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Case study: The difficulty of correct reference values when evaluating the effects of drought stress: a case study with *Thymus vulgaris*

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Summary

Medicinal and spice plants grown under semi-arid conditions frequently contain higher concentrations of relevant secondary metabolites than plants cultivated in moderate regions. It is well established that this phenomenon is due to the impact of drought stress. However, in principle, the increase in natural product concentration could be caused by two quite different effects. Firstly, it could be caused simply by a change of the reference values: typical stress-related reduced growth frequently results in a lower biomass of the stressed plants. In consequence – provided that the rate of natural product biosynthesis remains constant – this results in an enhanced concentration in the stressed plants. Secondly, there is a genuine stress-related increase in the rate of biosynthesis of secondary metabolites.

In the first case, the total amount of secondary metabolites remains constant, while in the second case, it increases. Accordingly, a thorough differentiation requires a reliable quantification and comparison of the relevant factors. This especially accounts for the total biomass, which generally is far less in drought stressed plants than in the well-watered ones. Consequently, in all studies, where data on the total biomass are lacking, it is not feasible to evaluate reliably the total amounts of secondary metabolites only on the bases of the concentrations determined. Unfortunately, in most of the corresponding literature published so far, these data are missing, and thus, it is not possible to decide whether the drought stress-related increase of secondary metabolites is due to a genuine increase in biosynthesis or whether it is just due to a change of the relevant reference values, i.e., the total biomass. In this study, the relationships of these factors in well-watered and drought-stressed thyme plants are examined and discussed.

Introduction

It is well known that spice plants grown under dry climate conditions are more aromatic than plants cultivated in moderate climates. In the same manner, medicinal plants from semi-arid areas such as the Mediterranean region exhibit a greater concentration of relevant secondary metabolites than those grown in Central or Northern Europe (Kleinwächter and Selmar, 2015). This phenomenon is caused by drought stress. There are two possibilities. In one case, because of water shortage, the stomata of the leaves are closed and the uptake of CO_2 decreases significantly. As result, the consumption of reduction equivalents ($NADPH+H^+$) for CO_2-fixation via the Calvin cycle declines considerably, generating a large oxidative stress and an oversupply of reduction equivalents. As a consequence, all metabolic activities that consume reduction equivalents increase. Accordingly, the synthesis of reduced compounds, such as isoprenoids, phenols or alkaloids, is enhanced (Selmar and Kleinwächter, 2013). In the second case, drought-stressed plants generally exhibit reduced growth resulting in a lower biomass compared to well-watered plants. In consequence – provided that the rate of natural product biosynthesis remains constant – this results in an enhanced concentration of these substances in the stressed plants. In principle, in the first case, the total amount of secondary metabolites per plant increases, whereas in the second case it remains constant. Unfortunately, these coherences are much more complex, as both effects may co-occur. At an extreme, the higher rate of biosynthesis of secondary plant products could be compensated or even overcompensated by the lower biomass of the plant. As a result, the total amount of secondary metabolites will remain unchanged or will even be lower in the stressed plants, although the concentration is strongly enhanced (Gershenzon, 1984; Selmar and Kleinwächter, 2013). Accordingly, thorough differentiation between these effects requires a reliable quantification and comparison of all relevant factors, especially the total biomass that is strongly influenced by drought. In most past and current literature on secondary metabolite production these data are lacking, and the total amounts of secondary metabolites cannot be estimated. Accordingly, no deduction on putative changes in the rate of biosynthesis could be made. Unfortunately, in most of the corresponding literature published so far, these data are missing, and thus, it is not possible to decide whether the drought stress-related increase of secondary metabolites is only due to a genuine increase in biosynthesis or it is just due to a change of the relevant reference values, i.e., the total biomass. As consequence, solid statements on increase of biosynthetic activity require a more precise definition, because the enhancement may refer either to the biosynthesis in the entire plant (total biosynthesis) or to the specific activity, calculated per gram biomass (specific rate of biosynthesis). This could be illustrated as follows: Due to massivly reduced growth, the total amount of natural products is far lower in stressed plants in comparison to the well-watered controls. Consequently, overall biosynthesis of natural products per plant is strongly reduced. Since this biosynthesis is achieved by a much smaller plant, the rate of biosynthesis per gram biomass may nonetheless increase significantly.

