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# MECHANISM OF BRACT REDDENING IN GUZMANIA

**JIANXINLIU[1,2*], GUOFUWANG[1], SHUYUCAI[1] , SHAOQUANHU[1] ANDWEIYONGWANG[2]**

[1]*Yuanpei College, Shaoxing University, Shaoxing, 312000, Zhejiang, P.R. China*
[2]*Flower Research and Development Center, Zhejiang Academy of Agricultural Science, 311202, Hangzhou, P.R. China*
[*]*Corresponding author's e-mail: liujianxin@usx.edu.cn*

**Abstract**

To clarify the mechanism of *Guzmania* bract reddening, we conducted the following investigation from three aspects. First, we determined the pigments contained in the red *Guzmania* bracts, observed tissue anatomy, and performed the color reaction test, spectral scanning analysis, and HPLC (High Performance Liquid Chromatography) analysis of the bracts. The results confirmed that the red substance distributed in the fence tissue of the bracts is anthocyanin (a flavonoid substance with an absorption wavelength of 510nm) composed of cyanidin (red) and pelargonium (orange-red), the yellow substances distributed throughout the mesophyll tissue of the bracts are mainly flavones (absorption wavelengths of 291nm and 328nm) and carotenoids (absorption wavelengths of 438nm and 467nm), and the green substance distributed in sponge tissue of the bracts is chlorophyll (absorption wavelengths of 440nm and 468nm). Second, to explore the molecular mechanisms of changes in flavonoids and chlorophyll, we used HPLC, RT-Qpcr (Quantitative Reverse Transcription Polymerase Chain Reaction), and other methods to detect changes in flavonoids and chlorophyll contents and the gene expression of key enzymes for corresponding metabolic processes. The results confirmed that bract reddening was caused by accumulated flavonoids and reduced chlorophyll content, while the reduction in chlorophyll was caused by a decrease in chlorophyll synthesis and a significant increase in chlorophyll degradation. Finally, we examined dynamic changes in ethylene release from the bracts and expression patterns of key enzymatic genes in ethylene biosynthesis. It was confirmed that the biosynthesis of ethylene increased in the bracts, further promoting the process of bract reddening. This study on the mechanism of bract color formation has practical significance for improving bract color and ornamental quality through breeding and guiding production practices.

**Key words:** Flavonoids, Chlorophyll, Ethylene, Anthocyanin, Ornamental Bromeliads.

## Introduction

Guzmania Ruiz & Pav., a kind of perennial green plant native to tropical and subtropical regions of the Americas, is a high-grade flower belonging to the *Bromeliaceae Juss*. family. The main ornamental parts are colored bracts (Shen *et al*., 2013). Floral bracts have a variety of colors, shapes, and scents to ensure successful reproduction, in addition to their function to protect the flowers (Elabon *et al*., 2011; Neves *et al*., 2020). The colored bracts of 'Ostara,' aprincipal variety of Guzmania, are red. Formation of the red portion is often accompanied by the fading of the green part of a bract, and eventually, red coloration diffuses further throughout the entire bract. The bract is a specialized leaf or leaf-like part. Pigments related to leaf color include chlorophyll, flavonoids, carotenoids, water-soluble alkaloids, and their derivatives (Zhao *et al*., 2003; Tanaka *et al*., 2008; Tanaka *et al*., 2009; Viniciu*set al*., 2019). Moreover, leaf color is also affected by leaf pH and environmental factors such as light and temperature (Zhao *et al*., 2005). Research on some colored plants revealed that the depth of leaf color is often determined by anthocyanin/chlorophyll ratio (Hu *et al*., 2007; Ji *et al*., 2010). Anthocyanins are flavonoid substances whose proportion can change the final colors of flowers or leaves to a great extent and reflect most red, blue, purple, and red-violet colors (Gillian & CooPer-Driver., 2001; Tanaka *et al*., 2010). For example, pelargonidin, cyanidin and delphinidin show orange-red, red and purple color, respectively. The flowering process of plants was often accompanied by significant changes in endogenous hormones. For example, in many plants, ethylene has the effect of promoting flowering. In the commercial production of Guzmania, many means of increasing ethylene content are often used to promote early flowering (Kuan *et al*., 2005; Danijela *et al*., 2006; Yuri & Jose, 2006). Therefore, the release of endogenous ethylene may be related to flowering in Guzmania.

At present, metabolic mechanisms such as flavonoid biosynthesis and carotenoid biosynthesis related to flower color formation have been researched in-depth, but relatively few studies have been conducted on leaf color. Moreover, these studies have mainly focused on leaf color changes of plants rather than on the colors of the bracts, which are specialized leaves in plants. Only individual species have been studied, for example, malvidin 3-rutinoside related to bract color formation in *Curcuma alismatifolia* (Nakayama *et al*., 2000) and the separation of bract anthocyanidins in poinsettia (Wang & Wei, 2006).

