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# INHERITANCE STUDY FOR DIFFERENT QUALITATIVE TRAITS IN GRAIN AMARANTH

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## ABSTRACT

Grain Amaranthus, is a highly potential pseudocereal having the capability to ensure wholesome nutrient availability and an ability to cope up with the degrading environment for which it can be considered as a blessing for the future. The present study was conducted in Instructional-cum-Research Farm, Raj Mohini Devi College of Agriculture and Research Station, IGKV, Ambikapur, Chhattisgarh, from*Rabi* 2020 to *Rabi*2022-23 for crossing of parents, evaluation and selfing of $F_1$ population and evaluation of $F_2$ population respectively, to identify the pattern of inheritance of 9 important morphological traits in the $F_2$ segregating population of a cross between two well known, cultivated varieties of grain amaranth i.e., Suvarna X GA2. In the $F_1$ generation, the observed traits included green leaf and stem colour, red leaf margins, red panicle colour, an erect and flat inflorescence, a spreading growth habit, and late flowering. These phenotypes suggest that these characteristics are dominant over their respective contrasting traits. In the $F_2$ generation, the inheritance pattern for various traits exhibited complex genetic interactions. Leaf colour and flowering time demonstrated supplementary gene action, while panicle colour and inflorescence shape followed polymeric gene action. Plant growth habit and the presence of spines on the inflorescence showed inhibitory gene action. Additionally, the presence of red leaf margins, stem colour, and inflorescence compactness adhered to Mendelian segregation patterns. These results provide insights into the genetic mechanisms underlying these phenotypic traits, highlighting the involvement of multiple gene interactions in the expression of plant morphology and flowering characteristics.

***Key words-*** pseudocereal, morphological traits, supplementary gene action, polymeric gene action, mendelian segregation pattern

Amaranth (*Amaranthus sp*.) is a self-pollinated, diploid (2n=32 and 34), herbaceous $C_4$ plant from the family *Amaranthaceae* and subfamily *Amaranthoideae*. Recognized as a nutrient-dense yet underutilized pseudocereal, its origins are traced to South and Central America (Sauer, 1967). During the fifteenth century, grain amaranth was a staple food for the indigenous populations of Central and South America, as well as for Indian communities. However, its cultivation declined significantly after Spanish colonizers prohibited its production due to religious restrictions. Recently, the crop has regained attention due to its potential to meet growing nutritional demands accompanied with degrading climatic and soil conditions and with lowest input, Grain amaranth has been recognized as the food of the future due to its potential nutritional value. Commercial cultivation for human consumption began in Mexico and several South American countries (Berghofer and Sochoenlechner, 2002). Amaranth exhibits strong performance under both optimal and stress conditions, demonstrating a high degree of adaptability. With increasing consumer demand, grain amaranth has the potential to become a highly productive cereal crop with significant yield prospects (Pandey, 2009).

To produce improved varieties meeting all the future demands with little effort it is very important to study the inheritance pattern of various yield related characters. A few works conducted by various people in Grain Amaranth includes the study of gluten content in endosperm, caused by a single gene, with the non-glutinous allele being dominant, study on the inheritance of starch properties in the perisperm of *A. hypochondriacus* by Okuno and Sakaguchi's (1982), Coons (1982) reported that seed coat colour in grain amaranth is governed by a single gene, with black being dominant over white. In a genetic study of six color traits, Kulakow et al. (1985) found that seed coat colour in *Amaranthus hypochondriacus*and *A. caudatus* is controlled by two loci. To better understand segregation ratios in grain amaranth, further research is needed to characterize cultivars for germplasm preservation and identify genetic markers for crop improvement. This experiment was designed to study the segregation of major alleles, which could also aid in identifying linkage groups and understanding the crop's genome organization.

## METHODS AND MATERIALS

The research was conducted in Instructional-cum-Research Farm, Raj Mohini Devi College of Agriculture and Research Station. Ajirma Ambikapur (C.G.) 497001. The crossing of parents to generate $F_1$ population was conducted in *Rabi* 2020-21, The $F_1$ population was planted and evaluated and selfed in *Rabi* 2021-22 and the $F_2$ population was planted and evaluated in *Rabi* 2022-23

Plant Material and Parental Genotypes

This study was conducted using two popular grain amaranth (*Amaranthus*) varieties as parental genotypes: *Suvarna* belonging to *A. cruentus* (2n = 34) and GA-2 belonging to *A. hypochondriacus* (2n = 32). These materials were obtained from AICRN on Potential Crops through Section of Genetics and Plant Breeding, RMD College of Agriculture and Research Station, Ambikapur, where they were maintained for research purposes.These two species exhibit distinct morphological characteristics, making them ideal candidates for studying the inheritance of multiple traits. The selection of *A. cruentus* and *A. hypochondriacus* for hybridization was based on their agronomic importance, with *Suvarna* being widely known for its high grain yield and GA-2 valued for its superior drought tolerance and seed quality (Das, 2020; Yadav*et al*., 2023). These parental lines differed in 7 key phenotypic traits, including leaf colour, panicle colour, flowering time, plant growth habit, red leaf margin, inflorescence shape, and inflorescence spiney (Table 1). These differences enabled a comprehensive study of genetic inheritance across multiple loci.

