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A BRIEF REVIEW ON SECONDARY METABOLITES BIOSYNTHESIS REGULATION: APPLICATION IN *GYNURA PROCUMBENS* IN GLASSHOUSE CONDITION

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ABSTRACT Secondary metabolites biosynthesis regulation study of herbal plants plays a significant role in the discovery of phytochemical compounds with a wide range of uses. These phytochemical compounds are important nature-derived drugs such as antibiotics, agrochemicals substitutes (allelopathy), pigments, and medicinal immuno-suppressor. Generally, there is a firm scheme for regulating secondary metabolites based on their components, function and synthesis: alkaloids, enzyme, fatty acid, phenolic, polyketides, polypeptides, steroids, and terpenoids. Therefore, with the increasing in economic importance of these valuable compounds has led to a great interest in secondary metabolism, mainly the possibility of modifying and regulating the production of metabolites by means of biotechnological and agricultural practice. This review presents information about the production regulation of bioactive compounds under various stress conditions, particularly on abiotic influence such as water supply and fertilizer application that could be included in *G. procumbens* production systems. Brief analysis was done by reviewing various collected works and material from articles in related issues. Therefore, it will permit quantitative comparison of studies to address intended questions based on procedures, study systems, locations and scale of used.

Keywords: Biosynthesis, glasshouse, *Gynura procumbens,* metabolites, nitrogen, potassium, review, water

ABSTRAK Kajian berkenaan peraturan biosintesis metabolit sekunder dalam tumbuh-tumbuhan herba memainkan peranan yang signifikan dalam penemuan sebatian-sebatian fitokimia dengan pelbagai kegunaan. Sebatian-sebatian ini adalah bahan ubatan penting alam semula jadi yang diperolehi seperti antibiotik, pengganti agrokimia (alelopati), pigmen, dan ubatan imun-penindas. Secara umumnya, terdapat satu bentuk sistem yang boleh mengawalatur penghasilan dan pengeluaran metabolit sekunder ini berdasarkan komponen, fungsi dan sintesis: alkaloid, enzim, asid lemak, fenolik, poliketide, polipeptide, steroid, dan terpenoid. Oleh itu, dengan peningkatan dalam kepentingan ekonomi sebatian berharga ini telah membawa kepada minat yang besar dalam penghasilan metabolit sekunder, terutamanya aspek mengubahsuai dan mengawalatur pengeluaran metabolit melalui amalan bioteknologi dan pertanian. Artikel ini membentangkan maklumat mengenai mekanisme pengeluaran sebatian bioaktif dalam pelbagai keadaan dan pengaruh, terutamanya mengenai pengaruh abiotik seperti bekalan air dan penggunaan baja yang boleh digunapakai dalam sistem pengeluaran *G. procumbens*. Analisis ringkas telah dilakukan dengan pengumpulan bahan dan material daripada artikel dalam isu-isu yang berkaitan. Oleh itu, artikel ini boleh digunakan dalam melakukan perbandingan kajian kuantitatif yang melibatkan persoalan yang sama seperti prosedur, sistem kajian, lokasi dan skala yang digunakan.

INTRODUCTION

Gynura procumbens (Lour.) Merr. (family Asteraceae) (*G. procumbens*) was largely distributed from Africa to Australia and South East Asia. To date, the highest diversity was found in Southeast Asia (SEA) including Indonesia, Malaysia and Thailand for medicinal purposes (Saiman, Mustafa, Schulte, Verpoorte, & Choi, 2012; Sekar et al., 2014). Generally, *G. procumbens* was described as an evergreen shrub or perennial herbs with a purple fleshy stem and tint. The plant was classified as tropical herbaceous medicinal plant and able to grow approximately at 10-25 cm tall (Bhore & Vaishana, 2010; Tan, Chan, Pusparajah, Lee, & Goh, 2016).

Typically, the plant was used in various health ailments such as blood hypertension reduction, cancer (anti-carcinogenic), constipation, diabetes mellitus, eruptive fevers, kidney disease, migraines, rash, and urinary infection (Adnan & Othman, 2012; Arifullah, Vikram, Chiruvella, Shaik, & Abdullah Ripain, 2014; Duñg & Loi, 1991; Hassan, Yam, Ahmad, & Yusof, 2010; Hoe, Lee, Mok, Kamaruddin, & Lam, 2011; Jarikasem et al., 2013; Kaewseejan, Puangpronpitag, & Nakornriab, 2012; Mou & Dash, 2016; Rahman & Al Asad, 2013; Shwter et al., 2014). The plant's benefits have also been supported by the reports of isolation and identification of numerous possible bioactive compounds such as flavonoid, glycoside, phenolic, saponins, sterol, tannins, and terpenoids from the leaf extract (Afandi, 2015; Altemimi, Lakhssassi, Baharlouei, Watson, & Lightfoot, 2017; Arulselvan et al., 2014; Hew, Khoo, & Gam, 2013; Iskander, Song, Coupar, & Jiratchariyakul, 2002; Liew, Stanbridge, Yusoff, & Shafee, 2012).

Despite greater medicinal value, however, its phytochemical properties and biosynthesis mechanisms specifically on *G. procumbens* have not been well studied until recently, except, on other Gynura species and variety in general (Dewick, 2002; Jimenez-Garcia et al., 2013; Julsing, Koulman, Woerdenbag, Quax, & Kayser, 2006; Ramawat, Dass, & Mathur, 2009; Xue & Zhang, 2017).

Due to the tradition, current application and potential future benefits of alternative medicine, there should be a study to be conducted to investigate the regulation of phytochemical production or secondary metabolites synthesis of *G. procumbens* to optimize the yield and mass of the plant's active compounds.

Therefore, alternatives can be delivered in two different scales, which is either in the laboratory or in the glasshouse work. Working in the laboratory will be translated into plant biotechnology by means of *in vitro* cell or organ cultures and genetic transformation (Nurisa, Kristanti, & Manuhara, 2017; Saiman et al., 2012).

Meanwhile, working in the glasshouse will be translated into agricultural means using micro environment or micro climate and abiotic elements (Jamaludin, Abd Aziz, Ahmad, & Jaafar, 2015). Indeed, both approaches are potentially significant and could be used to optimize the synthesis, accumulation and production of the desired metabolites in *G. procumbens*.