Since bracts are the main ornamental parts of bromeliads, investigation of the mechanism of bract color formation will have practical significance for improving bract color and ornamental quality, thus guiding commercial Guzmania production. Moreover, it also has great significance for exploring mechanisms of bract color formation in other ornamental bromeliads and bract plants.

## Materials and Methods

The Guzmania Ruiz & Pav. variety 'Ostara' was used as the test material, acquired from the Bromeliaceaegermplasm resource nursery of Flower Research and Development Center, Zhejiang Academy of Agricultural Science.

**Anatomical and biochemical analyses of red bracts**

**Anatomical analysis of red bracts:** Cross-section tissues of green bracts, semi-red bracts, and full red bracts were cut by freehand slicing and observed under a microscope (Upright microscope Olympus BX51TF, eyepiece x20, objective lens x10).

**Biochemical analysis of red bracts**

**Color reaction test of red bracts:** Qualitative reaction to pigment composition was tested according to Bai Xinxiang's method (Bai *et al.,* 2006). Specifically, red bracts (0.100 g) were ground into powder and were incubated in 5mL petroleum ether, 10.0% hydrochloric acid, and 30.0% ammonia water in test tubes with stoppers. Quickly ground to homogenate and filter. Finally, the color of the filtrate was observed and recorded.

**Spectral scanning analysis of red bract pigments** (Bai *et al.,* 2006)**].**

**Detection of chlorophyll:** Fresh red bracts (0.100 g) were ground into powder and extracted with 5 mL acetone: ethanol mixture (acetone: ethanol = 9:1, volumetric ratio) and then scanned at 400~700 nm with Spectra Max® Plus 384 microplate reader from Molecular Devices in the United States. The light path of the cuvette was 1cm (Zhao *et al.,* 2004).

**Measurement of carotenoids:** Freshred bracts (0.100 g) were ground into powder and extracted with 10 mL petroleum ether acetone mixture (petroleum ether: acetone = 1: 1, volumetric ratio). Then the samples were scanned in the range of 200~700 nm wavelength as described previously (Zhao *et al.,* 2004).

**Detection of flavonoids:** Red bracts (0.100 g) were sampled and immersed in 2 mL hydrochloric acid: methanol solution (HCl: MeOH = 1: 99, volumetric ratio) for 24 h in the dark and at room temperature (approximately 25°C). Subsequently, the extraction solution was diluted to 10 mL and scanned in the range of 220-600 nm wave length (Zhao *et al.,* 2004)

**HPLC analysis of pigments in red bracts:** The HPLC method was used to detect related products in flavonoid metabolic pathways of green bracts, semi-red bracts, and red bracts (Zhao *et al.,* 2017). The extraction process was as follows: each sample was acidified with 2MHCl in 80% aqueous acetone solution and homogenized for 5 min. Each supernatant was collected by centrifuging 5 times (×5000rpm) and then concentrated in a vacuum rotary evaporator. Each extraction solution was finally fixed to 10 mL with 2MHCl acidified water.

Catechin was used as a standard reference to determine the content of cyanidin, pelargonium, delphinidin, flavonoids, phenol, naringenin, aromadendrin, dihydroquercetin (intermediate product), dihydromyricetin (intermediate product), quercetin, myricetin, kaempferol, etc. The instruments used included a 1525 Waters Binary Pump 1525, a 2707 autosampler, a 2998 PAD detector, a 1500 Temperature Control Box, and a Sun FireTMC18 detection column (4.6 mm × 250 mm, 5μm). The mobile phase composition was A-0.1% trifluoroacetic acid (water phase) and B-acetonitrile. The gradient elution procedure was as follows: 0-3 min, 5% B; 3-20 min, 5% -25% B; 20-42 min, 25% -95% B; 42-45 min, 95% B; 45-50 min, 95%-5% B; 50-60 min, 5% B. The flow rate was 1.0 mL/min$^{-1}$, and the sample volume injected was 20 μL.

**Dynamic changes in chlorophyll content, flavonoid pigment contents, and expression patterns of key genes in metabolic pathways during the bract reddening process.**

**Dynamic changes in chlorophyll content, flavonoid pigment contents:** Chlorophyll content of the green, half red, and full red bracts of 'Ostara' flowering plants were determined used SPAD-502 Chlorophyll Tester (Konica, Japan), and that of green leaves were also measured as a control. Randomly took 5 points for each material to measure (exclude edges, stains, and other abnormal parts), take the average, and then repeated parallel sampling and testing 3 times, and finally performed statistical analysis.

