The specific responses to mechanical wound in leaves and roots of *Catharanthus roseus* seedlings by metabolomics

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**ABSTRACT**

The mechanical wound is one of the unavoidable threats to survival of plants. More researchers focus on the effect of mechanical wound to the over-ground tissues. And the effects of wound to roots were frequently ignored, although it is an important organ for plant growth. In our studies, the metabolomics study was performed to reveal the mechanical wound effects in *Catharanthus roseus* on roots and leaves by combining gas chromatography-mass spectrometer (GC-MS), liquid chromatograph-mass spectrometer (LC-MS) and statistical analyses. The metabolic response of TIAs and PCs in plants to wound was most active at 0.5 h after treatment via Q value analysis. At this time point, then significantly responsive primary metabolites and specific secondary compounds (TIAs and PCs) were screened by PLS-DA score plot. In this case, the treatments of CK, LT (wound to leaves) and RT (wound to roots) were clearly distinguished. The targeted compounds include 8 sugars, 4 TIAs and 12 PCs and they displayed specific responses to CK, LT and RT treatments. Under RT group, plants invest more resources on the local responses using TIAs and the color reactions to regulate wound close using PCs. Whereas, LT group might lay emphasis on systemic responses via TIAs induced by SA (salicylic acid) and gallic acid. Our studies provide some basic data for further investigations of the defensive mechanism on roots treated by mechanical wound.

1. Introduction

Throughout their life cycles, plants are stressed by a wide range of external environmental factors, including wind, rain, microorganisms and insect herbivores, and all of them accompanied with mechanical wound (Savchenko et al. 2013). Damaged tissue opens the passageway for pathogen and results in spread of disease into whole plant (Asai et al. 2017). Plants have evolved multiple mechanisms to defend themselves from a wide range of attackers. For instance, local and systemic location were included in the process of wound repair to prevent the penetration of infection (Rasmann and Agrawal 2009). The biosynthesis of chemical compounds, mainly secondary metabolites whose production is triggered by interference factor, is a common defense strategy (Rasmann and Agrawal 2009; Vasyukova et al. 2011). Secondary metabolites are considered to biosynthesize specially to adapt to environmental stresses (Nakabayashi and Saito 2015). They were produced by a metabolic pathway, and showed different tendencies according to different conditions regulated by hormone, stress and growth etc. Most secondary metabolites represent adaptive traits that have gone through continuous diversification during evolution in order to protect plants against UV-B, drought, metal viruses, bacteria, fungi, herbivores and mechanical wound (Vilarino et al. 2005; Chludil et al. 2008; Leicach et al. 2010). These defenses operation is still over-speed even under nutrient-limited conditions, and have evolved as a cost-saving strategy for plant growth (Rasmann and Agrawal 2009; Savchenko et al. 2013). Many plant secondary metabolites have toxic effects on a variety of herbivores and pathogens, while other plant defenses appear to have indirect effects upon pests and pathogens (Tebayashi et al. 2000; Sirvent et al. 2003). The induction of secondary metabolites, such as phytoalexins, has been documented in response to biotic or abiotic challenges in a number of plant species (Sirvent et al. 2003). On the other hand, some hormones like SA, which belongs to secondary metabolites, has been reported to elicit plant immune systems such as systemic acquired resistance (SAR) or hypersensitive response (HR) to regulate the mechanical wound (Loake and Grant 2007).

Metabolomics approaches have been in the spotlight as a powerful tool to gain comprehensive information of metabolic network and to identify significantly different metabolites related to defense mechanisms. It was increasingly used in quantifying and interpreting the occurrence and the abundance of metabolites in the context of systemic biology (Scherling et al. 2010; Wang et al. 2016; Chen et al. 2017). The sensitive, rapid and accurate attribute of mass spectrometry that coupled to the separated characteristics of gas chromatography or liquid chromatography enable approaches of high-throughput metabolite profiling (Hubert et al. 2015). GC-MS analysis predominantly focuses on the identification and quantification of small polar and volatile components, e.g. primary metabolites such as amino acids, sugars and organic acids, whereas LC-MS is applied for the analysis of larger semi- and non-volatile and thermally unstable compounds, such as secondary metabolites.
(Scherling et al. 2010; Weckwerth 2011). In addition, a class of compounds could be detected via the construction of standard substance, that is targeted detection, and the compounds in a certain category also could be detected, that is untargeted detection. Metabolomics has become a central element of plant systems biology research, and has been successfully applied to many studies of biological filed (Noctor et al. 2016; Chen et al. 2017; Cohen and Amir 2017). In our studies, the different strategies were used to screen, detect, qualify and quantify the primary and specific secondary metabolites in response to wound.