Abiotic control such as carbon dioxide (CO_2) elevation, light intensity, nitrogen (N) and potassium (K) fertilization, and water supply under glasshouse condition becomes popular approaches in agricultural sector since it provides and exhibits direct fast-growing material, denotes a simple and easy technique to monitor. These were reported by Astuti, Rogomulyo, & Muhartini (2011); Dunford & Vazquez (2005); Ghasemzadeh, Nasiri, Jaafar, Baghdadi, & Ahmad (2014); Ibrahim & Jaafar (2012); Ibrahim, Jaafar, Karimi, & Ghasemzadeh (2012, 2013, 2014); Ibrahim, Jaafar, Rahmat, & Rahman (2011); Jaafar, Ibrahim, & Fakri (2012); Jamaludin et al., (2015); Pradnyawan, Mudyantini, & Marsusi (2005).

Therefore, an abiotic control under glasshouse condition could work as a preferable system in conducting study of regulation and effects of certain elements in *G. procumbens* secondary metabolites synthesis. In view of that, a brief review has been performed on various herbal medicinal plants phytochemical's production and their potential application in *G. procumbens*. The reviews are focus on: *G. procumbens* plants; the pathway in which the biosynthesis is involved; and, also the acting mechanism of selected abiotic elements under glasshouse condition in regulating secondary metabolite synthesis.

This review not only provides researchers with the information pertaining to *G. procumbens* plants, but also might be useful to other herbal medicinal plants. The papers reviewed in this article are selected from various journals due to their reliable reputation.

MANIFESTATION OF SECONDARY METABOLITES

The development of herbal medicinal plants for potential bioactive compounds production was manifested by research and agronomic management challenges considerations (Jeong & Kim, 2015). Numbers of compounds from various plant parts such as flower, fruits, leaves, roots, stem, and tuber have been reported. These metabolites include diverse types of economically important compounds, including; allelopathy, elicitors, enzyme, immunomodulatory agents, pheromones, pigments, and toxins in animals, humans and plants (June et al., 2012; Kaewseejan et al., 2012; J. E. Li, Wang, Zheng, & Li, 2017).

Since the production of these products requires a greater mass of natural ingredients, therefore the demand for alternative bases derived primarily from quality medicinal plant production is increasing significantly (Briskin, 2000). On top of that, the importance of knowing the effects of nutrient level on the plantation and identifying the mechanism of regulation pathways in plant's secondary metabolite production for producing better and sustainable yields of the plants is timely (Boroomand & Grouh, 2012; Mohamad Bukhori, Jaafar, & Ghasemzadeh, 2015).

THE POTENTIAL OF GLASSHOUSE CONDITION STUDY

To cope with the target of enhancing and sustaining the plant yield and important secondary metabolite production in *G. procumbens*, is to domesticate the study under glasshouse condition. Nevertheless, there has no comprehensive documentation on standard protocol or practice for water supply and fertilization or the best harvest time for better yield and higher production of secondary metabolites in *G. procumbens* except on *Gynura bicolor* (Table 1).

Table 1: Compound biosynthesis under different parameters in *Gynura bicolor*.

Variety	Study Parameter	Report
Gynura bicolor	Alteration of polyphenol biosynthesis in leaves when induced by infrared (IR) irradiation.	Fukuoka, Suzuki, Minamide, & Hamada, 2014
Gynura bicolor	Alteration of anthocyanin and non-flavonoid polyphenol biosynthesis in leaves when exposed to different light quantum.	Fukuoka et al., 2014
Gynura bicolor	Induction of anthocyanin accumulation in cultured roots by methyl jasmonate.	Shimizu, Maeda, Kato, & Shimomura, 2011

The concentration of secondary metabolites such as total flavonoids and phenolic compound are very much influenced by agronomical practices, especially water supply, N and K fertilization (Jaafar et al., 2012). With regards to carbon: nutrient balance (CNB) hypothesis, whenever N or K resource availability in the growth media is decreasing, the low resource would limit the plant growth more than the photosynthesis; under this situation plants will allocate the extra carbon (C) which cannot be used for growth to the production of carbon-based secondary metabolites (CBSM) (Fonseca, Rushing, Rajapakse, Thomas, & Riley, 2006; Marchese, Ferreira, Rehder, & Rodrigues, 2010).

This information is vital for optimizing the production of *G. procumbens* under glasshouse condition, which data can also be simulated for production under different growing conditions. Therefore, a glasshouse study could be conducted primarily to understand the production of plant secondary metabolites, particularly the lead compounds activities, under different water level supply, N and K fertilization regimes; and to determine the right harvesting time for optimum production of the secondary metabolites.

STIMULATION OF PLANT SECONDARY METABOLITES PRODUCTION

Plants possess metabolic pathways leading to a wide arrays of compound products which commonly effectively reacting to stress environment imposed by biotic and abiotic factors (Heydarizadeh, 2016; Kennelly, O'Mara, Rivard, Miller, & Smith, 2012). These pathways regularly started from essential primary metabolism pathways with initial gene duplication which sometimes leads to altered transcriptional and translational genes of new functions and diversified roles in new pathways (Jimenez-Garcia et al., 2013; Julsing et al., 2006). The occurrence is a basic part of plants developmental program and marks the onset of developmental stages (Hunt, 2003).

On a molecular basis, a determined spatial and temporal control of gene expression warrants the correct synthesis and accumulation pattern of various compounds by featuring the ontogeny and circadian clock-controlled gene expression of the regulatory transcription factors for compound production in respective developmental stages of the plants (Ruffel, Krouk, & Coruzzi, 2010).

On the other hands, plant secondary metabolites are denoted to have primary function in interacting with environment for defense and adaptation (Mazid, Khan, & Mohammad, 2011). For example, in higher plants, a wide range of secondary metabolites are mainly synthesized from primary metabolites (carbohydrates, lipids, nucleic acids, and proteins). They are required in plant defense reactivity against herbivores and pathogens by conferring protection against environmental stresses (alkalinity, disease infection, drought, light, nutrients, salinity, and temperature) (Michalak, 2006). On that note, we are beginning to understand their essential role in plant growth and development.

On top of that, the accumulation or secretion of these metabolites is also subjected to various stresses factor including elicitors or signal molecules (Ruffel et al., 2010), where, the production of the compounds is often low (less than 1% dry weight) and depends greatly on the physiological and developmental stage of the plant (Jaafar et al., 2012).

To date, elicitor and precursor have been widely used to increase the production as well as to induce *de novo* synthesis of secondary metabolites by means of *in vitro* plant cell cultures (Tu et al., 2016). However, glasshouse condition seems to work best for domesticating the study of abiotic influence on secondary metabolites production since the agricultural cultivation and natural habitat have shown similar responses to manipulations (Massad, Fincher, Smilanich, & Dyer, 2011).

