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EFFECTS OF PHOTOPERIOD, LOW TEMPERATURE AND N NUTRITION ON VSP ACCUMULATION IN TAPROOT OF ALFALFA

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Abstract

In *Medicago sativa* L., vegetative storage protein (VSP), specifically accumulated in taproot, are strongly involved in nitrogen storage. How the accumulation of such VSPs is regulated remains largely unknown. Experiments were designed with non-nodulated alfalfa to determine if length of the photoperiod, a decrease of temperature, or high availability of mineral nitrogen may induce the accumulation of VSPs. ^{15}N labelling was used to quantify nitrogen uptake and its further relative translocation within the plant while VSPs accumulation was analysed by ELISA quantification. Results showed that environmental factors such as shortening daylength or low temperature changed biomass allocation within the plant by reducing shoot growth. As a consequence, short days promoted the relative N allocation to taproot whereas VSP accumulation showed a higher trend. On the other hand, low temperature, changes in N source or availability in the nutrient solution, may lead to a higher influx of nitrogen and a higher soluble protein relative concentration in taproot while VSP abundance remained low.

Keywords: Alfalfa, N partitioning, N accumulation, Vegetative Storage Protein, photoperiod, temperature, N fertilization.

Introduction

In perennial forage plants such as alfalfa (*Medicago sativa* L), mobilization of N reserve pools (mainly represented by taproot soluble proteins) previously accumulated in storage organs are needed to palliate a transient deficiency in N uptake or symbiotic fixation of N_2 during critical period of growth or development (Volenec *et al.*, 1996). Three taproot polypeptides of 32, 19 and 15 kDa possessing features consistent with those of VSP were characterized in alfalfa taproot (Volenec *et al.*, 1996). These polypeptides represent approximately 40% of total taproot soluble proteins, are located mostly in vacuoles of parenchyma cells of wood rays and bark of taproot (Avice *et al.*, 1996), and show a preferential pattern of mobilization / re-accumulation after cutting and in spring when shoot regrowth is resumed. The fact that specific deposition of VSP occurs during autumn-winter transition in perennial species like poplar (*Populus deltoides*) or alfalfa, prompt the question of putative involvement of photoperiod length and/or low temperature effect in this process. In poplar, exposure to short day treatments or low temperatures significantly induced a VSP accumulation (Van Cleve and Apel, 1993). Higher N availability also induces the VSP synthesis in soybean leaves (Staswick, 1994) or in poplar bark tissues (Van Cleve and Apel, 1993). Surprisingly, little is known about the signals involved in the regulation of VSP expression in taproot. This paper presents a first study on the identification of putative environmental factors (photoperiod, low temperature, N feeding) that modulate N partitioning and subsequently VSP accumulation in alfalfa.

Material and methods

After 4 months of growth under greenhouse conditions (16h/20°C-day and 8h/18°C-night, 160 □moles photons m^{-2} s^{-1}), non-nodulated alfalfa plants were defoliated and transferred hydroponically on a nutrient solution containing 1 mM of KNO_3 in growth chamber. After two weeks of regrowth during which N reserves were progressively depleted, treatments were applied during the following 35 (Experiment 1) or 21 day (Experiment 2) periods corresponding to the accumulation of storage compounds. In experiment 1, the nitrogen source was labelled with 2.5 atom % ^{15}N excess and the treatments were: long-days (16 h light-8 h darkness) at 20 °C day-18 °C night (**LD**) or 5 °C day-5 °C night (**LD / 5 °C**); short days (8 h light-16 h darkness) at 20 °C day- 18 °C night (**SD**) or 5°C day- 5°C night (**SD / 5 °C**); high nitrogen feeding (**High N**, 5 mM KNO_3). For LD, LD/5 °C, SD, SD/5 °C experiments, nutrient solution contained 1 mM KNO_3. In experiment 2, plants were subjected to sources and levels of mineral N (2 mM KNO_3, 1 or 20 mM NH_4NO_3, 2 mM NH_4Cl). Plants were harvested the first day of treatment (**T0**=control plant, *ie* 15 days after shoot cut) and after 35 (T0+35days, Experiment 1) or 21 days (T0+21days, Experiment 2) of treatment. Details about plant harvest and sample preparation for N, ^{15}N and protein analysis have been described by Avice *et al.* (1996). VSP quantification were performed by ELISA (Noquet *et al.*, 2000). The effects of treaments were analyzed by means comparison using t-test.