*Catharanthus roseus* has tenacious vitality and is well known to be an important model medicinal plant for secondary metabolites studies. The most attractive values of *C. roseus* is able to produce more than 130 kinds of TIAs (terpenoid indole alkaloids), which are believed to protect the plants from biotic and abiotic stresses (Sottomayor and Barceló 2006; Chen et al. 2017). Some of these TIAs, in their natural or semisynthetic forms, are of pharmacological importance, as exemplified by the anticancer agents vinblastine, vincristine and vindoline, the antihypertensive ajmalicine, and the sedative serpentine as well as the antihypertensive compounds ajmalicine and serpentine (Sottomayor et al. 2008; Ferreres et al. 2011). Given their potential medicinal applications, alkaloids have long attracted the attention of phytochemists and pharmacologists (Peebles et al. 2009). In addition, Phenolic compounds (PCs) are also one class of important metabolites in *C. roseus*, obscured by the light of TIAs (Mustafa and Verpoorte 2007; Liu et al. 2016). PCs represent an abundant and wide class of natural products to fight with biotic and abiotic stresses, deterrent herbivore, and regulate signaling among plants (Ferreres et al. 2008). For humans, the PCs of plants are constituents of several plant-derived drugs. Recently, they have attracted much attention due to their implication in protection against cancer, cardiovascular and neurodegenerative diseases, associated to their antioxidant activity (Valentão et al. 2001; Sousa et al. 2008). In addition, the accumulation of PCs and TIAs could interact with each other, as plant defense is a complex system (Mustafa and Verpoorte 2007).

Recently, researchers pay more attention to the response and regulation mechanism of plants treated by mechanical wound to the aboveground tissues contrasting to the belowground ones (Couture et al. 2013; Savatin et al. 2014). In this work, we attempted to compare and reveal the whole-plant or tissue-specific responses of specific metabolites after different wounding applications, including control group (CK), root-treated group (RT) and leave-treated group (LT). Metabolomic were employed to perform the significantly different metabolites analysis. We also analyzed the trend of pathway enriched by differential metabolites and revealed the relationship between resource tradeoff and response mechanism. Our results showed that LT and RT plants adopt completely different ways to cope with mechanical wound. The RT plants increased TIAs mainly in roots, displaying a local and instantaneous response. In contrast, the LT plants displayed large fluctuation of TIAs mainly in stems, indicating occurrence of a systematic response. For PCs, the pathway of hesperetin, quercetin-3-O-rhamnoside, obviously increased in RT group. The other pathways (gallic acid, daizein and salicylic acid) were activated significantly in LT group. Their possible functions of TIAs and PCs were also discussed in our work.

2. Materials and methods

2.1. Plant materials, treatment time and collection of sample

The *C. roseus* seeds were planted in pots containing perlite and kept moistened until the seeds had germinated, and then irrigated with 1/2 strength Hoagland solution (pH 5.9-6.0) (Chen et al. 2017). Seedlings were grown in pots for 2 weeks and were then potted into 10cm pots cultivated with hydroponics, in growth cabinets (S10H, CONVIRON, Canada) with a 14 h/10 h light (irradiation of 450 µmol m⁻² s⁻¹ and temperature of 28°C)/dark (without irradiation and temperature of 25 °C) regime, and with humidity of 60%. Pots were randomized among different growth cabinets until the plants had been growing for 10 weeks (about 8 pairs of leaves). Then plants were randomly assigned to three groups (15 plants for each group). The three grouped plants were separately used for control (CK) or wounding treatment to leaves (LT) or roots (RT). The wounding treatment was carried out by sterilized scissors to cut around 50% of each leaf or root shown as Figure 1. The time-dependent responses of TIAs and PCs in total plants were firstly detected 0, 0.5, 1, 3 and 5 h after treatments, respectively. There were three replicates with 5 plants per replicate for each time point. We found that the time point mostly influencing metabolic changes was 0.5 h after treatment via Q value analysis.

Subsequently, we repeated the treatments (CK, LT and RT) at this time point, and collected 15 plants of each treatment for further tissue-specific metabolic responses investigation. These samples were separated into leaf, stem and roots for metabolic analysis, including CK-leaf, CK-stem and CK-root in control (CK) group, LT-injured leaf, LT-uninjured leaf, LT-stem and LT-root in LT group; RT-leaf, RT-stem and RT-root in RT group. Each kind of sample was about 1 g (fresh weight) and repeated six times. All the samples were immediately frozen in liquid nitrogen and stored at −80°C.

