

**ARTIFICIAL INDUCTION AND CHARACTERIZATION OF VARIEGATION IN  
RADERMACHERA SINICA AND PORTULACARIA AFRA**

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**ABSTRACT**

*Leaf variegation is a biological phenomenon characterized by different coloured sectors within same leaf with normal green tissues due to variations in pigment accumulation or chloroplast development. Variegation arises through genetic mutations, plastid genome instability, chimeric tissue organization, physiological stress through environmental and chemical factors affecting pigment biosynthesis. Mutations in nuclear genes regulating chloroplast development can produce characteristic green-white mosaic patterns within leaves. Leaf variegation is significant in horticulture as variegated ornamental plants are highly valued in the global horticultural market. The unique colour patterns enhance aesthetic appeal in landscaping and indoor plant collections. Several studies describe chemical induction of chlorophyll-deficient tissues using antibiotics, mutagens, or herbicides. Reproducible protocols for generating stable variegated patterns across plant species are limited. Exploring chemical treatments and mutation-inducing methods is an important research direction in plant biotechnology. A multidisciplinary approach incorporating chemical treatments, mutagenesis experiments, pigment analysis, and computational gene analysis provides comprehensive understanding of the mechanisms underlying pigment instability and variegation. The study includes Radermachera sinica, a tropical ornamental plant widely grown indoors due to its glossy compound leaves and compact growth habit as well as Portulacaria afra, a succulent plant widely cultivated ornamental indoor plant due to its thick fleshy leaves. The plant mutagenesis was understood and applied in ancient India. In ancient treatises the Sanskrit term “drumavichitrikaranam” is described as drumava means a ‘tree’ and chitrikaranam means ‘to make appear extraordinary’. It indicates an alteration in the natural trait of the tree and the term can be parallel to plant mutagenesis.*

**Keywords:** mutagenesis, horticulture, variegation

**INTRODUCTION**

In ancient India, the term ‘drumavichitrikaranam’ was applied and described in several treatises. It is synonymousto the term plant mutagenesis in modern science. The Sanskrit word “drumavichitrikaranam” is composed of two words, drumava means a tree and chitrikaranam means to make (it) appear extraordinary. It indicates an alteration in the natural trait of the tree. The Vedic sage Kaśyapa introduced the idea of drumavichitrikaranam. It was described in Harivamśapurānathat sage Kaśyapa transformed a Mandāra tree (*Erythrinavariegata*) into a Kovidāra tree (*Bauhinia variegata*) (Ranganathan, 2018).

Ranganathan (2018) evaluated ancient texts to understand various purposes for plant mutations in ancient India.

1. Sūrapāla’s Vrikshāyurveda (1000 CE)
2. Chavundarāya’s Lokopakāra (1025 CE)
3. Someśvaradeva’s Mānasollāsa (1131 CE)
4. Śārngadhara’s Upavanavinoda (1300 CE)
5. Chakrapāni Mīśra’s Viśvavallabhavrksāyurveda (1580 CE)
6. Basavarāja’s Śivatattvaratnākara (1694-1714 CE)

Many treatises explain some procedures of drumavichitrikaranam in the chapter of agriculture while certain treatises contain a separate chapter on drumavichitrikaranam. In ancient India some of the principles in drumavichitrikaranam were used to alter the colour of fruits or flowers (Ranganathan, 2018).

Variegation in plants means you’ll see streaks or patches of different colours—think green, white, yellow, or even red—showing up on the same leaf or stem (Zhou *et al.*, 2024; Chen *et al.*, 2012). This happens because pigments aren’t evenly spread out. The patterns you notice show up mainly due to changes in the amounts of chlorophyll, carotenoids, and anthocyanins, which create that mosaic effect. Some areas turn completely white when there’s no chlorophyll, others lose pigment and look pale or yellow, and spots rich in anthocyanin take on red or purple hues.

Plants can end up variegated for lots of reasons. Sometimes, it’s locked in their genes—mutations in either the nuclear or the plastid DNA can mess up chloroplast formation or pigment production.