Results and Discussion

Regardless of temperature regime (20 or 5°C), short day application (SD= 8h light) leads to a decrease in total growth (Figure 1A). At 20 °C, SD strongly reduced shoot regrowth, while at 5 °C shoot-root ratio is not affected by duration of photoperiod (Figure 1A). In comparison with LD treatment (20 °C), SD reduced N uptake (p<0.05) but increased N allocation to root tissues and more particularly in taproot (18% *vs* 11%, Figure 1B). This preferential N allocation to taproot with SD application led to a low but significant (p<0.07)

accumulation of VSPs (8.9 $mg.g^{-1}DW$ for SD *vs* 7.4 for LD, Figure 2B) without significant difference in soluble protein concentrations (Figure 2A). Similarly, shortening photoperiod caused an accumulation of storage protein and corresponding transcript level in poplar (Van Cleve and Apel, 1993).

For a given photoperiod treatment, low temperature did not modify the total dry matter but reduced the shoot-root ratio (Figure 1A) and increased N translocation to roots (Figure 1B). In comparison with LD plants, SD together with low temperature led to a reduction in uptake and to a preferential N distribution to the root tissues (48% for SD/5°C *vs* 33% for LD, Figure 1B). Surprisingly, despite increased allocation to the roots and a significant accumulation of soluble protein (62 $mg.g^{-1}DW$ for LD/5°C *vs* 38 for LD, Figure 2A), VSPs were virtually undetectable (Figure 2B). These results were not consistent with previous studies on white clover or poplar (Van Cleve and Apel, 1993) showing that a reduction of temperature induced VSP accumulation. Therefore, it can be suggested that low temperature, as applied under our experimental conditions, was probably closer to a cold stress than to the real progressive variations of temperature observed during late autumn and winter.

In comparison with LD plants, the increase of N availability (5 mM) doubled the total dry matter without differences in i) shoot-root ratio (Figure 1A), ii) relative N partitioning within the plant (Figure 1B) or iii) N storage as VSP (Figure 2B). In addition, our results show that level of VSP accumulation in alfalfa taproot is not modulated by N source (KNO_3, NH_4NO_3 or NH_4Cl; Figures 2C and 2D) or N concentrations (1, 5 or 20 mM; Figure 2). These data are in contradiction with results observed in soybean (Staswick, 1994) or poplar (Van Cleve and Apel, 1993). Nevertheless, in young alfalfa seedlings, Kalengamaliro *et al.* (1997) have reported that increasing N fertilization (10 mM NH_4NO_3) stimulates the total plant growth but have no consequences on soluble protein or VSP accumulation.

From these results, it seems that N storage in protein form is not a priority for the plant when conditions of culture are optimal and N fertilization is ample. It follows that a transient deficiency in mineral N may affect the partitioning of N and its possible accumulation in VSP. Our study also shows that short photoperiod or low temperature changes the relationships between source and sink organ and induces a preferential N allocation towards root tissues. However, this increased root N allocation is not systematically concomittant with an accumulation of VSP, suggesting that the control and regulation of N flows towards VSP synthesis is subjected to different internal and external signals. Amongst endogenous factors, it is now well established that jasmonic acid play a preponderant role in the induction of VSP genes of soybean (Staswick, 1994). Recent results in alfalfa report that root application of methyl jasmonate induces a specific N translocation in taproot and a significant accumulation of VSP (Noquet *et al.*, 2000). Further study is needed to assess the effect of photoperiod and putative internal factors on N traffic in plant and VSP accumulation. Under this perspective, work is underway to isolate an alfalfa VSP cDNA in order to study at molecular level the regulation of VSP storage as influenced by environmental and endogenous signals.