2.2. Sample preparation

2.2.1. Detection of primary metabolites

The preparation of samples consulted the method of Chen et al. (2017). Stored material cooled with liquid nitrogen. Sample (60 ± 5 mg plant tissues) extracted in 360 µL cold methanol and 40 µL 0.3 mg mL⁻¹, 2-chlorophenylalanine (dissolve in methanol), and homogenized (Tissuelyser-192, Shanghai, China). After ultrasonication for 30 min, 200 µL chloroform and 400 µL water were added to the sample. Vortexed for 2 min sonicated for 30 min, the sample was centrifuged at 10,000 g for 10 min at 4°C. 400 µL supernatant were collected to a glass sampling vial for vacuum-dry. Then, 80 µL methoxamine-mine (15 mg mL⁻¹ in pyridine) was added to the vitreous vial, vortexed 30 s at 37°C for 90 min, followed by 80 µL BSTFA (1% TMCS) and 20 µL n-hexane at 70°C for 60 min. Derivative sample in liquid form was injected into the Agilent 7890A-5975C GC-MS system (Agilent Corporation, USA) with a split ratio of 30 to 1. Separation was carried out on a non-polar DB-5 capillary column (30 m × 250 µm I.D., J&W Scientific, Folsom, CA), high purity helium as the carrier gas.
at a constant flow rate of 1.0 mL/min. Injected and ion source temperature was set to 260°C and 230 °C, respectively. The model was electron impact ionization (-70 eV) at full scan mode (m/z 30-600), acquisition rate was 20 spectrum/second in the MS setting. The QC sample was prepared by mixing aliquots of the samples to be a pooled sample, and analyzed according to the method of samples. Obtained MS data were analyzed by ChromaTOF software (v 4.34, LECO, St Joseph, MI). Alignment with Statistic Compare component, the CSV file was obtained including sample information, retention time and peak intensities. The data set was normalized using the sum intensity of the peaks in each sample.

### 2.2.2. Detection of secondary metabolites

Secondary metabolites were extracted from the mixture of 20 mL absolute methanol (analytical grade) and 1.0 g fresh tissues for 40 min. The extract was centrifuged at 8000 rpm for 10 min. The alkaloids were determined by HPLC-MS (Ultra-performance LC, Waters, Japan; MS, AB SCIEX, USA) using ACQUITY UPLC BEH C18 Column (1.7 μm, 2.1 mm × 50 mm), and CH3CN/H2O and 0.05 mol L−1 ammonium acetate standard solvent system. The TIAs content was measured based on the method of Chen et al. (Chen et al. 2018). Retention times were serpentine (3.49 min), tabersonine (2.91 min), vindoline (3.37 min), vinblastine (2.81 min), loganin (0.76 min), vincamine (1.99 min) and vincristine (2.49 min). Injection volume was 10 mL; flow rate was 1 mL min−1. The alkaloids content was quantified by tagged of the level from 10E-7 to 10E-2 mg L−1 based on peak area, respectively. Obtained data were analyzed and normalized by AB analyst. The content of detected compounds was determined by the standard curve used peak area.

The PCs were qualitative based on phenolic metabolic pool created by LC-qTOF-MS (Ultra-performance LC, Waters, Japan, MS, AB SCIEX, USA) that contain 32 general PCs, that is 3-4Hydroxybenzoic acid, 2,5-Dihydroxybenzoic acid, apigenin, caffeic acid, catechin, chlorogenic acid, cinnamic acid, daidzein, ferulic acid, galangin, gallic acid, genistein, hesperetin, hesperidin, kaempferol, liquiritigenin, L-phenylalanine, isosquirten, kaempferol, luteolin, myricetin, myricitrin, naringenin, marigenin, p-coumaric acid, pectinidin, p-hydroxycinnamic acid, queretin queretin-3-O-rhamnoside, rutin, salicylic acid, syringic acid, vanillic acid. The chromatographic conditions were A%:0.05% formic acid water, B %:0.05% formic acid acetonitrile, m/z 120-1200, positive ion scanning mode, leucine enkephalin was labeled as an internal standard, using ACQUITY UPLC BEH C18 Column (1.7 μm, 2.1 mm × 50 mm). Obtained data were analyzed and normalized by Waters Masslynx.