Other times, you get a “chimeric” effect, with different genetic layers in the same tissue forming a patchwork look (Yu *et al.*, 2007; Zhou *et al.*, 2024). There’s also structural variegation, where the anatomy of the leaf itself changes how light bounces off. And honestly, the environment plays a part too. Excess light, drastic changes in temperature or oxidative stress can all damage chloroplasts and make pigments disappear (Aluru *et al.*, 2009; Sato *et al.*, 2015).

Variegation usually traces back to problems with chloroplast creation or pigment-making pathways. Certain nuclear genes—like VAR2, IMMUTANS, CLA1, and PDS—are central to how chloroplasts develop. VAR2 makes an FtsH protease that helps regulate thylakoid proteins; IMMUTANS is crucial for a step in carotenoid biosynthesis; PDS does carotenoid desaturation; and CLA1 supports the MEP pathway, which is key for making chlorophyll and carotenoids. When these genes don’t work right, chloroplasts can’t form properly and pigments don’t accumulate, so you get that classic variegated pattern (Chen *et al.*, 2000; Wu *et al.*, 1999; Yu *et al.*, 2007; Zhou *et al.*, 2024).

Chemical treatments can trigger variegation by interrupting how plants make pigments and keep chloroplasts stable. Things like 2,4-D, gibberellins, borax, colchicine, trichloroacetic acid, and hydrogen peroxide mess with hormones, shift cellular metabolism, and cause oxidative stress. That is how they break down pigments or throw plastid development off track. Using these chemicals gives researchers a predictable way to create variegation in the lab.

Variegated plants matter a lot in ornamental horticulture and plant science. Their unique patterns make them more valuable for growers, sure, but they also help researchers study things like chloroplast development, pigment pathways, and how the cell nucleus talks with plastids (Zhou *et al.*, 2024; Yu *et al.*, 2007). Most research sticks to the usual model plants, and we don’t see much attention on other ornamentals.

The study steps into that gap by looking at variegation in two very different ornamental species: *Radermachera sinica* and *Portulacaria afra*. These plants react differently to stress and have distinct physiologies, so comparing them sheds light on how variegation works across species. The main goal is to dig into the molecular, biochemical, and physiological details behind variegation and to figure out how to reliably induce and stabilize these patterns using chemical treatments.

## MATERIAL AND METHOD

### 2.1 Experimental Design

The six chemicals—2,4-D, hydrogen peroxide, TCA, borax, gibberellic acid, and colchicines were tested at three concentrations to study their effect on variegation in *Radermachera sinica* and *Portulacaria afra*. For *R. sinica*, the plants in rows were lined up, setting up duplicates for each treatment and including a control group. With *P. afra*, a completely randomized design, running each treatment in triplicate was used.

### 2.2 Preparation and Application of Chemical Treatments

All working solutions were prepared fresh, making up to 100 mL with distilled water. Those were sprayed directly onto the young leaves. Each treatment was given on regular intervals: borax, gibberellin, and hydrogen peroxide treatment was given every week while TCA, colchicine and 2,4-D were sprayed every two weeks.

### 2.3 Pigment Analysis

The colorimetric method was used to measure pigment levels. 0.5 grams of fresh leaf tissue was homogenized in a petroleum ether–acetone mix (1:1), filtered the mixture, and adjusted the final volume to 10 mL (using a 4:1 ratio). Then, absorbance was recorded at 645 nm and 655 nm for chlorophyll measurement, and at 480 nm and 495 nm for carotenoids measurement. The pigment concentrations are expressed as mg per gram of fresh weight.

### 2.4 In-silico Gene Analysis

The variegation-related genes were analyzed using in-silico approaches. The candidate genes were selected from *Arabidopsis thaliana* and *Portulacaria afra*. Using BLAST and domain analysis, homologous sequences and conserved domains were searched. These molecular findings were matched with phenotypic changes observed in the plants.