For instance, stresses in nutrient have a marked effect on phenolic levels in plant tissues (Michalak, 2006). Meanwhile, exposure to drought has leads to cellular dehydration, which eventually causes osmotic stress and removal of water from the cytoplasm to vacuoles. Apart from that, plants under water stress also exhibit increased accumulation of abscisic acid (ABA) which triggers changes in phenolics and terpenoid (Z. Li, 2002; Marchese et al., 2010).

Therefore, these will implicate certain levels of genes expression that shown a response and reactive to nutrient, temperature and osmotic stress, where, deficiencies in water supply, incorrect N and K fertilization directly affect the accumulation of phenylpropanoids and lignification and also increasing phenolic concentrations as well as increasing the 3-fold in anthocyanidins level and simultaneously doubling of quercetin-3-*O*-glucoside and regulate anthocyanin production in plants (Ghasemzadeh et al., 2014).

BIOSYNTHESIS OF PHENOLIC COMPOUNDS

A phenolic is one of the most important phytochemical groups in *G. procumbens*. This compound is characterized by at least one aromatic ring (C6) bearing one or more hydroxyl groups and mainly synthesized from cinnamic acid which is formed from phenylalanine by the action of L-phenylalanine ammonia-lyase (PAL), the branch point enzyme between shikimate pathway (primary) and phenylpropanoid pathway (secondary) metabolism (Figure 1; 4CL, 4-coumaroyl:CoA-ligase; ANS, anthocyanidin synthase; C4H, cinnamate-4-hydroxylase; CHI, chalcone isomerase; CHS, chalcone synthase; DFR, dihydroflavonol reductase; F3H, flavanone 3-hydroxylase; FLS, flavonol synthase; FS, flavone synthase; and PAL, phenylalanine ammonia-lyase) (Cheynier, Comte, Davies, Lattanzio, & Martens, 2013).

In fact, PAL diverts phenylalanine from protein synthesis (in primary metabolism) towards the production of *trans* cinnamic acid and other phenolic compounds. PAL is mainly located in the epidermal cells. Its activity is very high at the start of plant’s development, maximized during the plant’s growth phase; then started to decrease and beginning to low during maturation (Ghasemzadeh et al., 2014).

These vital reports at least would facilitate the study objective in which the practical or efficient water supply, fertilization level and the best harvest time for better yield and high production of secondary metabolites in *G. procumbens* to be noted.

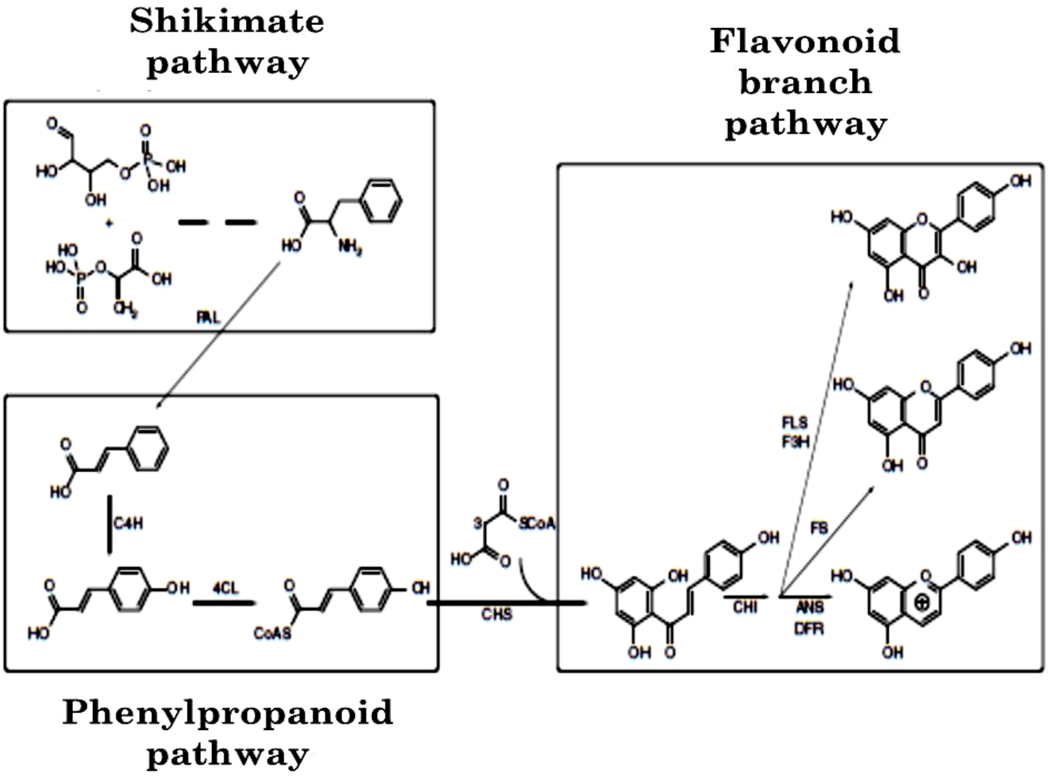


Figure 1: The schematic of major branch of (poly)phenol biosynthesis pathways. (Figure by Cheynier et al., 2013).

This primary pathway also leads to the three aromatic amino acids including L-phenylalanine, L-tyrosine, and L-tryptophan (Figure 2; PEP, phosphoenolpyruvate; and E4P, erythrose 4-phosphate).