Hybridization and Emasculation Procedure

Hybridization was carried out between Suvarna (female parent) and GA-2 (male parent). To ensure controlled pollination, emasculation of the female parent (*Suvarna*) was performed manually during the late evening hours. This method was chosen based on the observation that *Amaranthus* flowers are most responsive to emasculation at this time due to the natural closing of anthers, reducing the risk of self-pollination (Jha and Ohri, 2021). Emasculation involved the manual removal of anthers from unopened flower buds using fine forceps. The emasculated flowers were then bagged with butter paper bags to prevent contamination from undesired pollen. The following morning, controlled pollination

was performed using fresh pollen from the male parent (GA-2). The bagged flowers remained undisturbed for several days to ensure fertilization and seed development.

Confirmation of Hybridity Using SSR Markers

To verify the success of hybridization and confirm the hybridity of $F_1$ plants, molecular marker analysis was conducted using Simple Sequence Repeat (SSR) markers. SSR markers are highly polymorphic, codominant, and efficient in distinguishing between homozygous and heterozygous individuals (Sharma *et al*., 2023). DNA was extracted from fresh leaf tissue using the cetyltrimethylammonium bromide (CTAB) method (Doyle and Doyle, 1990). PCR amplification was performed using SSR primers specific to *Amaranthus* species, and the amplified products were resolved on a 2% agarose gel. The presence of heterozygous bands confirmed the hybridity of the $F_1$ population. The SSR analysis revealed that $F_1$ plants were heterozygous and phenotypically uniform, indicating successful cross-hybridization and expression of dominant traits.

$F_2$ Generation development and field experimentation

$F_1$ hybrids were allowed to self-pollinate to produce the $F_2$ generation. The $F_2$ population was grown under field conditions at the Instructional-cum-Research Farm, Raj Mohini Devi College of Agriculture and Research Station. Ajirma Ambikapur (C.G.) 497001 Standard agronomic practices, including irrigation, fertilization, and pest management, were followed throughout the growing season to ensure optimal plant development (Singh et al., 2022). The experimental field was divided into multiple blocks, each representing different replications of the $F_2$ population to minimize environmental variability. The population size was large enough to achieve accurate segregation ratios, with over 300 $F_2$ individuals scored for each trait.

Phenotypic Scoring of Traits in $F_2$ Generation

The phenotypic traits of the $F_2$ population were scored at specific growth stages under natural field conditions. The timing of trait expression was critical for accurate evaluation, as certain traits are more easily distinguishable at particular developmental stages (Table 2). Phenotypic scoring was conducted as follows:

Leaf Colour and Leaf Marks: Leaf colour was scored between 4 to 8 weeks after emergence, as the expression of the trait is most pronounced during this period. Beyond this window, the intensity of leaf colouration fades due to environmental factors and plant maturity. The colour variation among green, red, and dark leaves was visually recorded.

Flowering Time: Flowering time was recorded at the panicle initiation stage, with early and late flowering individuals noted. This trait is influenced by both genetic and environmental factors, and scoring at the precise stage of panicle initiation minimized discrepancies.

Panicle Colour, Plant Growth Habit, Inflorescence Shape, and Inflorescence Spininess: These traits were scored at the maturity stage, when full phenotypic expression was evident. Panicle colour was categorized into red, green, and intermediate types, while growth habit was noted as spreading or erect. Inflorescence shape was classified into erect, curved, or bushy types, and inflorescence spiney was recorded based on the presence or absence of spines.

