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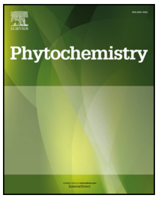

Impact of drought and salt stress on the biosynthesis of alkaloids in *Chelidonium majus* L.

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ABSTRACT

When plants are exposed to various stress situations, their alkaloid concentration frequently is enhanced. This well-known phenomenon is presumably due to a passively enhanced rate of biosynthesis, caused by greatly elevated concentrations of NADPH in stressed plants. Here, we used *Chelidonium majus* L. plants, which accumulate high concentrations of dihydrocoptisine in their leaves, to study the impact of drought and salt stress on the biosynthesis and accumulation of alkaloids. In comparison to well-watered controls, in the transcriptome of the gene encoding the key enzyme in alkaloid biosynthesis, stylopine synthase, is enhanced in stressed *C. majus* plants. If we presuppose that increased transcript levels correlate with increased enzymatic activity of the gene products, these data indicate, for the first time, that stress-related increases in alkaloid concentration might not only be caused by the well-known stress-related passive shift, but may also be due to an enhancement of enzymatic capacity.

1. Introduction

Higher plants are continuously exposed to various biotic and abiotic stresses. During the course of evolution, plants have developed many mechanisms to overcome the unfavorable conditions that result from stresses. Plant responses to stress generally involve alteration of metabolism and/or modification of gene expression (Madhava Rao et al., 2006). Lack of water is one of the most common stresses experienced by plants. The related water shortage drastically impairs entire plant metabolism. Specific metabolic responses vary markedly between plant types and result in the characteristic differences of certain plant species, such as their sensitivity to drought or high salinity (Chaves, 1991). In addition to the essential reactions to prevent massive water loss, e.g. by closure of stomata, or to avoid damage caused by osmotic imbalances, specialised metabolism is altered (Fig. 1).

Many studies report that accumulation of natural products such as simple or complex phenols, terpenes or alkaloids is increased under drought stress (Selmar and Kleinwächter, 2013a). There is no doubt that drought stress consistently enhances the concentration of specialised plant products (Selmar and Kleinwächter, 2013b). However, growth is significantly reduced in drought-stressed plants as well; thus, the reduction in biomass could result in the increased concentration, even without any enhancement of the overall production of specialised plant products (Paulsen and Selmar, 2016). Therefore, stress-related changes in the concentration of natural products could be due to various mechanisms (Fig. 1).

Only those studies in which the changes in the overall content of natural products per plant are known, are reliable for conceiving the impact of drought or salt stress on biosynthetic capacity. Unfortunately, only few studies are available in which the total content of specialised plant products per entire plants had been analyzed (Selmar and Kleinwächter, 2013a). For examples, in *Hypericum brasiliense* Choisy grown under drought stress, both the concentration and the total amount of the phenolic compounds was drastically enhanced in comparison to control plants (Nacif de Abreu and Mazzafera, 2005). In the same manner, in stressed peas (*Pisum sativum* L.), the overall content of anthocyanins was about 25% higher than in plants cultivated under standard conditions (Nogues et al., 1998).

According to Selmar and Kleinwächter (2013a), a strongly elevated ratio of NADPH to $NADP^+$ leads to a passive increase in all biosynthetic reactions consuming the reduction equivalents. As result, the rate of biosynthesis of specialised plant products is enhanced - even if no changes in biosynthetic capacity, i.e., the enzyme activity occur. However, in addition to such passive changes, in some cases an active enhancement of the biosynthetic ability also occurs due to up-regulation of the corresponding genes (Fig. 1). In this context, it is worth

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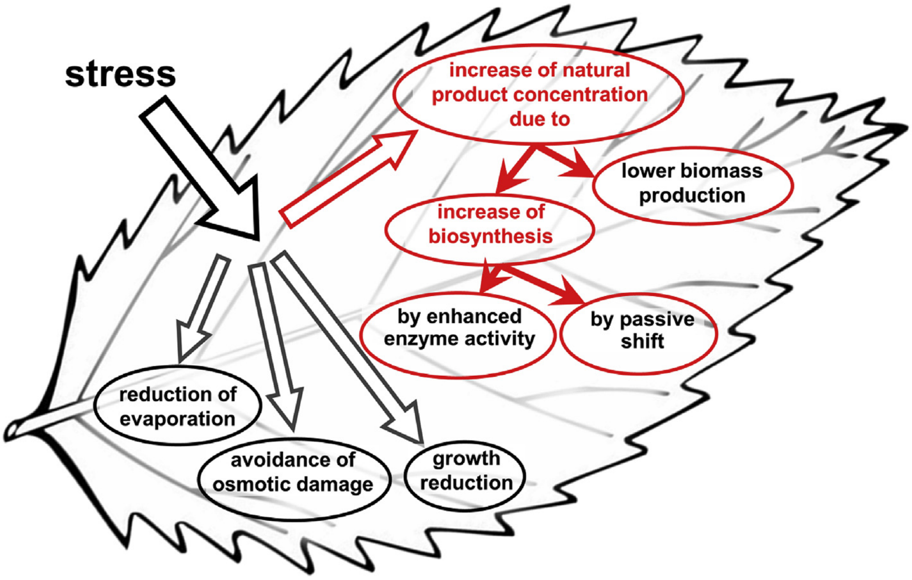

