

## Watering and nitrogen and potassium fertilization: The significance of abiotic control on *Gynura procumbens* (Lour.) Merr herbs in Malaysia for better growth and secondary metabolite enrichment

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**Abstract.** Environmental changes have led to cellular adjustment and adaptation in plant growth. External factors have, for example, influenced the growth pattern of *Gynura procumbens* plants and led to production of specific secondary metabolite internally for the purpose of differentiation and conditional interaction. These developmental patterns and production of metabolites are expressional characteristics of the plant, and so growers can have only a restricted range of movement or limited control over their reaction to environmental changes compared to their reaction to human or animal interactions. Even though metabolite production is pervasive among the plants, the need to explore abiotic control strategies for regulating the patterns of growth of *Gynura procumbens* as well as their accumulation of metabolites has been shown to be significant in recent studies of plant-abiotic interactions.

**Keywords:** Abiotic, growth, *Gynura procumbens*, herbs, metabolite

### INTRODUCTION

#### **Conventional value of *Gynura procumbens*.**

Traditionally, Malaysia has had an extensive array of herbal medicinal plant species and traditional medical systems. More than 2,000 medicinal plant species have been recorded locally with herbal products forming the most important components (Samah *et al.*, 2010; Sekar *et al.*, 2014). A review of ethno medical information related to medicinal plants in Malaysia produced a list of most important herbal medicinal plants used for various illnesses, including *Gynura procumbens* (*G. procumbens*), *Eurycoma longifolia*, *Labisia pumila*, *Ficus deltoidea* and *Zingiber officinale* Roscoe. The treatments included aphrodisiac, anti-diabetic, anti-microbial, anti-pyretic, anti-oxidant, anti-inflammatory, anti-cancer and anti-diuretic ingredients (Arifullah *et al.*, 2014; Keng *et al.*, 2009; Sekar *et al.*, 2014).

**Modern value of *Gynura procumbens*.** *G. procumbens* was listed as a high-value herbal plant under the Agricultural National Key Economic Area (NKEA) Initiative for Herbal Subsector in 2010 (MOA, 2015).

Later, the Global Information Hub on Integrated Medicine, Malaysia (Globinmed) was also set up in line with the significantly increased role of traditional and

complementary medicine production called for by the Malaysian government. The Globinmed has promoted the importance of medicinal plants by compiling the Malaysian Herbal Monograph (MHM) database in order for the interested parties to have a point of reference. The corporation also extended the initiative up to the South East Asean (ASEAN) region by establishing the ASEAN Task Force on Traditional Medicine (ATFTM). This step was positive since the interest in using and producing products based on herbs, especially *G. procumbens*, was shared by the ASEAN community in Thailand, Philippines, Indonesia and Malaysia (Globinmed, 2015).

All this while, the plant was planted and consumed domestically as a folk medicine because of their medicinal properties, including reported anti-diabetes, anti-hypertension and anti-cancer effects (Adnan and Othman, 2012; Rahman and Al Asad, 2013). Recently, the Economic Transformation Programme (ETP)

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under the Performance Management & Delivery Unit (PEMANDU) at the Prime Minister's Department has projected that the high-value herbal production and trading will contribute to the gross national income (GNI) by the year 2020 at the level of RM2.2 billion. Consequently, a few agrobiotechnology-based companies, namely Biotropics Malaysia Berhad, Natureceuticals Sdn. Bhd. and Bioalpha International, were chosen to spearhead a project focusing on developing and cultivating high-value local herbal plants such as *Euricoma longifolia*, *Labisia pumila*, *Orthosiphon stamineus* and *G. procumbens*, which are in high demand pertaining to this projection (PEMANDU, 2015).