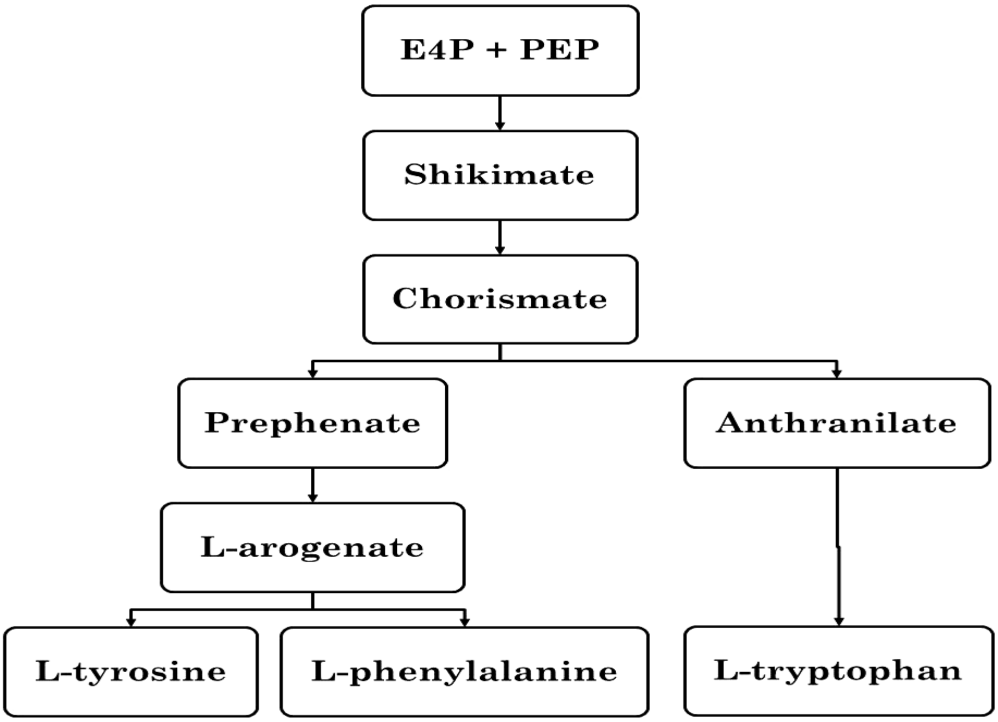


Figure 2: The schematic of primary pathway of the three aromatic amino acids synthesis. (Figure by Herrmann, 1995).

In plant cells, initially, chloroplasts will fix carbon dioxide (CO_2) through Calvin cycle into glyceraldehyde-3-phosphate (G3P), which eventually transformed and accumulated as carbohydrates storage followed by degradation whenever requires either by glycolysis (main products: G3P, phosphoenolpyruvate (PEP) and pyruvate) or via the oxidative pentose phosphate (OPP) pathway (main products: erythrose-4-phosphate (E4P) and G3P) into more simple molecules (Figure 3; DHQ, 3-dehydroquinic acid; DHS, 3-dehydroshikimic acid; E4P, erythrose-4-phosphate; G3P, glyceraldehyde-3-phosphate; and PEP, phosphoenolpyruvate) (Ossipov, Salminen, Ossipova, Haukioja, & Pihlaja, 2003; Shitan, 2016).

Initially, the first branch of the pathway located between the acetate-malonate and shikimate pathways. Both pathways are essential for the biosynthesis of phosphatidic acids (PAs), meanwhile ellagitannins (ETs) only rely solely on the shikimate pathway. In a condition where if significant levels of glycolytic PEP are directed into the shikimate pathway (together with E4P), therefore, the production of pyruvate for the needs of acetate-malonate pathway is significantly reduced. This has resulted direct negative effects on PA biosynthesis, since they would need malonyl-CoA as one of their building blocks. However, most of plant’s tannins rely on the efficient function of the shikimate pathway (Figure 3) (Ossipov et al., 2003).

Next, the second major branch point located at 3-dehydroshikimic acid (DHS). This is the main precursor for the gallic acid synthesis, the primary building block of all HC-toxin synthetase (HTs). Efficient production of gallic acid, however, will be negatively affects the synthesis of shikimic acid and its products: caffeic and coumaric acid derivatives, flavonoids, and PAs.

Finally, the hydrolysable tannin pathway contains the third major branch point at pentagalloyl glucose, which is the precursor for both gallotannins (GTs) and ETs (Figure 3) (Ossipov et al., 2003).

