100.00	305	305	16
	Accepted	P≥0.05	χ^2 =	5.90	
Stem girth with no. of internodes/primary					
Thin stem girth with less internodes	Sg _ In _	63.93	195	172	9
Thin stem girth with more internodes	Sg _ in in	11.48	35	57	3
Thick stem girth with less internodes	sgsg In _	17.70	54	57	3
Thick stem girth with more internodes	sgsg in in	6.89	21	19	1
Total		100.00	305	305	16
	Accepted	P≥0.95	χ^2 =	0.00678	

Considering the two factors, genetic cross combination of stem girth with other characters, the dihybrid ratio was found to be identical to the Mendelian dihybrid ratio of 9:3:3:1. Stem girth produced frequencies of combined traits in the F_2 population as per 9:3:3:1ratio with high degree of acceptability (P≥0.95) with number of internodes per primary, number of secondary shoots per primary and leaf area. The other phenotypic traits which expressed the association with stem girth in 9:3:3:1 ratio to 50% level of acceptance (P≥0.50) were the number of primary shoots per plant and leaf length. The same dihybrid ratio of 9:3:3:1 was acceptable at 30% probability level (P≥0.30) when stem girth was combined with primary thickness. The dihybrid cross combination of stem girth with length of primary expressed its acceptance in 9:3:3:1 ratio at 5% probability (P≥0.05). Apart from these, stem girth in combination with internodal length of primaries and leaf breadth produced a ratio fitting to the Mendelian ratio of 9:3:3:1 with a low level of acceptability (P≥0.02) (Table 3b). The above results indicated that both the parents involved in the dihybrid mating had homozygous allelic pairs where, Cauvery as one of the parents had acquired dominant Caturra genes and CxR had acquired recessive ones. It is evident from the dihybrid ratio expressed in the F_2 progeny in combination of several phenotypic characters that the Caturra mutant genes had normal course of gamete formation and character association as observed by Mendel during his experimentation.

The results of the present study revealed that coffee cultivars of commercial importance possess two types of genetic traits known as dependent and independent characters. The dependent characters follow the assortment of characters along with closely related characters and express varying degrees of expression. Therefore, the frequency of the occurrence of such phenotypic traits could not considerably match with the expected frequency of the same traits at high probability level while, it matched at low probability. The genetic behavior of independent traits exhibited the genetic segregation in accordance with the Mendel’s law of independent assortment giving goodness of fit to the dihybrid ratio of 9:3:3:1 with high level of statistical confidence (P≥0.50 up to 0.95) (Table 3c).

Table: 3c. *Dihybrid ratio in F_2 population of Cauvery x (CxR) crosses*

Stem girth with internodal length of primaries	**Allelic symbol**	**% plants**	**Observed value**	**Expected value**	**Ratio**
Thin stem girth with short internodal length	Sg _ Li _	64.92	198	172	9
Thin stem girth with long internodal length	Sg _ li li	10.49	32	57	3
Thick stem girth with short internodal length	sgsg Li _	11.15	34	57	3
Thick stem girth with long internodal length	sgsg li li	13.44	41	19	1
Total		100.00	305	305	16
	Accepted	P=≥0.02	χ^2 =	8.80	
Stem girth with no. of secondaries/primary					
Thin stem girth with less no. of secondaries	Sg _ S _	53.77	164	172	9
Thin stem girth with more no. of secondaries	Sg _ s s	21.64	66	57	3
Thick stem girth with less no. of secondaries	sgsg S _	14.10	43	57	3
Thick stem girth with more no. of secondaries	sgsg s s	10.49	32	19	1
Total		100.00	305	305	16
	Accepted	P=≥0.95	χ^2 =	0.00292	
Stem girth with leaf length					
Thin stem girth with less leaf length	Sg _ Ls _	41.31	126	172	9
Thin stem girth with more leaf length	Sg _ lsls	34.10	104	57	3
Thick stem girth with less leaf length	sgsgLs _	11.48	35	57	3
Thick stem girth with more leaf length	sgsglsls	13.11	40	19	1
Total		100.00	305	305	16
	Accepted	P=≥0.50	χ^2 =	1.20	
Stem girth with leaf breadth					
Thin stem girth with less leaf breadth	Sg _ Lb _	46.89	143	172	9
Thin stem girth with more leaf breadth	Sg _ lblb	28.52	87	57	3
Thick stem girth with less leaf breadth	sgsgLb _	14.43	44	57	3
Thick stem girth with more leaf breadth	sgsglblb	10.16	31	19	1
Total		100.00	305	305	16
	Accepted	P=≥0.02	χ^2 =	9.40	
Stem girth with leaf area					
Thin stem girth with less leaf area	Sg _ La _	50.82	155	172	9
Thin stem girth with more leaf area	Sg _ la la	24.59	75	57	3
Thick stem girth with less leaf leaf area	sgsg La _	15.74	48	57	3
Thick stem girth with more leaf area	sgsg la la	8.85	27	19	1
Total		100.00	305	305	16
	Accepted	P=≥0.95	χ^2 =	0.00763	

The study also exposed the mystery of coffee genetic composition and showed that the morphological characters such as leaf length and breadth and shoot length either of primary or secondary are very sensitive to seasonal variation/weather conditions. The genes controlling these traits were observed to fall susceptible to the environmental circumstances in spite of their genetic dominance. Changes in the morphology of such characters are easily perceptible when compared to the characters that are not frequently influenced by the environment. Genetic analysis of dihybrid cross combinations with χ^2 test showed that the characters such as leaf length, leaf breadth and primary length expressed notable deviation from expected frequencies of the dihybrid ratio of 9:3:3:1.

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References

[1] C. S. Srinivasan, A. Kumar, V. S. Amaravenmathy and A. Santaram, "Robusta-like Coffee Plants with Arabica-like Cup Quality- Myth or Possibility,"ASIC,20th International Conference on Coffee Science, Bangalore, October 2004, pp. 787-799

[2] M. S. Sreenivasan, A. Santaram and N. S. Prakash, "Tetraploid inter-specific hybrids in Coffee breeding in India," ASIC, 15thColloque, Montpellier. 1993, pp. 226-233

[3] R. L. Narsimhaswamy and S. Vishveshwara, "Report on hybrids between some diploid species of *Coffea* L." Indian Coffee, 1961, 25 : pp. 101-111

[4] R. L. Narsimhaswamy and S. Vishveshwara, "Progress report on hybrids between diploid species of *Coffea* L." Turrialba, 1967, 17 : pp. 11-17

[5] C. C. Chinnappa, "Interspecific hybrids of *Coffea canephora* and *Coffea arabica,*" Current Sci, 1968, 37: pp. 676–677.

[6] C. A. Krug, In Modern Coffee Production by Haarer, A.E. London, Leonard Hill (Books), Limited, 9 Eden Street, N.W.1, 1956, pp. 495.

[7] C. A. Krug, J. E. T. Mendes, A. Carvalho and A. J. T. Mendes, "A new type of coffee, "Bragantia, 1950,10 (1), pp. 11-25. [In Portuguese, English summary.]

[8] C. A. Krug, and A. J. T. Mendes, "Cytological observations in *Coffea* – IV," J Genet, 1940, 39, pp. 189–203.