

Apex development and stem morphology of vernalized and regrowing tillers of timothy (*Phleum pratense* L.)

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INTRODUCTION

Timothy (*Phleum pratense* L.) is a common forage grass species in mixtures for silage in Nordic conditions, since it has good overwintering capacity and the harvested biomass is palatable to livestock.

The accumulation of flowering stems and progressive lignification during maturation of these stems reduces the digestibility and so there is a negative correlation between the quantity and quality of grass biomass. The transition of apex from vegetative to reproductive stage is considered to be necessary for the formation of true stem and initiation of lignification processes. In timothy, the canopy structure of spring growth consists mainly of flowering and stem forming tillers whereas in the regrowing sward only few flowering stems can be found (Pakarinen et al, 2008). The spring growth is affected by vernalization which induces flowering. The regrowth, however, consist of non-vernalized tillers and the stem elongation and flowering is affected by shortening day length.

Whereas many *Lolium* and *Festuca* ssp. require vernalization and long days for flowering, timothy flowers without vernalization. However, timothy responds to day length and flowering tillers develop as the critical day length is exceeded (Heide 1982). As the accumulation of flowering stems reduces the digestibility of forage grass biomass, the control of flowering is one of the targets in forage grass breeding.

Here we have studied the development of elongating, yield forming tillers in timothy with special attention to the relationship between apex development and lignification and to the role of vernalization signals in this process.

RESULTS AND DISCUSSION

Apex development and stem morphology

A reduction in the digestibility of forage biomass is thought to be a result of extensive lignification of flowering stems (Akin 1989).

In this study, a lignified sclerenchyma ring was found at very early developmental stages of elongating tillers, already at apex stage A1 (Figure 1). The height of elongating tillers with vegetative and reproductive apices was the same, and we conclude that lignification was required for mechanical

support of the stem.

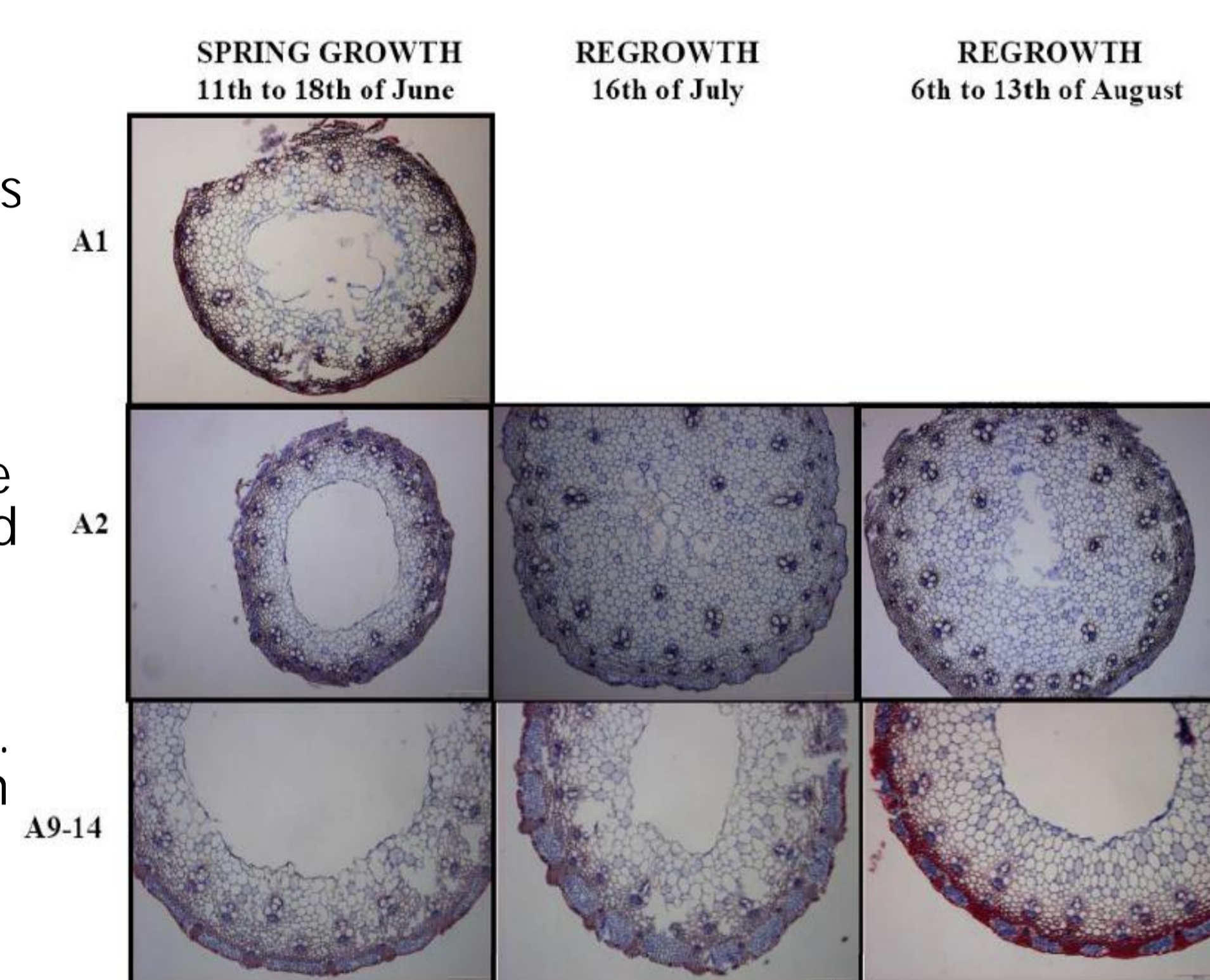


Figure 1. An example of the morphology of stems in spring growth and regrowth at different developmental stages of the apex. A1 to A2 - vegetative stage, and A9-A14 reproductive stages of apices (Sweet et al. 1991). Samples were stained with conventional safranin-alcian blue stain for lignin (red) and polysaccharides (blue).