In this paper, these complex coherences and problems are discussed and outlined on the basis of a case study involving well-watered and drought stressed thyme plants.

Consequences of different biomasses for the estimation of stress-related effects

Thyme plants were cultivated for nine weeks under well-watered as well as under drought stressed conditions (for details see Kleinwächter et al., 2015). As expected, drought stress had a strong effect on plant growth: the biomass of the stressed plants was markedly lower compared to those of the well-watered ones. At the end of this experiment, the biomass of the stressed plants accounted for only two-thirds of the biomass of the well-watered plants (Tab. 1; Kleinwächter et al., 2015).

As assumed, the concentrations of terpenes (mg/g d.w.) are increased in the drought stressed plants compared to the well-watered controls (Tab. 1), putatively due to the stress related lesser biomass. In contrast, the total amount of terpenes per plant is markedly reduced. At the first glance, drought seems to cause a decline of the overall

Tab. 1: Biomasses, concentrations and total amounts of terpenes in well-watered (≙100 %) and drought stressed thyme plants after nine weeks of cultivation under the respective conditions (KLEINWÄCHTER et al., 2015

	Well-watered thyme	Drought stressed thyme
Biomass	17.45 g d.w. **100 %**	11.63 g d.w. **67 %**
Concentration of terpenes	6.42 mg/g d.w. **100 %**	7.44 mg/g d.w. **116 %**
Total amount of terpenes	112 mg/plant **100 %**	87 mg/plant **78 %**

terpene biosynthesis. However, further comparison revealed that the total content of terpenes of the stressed plants accounts for 78 % of the content in the well-watered plants – a quota that is much higher than the share of the biomass (67 %). Assuming that the specific rates of terpene biosynthesis (mg/g d.w.) are the same in stressed as well as in well-watered plants, the corresponding quota should be equal. Obviously, the specific rates of biosynthesis change in the course of the experiment, and a more detailed evaluation with special emphasis on the specific rates of biosynthesis is required.

The specific rate of terpene biosynthesis (given in milligram terpenes per gram dry weight) can be calculated from the increase in the total amount of terpenes referred to the average biomass of these plants during the particular time span. The corresponding data for such an approach are compiled in Fig. 1. For a period up to three weeks, there are almost no differences in the growth of stressed and control plants, evidant by nearly the same biomass (Fig. 1A). However, in the second phase of the experiment, the biomass of the stressed plants is markedly lower than that of the well watered ones. The inhibiting effect of moderate drought stress on plant growth became visible only after six weeks. In contrast, and in accordance with our previous experiments, the total content of terpenes in the stressed plants is slightly enhanced after three weeks (Fig. 1C). In the same manner, also the concentration of terpenes in the stressed plants is significantly enhanced (Fig. 1B). However, due to the massive growth reduction in the second phase of the experiment, time dependent increase in the total amount of terpene is also reduced. As consequence, after six weeks, the number of terpenes is nearly the same in stressed and control plants. Yet, after nine weeks, it is far lower in the stressed plants. Nonetheless, in all cases the concentration of terpenes is enhanced in the stressed plants.

These complex coherences can easily be comprehended by the rates of biosynthesis displayed in Fig. 1D. Indeed, as expected, in the first phase of stress the rate of biosynthesis is markedly higher in the drought stressed plants than in well-watered ones. This enhancement is putatively due to the stress-related increase of reduction status as outlined by SELMAR and KLEINWÄCHTER (2013). In the course of the experiment, the rates of biosynthesis decrease in either case. This obviously reflects the well known decline of terpene biosynthesis in the course of leaf maturation (e.g., VORIN and BAYET, 1996). In consequence, within the period of four to six weeks, the rates in stressed and control plants are nearly the same. However, the reduction of the biosynthetic rate in drought stressed plants proceeds in the last phase of the experiment. It is very likely that the stressed plants have – at least in part – adapted to the stress situation and the reduction status has normalized. Finally, the rate of biosynthesis in thyme plants under drought stress is only 60 % that of the controls. This strong decrease may be due to putative stress-related damages.