**Valuable phytochemical properties of *Gynura procumbens*.** Conventionally, the plant is used in the treatment of various health ailments such as eruptive fevers, rash, kidney disease, migraines, constipation, blood hypertension reduction, diabetes mellitus, urinary infection, and cancer (Adnan and Othman, 2012; Dung and Loi, 1991; Hew *et al.*, 2013). However its phytochemical properties have not been well studied until recently. Several researchers (Bhore *et al.*, 2010, Hoe *et al.*, 2011, Hoe *et al.*, 2007, Jarikasem *et al.*, 2013, June *et al.*, 2012, Kaewseejan *et al.*, 2015, Kaewseejan *et al.*, 2012, Keng *et al.*, 2009 and Lee *et al.*, 2012) have reported positive effects of extracts from this plant. Extracts had an enhancing effect on glucose uptake in adipocyte cell lines and suggested that the anti-diabetic action of this plant might be mediated through the stimulation of glucose uptake. Crude ethanolic extracts showed anti-inflammatory properties, and steroids might be one class of anti-inflammatory compounds found in this species. The leaf extracts significantly suppressed elevated serum glucose levels (anti-hyperglycemia) and reduced serum cholesterol and triglyceride levels in diabetic patients (anti-hyperlipidaemic). The n-butanol extracts of this plant could reduce the blood glucose levels in type-2 diabetes. Two compounds, 3,5-di-*O*-caffeoylquinic acid and 4,5-di-*O*-caffeoylquinic acid, identified from this plant, were found to inhibit the replication of virus particles through anti-herpes simplex virus, anti-proliferative on human mesangial cell, anti-oxidative and anti-ulcerogenic properties. Kaempferol-3-*O*-rutoside and astragalins contained potential anti-oxidant components. Finally, polyphenolic flavonoid compounds with potential beneficial effects with anti-allergic, anti-platelet, anti-viral, anti-inflammatory, anti-tumor, and anti-oxidant activities.

The benefits of *G. procumbens* have also been supported by studies that have shown isolation and identification of several possible active chemical constituents such as phenolic, flavonoid, saponin, sterol glycoside tannin and terpenoid compounds, including proteins from the leaf extract (Afandi *et al.*, 2014; Arulselvan *et al.*, 2014; Hew and Gam, 2010; 2011; Iskander *et al.*, 2002; Liew *et al.*, 2012).

**Value projection.** The use of herbal medicinal plants and their related natural products has become increasingly important worldwide over the past two decades. As stated by Bhore and Vaishana (2010), global trade of natural plant products is projected to triple by the year 2020, with the herbal medicine market expected to grow by 10 to 30%. On the domestic front, the herbal industry of Malaysia was also estimated to grow at the rate of 15% annually; the market value was projected to rise from RM7 billion in 2010 to almost RM29 billion in 2020 (Ahmad and Othman, 2009).

On top of that, according to the World Health Organization (WHO), almost 80% of the populations living in developing countries including Malaysia are still using traditional medicines derived from plants for their primary health care needs (Bodeker *et al.*, 1997). At present, despite the necessity of synthetic drugs and antibiotics for some medical practices, a leading contribution to the pharmaceutical industries is still provided by plants (Parvin, 2014). As the production of these products requires a greater mass of natural ingredients compared with those from artificial chemical bases, the demand for alternative bases derived mainly from quality medicinal plant production is growing significantly (Briskin, 2000). The importance of producing high quality resource materials is thus great, and the study of abiotic control of *G. procumbens* growth for the purpose of establishing a good agricultural protocol to produce better and sustainable yield of the plants is timely.

*G. procumbens* is also important for study, since their phytochemical and ethnobotanical importance are projected to be highly valuable (Curtiss, 2013; Hassan *et al.*, 2010; Hassan *et al.*, 2008; Keng *et al.*, 2009; Puangpronpitag *et al.*, 2010; Whysall, 2013). On the other hand, the plants are manifestly open to simple cultivation by potential farmers and producers (Bhore and Vaishana, 2010; Pallez and Dole, 2001).