Expression of VRN1 and VRN2 homologues

We also studied the expression of two flowering genes, which regulate the transition to reproductive stage in elongating tillers in controlled greenhouse conditions and in field experiments.

We found that the induction of *VRN1* and *VRN2* expression required vernalization signal, and that the expression peaked at apex stage A3, when the floral structures start to develop. In field conditions, the expression of both *VRN1* and *VRN2* peaked in June when the day length was the longest, 20 hours (Figure 2). These observations indicate that the expression level of *VRN1* and *VRN2* was also dependent on day length and light intensity. The studied genes were active only in vernalized tillers, and not in elongating tillers in the regrowth. This indicates a similar low-temperature requirement of *VRN1* induction in timothy as has been reported earlier in barley (Sasani et al. 2009).

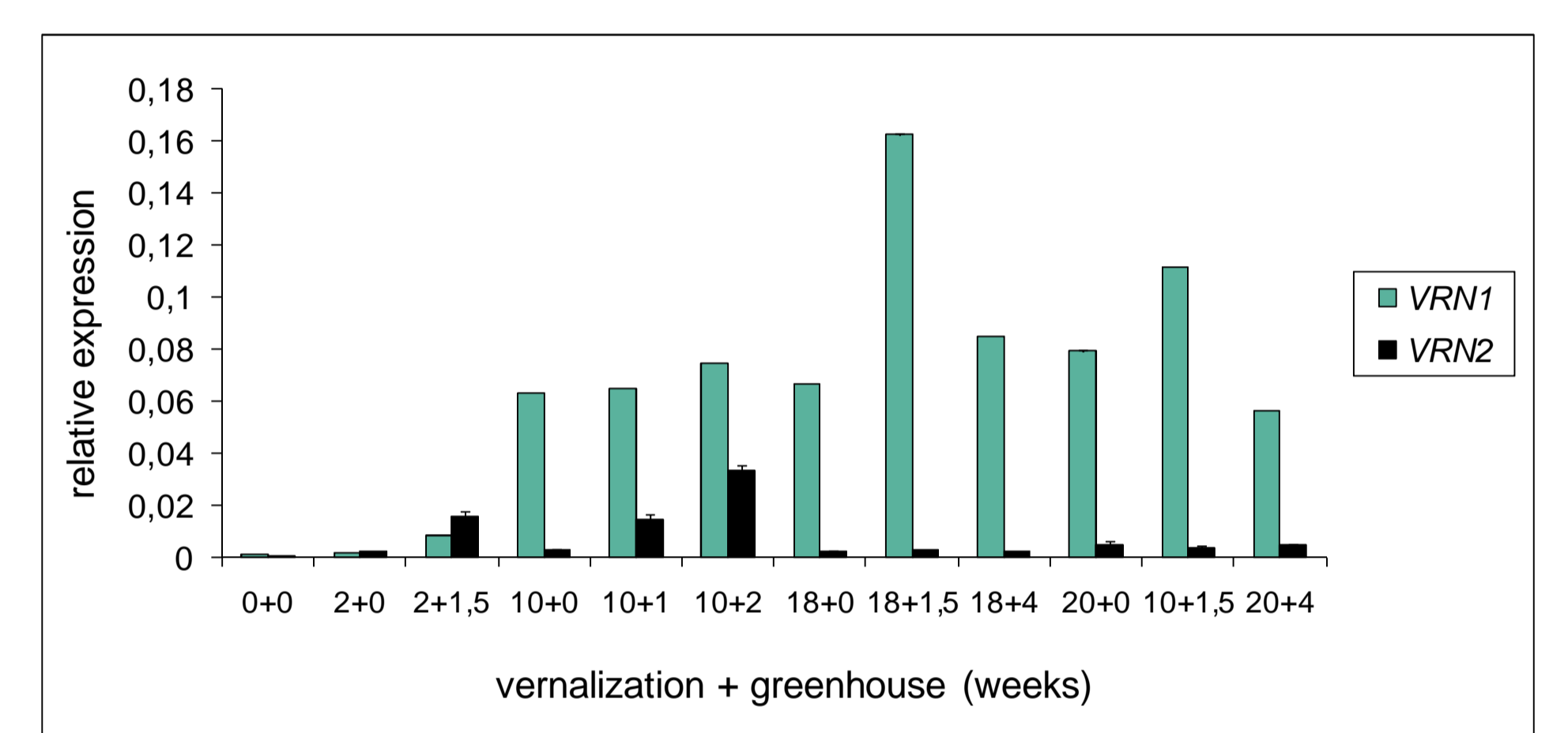


Figure 2. Relative amounts of putative *VRN1* and *VRN2* orthologue transcripts as compared with *Actin* transcript levels in the leaves of field grown timothy plants grown at different developmental stages. (Seppänen et al. 2010).

CONCLUSIONS

The transition of apex from vegetative to generative stage is commonly considered to occur prior to intensive lignification of stem. However, in those studies, the exact developmental stage of the apices has not been accurately monitored. Our results reveal that the formation of a lignified sclerenchyma ring that efficiently reduces the digestibility of the stem develops in vernalized tillers which apices are still at vegetative stage. The vegetative tillers could be as tall as the reproductive tillers and it was concluded that the lignification of the stem was not related to apex development but rather to a requirement for mechanical support. In the regrowing tillers the lignification occurred somewhat later. Both *VRN1* and *VRN2* homologues required a vernalization signal for expression, so the development of yield forming tillers in the regrowth was regulated independently of the studied genes.

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