Variations in osmo-regulatory and anti-oxidative status and their links with total nitrogen and phosphorus concentrations in lucerne during re-growth

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ABSTRACT

This study aimed to find out the changes in osmo-regulatory and anti-oxidative status during lucerne re-growth, elucidating their possible links with total nitrogen (N) and phosphorus (P) concentrations. The concentrations of osmo-regulatory substances, the activities of anti-oxidative enzymes and malondialdehyde concentration tended to increase after mowing. Total N and P concentrations decreased from 13 to 18 d after mowing. All responses were organ-specific. There were negative correlations of total N and P concentrations with osmo-regulatory and anti-oxidative status mostly in stems.

Key words: Alfalfa, Anti-oxidation, Cutting, Nutritional status, Osmotic adjustment, Temporal variation.

INTRODUCTION

Mowing and/or grazing impose remarkable impacts on the growth and survival of forages or grasses and as a consequence, the sustainability of the grassland is also influenced (von-Caemmerer and Farquhar 1984; Lu et al. 2016). Removal of the aboveground parts through mowing and/or grazing influences the photosynthetic functions of residues, resulting in changes in carbon fixation and reallocation within the plant (Yang et al. 2013). Mowing leads to increase in photosynthetic rate of the residues and then the rate keeps higher for a long period afterwards (Heichel and Turner 1984; von-Caemmerer and Farquhar 1984). While the rate and total amount of respiration sharply decreased shortly after mowing and then gradually increased in the residues (Hou 2001; Yang et al 2013). These finally not only produces more substance and energy but also changes the source-sink relationship (Thomson et al. 2003; Yang et al. 2013), helping the re-growth of the residues. Mowing may also lead to decrease in CO2 assimilation rate and carbohydrate content in the residues (Orodho and Trlica 1990; Asseng and Hsiao 2000). The re-growth largely depended on nitrogen pools in residues and roots, while carbon pools contributed little to the re-growth (Meuriot et al. 2004; Ourry et al. 1994).

Mowing and/or grazing also leads to change in nutritional status (i.e. N and P) in the plant possibly due to redistribution of nutrients within the residues and roots (Richards and Calldwell 1985; Yang et al. 2013). During re-growth, reserved compounds was activated and reallocated among residues and roots, including C- and N-contained compounds (Ourry et al. 1994; Agrell et al. 1999; Rong et al. 2000; Wang et al. 2003). Ourry et al. (1994) found that about 68% N in the roots and crown was translocated to regrowing shoots, and the amount of N available in roots and crown at the beginning of re-growth was significantly and positively correlated with the amount of N mobilized to new tissues, while no similar correlation was found for the starch.

The functioning of a plant is guaranteed by cell viability and structure integration which are controlled by osmo-regulatory and anti-oxidative status. Artificial disturbances (i.e. mowing and grazing) leads to decrease in leaf malondialdehyde (MDA) content (Fahnestock and Detling 2000), and increases in proline content and anti-oxidase activity in 10 h after mowing (He et al. 2009). These researches showed clues in finding possible links of photosynthesis change and compounds translocation with osmo-regulatory and anti-oxidative status. However, there were few studies to elucidate the links of osmo-regulatory substances and antioxidant enzymes with nutritional status during re-growth.

Lucerne (Medicago sativa) can survive over 20 years (Bennett 2012) and can be cut 2-5 times annually. So understanding the physiological mechanisms during re-growth is important to sustainable use of lucerne grassland. This study aimed to test the hypothesis that osmo-regulatory and anti-oxidative status might be correlated with total N and P concentrations during re-growth. The specific objectives were to find out: 1) How osmo-regulatory and anti-oxidative status and total N and P concentrations change during re-growth? 2) Whether there was a relationship of total N and P concentrations with osmo-regulatory and anti-oxidative status during re-growth? 3) Do the trends differ among roots, stems and leaves?

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MATERIALS AND METHODS

Experimental design: The experiment was conducted in a greenhouse. The soil from local farming fields was placed in plastic pots (diameter 30 cm, height 35 cm). During the experiment, water status in the soil was kept at 60% of the maximal field water capacity (30%) with the weighing method. Uniform seeds of lucerne (*Medicago sativa* L. cv Longdong) were sown into the pots on 25 April, 2013. On 30 July, lucerne in half of the pots was cut to the ground as mowing treatment and the rest pots were non-mowing treatment.

Sampling: The plants were sampled on 5, 12 and 17 August (6, 13 and 18 d after mowing, respectively) and samples were divided into roots, stems and leaves. Under mowing treatment, stems and leaves were not sampled on 5 August due to lack of enough materials. Fresh samples were immediately stored in the -80°C refrigerator for measurements of the activities of anti-oxidant enzymes and the concentrations of osmotic adjustment substances. Other samples were oven-dried and then ground uniformly for measurements of nitrogen and phosphorus concentrations.