In addition, the content of flavonoids was determined by the HPLC method, with catechin as the standard reference. The specific aspects are the same as the previous HPLC analysis of pigments in red bracts (Liu *et al.,* 2016).

**Expression patterns of key genes in metabolic pathways during the bract reddening process**

**Expression patterns of key genes in flavonoid metabolic pathways during the bract reddening process:** Three key genes in the metabolic pathway of flavonoids were screened out: CHS (chalcone synthase gene, GenBank: KX364270) (Hu *et al.,* 2012; Wiriyaampaiwong *et al.,* 2012; Zhou *et al.,* 2020), F3'H (flavanone 3-β-hydroxylase gene, GenBank: KX364271) (Han *et al.,* 2010; Hu *et al.,* 2015) and DFR (Dihydroflavonol 4-reductase gene, GenBank: KX364272) (Shimadal *et al.,* 2005; Zhang *et al.,* 2015), and then performed RT-qPCR test to detect their expression changes during the process of the bracts reddening process (Liu *et al.,* 2006; Hou *et al.,* 2011; Liu *et al.,* 2017a). The experiment process was as follows:

**Total RNA and cDNA acquisition:** Trizol reagent (Beijing Dingguo Changsheng Biotechnology Co., Ltd., NEP019-2, China) was used to extract total RNA from tested tissues (including green, half red, full red bracts, and green leaves as controls). Then used ultraviolet spectrometry and agarose gel electrophoresis to detect its concentration, purity, and integrity characteristics. Finally, after using DNase to eliminate potential DNA contamination, used TOYOBO Reverse Transcription Kit to reverse-transcribed total RNA into cDNA.

**RT-qPCR reaction:** First, design quantitative PCR primers as shown in Table 1, in which the housekeeping gene actin was used as an internal reference (Nakayama *et al.,* 2000).

The cDNA obtained above was used as a template for ordinary PCR amplification. After the amplified products were identified by agarose gel electrophoresis, they were diluted 5 times successively with a 10-fold concentration gradient and used as a template for the subsequent common PCR reaction. The common PCR reaction was performed to prepare the standard curve of each target gene (System 9600, Perkin Elmer, USA). Finally, RT-qPCR was performed on the PRISM 7700 Real-time PCR System (Applied Biosystems, USA) to detect the expression changes of each gene. The RT-qPCR reaction system (25ul) includes 1ulcDNA, 0.5ul primer-F (20pmol/ul), 0.5ul primer-R (20pmol/ul), 12.5ul2×mix, 1ulSybr Green I(10×), and 9.5ul dd$H_2O$. The corresponding PCR program is shown in Table 2.

**Expression patterns of key genes in chlorophyll metabolic pathways during the bract reddening process:**The experiment first screened out key genes *GTS* (glutamyl-tRNA synthetase, GenBank: KP144289) and *UROS* (uroporphyrinogen-III synthase, GenBank: KP144288) (Ohmiya *et al.,* 2014) in the process of chlorophyll synthesis and key gene *PPH* (pheophytinase, GenBank: KP723523) (Nancy *et al.,* 2009; Schelbert *et al.,* 2009; Asumi *et al.,* 2010) in the process of degradation (Liu *et al.,* 2016), and then performed RT-qPCR reaction on these three genes to detect their expression changes during the process of bract reddening. The designed RT-qPCR primers and corresponding procedures are also shown in Table 1 and Table 2, respectively. The specific test process was the same as previously noted.

**Dynamic changes in ethylene release during bract reddening and real-time quantitative expression analysis of *ACO* (ACC oxidase gene), a key enzyme gene in ethylene biosynthesis** (Kazumi *et al.,* 2007; Yang & Bradford, 2008; Liu *et al.,* 2017b; Saleem *et al.,* 2018).

**Measurements of dynamic changes in ethylene release levels:** Ethylene content released from leaves or bracts in vitro was measured with an ethylene measuring instrument (YUANTESKY2000-C2H4, Shenzhen, China). The specific method of ethylene content determination was as follows: cut 10 leaves or bracts from each test sample, and trimmed their incisions surface to ensure that their total weight and incision area were the same. Then put them in a closed glass container (about 600ml volume of space). Finally, measured and recorded the released ethylene content at intervals of 1 h until the released ethylene content becomes 0. Repeated the test 3 times and perform statistical analysis using Excel 2007.