## RESULTS

### 3.1 Initial Pigment Content in Control Plants

Control plants of *Radermachera sinica* and *Portulacaria afra* exhibited stable and uniform pigment composition prior to chemical treatment. Comparative analysis indicated that *Radermachera sinica* possessed relatively higher total chlorophyll and  $\beta$ -carotene content, whereas *Portulacaria afra* showed comparatively

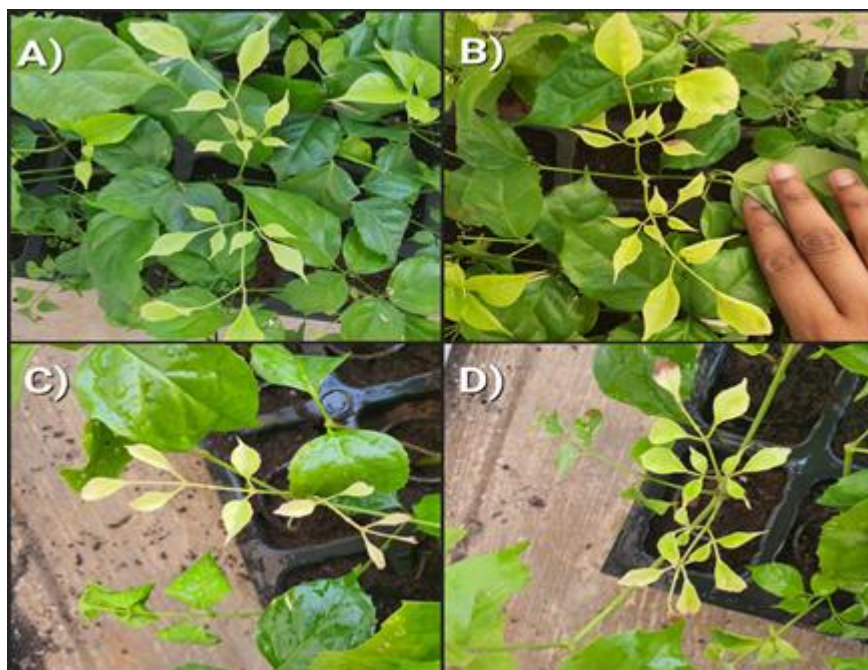
lower baseline pigment levels. The uniformity in pigment composition across replicates confirmed the physiological stability of the plants and provided a reliable baseline for assessing treatment-induced changes.

### 3.2 Morphological and Visual Changes Following Chemical Treatment

#### 3.2.1 *Radermachera sinica*

In *Radermachera sinica*, chemical treatments induced distinct pigmentation changes primarily in young and developing leaves. Early responses, observed within 7–10 days of treatment, included a gradual reduction in green pigmentation, resulting in yellowish to pale white coloration. These effects were particularly prominent under high concentrations of gibberellin and medium concentrations of 2,4-D.

In several cases, the discoloration was uniform across the leaf lamina, indicating a generalized reduction in chlorophyll content. With continued exposure, irregular pale or white patches developed, which in certain treatments progressed into brown, necrotic regions, especially under borax application. Additionally, localized red pigmentation was observed along leaf margins and tips in plants treated with gibberellin and 2,4-D, resulting in multicoloured variegation patterns.



**Figure 1:** Pigmentation and morphological changes observed in *Radermachera sinica* following chemical treatments: (A) uniform chlorophyll reduction in young leaves, (B) effect of gibberellin treatment on leaf coloration, and (C–D) variegation patterns induced by 2,4-D treatment.

#### 3.2.2 *Portulacaria afra*

Chemical treatments on *Portulacaria afra* mostly changed the plant's shape instead of causing major pigment loss. When exposed to high levels of borax, new leaves came out distorted, looking quite different from those on untreated plants. Gibberellin pushed the plant's internodes to stretch out, so the leaves turned longer, thinner, less juicy, and shifted to a lighter green or even yellowish tone. Hydrogen peroxide and trichloroacetic acid didn't do much to colour, but they did cause slight discoloration and created some irregularities inside the tissues, especially in younger leaves. In every case, stronger concentrations led to more drastic changes.



**Figure 2:** Morphological alterations observed in *Portulacaria afra* following chemical treatments: (A) leaf shape distortion under high concentration of borax, and (B) changes in growth pattern and plant architecture under gibberellin treatment.