If indeed the metabolic processes and coherences outlined above are responsible for various effects in the course of the different phases of drought stress must be verified by basic experiments. However, with respect to sound estimation of stress-related changes on secondary metabolism, especially for the evaluation of its impact on food quality, the results outlined in this paper clearly demonstrate the importance of the determination of both, concentration and total amount of the natural product of interest. Only if both, the rate of plant biomass production and the biosynthetic rate of secondary metabolite production throughout growth of the plants are considered, it will be possible to deliberately apply drought stress as a means of modifying the quality of commodities derived from spice and medicinal plants.

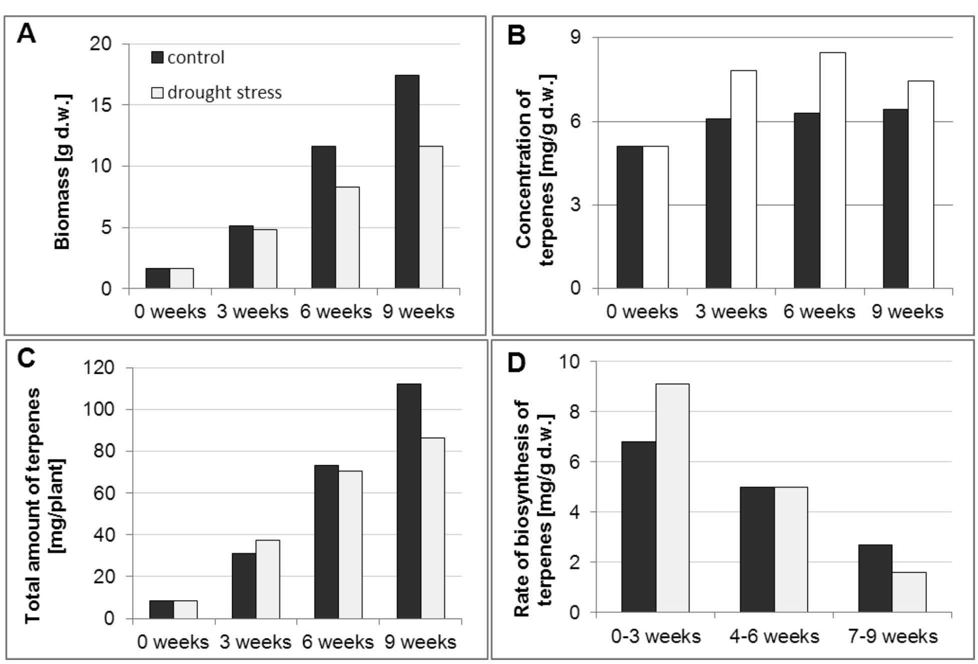

Fig. 1: Biomass (A), concentration of terpenes (B) total amounts of terpenes (C) and rates of biosynthesis of terpenes (D) in well-watered and drought stressed thyme plants

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References

GERSHENZON, J., 1984: Changes in the levels of plant secondary metabolites under water and nutrient stress. In: Timmermann, B.N., Steelink, C., Loewus, F.A. (eds.), Phytochemical adaptions to stress – recent advances in phytochemistry, 18, 273-320. Plenum Press, New York.

KLEINWÄCHTER, M., PAULSEN, J., BLOEM, E., SCHNUG, E., SELMAR, D., 2015: Moderate drought and signal transducer induced biosynthesis of relevant secondary metabolites in thyme (*Thymus vulgaris*), greater celandine (*Chelidonium majus*) and parsley (*Petroselinum crispum*). Ind. Crop. Prod. 64, 158-166.

KLEINWÄCHTER, M., SELMAR, D., 2015: New insights explain that drought stress enhances the quality of spice and medicinal plants: potential applications. Agron. Sustain. Dev. 35, 121-131.

SELMAR, D., KLEINWÄCHTER, M., 2013: Stress enhances the synthesis of secondary plant products: the impact of stress-related over-reduction on the accumulation of natural products. Plant Cell Physiol. 54, 817-826.

VORIN, B., BAYET, C., 1996: Developmental changes in the monoterpene composition of *Mentha* × *piperita* leaves from individual peltate trichomes. Phytochem. 43, 573-580.

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