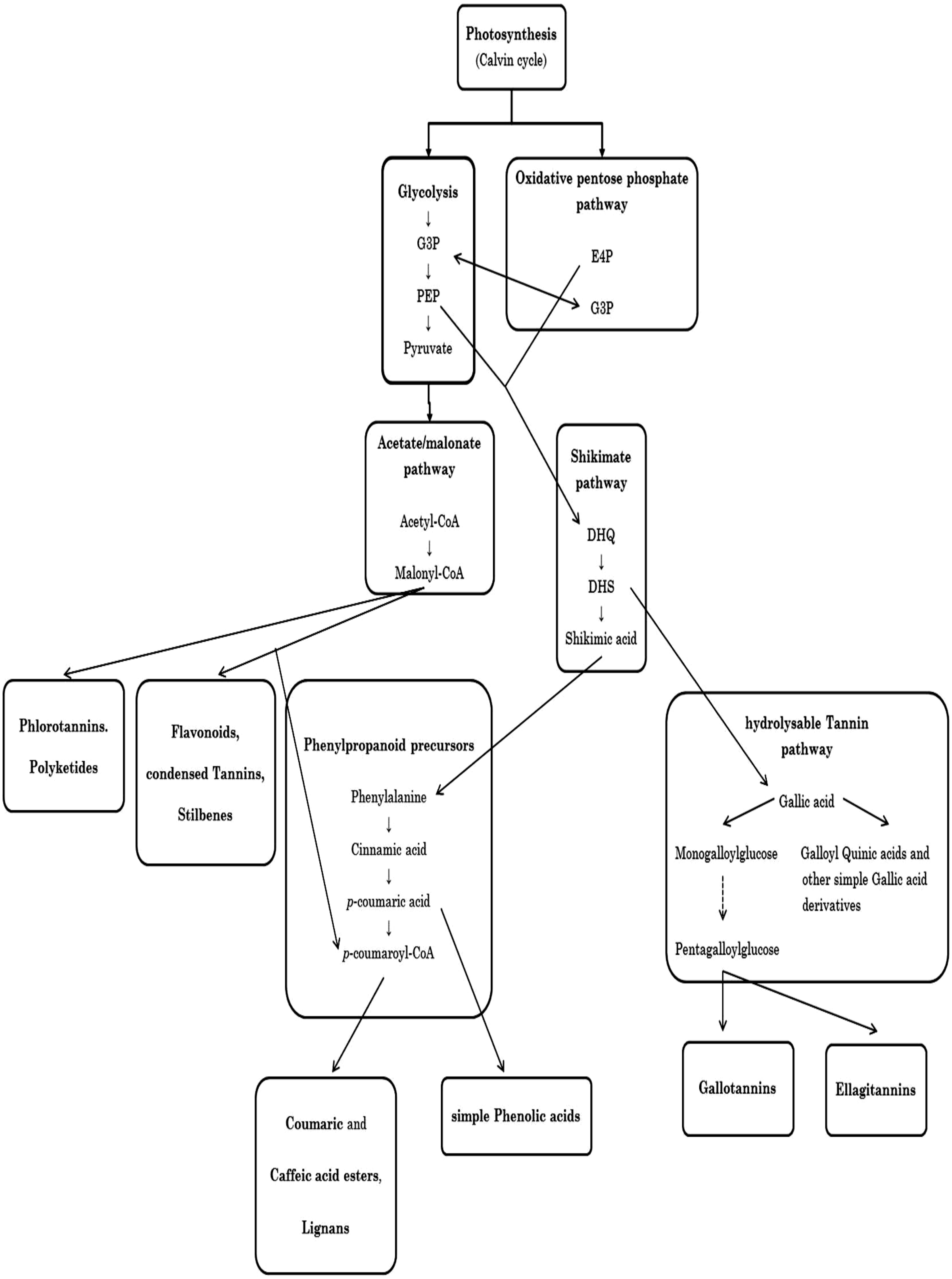
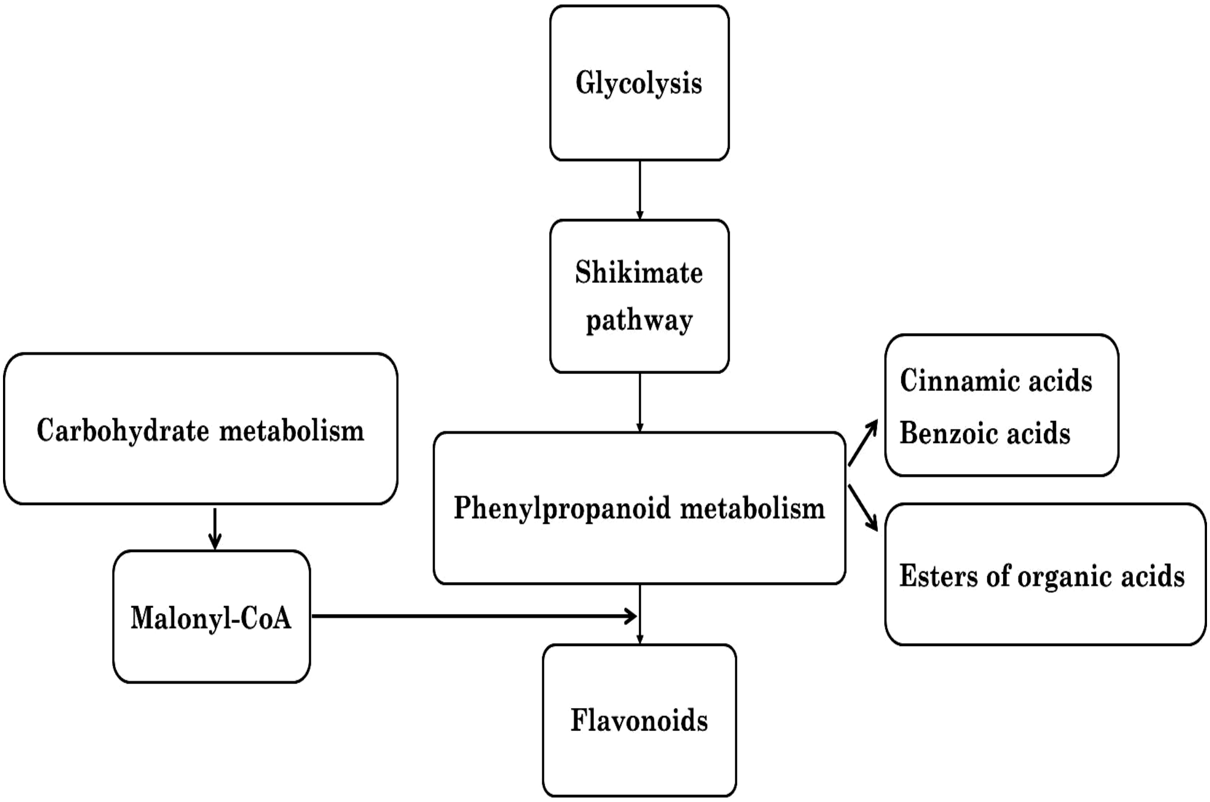


Figure 3: The schematic of general phenolic biosynthesis pathway. (Figure by Ossipov et al., 2003).

The significance of this pathway to be considered when applying environmental stress parameter in the study was supported by the report, which, in normal plant growth conditions, 20% of C fixed by plants will flows through this pathway (Figure 4) (Michalak, 2006). Hence, determining the proper growth of plants will direct the right direction of C flow into the correct biosynthesis pathways of flavonoid, phenolics and tannins in *G. procumbens*.

Figure 4: The schematic of the main groups of phenolic compounds biosynthesis pathways (Figure by Michalak, 2006).



On top of that, phenols are also divided into numerous different groups, determined by the number of constitutive C atoms in concurrence with the structure of the basic phenolic skeleton such as simple phenol, benzoquinones (C6); phenolic acid (C6-C1); acetophenone, phenylacetic acid (C6-C2); hydroxycinnamic acid, coumarin, phenylpropanes, chromones (C6-C3); naphthoquinones (C6-C4); xanthones (C6-C1-C6); stilbenes, anthraquinones (C6-C2-C6); flavonoids, isoflavonoids, neoflavonoids (C6-C3-C6); bi-, triflavonoids ((C6-C3-C6)$_{2,3}$); lignans, neolignans ((C6-C3)$_{2}$); lignins ((C6-C3)$_{n}$); catechol melanins ((C6)$_{n}$); and condensed tannins ((C6-C3-C6)$_{n}$) (Lattanzio, 2013).

In addition, the catalysis and an enhancement of phenylpropanoid metabolism as well as the observation of other compounds synthesis including phenolic can be observed under various environmental stress conditions and factors as in the following example of cases (Aminifard, Aroiee, Nemati, Azizi, & Jaafar, 2012; Ghasemzadeh &

Ghasemzadeh, 2011; Ghasemzadeh et al., 2014; Ibrahim et al., 2013, 2014, 2011; Jaafar et al., 2012; Michalak, 2006; Muradoglu et al., 2015; Smith, Wu, & Green, 1993) (Table 2);

Table 2: The effects in compound biosynthesis under various environmental influences (Boroomand & Grouh, 2012; Cheynier et al., 2013; Ferreyra, Rius, & Casati, 2012; Ibrahim et al., 2013; Jaafar et al., 2012; Texeira, de Carvalho, Zaidan, & Klein, 1997).