### 3.3 Effect of Chemical Treatments on Pigment Content

The pigment analysis of leaves of *Portulacaria afra* was carried out. Leaves of *Radermachera sinica* were not mature enough. Chlorophyll and carotenoid levels were changed drastically depending on the treatment. High gibberellin led to the lowest chlorophyll, while medium doses of 2,4-D showed higher levels of chlorophyll. When used at high concentrations, hydrogen peroxide and borax boosted  $\beta$ -carotene.

**Table 1:** Effect of chemical treatments on chlorophyll content (chlorophyll a, chlorophyll b, and total chlorophyll; mg/L) in *Portulacaria afra* after treatment.

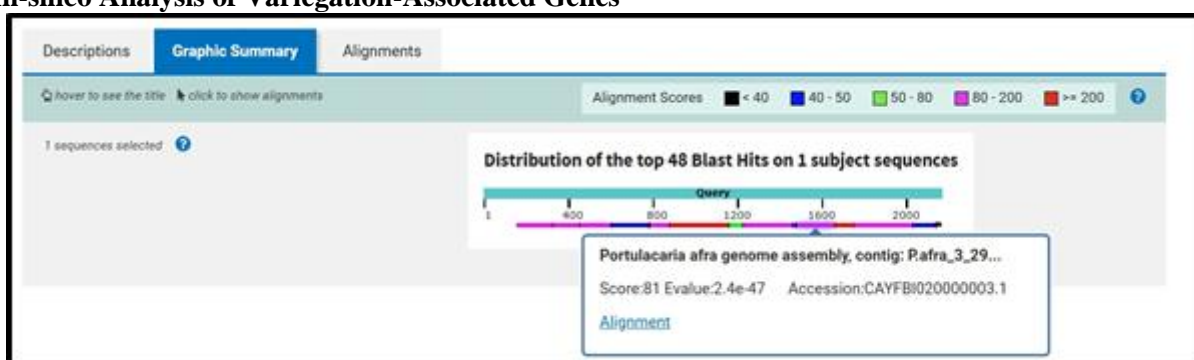
Treatment	Concentration	Chlorophyll a (mg/L)	Chlorophyll b (mg/L)	Total Chlorophyll (mg/L)
H <sub>2</sub> O <sub>2</sub>	High	1.98	0.21	2.25
	Medium	1.86	0.94	2.80
	Low	2.89	0.44	3.98
Colchicine	High	1.77	0.86	3.68
	Medium	1.70	1.13	2.87
	Low	1.77	1.01	2.87
Borax	High	1.14	1.50	2.69
	Medium	1.21	1.24	2.10
	Low	1.77	1.20	2.98
2,4-D	High	1.18	0.97	2.35
	Medium	2.66	1.15	5.38
	Low	1.54	1.12	2.90
GA <sub>3</sub>	High	0.30	0.09	0.40
	Medium	0.15	0.51	0.68
	Low	0.41	1.05	1.17
TCA	High	1.52	0.48	2.00
	Medium	1.75	0.79	2.54
	Low	2.17	1.04	3.21

**Table 2:** Effect of chemical treatments on  $\beta$ -carotene content (mg/L) in *Portulacaria afra* after treatment

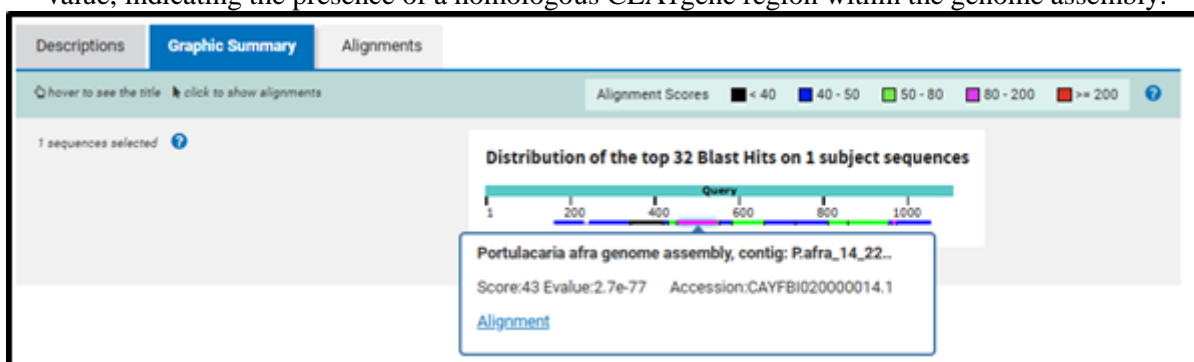
CHEMICALS	CONCENTRATION	$\beta$ -CAROTENE
H <sub>2</sub> O <sub>2</sub>	High	8.40
	Medium	1.50
	Low	1.80
	High	7.30