## THE NEED FOR FURTHER INVESTIGATION

*G. procumbens* extract has been shown to possess certain pharmacological activities such as anti-oxidant, anti-cancer, anti-diabetic, anti-hypertensive and hepatoprotective properties and it can lower the occurrence of chromosomal aberration (Gracilla and Bagunu, 2014; Liu *et al.*, 2010; Rahman and Al Asad, 2013; Sekar *et al.*, 2014; Shwter *et al.*, 2014). Thus, this plant has exhibited similar or possibly greater value than other common herbal medicinal plants used to derive medicinal products, such as *Clinachantus sp.*, *Ficus sp.*, *Morinda sp.*, *Orthosiphon sp.* and *Zingiber sp.* On top of that, if the mode of cultivation of *G. procumbens* is less critical, which will then translate into lower planting and production costs, making this plant a promising asset to the Malaysian herbal medicinal industry.

However, the common problems experienced by the herbal planter include watering effectiveness and nutrient application. Watering ineffectiveness may cause rotting roots, so the capability of plants to imbibe available nutrients from the growth media will be inefficient. Meanwhile, imbalance in nutrient supply may cause imperfect cell division and differentiation; thus, the metabolic rate will be disrupted and could result in senescence and abscission of leaves and lead to dying plants (Bhore and Vaishana, 2010; Keng *et al.*, 2009; Pallez and Dole, 2001; Parvin, 2014; Thomas, 2013).

The local entrepreneur or herbal planter venturing into cultivating this plant, due to its promise and validation from published reports, could trade products such as exported plantlets, drink-tea, edible ornamental plants, or processed pharmaceutical products such as supplements pills and capsules or even potentially dietary supplements. Maximising the effects of mixed fermented medicinal plants (FMP) and antibiotic growth promoter (AGP) on growth performance in broilers definitely requires elite planting materials to produce healthy plant structures rich in valuable compounds (Jeong and Kim, 2015; Keng *et al.*, 2009). It is therefore important to investigate environmental factors affecting plant material yield in *G. procumbens* in order to produce an effective growth and secondary metabolite production. Therefore, studies to explore the effects of factors on plant growth and secondary metabolite production, and to define the relationships between plant growth and secondary metabolite production, are important.

## THE RESOURCE AVAILABILITY HYPOTHESIS

Many studies have given attention to establish the theories of primary physiological processes that are unique to plants such as photosynthesis and morphology to describe such theories in terms of their effectiveness (Hunt, 2003). However, refining the theory of interaction which diverts the environmental and secondary metabolites that are responsible for plant growth pattern and secondary metabolite production is expected to provide greater insight into understanding the total mechanism across the whole plant. In particular, generalizations derived from this understanding of mechanisms in natural systems would provide immediate applications to alter patterns of agriculturally controlled cultivation for the desired growth and metabolite production in plants. Therefore further study would enable us to extend the findings for specific factor(s) for maximizing *G. procumbens* yields through suitable manipulation.

Therefore several hypotheses, such as Carbon: Nutrient Balance (CNB), Growth Rate (GR), Growth -Differentiation Balance (GDB) and Optimal Defense (OD) (see Table 1), have specifically served as frameworks for investigating the ideas and theories pertaining to patterns of growth and metabolite production against environmental changes in plant (Fonseca *et al.*, 2006; Ramakrishna and Ravishankar, 2011). The predictions, tests and analysis of these hypotheses have somehow led to

**Table 1.** Theories in plant evolutionary ecology.

Hypotheses	Principal contribution
CNB	Served as an investigation of how resources affect phenotypic expression of plant growth and metabolite production, in studies concerned with allocation cost of formation and secretion
GR	Explained how the intrinsic growth rate of plants formed evolutionarily by resource availability affects metabolite production patterns.
GDB	Recognized the constant physiological trade-off between growth and differentiation at the cellular and tissue levels relative to the selective pressures of resource availability, including plant potential expression by the environment
OD	Functioned as the main framework for investigation of genotypic expression of plant defense, with the emphasis on allocation cost of metabolite-defense.

significant perplexity about the growth, development and metabolite production theory of plant systems (Fonseca *et al.*, 2006; Szakiel, *et al.*, 2011; Yazaki, 2005).