Unfavorable environment or stress conditions	Compound synthesis
Injured and/or infected plants, or under low temperatures and nutrient supply.	Induction of isoflavones and some other flavonoids.
Prevention of UV-B penetration into the deeper tissues of the plant.	Accumulation of UV-absorbing flavonoids and other phenolic compounds in vacuoles of epidermal cells.
Activation of root nodule bacterial genes.	Secretion of flavonoids from roots of *legumina*.
Response of wheat to Ni toxicity.	Induction of phenolic.
Response of maize to Al.	Induction of phenolic.
Exposure of *Phaseolus vulgaris* to Cd^{2+}.	Accumulation of soluble and insoluble phenolic.
Cu_2SO_4 sprayed to *Phyllantus tenellus* leaves.	Induction of phenolic more than the control plants.
Fulvic acids effect on fruit quality of *Capsicum annuum*.	Fruit's antioxidant activity, capsaicin, carbohydrate, carotenoids, and total phenolic contents were influenced, but ascorbic acid and total flavonoid contents were not affected significantly.
Toxicities of Cd in strawberry cv. Camarosa roots and leaves.	Affect the chlorophyll content and decreased nearly 30% of plant growth.
Enrichment of CO_2 on the nutritional quality of *Zingiber officinale*.	Increase level of CO_2 from ambient to elevated resulted in amino acids, cyanide, fructose, glucose, phytic acid, sucrose, tannin, and total carbohydrate content to increase; and reduction of total protein content in the leaf and rhizome.
CO_2 and light intensity impact on *Labisia pumila* Benth.	Influence the production of chlorophyll, malondialdehyde, and sugar content by the interactions between CO_2 and irradiance.
Organic and inorganic fertilizers impact on *Labisia pumila* Benth	Enhance the production of ascorbic acid, flavonoids, gluthathione, saponin, and total phenolics content by organic fertilizer compared to the use of inorganic fertilizer.
Changes in the production of primary and secondary metabolites in *Orthosiphon stamineus* Benth induced by ABA.	Influence the production of antioxidant activity, PAL activity, LOX inhibitory activity, and soluble sugars by; i. Enhance the production of flavonoids,

	H_2O_2, LOX inhibitory activity, O_2^{-}, PAL activity, sucrose, and total phenolics. ii. Increase the antioxidant capabilities (DPPH and ORAC). iii. Increase the production of antioxidant enzymes (APX, CAT, and SOD). iv. Reduce the net photosynthesis and stomatal conductance under high application rates of ABA.
CO_2 enrichment and foliar application of salicylic acid effect on ginger.	Increase the production of anthocyanin, apigenin, fisetin, morin, myricetin, naringenin, and rutin contents in leaves.
Foliar application of salicylic acid effect on *Zingiber officinale*.	Induce the synthesis of anthocyanin and fisetin, enhance the chalcone synthase (enzyme activity involving in flavonoid synthesis) and increase the protein activity.
Soil field water capacity impacts on secondary metabolites of *Labisia pumila* Benth.	As net photosynthesis occurs, the apparent quantum yield and chlorophyll content will be down-regulated under high water stress; therefore, the production of anthocyanin, flavonoids, and total phenolics will be up-regulated implying the imposition of high water stress.
N_2 fertilization effects on synthesis of primary and secondary metabolites in *Labisia pumila*.	Enhance PAL activity, reduce the production of soluble protein under low N_2 fertilization indicate more resources of amino acid phenyl alanine under low N_2 content stimulate the production of CBSM. This was manifested by high CN ratio in plants.

Environmental factors such as abiotic stresses will stimulate C fluxes from the primary to the secondary metabolic pathways. The event therefore will catalyze a shift of the available resources in favor of the synthesis of secondary products (Hill, Germino, & Alongi, 2011). In plants, normally they have limited resources to support their physiological processes; therefore, all requirements cannot be met simultaneously and resulted in more C will be diverted from growth toward secondary metabolism when plant growth is restricted by the physiological and/or ecological constraint as reported in the role of phenylalanine may shift from initiating protein formation to enhancing phenolic synthesis upon changes in water stress (Romagni, 2009).

An interesting link presented between primary and secondary metabolism has been proposed by Lattanzio (2013), which connects the accumulation of stress metabolite proline with the energy transfer toward phenylpropanoid biosynthesis through the oxidative pentose phosphate (OPP) pathway (Figure 5). In most plants, free proline will accumulate in response to the imposition of various abiotic stresses factors such as atmospheric pollution, heavy metal toxicity, high or low temperature, nutrient deficiency, pathogen infection, salinization, water deprivation, and ultraviolet (UV) irradiation (Caretto, Linsalata, Colella, Mita, & Lattanzio, 2015; Rahimi, Sayadi, Dashti, & Tajabadi, 2013).

Pertaining to this connection, it has also suggested that the value of stress-induced proline accumulation may be mediated mainly through the effects of its synthesis and degradation on the cellular metabolism's level. As proline synthesis is accompanied by the oxidation of nicotinamide adenine dinucleotide phosphate (NADPH), meanwhile, an increase in $NADP^+$/NADPH ratio is to be expected to enhance the activity of the OPP pathway providing precursors for the phenolic biosynthesis through the shikimic acid pathway. The alternating oxidation of NADPH by proline synthesis together with the reduction of $NADP^+$ by the two oxidative steps of the OPP pathway has serve to link both pathways and thus facilitate the continuation of high rates of proline synthesis during any stress exposure and lead to a simultaneous accumulation of phenolic compounds (Lattanzio, 2013).

These dynamic reports would apply best in *G. \procumbens* in which while the study is targeting to up-regulate the synthesis of valuable compounds whilst maintaining the growth of the plant, since the idea of manipulating the physiological and/or ecological constraint are adapted in the plantation. Primary and secondary metabolisms are strongly interconnected (Tugizimana, Piater, & Dubery, 2013). Sufficient nutrient resources such as N, phosphorus (P), K and water are required to support plant's physiological processes; and hence, the requirements can be met simultaneously, and C will be distributed evenly for growth and secondary metabolism once plant growth is not restricted by any physiological and/or ecological constraint (Fraser, Silk, & Rost, 1990; Zheng, 2009).