### 2.2.3. Data analysis and statistics

Partial least-squares-discriminant analysis (PLS-DA) analysis was performed with the help of SIMCA-P13 software package (Umetrics, Umeå, Sweden), after mean centering and unit variance scaling. Metabolic pathways were performed in the MBRole (http://csbg.cnb.csic.es/mbrole) and KEGG web (http://www.genome.jp/kegg/) to identify the top affected metabolic pathways and facilitate further metabolites interpretation. Pearson’s correlation coefficients were calculated by SPSS 17.0. Metabolite data were log2 transformed to improve normality and Min-Max Normalization. The Student’s t-test and Tukey’s test were used for mean value comparison. The metabolite pathway was created by Visor. The score of principal component ‘Q’ is an indicator of a comprehensive analysis, scientific evaluation of objective phenomenon.

\[
F_1 = a_{11}X_{11} + a_{21}X_{21} + \ldots + a_{p1}X_p \\
F_2 = a_{12}X_{12} + a_{22}X_{22} + \ldots + a_{p2}X_p \\
\vdots
\]

\[
F_p = a_{1m}X_{1m} + a_{2m}X_{2m} + \ldots + a_{pm}X_p \\
Q = (A_1F_1 + \ldots + A_pF_p) / (\lambda_1 + \lambda_2 + \ldots + \lambda_p)
\]

\(F_p\) is the value of the principal components. \(a_{ip}, a_{2p}, \ldots, a_{mp}\) \((i = 1, \ldots, m)\) is corresponding eigenvectors of the eigenvalues of the X covariance matrix \(\Sigma\), \(X_1, X_2, \ldots, X_p\) is the value of the original variables through standardized processing because in practice, indicators of dimension are often different, so the calculation must be to eliminate the influence of this to standardize original data.

### 3. Results

#### 3.1. The screen of significantly different metabolites

*C. roseus* tissue samples were collected following a time series ranging treated by sterilized scissors \((t = 0.5)\), until 5 h after. The Q value of secondary metabolites (TIAs and PCs) in 0.5 h was shown the most intense response in treated groups, compared to CK group (Figure 2). Therefore, 0.5 h was selected for subsequent experiments. In order to gain an

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**Figure 1.** The treated position of mechanical wound. (A) Control group, CK; (B) leaves treated group, LT; (C) roots treated group, RT.
insight into the metabolic variation caused by mechanical wound, a PLS-DA was performed in sugars, TIAS and PCs. The principal components covering 23.2% of total variance in data set of GC-MS, covering 77.2% of total variance in data set of TIAS and covering 39.6% of total variance in the data set of PCs (Figures 3 and 4). The two treated group are clearly separated by means of the second independent component of primary metabolites (Figure 5). We focus on the significantly different metabolites that could reflect the supply of energy from data of GC-MS. 8 significantly different metabolites of sugars (tagatose, fructofuranose, sorbose, galactitol, sucrose, mannose, mannopyranose and galactose), which are the direct supply of energy, were obtained according to their variable importance in the projection values (VIP, VIP >1) and p-values (p-value < .05) from 143 compounds (Table 1). These metabolites displayed substantial difference in the plant of LT group, RT group and CK group. The relative content of sugars appeared to be more consumable in treated group of mechanical wound compared to the samples in CK group (Figure 3). And sugars response to the mechanical wound was more obvious in the RT than them in LT (Figure 3).

3.2. Metabolic profiling

Treated groups were separated by the first independent component on TIAS, while they were separated by second independent component on PCs (Figure 4). Materials and energies followed to TIAS and PCs from shikimic acid, respectively (Figure 5). Concerning TIAS, 4 significantly different metabolites (tabersonine, tryptamine, catharanthine and vindoline) were obtained, and 12 significantly different metabolites (ferulic acid, myricetin, syringic acid, hesperetin, gallic acid, cinnamic acid, quercetin, daidzein, myricitrin, isoliquiritigenin and salicylic acid) were obtained on PCs; according to their VIP values (VIP >1) and p-values (p-value < .05) (Tables 2 and 3). Further detailed analysis was performed to understand which metabolites significantly involved in responses to mechanical wound. This initial
global fingerprinting analysis indicates that mechanical wound could change metabolic direction in a tissue-specific and time-dependent manner. The content of tryptamine, which is the upstream precursor metabolites of TIAs, enriched on the aboveground in RT and LT groups (Figure 5). The downstream TIAs accumulated primarily in stems and roots. Especially, in the RT group, TIAs content of roots was significantly higher than that in LT group (Figure 5). However, the content of TIAs almost have no changes in stems in RT group (Figure 5). These two observations

![Image of Figure 4](image1.png)

**Figure 4.** The PLS-DA score plot of secondary metabolites. (A) PLS-DA score plot of TIAs, (B) PLS-DA score plot of PCs, CK, control group, LT, leaves treated group; RT, roots treated group.