On top of that, variation in growth and secondary metabolite content of herbal medicinal plants can also arise due to environmental and genetic variables (Chaffai *et al.*, 2007). Moreover, the concept of phenotypic plasticity preceding genetic variation is also responsible for considerable variation in plant growth patterns and secondary metabolite production (Abrahamson *et al.*, 1988; Geelen *et al.*, 1976). The fact that both sources of variation are similar in magnitude is relevant to studies of herbal medicinal plant growth and secondary metabolite accumulation. Meanwhile, most of the studies looking at quantitative manipulated genotypes or the environment have been conducted deliberately to measure the response of plant growth and chemistry, but the environmental manipulations have not always controlled for genotype and *vice versa* (Yazaki, 2005). Interesting generalizations may emerge from this synthesis of environmental variation to external growth arrangement as well as internal metabolite secretion.

Thus, since the growth form and secondary metabolite production are important in modifying a plant's interaction and reaction to the environment, there is a need to conduct studies to examine the mechanism of variation in these parameters (Geelen *et al.*, 1976; Satoh and Flores, 1990). Previously, studies on secondary metabolite production were often linked to questions of the efficacies of plant growth and hypotheses about their evolution in different plant development conditions. Hence, future study should include a series of analyses to examine the impact of environmental variation in determining the quality of growth pattern and secondary metabolite properties of the plants.

This is because, hypothetically, environments affect plant growth arrangement and secondary metabolite production, supporting continued study of the CNB and GDB hypotheses (Kennelly *et al.*, 2012). In addition, herbaceous plants are more influenced and susceptible to the environmental variation compared to woody plants, by genetic variation (Ramakrisna and Ravishankar, 2011). Ironically, plant growth and secondary metabolite production in agricultural cultivation and natural habitats have shown similar responses to manipulations as plants in laboratory, greenhouse or field studies (Satoh & Flores, 1990). Hence, study into growth pattern and

secondary metabolite production should reveal an effective controlled strategy for a plant in abiotic mutualisms with the environments.

## DEVELOPMENT OF A STUDY DESIGN

Pertaining to the above mentioned hypotheses, a study incorporating the elements of water and nitrogen and potassium fertilizer would serve to determine the possible influential environmental factors on yield and phytochemical content of *G. procumbens*. These elements represent the CNB hypothesis which explains how resources affect phenotypic expression of plant growth and secondary metabolite production (Fonseca *et al.*, 2006).

The idea was supported by the fact that post-mitotic expansion during the cell cycle processes in plants is largely driven by water. This eventually affects growth and is a scalable property, from populations of cells, to phytomers, to individuals, and to the whole biomass (Thomas, 2013). Consequently, the supply of water will determine the developmental quality of the plants. On top of that, it is well established that an adequate nutrient supply, especially nitrogen and potassium, are required for optimal plant growth. If the nutrient is taken up beyond the required concentration it offers no further advantage for growth (Kennelly *et al.*, 2012). However, less is known about the response which leads to the occurrence of plant secondary metabolite production. Rationally, with a limited amount of nutrients available in the environment, plant growth and secondary metabolite production may compete for the nutrient, and a trade-off between plant growth and secondary metabolite accumulation will occur (Boroomand and Grouh, 2012; Szakiel, 2011). It is likely that proper manipulation of water, nitrogen and potassium availability may result in well-balanced distribution between plant growth and secondary metabolite production. Thus, the occurrence will provide a promising way to increase plant yield. Consequently, there will be an effect of nitrogen and potassium availability on plant growth and secondary metabolite production.

A clearer understanding of these hypotheses will facilitate further development of a well-designed study to address the gaps in understanding about the herbaceous plant mechanisms related to growth quality, metabolite production and environmental variation.