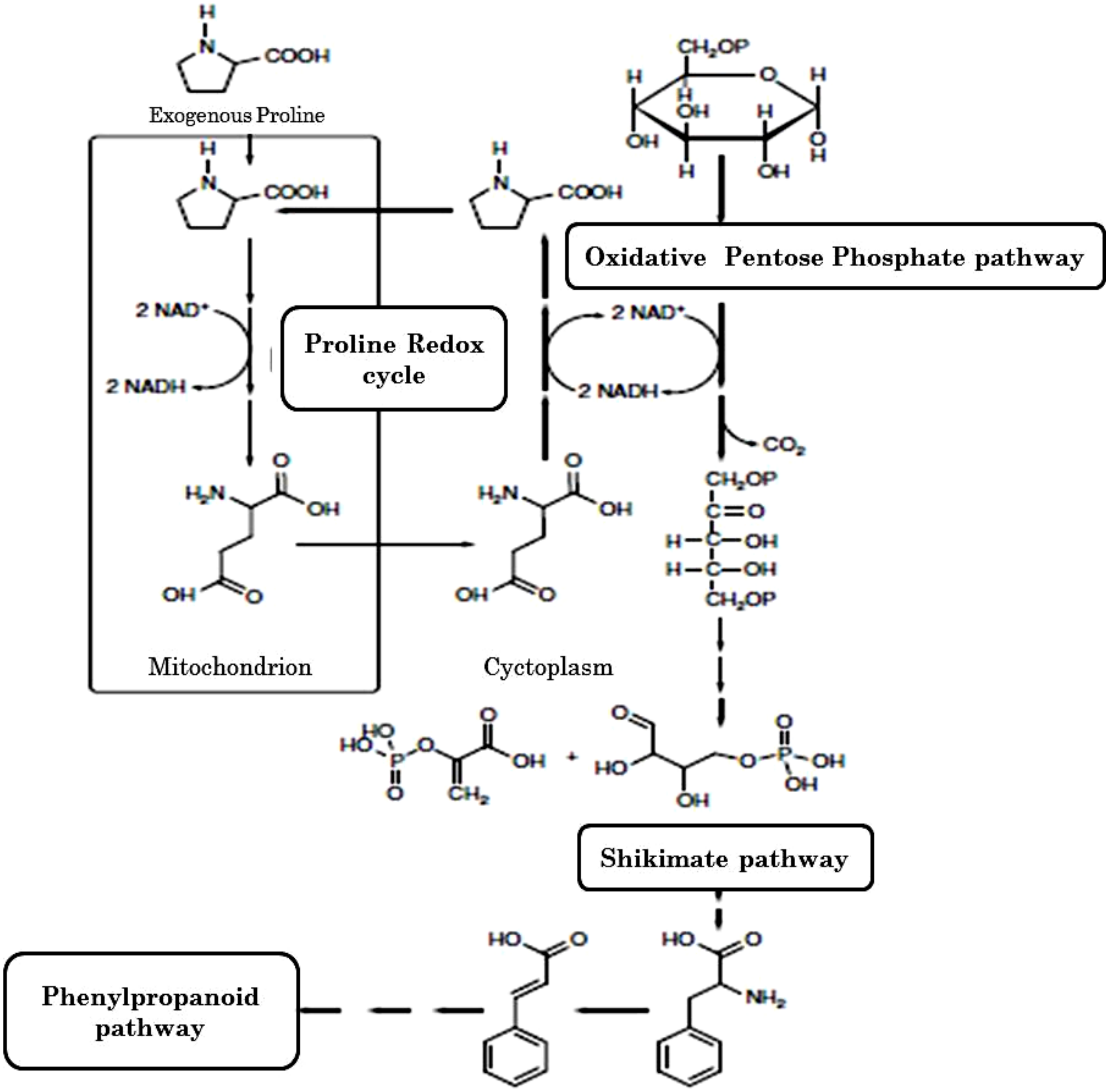


Figure 5: The schematic of oxidative pentose phosphate, phenylpropanoid, and proline redox cycle relationship pathway (Figure by Caretto, Linsalata, Colella, Mita, & Lattanzio, 2015).

Apart from that, the chalcone synthase enzyme (CHS) was also being discovered and reported to play as a key enzyme in flavonoid metabolism in plant cells (cortex and epidermal cells in the tip and elongation zone of the root), consistent with the accumulation of flavonoid end products at these sites (Ramawat et al., 2009). Also, has been reported that the CHS activity was significantly influenced by the plant age, where the lowest and highest activity levels of CHS were recorded in one and 6-month-old buds respectively. Apart from that, the CHS enzyme activity was also enhanced in one to 6-month-old seedlings in the leaves and buds respectively (Ghasemzadeh et al., 2014).

In contrast, with the increasing growth period from 6-month-old to one year, CHS enzyme activity was decreased significantly. The mechanism of this enzyme effect on flavonoid synthesis has reflected the initial reaction of PAL, in which the branch point enzyme between shikimate and phenylpropanoid pathway for diverting phenylalanine from primary metabolism to the production of *trans* cinnamic acid and other phenolic compounds, where its activity is very high at the start of development and beginning to decrease up to maturation (Ghasemzadeh et al., 2014).

The CHS might always present in the plant cells and will be activated at the protein level. Figure 3 and 4 has shown the flavonoids were derived from 4-coumaroyl-CoA and malonyl-CoA in the presence of CHS enzyme. This indicates that the CHS is an important enzyme for flavonoid biosynthesis. Therefore, CHS could be considered as a biochemical marker in evaluating the dynamic changes in flavonoid synthesis in plants (Ghasemzadeh & Ghasemzadeh, 2011; Ghasemzadeh et al., 2014).

Above all, most of the compound's C skeleton is derived mainly from carbohydrates synthesized by photosynthesis. The synthesis of numerous classes of secondary metabolites from primary metabolites is presented in Figure 6; DOX/MEP pathway, non-mevalonate pathway or deoxy-xylulose 5-phosphate/methyl-derithrol 4-phosphate pathway. Most secondary metabolites are synthesized through the two principal biosynthetic pathways: shikimic acid pathway will produce a group of aromatic amino acids, which in turn are converted into various compounds such as phenolics (lignins, quinones, and tannins) and alkaloids, and acetyl-CoA mevalonic acid pathway will lead to a vast array of terpenoids (Lattanzio, 2013).