![Image of Figure 5](image2.png)

**Figure 5.** The diagram of network comprised and the content of significant secondary metabolism after 0.5 h of mechanical wound. (A) The diagram of network comprised, (B) The content of significantly different metabolites of TIAs, (C) The content of significantly different metabolites of PCs. The grid was CK, control group; LT, leaves treated group; RT, roots treated group; from left to right, respectively. The content of metabolites from low to high indicated by the color of blue to red, respectively, imaginary line represent multi step reaction.
indicate that the responses of RT group were local and instantaneous, and these metabolites were direct to use for mechanical defense in producing parts. In contrast, there was sensitive response to mechanical stress in stems of LT group with lower content of TIAs in leaves and roots (Figure 5). It showed that LT group responses were systematic and time-consuming. PCs, another metabolites flux from shikimic acid, were mainly involved in five pathway of significantly different metabolites (Figure 5). Among them, the content of the pathway of hesperetin, quercetin-3-O-rhamnoside obviously increased in RT group, compared to other group. It also contains myricitrin, almost increased in RT group. And the other pathways (gallic acid, daizein and salicyic acid) were activated significantly in LT group (Figure 5). Ferulic acid was highlight in these metabolites. There is no overlap on these significantly different metabolites between RT group and LT group. It suggests that there is may be a different defensive response strategy between them.

4. Discussions

Mechanical wound is one kind of common challenge that plants always face to (Pankoke and Müller 2013). Among them, roots are also suffered damages from mechanical stress, although it deep buried underground. Our research mainly concerned about the metabolic responses of C. roseus to mechanical wound in roots or leaves, trying to compare their resulting difference. In contrast to the previous reports regarding wound induced metabolic response, we employed high-throughput method to give more comprehensive profiling of wound-affected metabolism in this study (Vasyukova et al. 2011). The GC-MS and LC-MS technologies were used to dissect the primary and secondary (TIAs and PCs) metabolites between LT and RT group plants.

Regarding to the primary metabolites, we obtained 8 sugars with significant variable values from 143 compounds, including tagatose fructofuranose sorbose, galactitol, sucrose,
Figure 5. Continued.
Figure 5. Continued.
Table 1. The significantly different metabolites of sugars.

| Significantly different Metabolites | VIP   | p-value |
|------------------------------------|-------|---------|
| Sugar Tagatose                     | 1.45  | *       |
| Fructofuranose                     | 1.30  |         |
| Sorbose                            | 1.23  |         |
| Galactitol                         | 1.15  | **      |
| Sucrose                            | 1.14  | *       |
| Mannose                            | 1.08  |         |
| Mannopyranose                      | 1.06  |         |
| Galactose                          | 1.02  |         |

Note: VIP, variable importance in the projection; Significantly: *p < .05, Extremely significantly: **p < .01.

Table 2. The significantly different TIAs.

| Significantly different metabolite | VIP   | p-value |
|-----------------------------------|-------|---------|
| Tabersonine                       | 1.24  | *       |
| Tryptamine                        | 1.14  | **      |
| Catharanthine                     | 1.09  | *       |
| Vindoline                         | 1.05  | **      |

Note: VIP, variable importance in the projection; Significantly: *p < .05, Extremely significantly: **p < .01.

mannose, mannopyranose and galactose (Table 1). The functions of sugars playing in this process are considered to mainly provide substrates for energy production and biosynthesis of secondary metabolites to increase plant resistance. We observed that wounding either to roots or to leaves decreased sugar availability in plants and it was assumed that the wounding treatments obviously consumed sugars for responsive process. The accurate correlation of primary metabolites, especially sugars, with wounding response is few reported and our results provide some potential targets for further investigation in this field. The detailed mechanisms remain to be investigated further.