**Table 1. Primers sequence for RT-qPCR reaction.**

| S. No. | Metabolism | Genes | Primer (5′-3′) | Product length (bp) |
|---|---|---|---|---|
| 1. | Chlorophyll synthesis and degradation | *ACTIN* | TTCCTCATCGTCGTCCC<br>CCAACCATCACTCCCGT’ | 217 |
| | | *GTS* | AAGCAACGAGTTTGTGGAGAA<br>GAACCAATACGATGATGAGGA | 375 |
| | | *UROS* | CACTGAGTTTGACTGGATTG<br>CTCTTGTGAGTTGATTTTGG | 234 |
| | | *PPH* | ACTCTCTTGGAGGCTTTG<br>GGGGTCGCTTATTTTACT | 218 |
| 2. | Anthocyanin synthesis | *ACTIN* | CGGCTATGTATGTTGCT<br>CTGCTGTGGTGGTGAAG | 225 |
| | | *CHS* | GACCTGGCAGAGAACAA<br>TCCCTAACGGAGTGAAT | 349 |
| | | *F3’H* | GAGAAGGACAAGGAGAATG<br>TGGACTGATAGCAGAAACA | 155 |
| | | *DFR* | AGGGGAGGCTGAGAGATT<br>TTGCTGGGATTGGAAAGA | 194 |
| 3. | Ethylene synthesis | *ACTIN* | TTCCTCATCGTCGTCCC<br>CCAACCATCACTCCCGT’ | 217 |
| | | *ACO* | GCCCCCGACCCCCTACT<br>CGCCGCCTTCTCAACCA | 193 |

Note: Actin was used as an internal control

**Table 2. RT-qPCR procedures.**

| S.No. | Metabolism | Genes | Pre denaturation | Denaturation | Annealing | Extension | Cycles | Final extending |
|---|---|---|---|---|---|---|---|---|
| 1. | Chlorophyll synthesis and degradation | *Actin* | 94°C, 2 min | 94 °C, 30 s | 53 °C, 30 s | 72 °C, 30 s | 35 | 72 °C, 10 min |
| | | *GTS* | 94 °C, 2 min | 94 °C, 30 s | 53 °C, 30 s | 72 °C, 30 s | 35 | 72 °C, 10 min |
| | | *UROS* | 94 °C, 2 min | 94 °C, 30 s | 47 °C, 30 s | 72 °C, 30 s | 35 | 72 °C, 10 min |
| | | *PPH* | 94 °C, 2 min | 94 °C, 30 s | 50 °C, 30 s | 72 °C, 30 s | 35 | 72 °C, 10 min |
| 2. | Anthocyanin synthesis | *Actin* | 94 °C, 2 min | 94 °C, 30 s | 50 °C, 30 s | 72 °C, 30 s | 35 | 72 °C, 10 min |
| | | *CHS* | 94 °C, 2 min | 94 °C, 30 s | 47 °C, 30 s | 72 °C, 30 s | 35 | 72 °C, 10 min |
| | | *F3’H* | 94 °C, 2 min | 94 °C, 30 s | 47 °C, 30 s | 72 °C, 30 s | 35 | 72 °C, 10 min |
| | | *DFR* | 94 °C, 2 min | 94 °C, 30 s | 53 °C, 30 s | 72 °C, 30 s | 35 | 72 °C, 10 min |
| 3. | Ethylene synthesis | *Actin* | 94 °C, 2min | 94 °C, 30s | 53 °C, 30s | 72 °C, 30s | 35 | 72 °C, 10min |
| | | *ACO* | 94 °C, 2min | 94 °C, 30s | 53 °C, 30s | 72 °C, 30s | 35 | 72 °C, 10min |

