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Is the C:N ratio a reliable indicator of C allocation to primary and defence-related metabolisms in tomato?

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ABSTRACT

Plant growth and defence are both fuelled by compounds synthesized from a common pool of carbon and nitrogen, implying the existence of a competition for carbon and nitrogen allocation to both metabolisms.

The ratio of carbon to nitrogen (C:N) of an organ is often regarded as a convenient indicator of growth and quality. The purpose of this work was to assess whether or not it is possible to extend its use to characterize the trade-off between growth and defence processes. Therefore, we calculated C:N ratios in the pool of resources and in the total plant, and correlated them to the concentrations of diverse compounds of the primary and secondary metabolisms in young tomatoes.

Plants were grown hydroponically at N availabilities either limiting (0.1 mM) or not (7 mM) for growth in two glasshouses maintained either under ambient or enriched (700 vpm) air CO_2. These conditions yielded a large array of C:N in fully developed leaves, developing leaves, stem and roots, sampled 27, 35 and 47 days after sowing. Growth parameters and tissue concentrations of primary metabolites (carbohydrates, starch), defence-related compounds (polyphenols, glycoalkaloids), lignin, nitrate, ammonium, C and N were analyzed. Net CO_2 exchange rate was also measured at the last sampling date.

Low N limited plant growth more than photosynthesis. The C:N in the resource pool was far higher than the total C:N. Starch was the most responsive compound, attaining high concentration under high C:N, whereas lignin remained stable. Chlorogenic acid, rutin, kaempferol-rutinoside and tomatine concentrations correlated positively to C:N. The same patterns were observed for most organs and molecules, except soluble carbohydrates in fully developed leaves whose concentration was not influenced. Among the organs, developing leaves showed the highest concentrations of secondary compounds and were the most responsive to C:N variations. Neither the biochemical nature of the compounds (C-based or N- containing metabolites) nor the calculation mode of C:N, influenced the patterns observed.

Within the range of N availabilities considered (up to N limitation but not deficiency), the C:N can be considered as a good indicator of the secondary compounds concentrations in organs, especially for those involved in the chemical defence.

1. Introduction

In their environment, plants frequently experience conditions such as low nutrient availability or pest and disease attacks, that limit their growth. During evolution, plants have developed efficient resource acquisition systems and defence processes that ensure their growth, their development and their survival in the ecosystems. At the metabolic level, however, growth and defence seem antagonistic. The former results from the primary metabolism that acquires and transforms resources into new tissues, while the latter rests on the secondary metabolism that produces a wide diversity of defence-related molecules (Croteau et al., 2000; Stamp, 2004). The secondary metabolism involves compounds that are not

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Abbreviations: C:N, ratio of carbon to nitrogen; C, carbon; N, nitrogen; CNB, carbon-nutrient balance; ODT, optimal defence theory; GDB, growth-differentiation balance; PCM, protein competition model; $C:N_r$, resource C:N ratio; $C:N_t$, total C:N ratio; CGA, chlorogenic acid; KR, kaempferol-rutinoside; dry wt, dry weight; LA, leaf area; N_{nit}, nitrate alone; N_{mix}, nitrate-ammonium mixture; HC, elevated CO_2; LC, ambient CO_2; HN, high N; LN, limiting N; [X], concentration of X; A_{net}, CO_2 exchange rate; das, days after sowing.

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necessarily vital to plants, but useful for their adaptation to the environment. However, this separation between metabolisms is not clear-cut. Furthermore, secondary and primary metabolites are synthesized from the same resources, mostly C, acquired from the environment. Thus plants face an endless dilemma between growth and defence (Mittelstrass et al., 2006). This is why productivity is viewed as the result of a trade-off between resource allocation to growth and to defence processes (Mittelstrass et al., 2006).

Four main hypotheses have been put forward to conceptualize this trade-off: the carbon–nutrient balance (CNB) (Bryant et al., 1983; Tuomi, 1992), the optimal defence theory (ODT) (McKey, 1974, 1979), the protein competition model (PCM) (Jones and Hartley, 1999), and the growth–differentiation balance (GDB) (Loomis, 1932; Herms and Mattson, 1992; Herms, 2002). They form a conceptual framework for the functional ecology of plant defence suggesting that plants make an effective use of costly *versus* beneficial investments towards defence *versus* growth processes. This trade-off is mainly conditioned by the relative availability of resources such as N and C (Herms and Mattson, 1992). The C:N ratio of the biomass is considered as a good indicator of this relative availability. It has been used to diagnose the growth status of plant organs (Grechi et al., 2007; Minden and Kleyer, 2011; Peng et al., 2011) and as a plant trait to assess the impact of the environment such as elevated CO_2 concentration (Lam et al., 2012; Sardans and Penuelas, 2012) or agronomic factors such as defoliation (Bazot et al., 2005) on the quality of plant tissues. In this paper, we envisage to extend its use to the diagnosis of the share of resources between growth and chemical defence. Very few studies considered the plant or organ C:N status as an indicator of chemical defence (Hoffland et al., 2000; Ibrahim et al., 2011) and none as a predictor of the balance between metabolisms. C:N is worth to be tested as an indicator of the trade-off between growth and secondary metabolism.