In contrast to the functions of sugars in wounding response, the secondary metabolites were considered to activate defense, via the supply of primary materials and the stimulation of external environment (Savchenko et al. 2013). They are a very important kind of compounds for plants. TIAs and PCs were two branches of secondary metabolites, that could respond to the mechanical stress, according to its coordination or competition via the trade-offs and distribution. The potential advantage of this "division of labor" is to ensure the most effective defense strategy that minimizes incurred damages at a reduced metabolic cost (Chehab et al. 2008). TIAs, which belong to that kind of secondary metabolites, are thought to function as toxins that can poison herbivores, insect and germs (Baldwin et al. 2001). Plants are able to sense the injured tissue as an altered self and induce responses similar to those activated by pathogen infection (Chen et al. 2017). TIAs, which play function as preventive metabolite, show a hysteresis response to mechanical wound compared to PCs do (Figure 2). Tryptophan, which is a limited factor of TIAs synthesis and widespread exists in plants, were extensively generated in aboveground in wounding treated group and used to downstream metabolites production. In addition, it has been reported it has involved in wound responses (Vázquez-Flota et al. 2004). Interestingly, the significantly different metabolites are mainly located at downstream pathway and basically accumulated in stems and roots. Among them, TIAs accumulation of LT group occurred obviously in the stems, while for RT group in roots (Figure 5). And there was no mark of TIAs in stems under RT group. It indicates that the responses of RT group to wounding take place at the site of injury, that is local responses, where the metabolites were directly used for mechanical defense (Sun et al. 2013). By contrast, the major site of action of these significantly different metabolites was stems in LT group. Systemic response adopted by LT group and need time to regulations (Sun et al. 2013). Therefore, different responses style was adopted between RT group and LT group in terms of TIAs accumulation.

It was noted that the responses of PCs were faster than TIAs (Figure 5). Gallic acid pathway was obviously activated in LT group. Gallic acid is reported to be the precursor of tannin, which is a class of complex polyphenolic compounds in plants (Chen et al. 2017). Tannin as a defensive substance could evaluate the defense level based on the content of itself (Barbehenn and Peter 2011). Together with gallic acid pathway respond quickly in LT group, while it was negatively responsive in RT group. It indicates that gallic acid specifically plays defense role in leaves injured by mechanical wound. Similar to gallic acid, daizein pathway also appeared these phenomena. It is worth noting that SA was roundly induced in LT group (Figure 5). SA is a signaling molecule of plant coping with disease resistance and also a stress signaling molecule inducing plant responses to abiotic stresses (Li et al. 2017). It could implement hypersensitive response and Systemic acquired resistance (SAR) strategy. Utilizing regulation of stomata closure and redox states, SA regulated the intensity and speed of response (Mittler et al. 2011). And more, it can effectively produce antibodies against viruses, fungi and bacterial diseases in plants (Denancé et al. 2013). It has been reported that SA actively participate in the regulation of mechanical injury in Arabidopsis leaves (Ogawa et al. 2010). In addition, SA can affect the distribution and production of secondary metabolites (Hwang et al. 2013; Li et al. 2015). In RT group, the strongest response was quer cetin pathway, and the metabolites in this pathway all have the effects of antioxidant and anti-inflammatory. In addition, the downstream metabolites anthocyanidin were involved in plant color reactions. Therefore, the accumulation of pigment may play the color reaction role to be beneficial for the closure of wounding stress. Moreover, hesperetin plays an important role to defense the invasion of germs under RT group.

After mechanical wound, plants should invest their resources in a way that maximizes their fitness, which may lead to tradeoffs in investment of metabolites. C. roseus must implement a strategy in limited resource. A tradeoff of allocation in the metabolites of the plant was manifested, when the accumulation of some metabolites changed. The secondary metabolites supply more abundance and defense
production in injured leaves [45]. LT group and RT group plants adopt completely different ways to cope with injuries, in our research. Local responses were employed by RT group, and systemic responses were adopted in LT group to produce TIA. Concerning about PCs, LT group focus on the investigation of SA-SAR defensive and gallic acid participate in physiological defense, while RT group emphasis on the color reactions to promote closure of wounding site. The wounding group activated different metabolites to complete mechanical response via tolerance of LT group (i.e. the ability to support a certain level of damage, also known as compensation) or evasion of RT group (i.e. biochemical changes related to the chemical defenses) (Gómez et al. 2012). They need to consume more plant energy and substrates to complete their mission. Besides, mobilized resources might be temporarily unavailable to other plant functions such as growth or other defense, and might thus be costly for the plant. Because many secondary metabolites are thought to function as toxins that can poison fungus or insect, plants actively invest limited resources to these metabolites for defense by tradeoff. Therefore, a tradeoff relationship should exist between response and resource availability at the whole-plant level.

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