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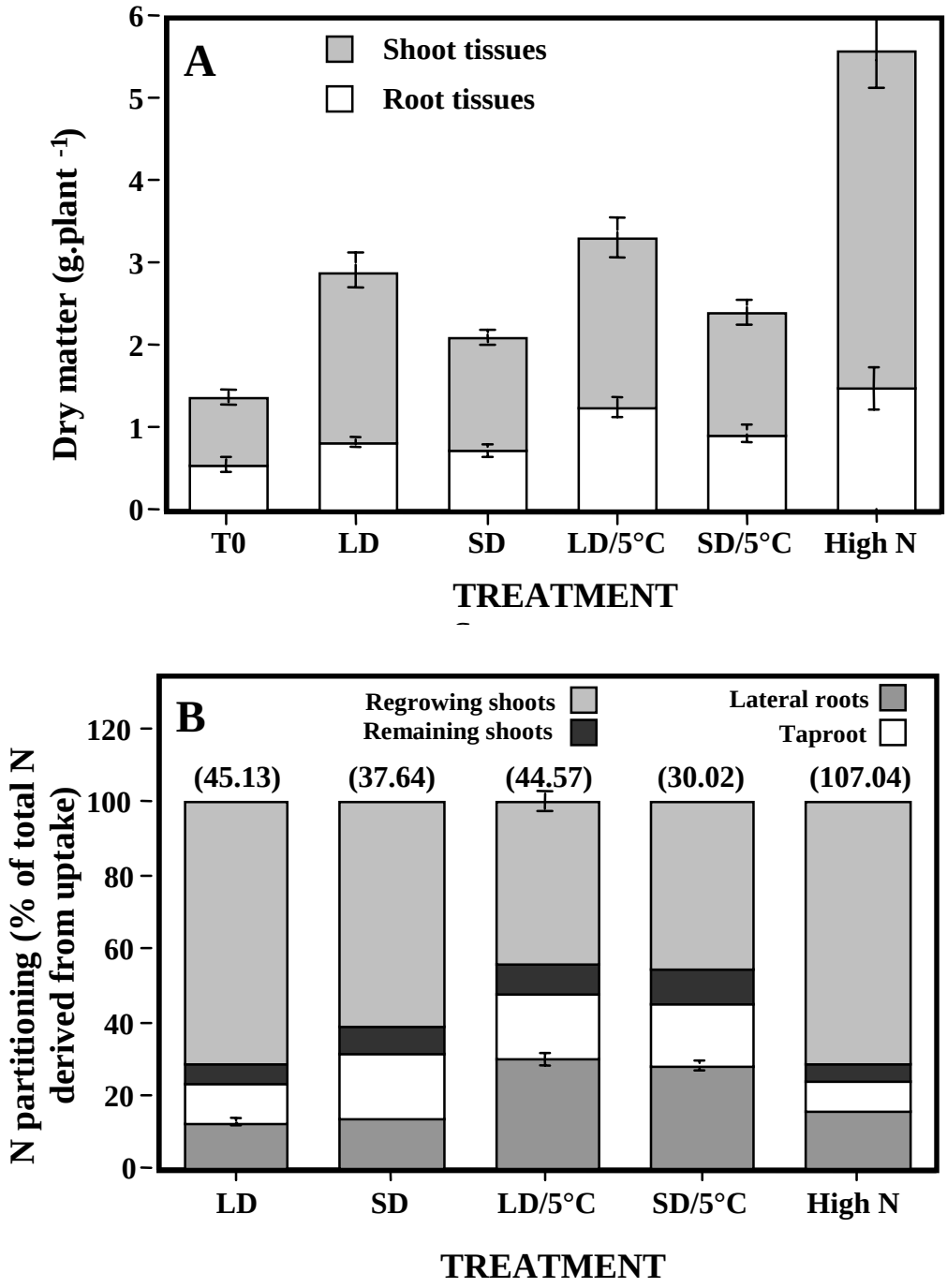

Figure 1 - Effects of photoperiod, temperature and N availability in the nutrient solution on total dry matter (**A**) and relative partitioning of N derived from uptake (**B**). Total N uptake in brackets. **T0 :** Plants harvested two weeks after defoliation, plants kept for 35 days with long day photoperiod (16 h light-8 h darkness) at 20°C day-18°C night (**LD**) or 5°C day-5°C night (**LD/5°C**); with short day photoperiod (8 h light-16 h darkness) at 20°C day-18°C night (**SD**) or 5°C day- 5°C night (**SD/5°C**); or with high nitrogen supply (**High N**, 5 mM KNO_3) with long day photoperiod (16 h light-8 h darkness) at 20°C day-18°C night. Vertical bars indicate the mean □ S.E. for n=3.