## ABIOTIC FACTORS: WATER, NITROGEN AND POTASSIUM FERTILIZER

Water is crucial for plants' productivity and quality. However, water requirements differ according to the plant's variety and growth media type (Mráz *et al.*, 2014), and total water requirement includes the water the plants use and also the losses due to evapotranspiration, water application, land preparation, and leaching processes during the growth period (Mbagwu and Osuigwe, 1985; Smith *et al.*, 1993).

Meanwhile, nitrogen (N) is classified as a macronutrient, required for plant cell development and necessary for all proteins, enzymes and metabolic processes involved in the synthesis and transfer of energy (Kennelly *et al.*, 2012). Relatively large amounts of N are absorbed from growth media. Apart from that, N is also part of chlorophyll, the green pigment of the plant which is responsible for photosynthesis reactions. An abundant source of N helps plants with rapid growth, increasing seed and fruit production and improving the quality of leaf and forage crops (MacAdam *et al.*, 1989; Pradnyawan *et al.*, 2005). Ordinarily, N comes from fertilizer application and from the air.

In addition, potassium (K) is an essential nutrient for plant growth. Since large amounts of K are absorbed from the root zone in the production of most agronomic crops, it is also classified as a macronutrient (Kennelly *et al.*, 2012). This nutrient is absorbed by plants in larger amounts than any other mineral element except N, and, in some cases, calcium (Ca). Potassium is associated with movement of water, nutrients, and carbohydrates in plant tissue. If K is deficient or not supplied in adequate amounts, the plant growth is stunted and yields are reduced. Availability of K helps in the protein building, photosynthesis, fruit quality and reduction of diseases (Ibrahim *et al.*, 2012; Rehm and Schmitt, 2002). This nutrient is important and supplied to plants by growth media minerals, organic materials and fertilizer.

### ***Effects of watering, nitrogen and potassium fertilization on growth and development.***

Negative watering effects can be classified into deficiency and excessive statuses. Generally, low water status (LW) in plants can occur either as a short-term (STD) or prolonged deficiency (PD). An STD might result in minor effects on the plant such as wilted leaves or shoots. These symptoms may be temporary, for example they can occur during the warmest part of the day when transpiration rates are high. Once the environmental stresses are relieved, the symptoms of LW may disappear (Mbagwu and Osuigwe, 1985; Santo *et al.*, 1999). In contrast, during PD, the damage sustained by plants may be severe. Plants may grow slowly or not at all, young leaves may not fully expand, or foliage may not grow as colourful as foliage developing when the plant is not

under LW. In severe cases, burning or marginal leaf necrosis can also occur.

Meanwhile, excessive soil moisture (ESM) can result in reduced oxygen (O<sub>2</sub>) availability to the roots. Therefore, a primary symptom of ESM is plant wilt. On top of that, ESM can also manifest as acute (AWE) or prolonged (PWE) water excess. During AWE, roots are subjected to low O<sub>2</sub> status, and cell weakening and/or death occurs. Symptoms include discolored and/or water-soaked and mushy roots. Under these conditions, roots can become weakened and predisposed to invasion by pathogens. The AWE effects also predispose plants to root diseases caused by certain pathogens that have flagella and are able to swim towards host roots. In contrary, PWE may result in plants becoming stunted and with underdeveloped shoots. In severe cases, bleeding cankers on stems can also occur. Adventitious roots may form at the root crown. Bark can split and wood may become water-soaked and discolored. Edema or corky, blister-like swelling can occur on the underside of leaves on plants growing in waterlogged soils. Edema can be worse during cloudy, overcast periods (Mbagwu and Osuigwe, 1985).