The use of the C:N ratio has been criticized because it is calculated from the total C and N contents (Stitt and Krapp, 1999; Fritz et al., 2006). Indeed, they are the mere concentration of various C and N forms making up compartments of different physiological nature, either resources (starch, nitrate, etc.) or metabolic (proteins) and non-metabolic (lignin) structures. Therefore, in this paper, we refined the C:N by considering these forms separately or together, thus defining two distinct C:N ratios: resource ($C:N_r$) or total ($C:N_t$).

We assess here whether or not the CN status at organ level can describe and predict the partitioning of resources to defence compounds. For this purpose, we correlated both C:N ratios to the concentrations of diverse primary metabolites (carbohydrates), lignin and some defence-related chemicals, either C-based (phenolic acids and flavonoids) or C&N-based (glycoalkaloids).

The primary metabolism was assessed by the dry weight and non-structural carbohydrate (soluble sugars and starch) accumulations. The secondary metabolism was characterized by the accumulation of a major glycoalkaloid in tomato (tomatine) and of soluble (chlorogenic acid, CGA; rutin and kaempferol-rutinoside, KR) and insoluble phenolics (lignin). These soluble secondary metabolites were chosen because they have been cited as typical phytoanticipins in tomato (Van Etten et al., 1994; Ruelas et al., 2006). Although not involved in chemical defence, lignin was also quantified because it is an end-product of the phenylpropanoid pathway in which flows a significant part of C. Moreover, lignin contributes to plant defence passively as a physical barrier for pests.

A first subsidiary objective was to investigate the within-plant variation of this relationship between CN status and resource allocation. Variations of quality/composition of biomass at a small spatial scale (i.e. organ scale) may influence host selection and subsequent success/failure of insect herbivores (Zangerl and Berenbaum, 1993) or other pests.

Our experimental strategy was to induce a wide range of C:N in young tomato plants (*Solanum lycopersicum* L.). They were grown hydroponically at two contrasting levels of N and CO_2 availabilities. The availability of both nutrient resources is commonly controlled by tomato growers. Furthermore, two forms of N nutrition (NO_3 alone *versus* a NO_3/NH_4 mixture) were utilized to extend the range of C:N ratios. Thus, a second subsidiary objective was to assess whether or not the addition of ammonium in nutrient solutions can modify the concentration of secondary compounds. Indeed, the forms of N nutrition change markedly the non-structural carbohydrate content in tomato (Horchani et al., 2010) but information lacks on a putative effect of ammonium on secondary metabolites of tomato and is scarce for other plants (Graham, 2002).

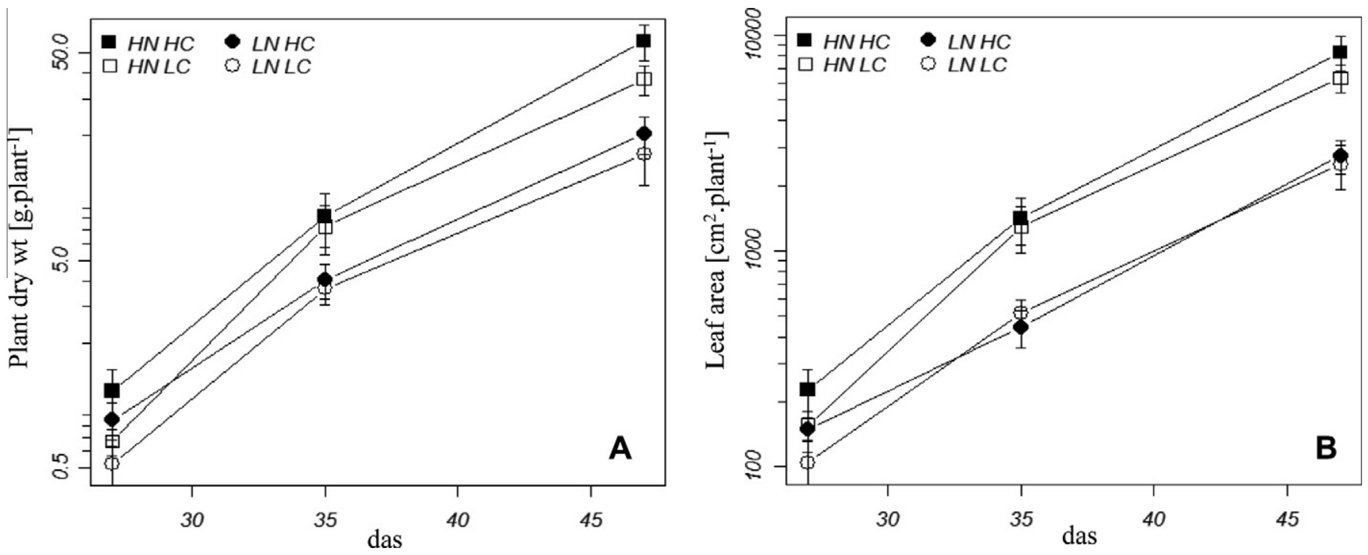

Fig. 1. Dry mass accumulation (A) in the tomato plants and total leaf area (LA) (B) over the three sampling times: 27, 35 and 47 days after sowing (das). HN: high [N], LN: low [N], HC: high [CO_2], LC: low [CO_2], nit: N provided as pure NO_3, mix: N provided as a NO_3/NH_4 mixture symbols are the means of three replicates and 2 N form treatments (nit and mix) that were pooled because N form was not significant. Vertical bars denote SE, $n = 6$.