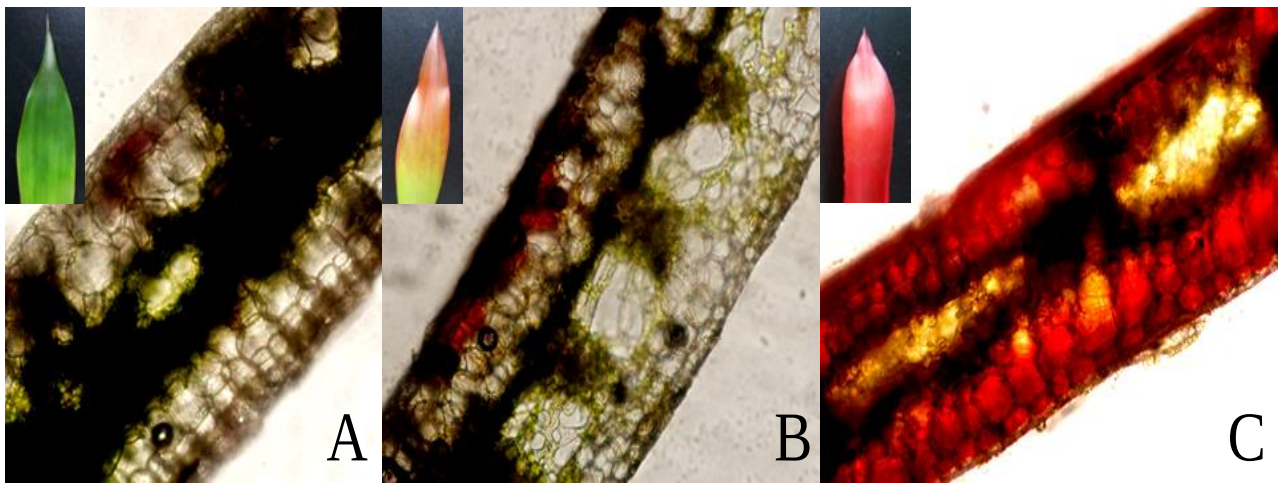



Fig. 1. Bracts of different colors and their cross sections (200×).
Note: A—green bracts; B—semi-red bracts; C—full red bracts; microscopic magnification 200 times

**RT-qPCRanalysis:** In this experiment, actin (GenBank: HQ184438) was used as an internal control (Liu *et al.*, 2012). The expression changes of ACO (GenBank: X972145) (Liu *et al.*, 2017c; Liao *et al.,* 2018) at bract reddening stages were determined by the RT-qPCR method. The primers and reaction procedures used for RT-qPCR are shown in Table 1 and Table 2, respectively. The specific test process was consistent with the previous one.

## Results

### Anatomic analysis of red bracts and biochemical analysis of chromogenic substances

**Anatomic analysis of red bract tissue structure:** Cross-sections of tissues of green bracts, semi-red bracts, and full red bracts were made by freehand and observed under a microscope (Fig. 1). Epidermal cells were colorless and transparent, and green ingredients (presumably chlorophyll) were distributed insponge tissue in green bracts (Fig. 1A). We then found yellow ingredients and red ingredients, which were distributed throughout the entire mesophyll tissue and fence tissue, respectively, during bract ripening (Figs. 1B and C).

### Biochemical analysis of red bracts

**Color reaction test of red bracts:** Color reaction test results are shown in Table 3. Treatment with petroleum ether resulted in a solution that was pale yellow and transparent (lemon yellow), indicating that the bracts contained trace amounts of carotene. Treatment with 10.0% hydrochloric acid resulted in orange-red solution, indicating the bracts contained anthocyanin. Treatment with 30.0% ammonia water yielded an earthy yellow solution, indicating that the bracts contained flavones pigments.

**Spectral scanning analysis of red bract pigments** (Bai *et al.*, 2006): The wavelength scanning analysis of red bracts using ultraviolet and visible light was performed, and the results are shown in Table 4. Three peak shapes of 291 nm, 328 nm, and 510 nm were found in the flavonoid detection results, which indicate the presence of flavonoids (291 nm, 328 nm) and anthocyanins (510 nm). In the detection of carotenoids, two peaks at 438 nm and 467 nm were found, indicating the presence of carotenoids. In the detection of chlorophyll, there were two obvious peaks at 440nm and 468nm, which were close to 438nm and 467nm, respectively, and both should represent carotenoids. In addition, there was no obvious absorption peak at 644-662nm, indicating that chlorophyll is basically absent.

**HPLC analysis of pigments in red bracts:** Phenol, flavonoids, cyanidin chloride, pelargonium chloride, delphinidin chloride, naringin, aromadendrin, dihydroquercetin (intermediate product), dihydromyricetin (intermediate product), quercetin, etc., were detected in *Guzmania* bracts by HPLC, and the results are shown in Table 5. According to the results, we know that contents of cyanidin and pelargonium in the green bracts are 0, while the contents of cyanidin and pelargonium in red bracts (including half red and full red) are very high, indicating that red bracts contain cyanidin and pelargonium. Since cyanidin appears red, and pelargonium appears orange-red, red bract color may be caused by cyanidin and pelargonium. In addition, during the period of bract reddening, the total contents of polyphenols and flavonoids are also growing.

**Dynamic changes in chlorophyll, flavonoid pigment content, and expression patterns of key genes in their metabolic pathways during the bract reddening process:** The results of changes in chlorophyll and flavonoid content during the bract reddening process was shown in Figure 2. It was known that green bracts contain the highest chlorophyll content, followed by semi-red bracts, and finally red bracts during the process of bract discoloration. As a control, green leaves had higher chlorophyll content than did the green bracts. Using catechin as a control, the flavonoid content measured by HPLC showed that red bracts had the highest flavonoids, followed by semi-red bracts and then green bracts, and the green leaves had the lowest flavonoid content.