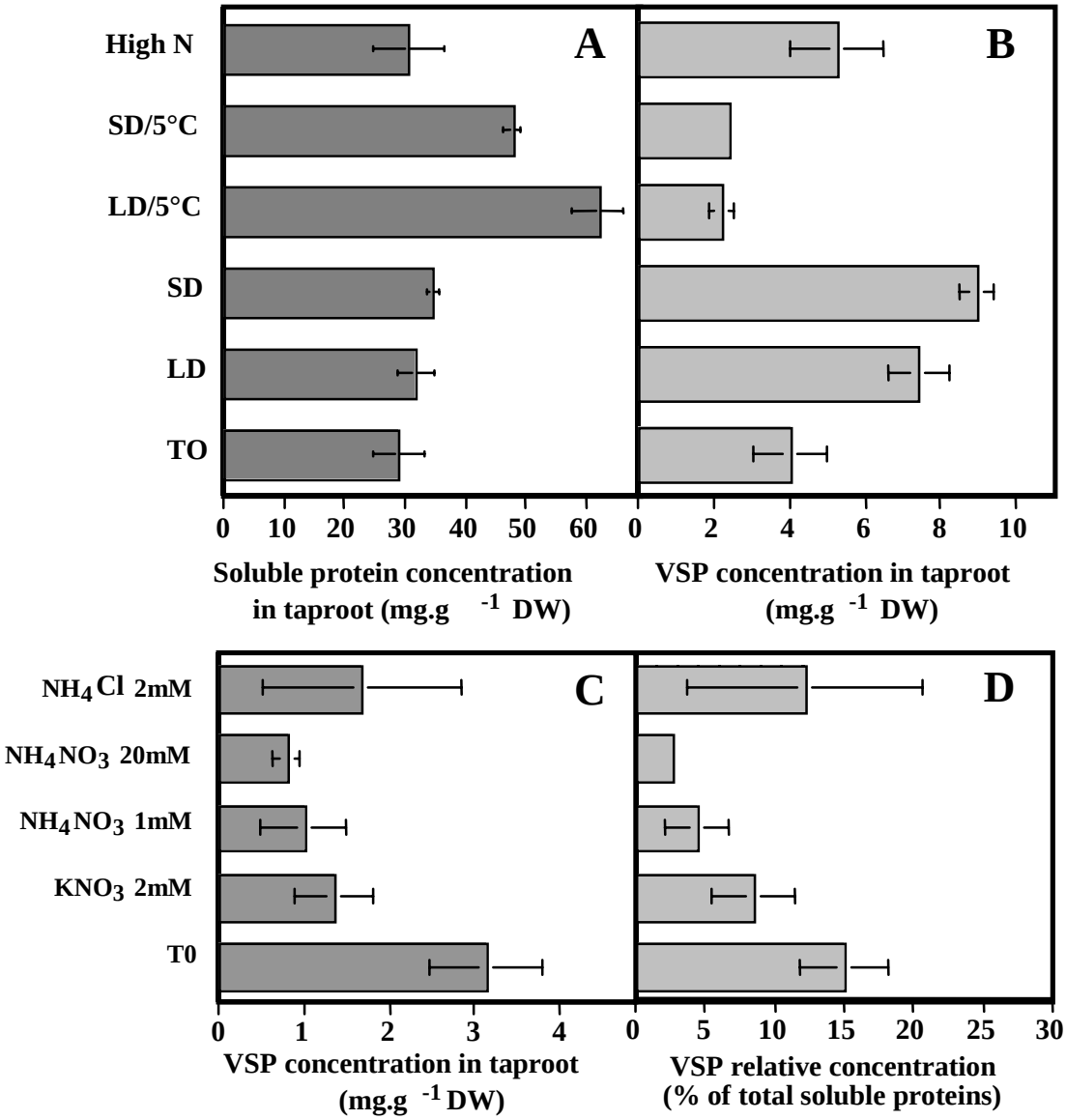

Figure 2 - Effects of photoperiod, temperature and N availability in the nutrient solution on taproot soluble protein contents (**A**) and VSP concentration (**B**). Plants were treated as explained in Fig.1. Vertical bars indicate the mean □ S.E. for n=3. *Experiment 2:* effects of N sources (NH_4Cl, KNO_3 or NH_4NO_3) and N concentration in the nutrient solution applied during 21 days on VSP concentrations (**C**) and VSP relative concentration (**D**). Vertical bars indicate the mean □extreme values for n=2.