Deficiency in N is a major limitation for non-leguminous agricultural plants such as herbal crops because N is quickly leached out of the soil once it is converted into NO<sub>3</sub><sup>-</sup> by soil microbes. Usually, plants can absorb N in two ionic forms, NO<sub>3</sub><sup>-</sup> and NH<sub>4</sub><sup>+</sup>. Therefore, the use of N fertilizer is higher than any other single macro- or micronutrient (Walker *et al.*, 2001). Nitrogen is important for the production of chlorophyll, the pigment that makes plant tissues green. Deficiency in N typically has a pale yellow colour (chlorosis) as a result of reduced chlorophyll production (Fonseca *et al.*, 2006; Szakiel, *et al.*, 2011). Nitrogen is also vital for many other plant physiological processes as a component of proteins synthesis. Thus, plants which are deficient in N may also appear stunted and display poor vigour.

Deficiency in K arises in the older leaves of the plant, and the nutrient is predominantly important in certain fruit production systems for its role in fruit quality. This nutrient plays a major role in cellular signaling and growth regulation in plants. It also functions in other processes such as photosynthesis (Armstrong, 1998). Other symptoms of K deficiency include necrosis on leaf margins, leaf curling and browning, and interveinal chlorosis, and plants which are deficient in K can also be prone to frost damage as well as certain diseases (Rehm and Schmitt, 2002). Usually, K is found in large quantities throughout the growth media and especially in soil. However, due to the effect of weathering, including temperature, moisture, and oxygen availability, growth media K levels may vary significantly as K availability is reduced by the presence of competing cations such as Ca<sup>2+</sup> and NH<sub>4</sub><sup>+</sup> (Gaj *et al.*, 2013). Potassium can also be readily leached from sandy growth media but can be applied in conventional fertilizers or with rock phosphate.

***Effects of watering, nitrogen and potassium fertilization on cellular differentiation.***

A decrease in soil water potential due to drought will decrease the rate of leaf expansion, whereas the roots and stems are much less affected. If the root water potential decreases suddenly, the response of leaf expansion is so rapid and large that it must be due to a change in the rate of expansion of existing cells, rather than to a change in the rate of production of new cells. However, when plants have grown for some time in soils of low water potential, smaller leaves with fewer cells are formed (Schuppler *et al.*, 1998). These observations suggest that reduced cell formation during water stress may limit final leaf size.

Therefore, further studies should be conducted to investigate the anatomical basis for the altered growth patterns seen in response to water stress. It is well known that roots growing under conditions of severe water stress exhibit much slower root extension rate than that seen in well-watered plants (Smith *et al.*, 1993). A significantly shorter growth zone has also been seen in these slower growing roots. The cellular growth patterns underlying these phenomena have not been well understood to date. It is not known whether the cell length profile over the growth zone of water-stressed roots resembles that of well-watered roots; cells merely stop growing sooner. If water deficit changes cell division rates as well as growth rates, then a more complicated pattern of cell lengths would occur (Fraser *et al.*, 1990; Mbagwu and Osuigwe, 1985).

Differentiation and growth of plant tissue is also primarily dependent on the composition and availability of nutrients in the growth medium, and especially on appropriate relative concentrations of hormones (Boroomand and Grouh, 2012). Therefore, the interaction of polyamines, plant hormones and growth in plant tissues seems to be more obvious than for other cellular growth requirements. Among the nutrients which affect the quality of herbal medicinal plants, N is most often indicated, and the application of high amounts of N results in high yields with better tissue growth and development (Khalid, 2012; MacAdam *et al.*, 1989). The findings of this proposed study will facilitate the evaluation of concomitant physiological processes, such as macronutrient metabolism in herbal medicinal plants.