Chi-Square ($\chi^2$) Analysis for Inheritance Pattern

Chi-square ($\chi^2$) tests were conducted to analyse the segregation ratios in the F1 and F2 generations, determining the mode of inheritance for each trait. The $\chi^2$ test for goodness-of-fit was performed according to the methodology suggested by Fisher (1936), to assess whether the observed segregation ratios deviated significantly from the expected Mendelian ratios. The $\chi^2$ value was calculated using the following formula:

The mathematical formula of $\chi^2$ analysis is given as follows:

$$N \qquad (Oi-Ei)^2$$

$$\chi^2 \text{value} = \sum_{I=1} \frac{}{Ei}$$

Where,

$E_i$ = expected frequency of $i^{th}$ class

$O_i$ = observed frequency of $i^{th}$ class

(n-1) = degree of freedom

n = number of factors studied

The calculated value of $\chi^2$ (Chi-square) was compared with tabulated values for (n-1) degrees of freedom. If the calculated value of $\chi^2$ was less than the table value of $\chi^2$ for (n-1) df at 5% level of significance, then the difference between observed and expected values were treated to be non-significant. The non-significant $\chi^2$ value indicated agreement between proportion of plants with parental traits and assumed ratio in $F_2$ population, thereby leading to acceptance of hypothesis. In the present study the $\chi^2$ calculated values were compared with $\chi^2$ table values on various degrees of freedom for $F_2$s as mentioned in Table 3

Statistical Software and Validation of Results

All statistical analyses were performed using the free online software GraphPad (Dotmatics). GraphPad software was chosen for its robust $\chi^2$ analysis capabilities and ease of use for analysing genetic data. The statistical results were validated by repeating the analysis for each trait, ensuring accuracy and reproducibility. The agreement between observed and expected segregation ratios was evaluated, and non-significant $\chi^2$ values supported the hypothesis of Mendelian inheritance for most traits (Table 3)

**RESULTS AND DISCUSSION**

Inheritance of Leaf Colour

The inheritance of leaf colour in this study demonstrated a classical Mendelian pattern, where all F1 plants exhibited green leaves, indicating that green is the dominant trait. The $F_2$ population segregated into green, red, and dark leaf phenotypes in a 9:3:4 ratio, which suggests that leaf colour is controlled by two supplementary genes, designated as *lc-g* (for green) and *lc-r* (for red). The interaction of these genes follows the pattern of complementary gene action, where the presence of both dominant alleles produces green leaves, while combinations involving recessive alleles result in either red or dark leaves. The observed 9:3:4 ratio confirms the influence of gene interaction, specifically supplementary epistasis, where one gene complements the expression of another. Similar gene interactions have been reported in crop plants like *Capsicum annuum* and *Solanum lycopersicum*, where colour expression is modulated by the interaction of multiple genes.

Inheritance of Panicle Colour

Panicle colour exhibited a complex inheritance pattern. In the $F_1$ generation, all plants had red panicles, signifying the dominance of red panicle traits. The $F_2$ population segregated into red, green, and light pinkish panicles in a 9:6:1 ratio, suggesting the involvement of duplicate genes with cumulative effects (*PR* and *PG*). This gene interaction is consistent with polymeric gene action, where the cumulative effect of both dominant alleles enhances red colouration, while the absence of one or both alleles leads to intermediate or green panicle colour. Polymeric gene action has been widely studied in *Oryza sativa* (rice) and *Zea mays* (maize) for traits such as grain colour and panicle pigmentation, where the combined action of genes produces cumulative phenotypic effects (Sharma *et al.,* 2021; Qiu *et al*., 2023).

Inheritance of Flowering Pattern

The flowering pattern of the $F_1$ plants indicated that late flowering is dominant. In the $F_2$ population, early, late, and intermediate flowering patterns were observed in a 9:3:4 ratio. This points to the involvement of two supplementary genes (*Fl-e* for early flowering and *Fl-l* for late flowering), where their interaction determines flowering time. The epistatic interaction of these genes modifies the expected Mendelian ratio, as

observed in other crops like *Arabidopsis thaliana* and *Triticum aestivum* (wheat), where flowering time is often regulated by multiple gene loci interacting to modulate phenotypic expression (Blümel *et al*., 2015; Sharma *et al*., 2022).

Inheritance of Plant Growth Habit

In the $F_1$ generation, all plants exhibited a spreading growth habit, indicating that this trait is dominant. In the $F_2$ generation, a 13:3 segregation ratio for spreading versus erect plants was observed, which suggests that plant growth habit is controlled by inhibitory gene action involving two dominant and recessive epistatic genes (*PGE* and *PGS*). This altered ratio is a hallmark of inhibitory epistasis, where one gene's expression is suppressed by another. Similar inhibitory gene action has been reported in cotton and *Glycine max* (soybean), where plant architecture is shaped by the interaction between growth habit genes (Naveed*et al*., 2023; Song *et al*., 2021).