Colchicine	Medium	0.40
	Low	7.10
Borax	High	4.20
	Medium	1.40
2,4-D	Low	5.90
	High	2.60
GA <sub>3</sub>	Medium	4.90
	Low	0.90
TCA	High	-0.60
	Medium	5.80
	Low	0.20
	High	0.90
	Medium	-0.50
	Low	6.40

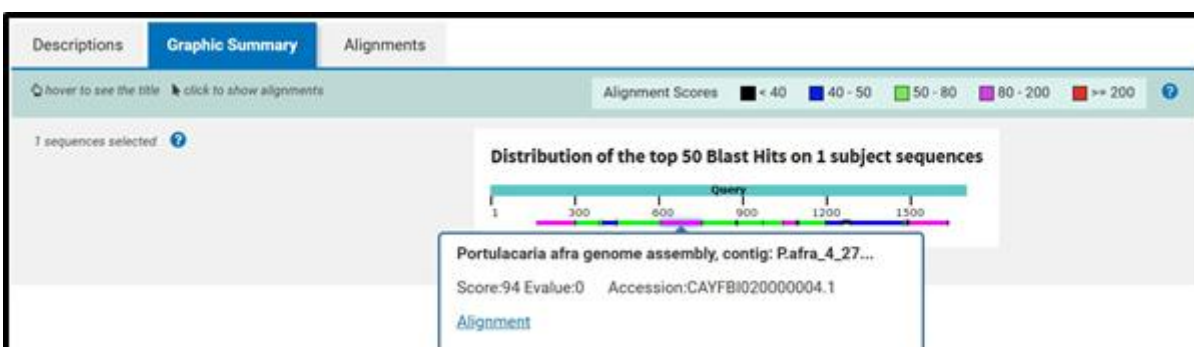
### 3.4 In-silico Analysis of Variegation-Associated Genes



**Figure 2 :** BLAST graphical summary showing alignment of the CLA1 gene sequence against the Portulacaria afra genome. The alignment exhibits high sequence similarity with a significant alignment score and low E-value, indicating the presence of a homologous CLA1 gene region within the genome assembly.



**Figure 4 :** BLAST graphical summary showing alignment of the IMMUTANS (IM) gene sequence against the Portulacaria afra genome. The result demonstrates significant sequence similarity with a high alignment score and low E-value, indicating the presence of a homologous IM gene region within the genome assembly.

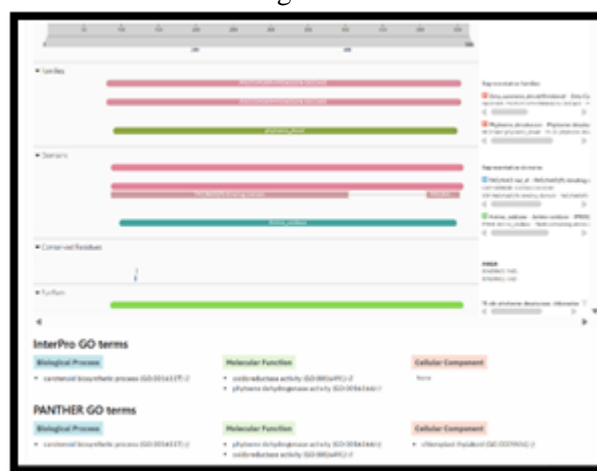


**Figure 3 :** BLAST graphical summary showing alignment of the PDS (phytoene desaturase) gene sequence against the Portulacaria afra genome. The alignment shows a high alignment score with an E-value of zero,





**Figure 9 :** Domain architecture of VAR2 protein showing conserved FtsH protease-related domains identified using InterPro.