However, the influence of K is another collective function played by nutrients in mitigating negative effects of abiotic stresses during the plant's growth. Plants provided with sufficient amounts of K are less vulnerable to water deficiency, low temperatures and pathogen attacks (Ibrahim *et al.*, 2012). Potassium yield-stimulating functions are different, influencing differentiation and growth of plant organs during the vegetation period (Lu *et al.*, 2012). This mineral element also shapes N management in high-yield cultivation. Since K is an indispensable component during the main stages of protein biosynthesis, therefore, its deficiency leads to a decrease of protein amount produced by a plant, and this effect occurs regardless of the N nutrition level and accumulation of non

-protein N (Gaj *et al.*, 2013 and Mudau *et al.*, 2005). Furthermore, K deficiency impedes N uptake and, as a result, the growth of the leaf assimilation surface, and reduces the uptake and transport of nitrates in plants. Many researchers have undertaken to evaluate the effects of K concentration on plant yields, but until now information on yield quality traits has been scarce, and mostly lacking in consistent data concerning the influence of K fertilization level on cultivated plants. In order to enhance this knowledge, a study should be conducted to determine the influence of differentiated fertilization with K on herbal medicinal plant yield and secondary metabolite production (Abdelaziz *et al.*, 2007).

***Effects of watering, nitrogen and potassium fertilization on secondary metabolite Production.***

Plants have evolved while constantly adapting to environmental stresses. They have adapted through their morphology, physiology and biochemistry. There has been recent increased interest in understanding the mechanisms of adaptation of plants to their adverse ecosystems. These adaptations may include both primary and secondary metabolism production (Dunford and Vazquez, 2005). Plants have evolved a wide range of secondary metabolites that prevent damage by herbivores, pathogens and competing species, attract pollinators, and protect them from abiotic stresses such as water and nutrient deficits. Advances in understanding the influences of the environment in the regulation of such compounds may lead to their advantageous manipulation in plants. The stresses already proven to alter the secondary metabolite accumulation include water deficit, nutritional deficiency, temperature extremes, insect and pathogen attacks, and mechanical damage (Jaafar *et al.*, 2012; Marchese *et al.*, 2010).

Usually, when conditions and resources are favourable, the priority of a plant is growth and development, decreasing the availability of carbon (C) to support secondary metabolism. However, when conditions and resources for growth and development are scarce, photosynthesis and growth are affected, but C at low levels can still be directed to support vital plant functions leading to a decrease in the production of secondary metabolites (Marchese *et al.*, 2010). Thus, factors that limit growth more than photosynthesis, such as moderate water-deficit, low temperatures, and moderate nutrient deficiency, can actually increase the C pool allocated to secondary metabolism, with little or no competition with growth and development (Smith *et al.*, 1993). Consequently, quantitative variations on secondary metabolism are directly linked to variations in the plant growth rate. Usually, limited availability of water has a negative effect on plant growth and development. However, a moderate water deficit has sometimes proved beneficial for the accumulation of biologically-active compounds in medicinal and aromatic plants. According to Gershenzon (1984), in herbaceous

plants and shrubs terpenes tend to increase under stress, particularly under moderate water deficit.

Accumulation of secondary metabolites often occurs in plants subjected to stresses including various elicitors or signaling molecules such as salicylic acid, jasmonic acid, or their derivatives (Ibrahim *et al.*, 2011). The production of these compounds is often low (less than 1% by dry weight) and depends greatly on the physiological and developmental stage of the plant (Ibrahim *et al.*, 2013). Several researchers have applied various elicitors for enhancement of secondary metabolite production in plant cell, tissue and organ (Hendawy and Khalid, 2011).

Meanwhile, environmental stresses such as pathogen attack, UV radiation, intense light, wounding, temperature or nutrient deficiencies often increase the accumulation of secondary metabolites, for instance phenylpropanoids. Nutrient stress, for example, has a marked effect on phenolic levels in plant tissues including *G. procumbens*, a phenolic-enriched plant (Akowuah *et al.*, 2009). This is because the concentrations of various secondary plant products are strongly dependent on the growing conditions and have impact on the metabolic pathways responsible for the accumulation of the related natural products (Li, 2002). For example, deficiencies in N directly influence the accumulation of phenylpropanoids, and K deficiency is also reported to increase phenolic concentrations (Astuti *et al.*, 2012; Fonseca *et al.*, 2006; Ramakrisna and Ravishankar, 2011).

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