Inheritance of Red Leaf Margin

The red leaf margin trait was inherited in a typical Mendelian manner. All $F_1$ plants exhibited red leaf margins, and in the $F_2$ population, the trait segregated in a 3:1 ratio for red to no leaf margin. This suggests that the trait is governed by a single dominant gene (*LMR*), consistent with classical Mendelian inheritance. Studies in *Brassica rapa* and *Capsicum spp.* have also reported single-gene control of pigmentation traits, where dominant alleles produce phenotypic colour expression (Hawk*et.al*.,1982; Singh*et al*., 2020).

Inheritance of Inflorescence Shape

The $F_1$ generation showed uniformly erect inflorescences, while the $F_2$ population exhibited a 9:6:1 ratio of erect, curved, and bushy inflorescences. This suggests that inflorescence shape is controlled by duplicate genes with cumulative effects, designated as *IE* (erect) and *IC* (curved). Such gene interactions, often described as polymeric effects, have been observed in *Sorghum bicolour* and *Panicum miliaceum*, where inflorescence architecture is determined by complex gene interactions that influence floral development (Arya *et al*., 2021).

Inheritance of Inflorescence Spines

The inflorescence spine trait followed inhibitory gene action, as indicated by the 13:3 segregation ratio in the $F_2$ population. All $F_1$ plants displayed flat inflorescences, while the $F_2$ generation included both flat and spiney inflorescences. This suggests that the trait is controlled by two interacting genes (*ISF* and *ISS*), with inhibitory effects that modulate the presence of spines. Inhibitory gene action has been extensively documented in *Helianthus annuus* (sunflower) and research has shown that the presence of spines on safflower leaves and bracts is controlled by a single dominant gene1. This means that the spiny phenotype is dominant over the spineless one (Pahlavani*et.al.,* 2004)

Inheritance of Stem Colour

Stem colour followed a simple Mendelian inheritance pattern, with all $F_1$ plants displaying green stems. In the $F_2$ population, a 3:1 ratio of green to red stems was observed, indicating that stem colour is controlled by a single dominant gene (*SCG*). Similar findings have been reported in *Solanum tuberosum* (potato) and *Glycine max* (soybean), where stem pigmentation is governed by single genes, reflecting classical Mendelian inheritance.

Inheritance of Inflorescence Compactness

The inflorescence compactness trait followed a 3:1 segregation ratio in the $F_2$ generation, with compact inflorescences dominant over spreading ones. This pattern suggests that inflorescence compactness is governed by a single dominant gene (*IC*). Similar single-gene control of inflorescence architecture has been observed in *Sorghum bicolour* and *Triticum aestivum* (wheat), where inflorescence compactness is an important agronomic trait (Li *et al*., 2022; Wang *et al*., 2023).

The obtained ratios of occurrence of various characters are represented in Table 3.

**CONCLUSION**

This study provides a comprehensive understanding of the inheritance of several key morphological traits in plants, revealing the involvement of supplementary, duplicate, and inhibitory gene actions in regulating phenotypic expression. The complex genetic interactions observed here, such

as epistasis and gene complementation, highlight the importance of genetic regulation in plant development. These findings align with existing research in model crops and offer valuable insights into trait improvement for breeding programs. Future studies could explore the molecular mechanisms underlying these gene interactions to further refine plant breeding strategies. Recent advances in genomics and molecular biology could accelerate the identification of genes responsible for these traits, aiding in the development of improved varieties with desirable agronomic characteristics.

In the cross between Suvarna and GA-2, the $F_1$ generation exhibited dominant traits, including green leaf and stem colour, red leaf margins, red panicle colour, an erect and flat inflorescence, a spreading growth habit, and late flowering. These traits were dominant over their contrasting counterparts. Upon analyzing the $F_2$ population, it was observed that leaf colour and flowering time followed supplementary gene action, with a segregation ratio of 9:3:4. Panicle colour and inflorescence shape exhibited polymeric gene action, segregating in a 9:6:1 ratio. However, Gupta and Gudu (1989) reported that inflorescence colour in *Amaranthus hypochondriacus* and *A. caudatus* followed normal Mendelian segregation ratios, considering two contrasting traits. Plant growth habit and the presence of spines on the inflorescence were found to follow inhibitory gene action, segregating in a 13:3 ratio. The remaining traits—red leaf margin, stem colour, and inflorescence compactness—followed standard Mendelian inheritance, segregating in a 3:1 ratio.

The gene symbols for these traits were designated in this study, as no previous work on inheritance patterns in this specific cross (Suvarna X GA-2) was available. This detailed segregation analysis provides valuable insights for genetic characterization, aiding in future breeding efforts and germplasm preservation. The findings will also contribute to identifying key genetic markers for improving agronomic traits in grain Amaranth.

A summary of the gene symbols and inheritance patterns is presented in Table 3.