**Table 3. Color reaction of red bracts.**

| Color reaction test | Color of the solution | Corresponding substances |
|---|---|---|
| A: petroleum ether treatment | Transparent light yellow (lemon yellow) | Carotene (Trace) |
| B: 10.0% hydrochloric acid treatment | Orange-red | Anthocyanin |
| C: 30.0% ammonia water treatment | Earthy yellow | Flavone pigment |

**Table 4. Spectral scanning analysis of red bracts.**

| Detection items | Scanning range | Peak wavelength |
|---|---|---|
| A: Flavonoid detection | 400~700 nm | 291nm<br>328nm<br>510nm |
| B: Carotenoid detection | 200~700 nm | 438nm<br>467nm |
| C: Chlorophyll detection | 220~ 600 nm | 440nm<br>468nm |

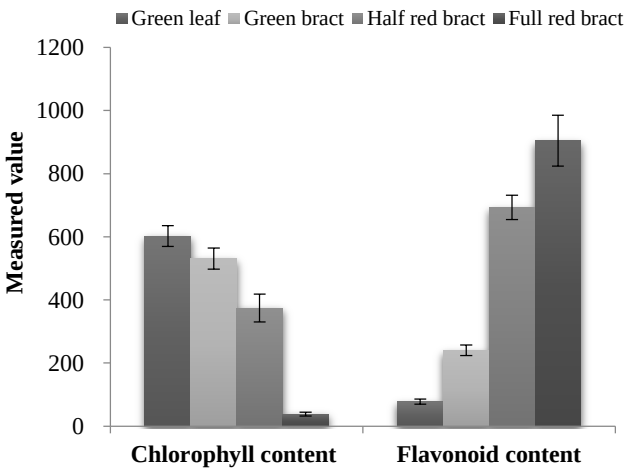



Fig. 2. Changes in chlorophyll and flavonoid content.
Note: Measured value of chlorophyll contene is 10 × fold SPAD value, and unit of Flavonoid content value is mg catechinequiv/100g FW.

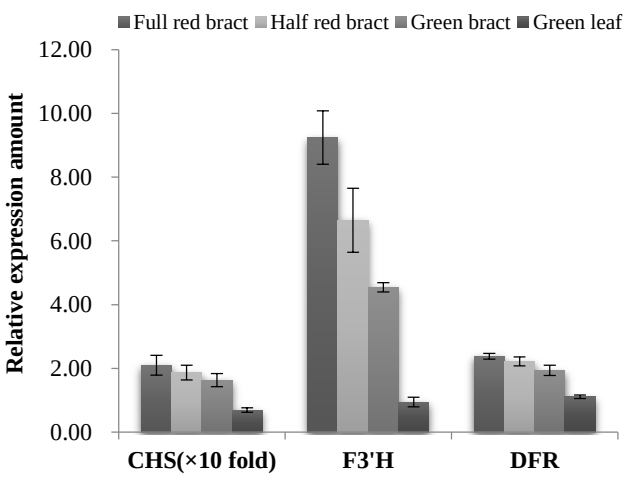



Fig. 3. Expression difference of *CHS, F3'H* and *DFR*.
Note: In order to make the chart look clearer, the expression of CHS has been increased by 10 times

The expression changes of key genes in anthocyanin biosynthesis during the bract reddening process was shown in Figure 3. It can be seen that the expression levels of three key enzyme genes *CHS*, *F3'H*, and *DFR* in anthocyanin biosynthesis all increased with the deepening of red in the bracts. Their expression levels were highest in the full red state, and green leaf as the control had the lowest expression level.

In terms of chlorophyll metabolism (Fig. 4), *GTS* and *UROS* expression levels of related to chlorophyll biosynthesis decreased significantly during bract discoloration. In contrast, expression levels of *PPH*, an essential enzyme gene for chlorophyll degradation, were significantly increased during the initial stage of bract discoloration. After bract reddening finished, it decreased to the lowest level, close to that of the green leaf state (Liu *et al.*, 2016).

**Dynamic changes in ethylene release during bract coloration and quantitative expression analysis of *ACO***

**Dynamic changes in ethylene release during bract coloration:** The dynamic change and total amount of ethylene released during bract reddening are shown in Figure 5 (Liu *et al.*, 2017c), ethylene released contents of green leaves and red bracts peaked in the first hour after separation from mother plants, and green bracts peaked during the second hour. Most ethylene in all stages was released in approximately 4h, and all were completely released after 21h. Summing the quantities of ethylene released at different times, it can be seen that the ethylene release content of red bracts was the highest, followed by green bracts, and then green leaves released the lowest amount.

**Quantitative expression analysis of *ACO*:** After the experiment on which making the amplification curve and dissociation curve of *ACO* and *actin* were finished by three parallel quantitative PCR reactions, their Ct values were obtained. By calculating the expression quantity of ACO based on *actin*, the expression levels of ACO in different reddening stages of the bracts were obtained (Fig. 6). The expression level of *ACO* was highest in red bracts, followed by green bracts, and the lowest expression was in green leaves.