Measurements: The MDA concentration was measured using a modified thiobarbituric acid method (Puckette et al. 2007). The proline was measured using a modified acid-ninhydrin method (Bates et al. 1973). Soluble sugar (SS) was measured using a modified anthrone method (Sánchez and others 1998). Soluble protein (SP) was measured using a modified coomassie brilliant blue method (Georgiou et al. 2008). Superoxide dismutase (SOD) was assayed on the basis of its ability to inhibit the photochemical reduction of nitro blue tetrazolium (Stewart and Bewley 1980). The catalase (CAT) was measured by the modified method of lodin titration (Kenten and Mann 1952). The peroxidase (POD) was assayed as the recorded absorbance increased at 470 nm by guaiacol method (Castillo et al. 1984). Total nitrogen (TN) in dried samples was measured using the semimicro-Kjeldahl method with a Kjeldahl auto-analyzer (KDN-102C, Shanghai, China). Total P (TP) was determined colorimetrically with a spectrophotometer (UV-2102 PCS, Shanghai, China).

Statistical analysis: The differences in concentrations or activities among mowing, days after mowing (DAM), and organs of lucerne were analysed using One-Way Repeated Measures ANOVAs. The effects of mowing, DAM, organ and their interactions were analysed using Three-Way ANOVAs. The linear correlations were analysed with the model $y = ax + b$ using SPSS 11.5.

RESULTS AND DISCUSSION

Changes in MDA concentration: The MDA concentration in lucerne was significantly affected by mowing, DAM, organ and mowing×DAM (Table 1). Generally, MDA concentration under mowing treatment was lower than that under non-mowing treatment, but on 18 d after mowing they almost...
equaled (Figure 1). During the re-growth, MDA concentration increased till 18 d after mowing under mowing treatment. The plant has developed various strategies responding to “mowing” stimuli, including short-term and long-term reactions. At both temporal scales, MDA concentration increases when a plant encounters adverse environments or artificial disturbances (Cavalcanti et al. 2004; He et al. 2012). The root, stem and leaf of a plant exert different functions and characterize variously, for instance, developing different physiological responses to the adverse conditions (Amos et al. 2005; Markoviæ et al. 2009). In this study, there was much more MDA in roots than stems or leaves, indicating that root cell would have been damaged more heavily due to more energy and protective substances may be allocated to the re-growth.

Changes in SS, proline and SP concentrations: The SS concentration was affected significantly by organ and mowing×DAM, proline concentration was affected significantly by mowing, DAM, organ and their interactions (except mowing×organ), and SP concentration was affected significantly by mowing, organ and their interactions (except DAM, mowing×organ) (Table 1). The SS, proline and SP concentrations under mowing treatment tended to be lower than those under non-mowing treatment, but on 18 d after mowing, SS and proline concentrations tended to be higher than those under non-mowing treatment (Figure 2). During the re-growth, SS, proline and SP concentrations increased till 18 d after mowing under mowing treatment (except that SP concentration decreased in leaves). The increases in concentrations of such substances under mowing treatment scavenge MDA and help to recover cell structure and function (Cavalcanti et al. 2004; He et al. 2012; Zhang et al. 2016). In addition, SS and proline concentrations were significantly higher in roots than leaves and stems, and SP concentration was lowest in stems and highest in leaves, indicating that these substances play vital roles in different organs for the re-growth.

Changes in POD, CAT and SOD activities: The POD activity was significantly affected by DAM, organ and mowing×DAM, CAT activity was significantly affected by mowing, DAM, organ and their interactions (except mowing, mowing×DAM), and SOD activity was significantly affected by organ, mowing×DAM, and mowing ×DAM×organ (Table 1). The activities of POD, CAT and SOD increased from 13 to 18 d after mowing as well as SS, proline, SP and MDA concentrations (Figure 3). The concentrations of osmotic adjustment-related substances and the activities of enzymes scavenging radical oxygen species (ROS) increased to maintain or help to recover cell structure and function (Cavalcanti et al. 2004; He et al 2012; Usha and Dadlani 2016; Zhang et al. 2016). Furthermore, the activities of CAT and SOD were highest in the leaves in this study, suggesting the leaf may be more efficient in scavenging ROS than in other
Figure 3: Activities of peroxide dismutase (POD, A), catalase (CAT, B) and superoxide dismutase (SOD, C) in the root, stem and leaf of lucerne under mowing and non-mowing treatments. The 6, 13 and 18 refer to the days after mowing. Different lowercase letters represent significant differences among organs at $P<0.05$. Different capital letters show significances among days after mowing.