Fig. 1. Effect of drought and salt stress on concentration and content of natural product accumulation.

mentioning that the stress-related changes in reduction/oxidation state in plants and the subsequent redox sensing and signalling is the bases for the regulation of the entire metabolic responses to the stress situation by changing gene expression and translation (e.g., Foyer and Noctor, 2003; Askari et al., 2006). Accordingly, stress induced increases of specialised metabolites might also be caused by active changes in the biosynthetic capacity by up-regulation of the biosynthetic enzymes under stress conditions. Unfortunately, until now, there has been only very limited information available. Nasrollahi et al. (2014) reported that drought stress-enhanced biosynthesis of triterpenoid saponins was correlated with increasing gene expression of sequalene synthase, β-amyrin synthase, lupeol synthase, and cycloartenol synthase. In *Salvia officinalis,* the stress-related increase of monoterpenes was accompanied by enhanced expression of the related monoterpene synthases (Radwan et al., 2017). No corresponding data has been reported on alkaloid biosynthesis, as of this writing.

It is well known that natural products are crucial for protection against pathogens or for repelling herbivores (Freeman and Beattie, 2008; Mithofer and Boland, 2012; War et al., 2012). However, since neither the extent of pathogen attack nor the pressure by herbivores is enhanced under drought stress, the question of the biological significance for stress-related enhanced biosynthesis arises. In this context the generation of isoprene, which is synthesized and emitted by leaves of numerous plants under stress conditions, may provide an explanation. The synthesis of a highly reduced compounds, such as isoprene significantly contributes to the dissipation of excess of photosynthetic energy (e.g. Fall 1999; Sharkey and Yeh, 2001) and also serves to cool the stressed leaves (Behnke et al., 2007). In addition to classical energy dissipating mechanisms such as non-photochemical quenching, photorespiration, and the xanthophyll cycle (Alscher et al., 2002; Asada, 1999; Chen et al., 2004; Pitzschke et al., 2006), the stress-related harmful over-reduced cellular conditions can also be decreased by enhancing the biosynthesis of highly reduced natural compounds. This assumption is underlined by the fact that at higher temperatures, the amount of the energy dissipated by isoprene emission can account for up to 25% of the energy supply required for photosynthesis (Magel et al., 2006). Yet, isoprene emission also cool the stressed leaves as well (Behnke et al., 2007).

There have been many reports that alkaloid concentrations are increased during stressful conditions (Çakir and Çebi, 2010; Kirk et al., 2010). For a more extensive review of this topic see (Selmar and Kleinwächter, 2013b; Kleinwächter and Selmar, 2015). However, until now, there has been little reported information regarding whether the stress-related synthesis of alkaloids is due exclusively to a passive shift as described above or also an active up-regulation of the corresponding biosynthetic enzymes.

As the major steps in the biosynthesis of benzylisoquinoline alkaloids in *Chelidonium majus* have recently been elucidated (Yahyazadeh et al., 2017) and dihydrocoptisine is the major alkaloid in *Chelidonium majus* leaves, we chose this plant as a model system to investigate the impact of drought and salt stress on the accumulation of alkaloids and changes in related biosynthetic capacity. In *C. majus,* dihydrocoptisine accounts for more than 94% of total alkaloid content in the aerial parts of the plant; however, upon tissue distraction (e.g. during drying), dihydrocoptisine is spontaneously converted to coptisine (Fig. 2).

In order to avoid analytical faults in the course of alkaloid quantification, plants were oven dried to ensure complete conversion of dihydrocoptisine to coptisine; the results were subsequently quantified using HPLC. In parallel, the transcript level of stylopine synthase gene, the key enzyme of the biosynthesis of benzylisoquinoline alkaloids in *C. majus,* was also estimated (Yahyazadeh et al., 2017).

In addition to the application of drought and salt stress, *C. majus* plants were also treated with methyl jasmonate (MeJA). As jasmonate is known to be part of numerous different signal transduction pathways (Riemann et al., 2015), and the application of external MeJA elicits an overall general and comprehensive level of stress (Kazan and Manners, 2008), we chose this approach as a positive control.

2. Results

2.1. *Drought stress induced changes in evapotranspiration*

In contrast to the well-watered controls, which were supplied every day with exactly the amount of water that had been evaporated (100%), drought stress was induced by a continuous decline in the daily water supply. To achieve mild drought stress, the daily supply was set at 80% of the daily water loss (denoted as −20%); severe drought stress was induced by reducing the daily supply to 60% of daily water loss, denoted as −40% (for details see Materials and methods). This approach resulted in a continuous decline in soil water content in the stressed plants, visible by the permanent decrease in pot weight. The soil water content of the controls was about 29% during the entire experiment; whereas, due to the reduction in watering, it constantly decreased in the stress trials, reaching 13% when moderate drought was applied and