**Table 5. HPLC analysis of pigments in red bracts.**

| Tissue | Phenol (mg GAEequiv /100 g FW) | Flavonoids (mg catechin equiv/100 g FW) | Cyanidin chloride (ug/g FW) | Pelargonium chloride (ug/g FW) |
|---|---|---|---|---|
| Green bract | 348.1 ± 6.3 | 73.02 ± 7.39 | 0 | 0 |
| Semi-red bract | 708.41 ± 6.87 | 653.45 ± 35.88 | 78.11 ± 0.56 | 2954.3 ± 28.68 |
| Full red bract | 877.46 ± 16.89 | 849.21 ± 75.49 | 76.68 ± 4.49 | 3511.46 ± 122.18 |

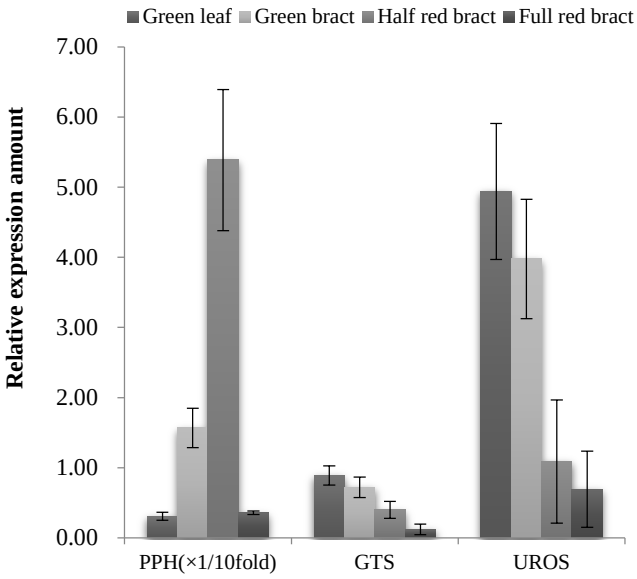



Fig. 4. Expression difference of *GTS, UROS* and *PPH***.**

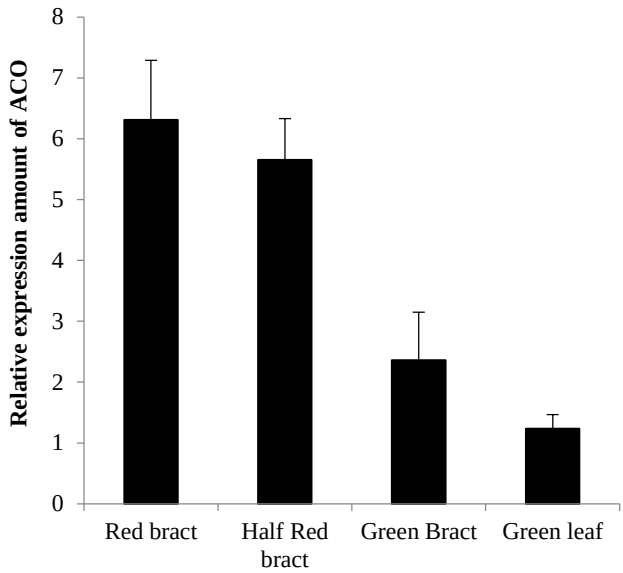
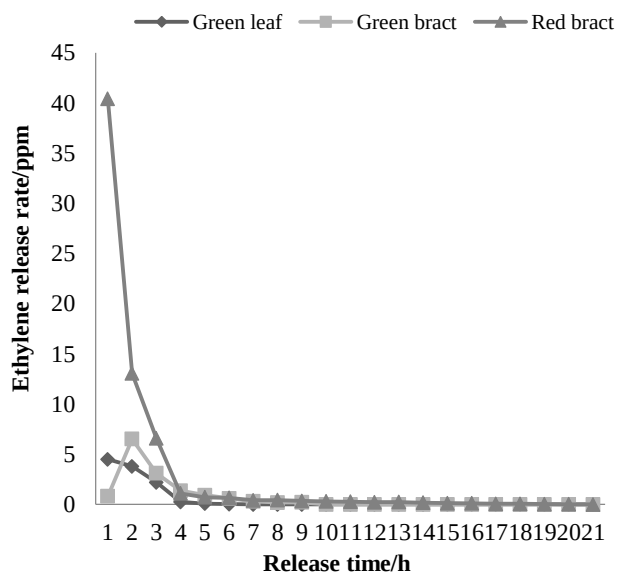



Fig. 6. Expression differences in *ACO* gene during flowering.