Figure 4: Concentrations of total nitrogen (TN, A) and phosphorus (TP, B) in the root, stem and leaf of lucerne under mowing and non-mowing treatments. The 6, 13 and 18 refer to the days after mowing. The asterisk (*) represents a significant difference between two treatments at $P<0.05$. Different lowercase letters represent significant differences among organs at $P<0.05$. Different capital letters show significances among days after mowing.

Table 2: Linear correlation coefficients between nutritional status and osmo-regulatory, anti-oxidative status, MDA concentration in roots, stems and leaves of lucerne during re-growth.

<table>
<thead>
<tr>
<th></th>
<th>SS</th>
<th>proline</th>
<th>SP</th>
<th>POD</th>
<th>CAT</th>
<th>SOD</th>
<th>MDA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Root</td>
<td>TN</td>
<td>-0.461</td>
<td>-0.603</td>
<td>-0.148</td>
<td>-0.263</td>
<td>-0.564</td>
<td>-0.322</td>
</tr>
<tr>
<td></td>
<td>TP</td>
<td>0.343</td>
<td>0.081</td>
<td>0.271</td>
<td>0.093</td>
<td>0.217</td>
<td>-0.137</td>
</tr>
<tr>
<td>Stem</td>
<td>TN</td>
<td>-0.785</td>
<td>-0.993***</td>
<td>-0.701</td>
<td>-0.945**</td>
<td>-0.876*</td>
<td>-0.997***</td>
</tr>
<tr>
<td></td>
<td>TP</td>
<td>-0.831*</td>
<td>-0.983***</td>
<td>-0.720</td>
<td>-0.981**</td>
<td>-0.843*</td>
<td>-0.986***</td>
</tr>
<tr>
<td>Leaf</td>
<td>TN</td>
<td>-0.220</td>
<td>-0.455</td>
<td>0.569</td>
<td>-0.802</td>
<td>-0.848*</td>
<td>0.585</td>
</tr>
<tr>
<td></td>
<td>TP</td>
<td>-0.381</td>
<td>-0.273</td>
<td>0.438</td>
<td>-0.847*</td>
<td>-0.926**</td>
<td>0.351</td>
</tr>
</tbody>
</table>

MDA, SS, SP, POD, CAT, SOD, TN and TP refer to malondialdehyde, soluble sugar, soluble protein, peroxidase, catalase, superoxide dismutase, total nitrogen and total phosphorus, respectively. The asterisk (*) represents a significant correlation at $P<0.05$, the double asterisks (**) represent a significant correlation at $P<0.01$ and the triple asterisks (****) represent a significant correlation at $P<0.001$. Parts of a plant, especially through the way involving CAT.

Changes in TN and TP concentrations: Element concentrations reveal the nutritional status in plant (Wright and Westoby 2003; Imaran and Gurmani 2011). The TN and TP concentrations were significantly affected by mowing, DAM, organ and the interactions (Table 1). They tended to be greater under mowing treatment than non-mowing treatment. The re-growth may involve two phases according to time after mowing, remobilization of stored carbohydrates and storage re-enhanced in both roots and stubble (Prud’homme et al. 1992; Meuriot et al. 2004). Nitrogen-
contained compounds changed similarly as carbohydrates did. Shortly after mowing, nitrogen uptake from roots is suppressed due to energy supply retardation and destruction of transpiration stream, the latter indicates that the force for pulling nutrients into the plant may not be enough. Generally, the concentrations of TN and TP in plants are flexible during plant re-growth (Yang et al. 2013). In this study, TN and TP concentrations decreased on 13 d after mowing (Figure 4), which may be due to dilution in the whole plant as the plant grows very quickly (Martiniello et al. 1997; Marković et al. 2009).

In this study, TN and TP concentrations were significantly higher in leaves than in stems and roots under mowing treatment, but more stable in roots than in stems and leaves (Figure 4), indicating that amounts of nutrients in roots were translocated to the aboveground part of the plant and root function might be affected due to shortage of energy supply, the absorption of root from soils was still guaranteed. However, in stems and leaves, most of N and P were derived from root storage, so it is supposed to increase after mowing and tend to be much flexible. At the later stages of re-growth, i.e. 18 d after mowing for lucerne, it dropped in the stems and leaves. This is because the rapid re-growth of shoot led to the dilution of such nutrients within stems and leaves.

**Linear correlations of TN and TP concentrations with osmo-regulatory and anti-oxidative status:** There were negative correlations of TN and TP concentrations with osmo-regulatory and anti-oxidative status in stems while in roots and leaves there was less correlation during re-growth (Table 2). The stems may have encountered much more serious stress after mowing stimuli than leaves and roots, and N- and P-containing compounds should also play roles the same as proline and anti-oxidative enzymes to maintain osmotic potential and to scavenge ROS.

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