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**Author contributions**

Madhuchhanda Mishra conceived and designed the study, conducted the experiments, analysed the data, and prepared the manuscript.

Suchismita Chatterjee- Prepared and critically reviewed the manuscript.

Preeti Sagar- Prepared and critically reviewed the manuscript.

A. K. Singh- Critically reviewed the manuscript.

Jitendra Kumar Tiwari- Designed the experiment, critically reviewed and approved the manuscript

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**Ethical approval-** This study did not involve human participants or animals. Ethical approval was therefore not required.

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a.







b.



$F_1$ GENERATION

c.



**$F_2$ GENERATION**

**Figure 1- a. Parents, b. $F_1$ and c. $F_2$**



(a) (b) (c)

**Figure 2- Plants with (a) dark green, (b) green and (c) red leaves**



**(a)** **(b)** **(c)**

**Figure 3- Plants with (a) green, (b) red and (c) intermediate (light pinkish) panicles**




**Figure 4 – Late and Early type plants**

Spreading




**Figure 5- Spreading and erect type plants**



(a) (b)

**Figure 6 – (a) having red leaf margin; (b) without red leaf margin**



**(a) (b) (c)**

**Figure 7 – (a) Erect; (b) Curved and (c) Bushy inflorescence**




**Figure 8 – Spines marked in case of spiney inflorescence**



**(a)** **(b)**

**Figure 9 – Plants having (a) Green stem; (b) Red stem**



(a) (b)

**Figure 10- Plants having (a) Compact inflorescence; (b) Spreading inflorescence**

***Table1*- Parents involved in the study for analysis of segregation ratio.**

| variety | species | Chromosome no. | Released from | remarks |
|---|---|---|---|---|
| Suvarna | *A. cruentus* | 2n = 34 | Banglore,1992 | Green, Plum, drooping and semi spreading panicle having green leaves and stem. |
| GA2 | *A. hypocondriacus* | 2n = 32 | S.K. Nagar, Gujrat, 2002 | Red, erect, compact panicle having redish leaves and stem. |

*Table 2*- **The $\chi^2$ analysis result of different characters**

| **Character** | **$F_1$ reaction** | **$F_2$ reaction** | | | | **Ratio** | **$\chi^2$ value** | **P value** | **df.** |
|---|---|---|---|---|---|---|---|---|---|
| | | No. of plants | | | | | | | |
| | | | | | Total | | | | |
| **Leaf Colour** | Green Leaf | 55(Red leaf) | 84(Dark leaf) | 142 (Green Leaf) | 281 | 3: 4: 9 | 4.421 | 0.1096 | 2 |
| **Panicle Colour** | Red panicle | 169 (Red panicle) | 97 (Green panicle) | 15 (Intermediate panicle) | 281 | 9: 6: 1 | 1.797 | 0.4072 | 2 |
| **Flowering** | Late | 147 (Late) | 85 (Intermediate) | 39 (Early) | 281 | 9: 3: 4 | 0.596 | 0.7424 | 2 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Plant Growth Habit** | Spreading | 234 (Spreading) | 47 (Erect) | | 281 | 13: 3 | 0.759 | 0.3837 | 1 |
| **Red Leaf Margin** | Having Red Leaf Margin | 201 (with Red Leaf Margin) | 80 (Without Red Leaf margin) | | 281 | 3: 1 | 1.903 | 0.1678 | 1 |
| **Inflorescence Shape** | Erect | 184 (Erect) | 81 (Curved) | 13 (Bushy) | 281 | 9:6:1 | 5.187 | 0.0747 | 2 |
| **Inflorescence Spiney** | Flat | 227 (Flat) | 54 (Spiney) | | 281 | 13: 3 | 0.023 | 0.8788 | 1 |
| **Stem Colour** | Green | 221(Green) | 60 (Green) | | 281 | 3: 1 | 1.994 | 0.1579 | 1 |
| **Inflorescence Compactness** | Compact | 202(Compact) | 79(Spreading) | | 281 | 3: 1 | 1.453 | 0.2280 | 1 |

**Table 3- various characters and designated genes responsible for the characters**

| CHARACTER | Gene designated as |
|---|---|
| 1. Leaf Colour | *lc-g* and *lc-r* |
| 2. Panicle colour | $P_R$ and $P_G$ |
| 3. Flowering time | *Fl-e* and *Fl-l* |
| 4. Plant growth habit | $PG_E$ and $PG_S$ |
| 5. Inflorescence shape | $I_E$ and $I_C$ |
| 6. Inflorescence spiney | $IS_F$ and $IS_S$ |
| 7. Stem Colour | $SC_g$ |
| 8. Inflorescence compactness | IC |