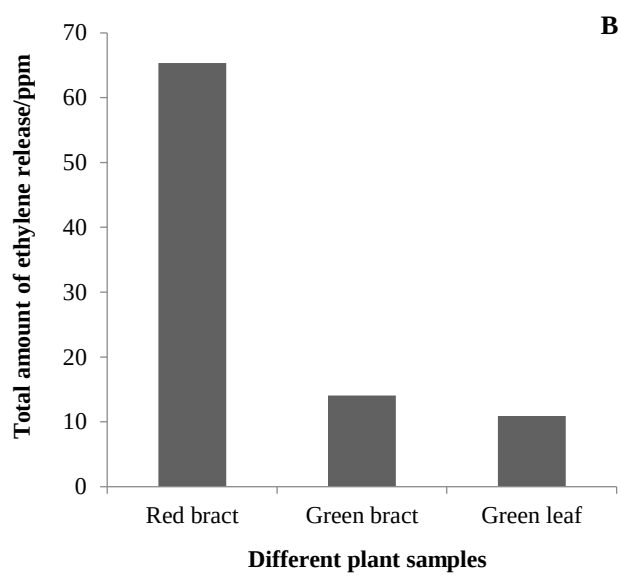




Fig. 5. Changes in ethylene release during bract reddening.

## Discussion

Our results suggest that anthocyanin (absorption wavelength of 510nm), composed of cyanidin (red) and pelargonium (orange red), contributes to the red color of *Guzmania* bracts and is mainly distributed in fence tissue. During ripening, the yellow substances distributed throughout mesophyll tissue are mainly flavones (absorption wavelengths are 291nm and 28nm) and carotenoids (absorption wavelengths are 438nm and 467nm). However, in the early stage, the green substance distributed in sponge tissue is chlorophyll (absorption wavelengths are 440nm and 468nm). The conclusions obtained in our study differ from those of previous research on bract color of bromeliaceous plants to some extent (Zhang *et al.*, 2005). As indicated, Zhang found anthocyanins mainly distributed between upper and lower epidermal tissues and fence tissue; nevertheless, anthocyanins were only distributed infence tissue, not in epidermal tissue in our results. Notably, the distribution of chlorophyll is similar, mainly inmiddle mesophyll tissue, that is, sponge tissue (Zhang *et al.*, 2005; Zhang *et al.*, 2019).

The dynamic changes of chlorophyll and flavonoid contents in the process of bract reddening were completely opposite. Along with the color change of the bracts from green to red, the chlorophyll content decreased gradually, while the content of flavonoids increased continuously. Consistent with previous conclusions obtained for various plants with colored leaves, the degree of leaf color darkening is often determined by anthocyanin/chlorophyll ratios (Zhao *et al.*, 2003; Zhao *et al.*, 2004; Tanaka *et al.*, 2009). Furthermore, the expression levels of *CHS, F3'H*and *DFR*, key enzyme genes in the anthocyanin synthesis pathway, increased gradually, from green leaves with the lowest expression to full red bracts with the highest expression. This trend indicates that coloration of the bracts is closely related to biosynthesis of anthocyanins. The downregulated expression of *GTS* and *UROS*, two key genes in chlorophyll biosynthesis, may suppress chlorophyll accumulation, while the upregulated

expression of *PPH*, the key degradation gene, may contribute to chlorophyll degradation, thus illustrating the internal reasons for the gradual decrease of chlorophyll content during bract reddening. More importantly, *PPH* plays a key role in this process (Liu *et al.,* 2016)

According to dynamic changes of ethylene release and expression patterns of ethylene biosynthetic gene *ACO,* it can be seen that the amount of endogenous ethylene exhausted increased, and ethylene biosynthesis also increased during bract coloration. Thus, we know that an increase in ethylene biosynthesis promotes the process of bract coloration. The result is consistent with previous statements that ethylene can promote rapid flowering of bromeliad plants and that it is a key factor inducing pineapple flowering (Kuan *et al.,* 2005). We propose that ethylene biosynthesis promotes the bract coloration process. This research on the mechanism of bract color formation is beneficial for improving bract color and ornamental quality and for guiding production practice.

## Conclusions

In this study, we have confirmed that the red substance distributed in fence tissue is anthocyanin (absorption wavelength: 510nm), composed of cyanidin (red) and pelargonium (orange-red). The yellow substances throughout mesophyll tissue are mainly flavones (absorption peaks at 291nm and 328nm) and carotenoids (absorption peaks at 438nm and 467nm), and the green substance distributed in sponge tissue is chlorophyll (absorption peaks at 440nm and 468nm). Furthermore, we also confirmed that bract reddening was caused by the accumulation of flavonoid content and decreased chlorophyll content. Notably, the decrease in chlorophyll content was due to reduced chlorophyll synthesis and enhanced chlorophyll degradation. Finally, we found that the enhancement of ethylene biosynthesis increased ethylene content in the bracts and then promoted bracts reddening.

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