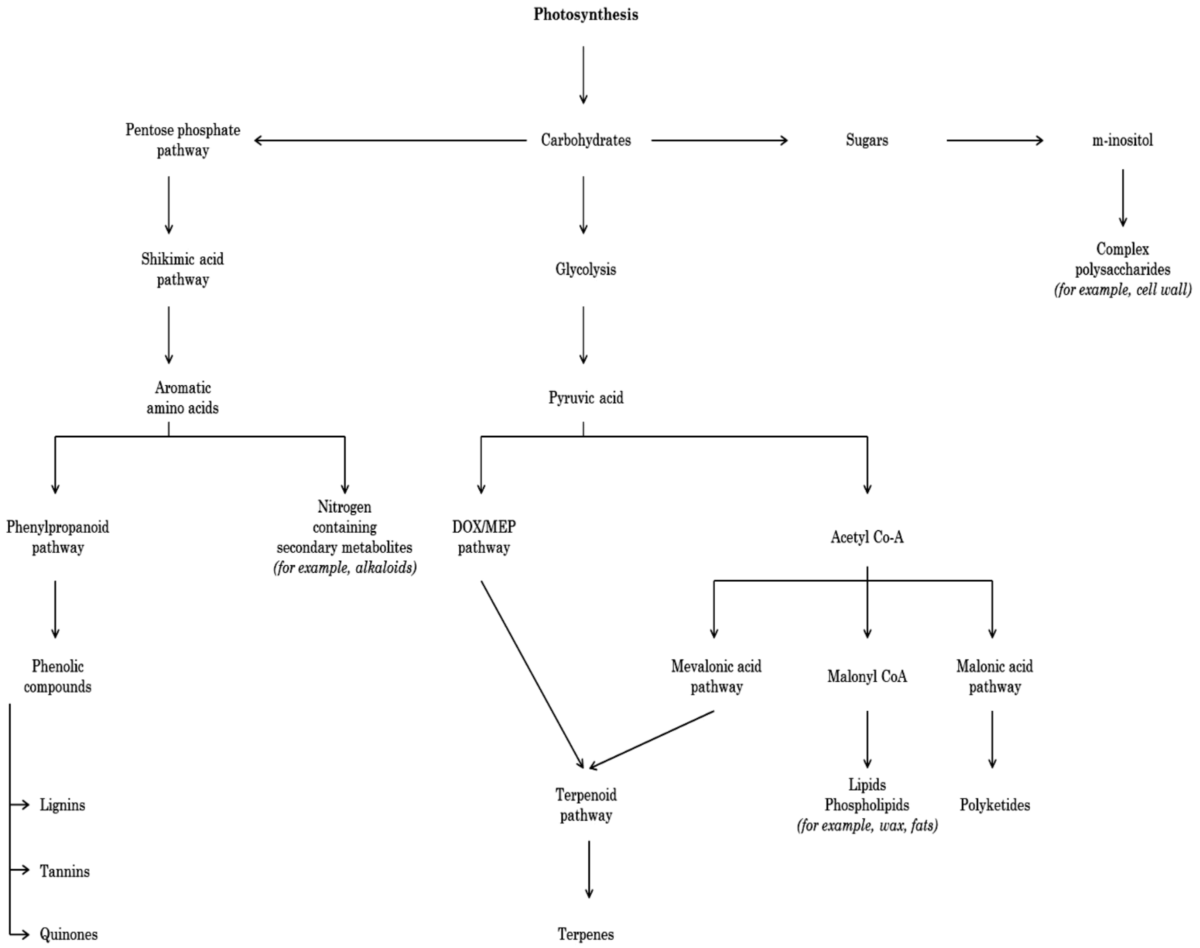


Figure 6: The schematic of principle biosynthetic pathways leading to secondary metabolites synthesis. (Figure by Ramawat et al., 2009).

SYNERGISM OF WATER, NITROGEN AND POTASSIUM IN SECONDARY METABOLITES BIOSYNTHESIS REGULATION OF PLANTS GROWN UNDER GLASSHOUSE CONDITION: APPLICATION IN *Gynura procumbens*

Manipulating water availability in plant propagation, acclimatization and cultivation system could result in diverse phytochemical profiles. Water stress increases the production of jasmonic acid and ABA, which results in stomata closure, and accumulation of sesquiterpene and tannins (Z. Li, 2002). Plants growing under reduced-water availability also had lower phenolic contents compared to plants which received continuous irrigation (Espírito-santo, Fernandes, Allain, & Reis, 1999; Szakiel, Pączkowski, & Henry, 2011).

Meanwhile, in an excessive N supply beyond the need of plant requirement could be presumably shifted into phenolics biosynthesis based on a carbon-use efficiency theory (Hill et al., 2011; Schuppler, He, John, & Munns, 1998), i.e. carbon accumulated/carbon depleted was significantly improved when plants had a combination of external sugars available compared with plants grown in a single hexose alone (Armengaud et al., 2009). Plants will avoid complete down-regulation of photosynthesis even though a large excess of external C fluxed through their cells. However, when N supply is taken deficient the optimal level, it will cause scleromorphism, stunting, and increased root to shoot ratio (RSR) (Hanudin, Wismarini, Hertiani, & Sunarminto, 2012; Walker, Burns, & Moorby, 2001).

As for K, the nutrient has least important in the chemical structure of the plant. The availability and movement of the nutrient in the plant will allow it to influence almost every aspect of plant growth (Mudau, Soundy, & du Toit, 2005). Potassium activates enzymes, controls plant turgidity, encourages root growth, helps in protein formation, strengthens stalks, transports sugar and starch, and involved in many other plant functions to provide regulatory roles in plants development and survival (Donald L. Armstrong, 1998; Gaj, Górski, & Przybył, 2012).

Ultimately, an important aspect to take into accounts in inducing plants to regulate water availability as well as varying nutrients supply is the potential of synergistic or antagonistic effect of multiple stress-producing environments such as the advantage of a water stress in increasing secondary metabolites could be by accompanied with undesired effects; a reduction in yield (Mbagwu & Osuigwe, 1985).

RATIONALE OF STUDY

Phenols are mainly synthesized through the shikimate pathway. Meanwhile, the shikimate pathway is a major biosynthetic route for both primary and secondary metabolism, beginning with the PEP and E4P and ends with the chorismate. Furthermore, the chorismate is an important branching point since it is the substrate for all subsequent products, and vast diversity of phenolic compounds is synthesized through these intermediate products (Lattanzio, 2013; Michalak, 2006).

At the same time, inorganic N and K ion (NO_3^- and K^+), imbibed by the plant roots and transported into the leaves was converted into NO_2^- by nitrate reductase and then NH_4^+ by nitrite reductase. The NH_4^+ is assimilated into glutamine by glutamine synthetase. The glutamine was then transferred an amino group to chorismate by aminotransferase (Hendawy & Khalid, 2011; Z. Li, 2002). The effects of phenylalanine and tyrosine on enzyme levels and activity showed that chorismate mutase P is probably related to phenylalanine biosynthesis and chorismate mutase T to tyrosine biosynthesis.

Abiotic factors, such as water, N and K fertilization could provide the main C source for the biosynthesis of the C-containing compound through efficient photosynthesis process (Abdelaziz, Pokluda, & Abdelwahab, 2007; Heydarizadeh, 2016). Thus, it is conceivable that supplying the right amount of water and nutrient to *G. procumbens* might increase the secondary metabolite biosynthesis and accumulation.

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