**Figure 10:** Domain architecture of PDS protein showing conserved oxidoreductase domains involved in carotenoid biosynthesis.

Domain analysis confirmed the presence of conserved functional regions, including Alternative Oxidase (AOX) domains in IMMUTANS, MEP pathway-related domains in CLA1, FtsH protease domains in VAR2, and oxidoreductase domains in PDS. These domains are associated with chloroplast development and pigment biosynthesis.

The study found that chemical treatments changed the shape and colour of *Portulacaria afra* in a way that depended on how much was used. These changes also showed up in the levels of chlorophyll and carotenoids. Computer analysis pointed to four main genes IMMUTANS, CLA1, VAR2, and PDS as important for chloroplast development and pigment production.

## DISCUSSION

The study shows that chemical treatments can trigger both morphological and pigmentation changes tied to variegation in *Portulacaria afra*. The plants reacted differently depending on the type and concentration of each treatment. When exposed to higher concentrations, we saw clearer signs of leaf chlorosis, bleaching, and structural twists. These visible changes matched up with measured drops in chlorophyll and shifts in carotenoid levels. Gibberellin (GA<sub>3</sub>) and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), in particular, caused significant chlorophyll loss. Gibberellin likely disrupts how chloroplasts differentiate, while H<sub>2</sub>O<sub>2</sub> causes oxidative damage to chloroplast membranes and photosynthetic parts, speeding up pigment breakdown.

Borax produced leaf distortion and moderate pigment changes, reflecting interference with cellular metabolism and membrane stability. Colchicine, by blocking mitosis, led to chimeric tissues and visible variegation but didn't cause heavy pigment loss. Trichloroacetic acid (TCA) also hampered plastid development, causing moderate shifts in pigmentation. With 2,4-D, the effects depended on how much was used: at moderate concentrations, chlorophyll content stayed relatively higher, a sign of its indirect hormonal effects on chloroplast development.

On the molecular side, variegation seems tied to disruptions in key nuclear genes that control chloroplast function and pigment biosynthesis namely IMMUTANS (IM), CLA1, VAR2, and PDS. These genes are central to carotenoid biosynthesis, chloroplast formation, and protein management. IMMUTANS makes a plastid terminal oxidase needed for carotenoid desaturation; PDS also serves in carotenoid production and photoprotection. CLA1 operates in the MEP pathway to provide pigment synthesis precursors, while VAR2 encodes an FtsH protease that ensures thylakoid protein quality. Computer analyses confirmed these genes exist in *Portulacaria afra*. BLAST searches and domain checks revealed conserved functional regions such as AOX, FtsH protease, and oxidoreductase domains, reinforcing their roles in pigment metabolism and chloroplast upkeep. These are predictions based on sequence homology. Real confirmation depends on further experiments examining actual gene expression or function.

Pigment and in-silico gene analyses focused solely on *Portulacaria afra*, since *Radermachera sinica* leaves were not at the right developmental stage and lacked comprehensive genomic data. Altogether, the results point to a network of factors oxidative stress, hormone balance, disturbed plastid development, and pigment synthesis driving variegation. By combining morphological observations, biochemical assays, and bioinformatics, we get a broad picture of variegation's triggers. Nonetheless, direct molecular studies are still needed to nail down the precise mechanisms.

### CONCLUSION

The study shows that chemical treatments can trigger noticeable changes in the appearance and colour of *Radermachera sinica* and *Portulacaria afra*, though the results vary depending on the treatment. *Radermachera sinica* reacted fast, with clear signs like leaf yellowing, bleaching, and patchy colouring. *Portulacaria afra*, on the other hand, changed both in shape and in its levels of chlorophyll and carotenoids clearly indicating that its pigment production and chloroplast health took a hit.

The in-silico work confirmed that *Portulacaria afra* has several important variegation genes IMMUTANS, CLA1, VAR2, and PDS all of which play a part in controlling pigments and developing chloroplasts. While the study did not experimentally prove how these genes work in the process, the physical, chemical, and computational observations together provide a solid initial research for artificially creating variegated plants.

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