Meristem activation in potato: impact on tuber formation, development and dormancy

R. Viola, M.A. Taylor & K.J. Oparka

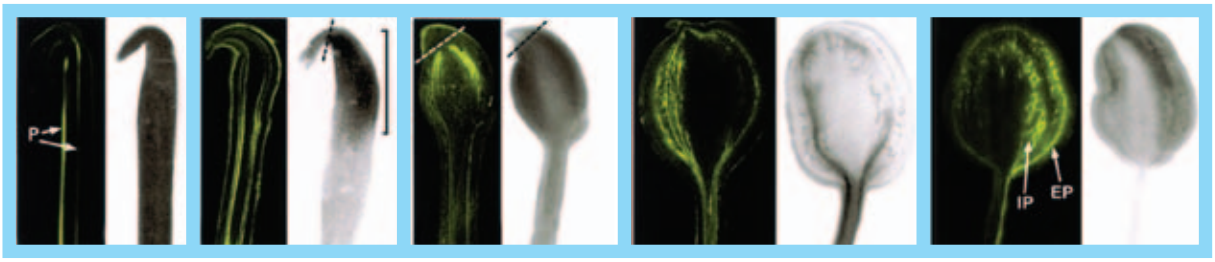

Figure 1 Unloading of CF (colour plates) and ^{14}C (autoradiographs) in potato stolons and tubers following treatment of leaves with CFDA or $^{14}CO_2$.

The life cycle of the potato tuber The life cycle of the potato tuber includes organogenesis, tuber development, dormancy and sprouting. This developmental sequence requires the co-ordinated control of a complex set of interlocked physiological processes and metabolic pathways and impacts directly on many qualitative and quantitative traits of the potato crop. Meristem activity plays a key role in this process. In elongating stolons and growing sprouts, the apical meristem acts as a sink for nutrients, which are sourced from the photosynthetic apparatus and tuber storage parenchyma respectively. Inactivation of the apical meristem, as a result of tuber induction, impacts greatly on the physiology of the entire plant, as the developing tubers subsequently become the largest sinks present. Increased cell division and expansion in the developing tuber is followed rapidly by a massive deposition of starch and storage protein. Following tuber maturation and during the rest phase, several tuber tissues undergo a functional and metabolic sink-source transition. The completion of these transitions coincides with the re-activation of meristem functionality at dormancy break.

Meristem activity and tuber formation We have adopted a multidisciplinary approach to investigate developmental changes occurring in the tuber (cv. Désiree) during its life cycle, focusing on the apical meristem[1]. Firstly we examined the physiological and metabolic changes accompanying tuber induction. Carboxyfluoroscein diacetate (CFDA) or $^{14}CO_2$ were applied to mature source leaves of potato plants to image changes in phloem unloading to the various regions during the tuber induction phase. Confocal images (colour plates) and autoradiographs (B&W plates) of stolons and tubers show unloading of CF and ^{14}C respectively (Fig. 1). In non-swelling stolon tips, the CF signal is restricted to the phloem (P) in both the stolon axis and hook regions, whilst radioactive assimilates appear distributed evenly across the stolon axis. Following tuber initiation, substantial unloading of CF and ^{14}C occurs from both internal (IP) and external (EP) phloem networks, suggesting the activation of cell-to-cell (symplastic) phloem unloading. In elongating stolons, substantial unloading of nutrients is observed in the apical region. However, irrespective of the developmental stage of swelling tubers, CF or ^{14}C were not found at the apex of the tuber within the dormant apical bud. Closer examination of the apical buds in growing tubers showed that although phloem connections were present immediately below, and leading into, the apical bud, no unloading of CF occurred in this region (Fig. 2).

These findings indicate that tuber formation is characterised by a switch from apoplastic to symplastic

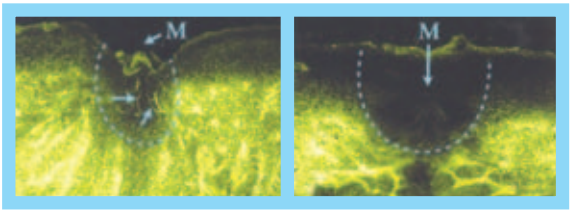

Figure 2 Close-up of CF unloading in the apical part of developing tubers. In the region containing the apical meristem (M), dye is apparent in the phloem (arrows) but does not unload. Abundant unloading is observed in the subtending swelling region.

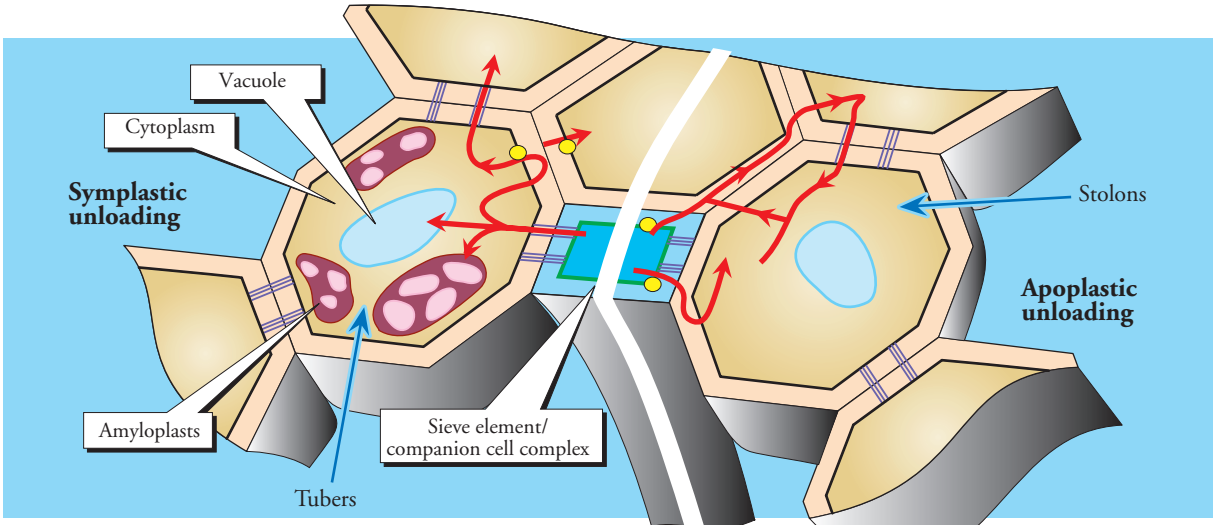

Figure 3 Schematic representation of phloem unloading in stolons and tubers.

unloading of assimilates in the subapical stolon region (Fig. 3). In elongating stolons, sucrose and other nutrients are unloaded from the phloem transport system into the apoplast. In the subapical region, sucrose is retrieved by parenchyma cells via sucrose transporters. In swelling stolons, this step is bypassed as a result of the establishment of symplasmic communication between the phloem system and the parenchyma cells, resulting in enhanced sink potential within stolon tips (Fig. 4) and a marked increase in the compartmentalisation of unloaded sucrose (Fig. 5). The latter may be directly responsible for the up-regulation of gene expression (starch and protein deposition) observed during tuber development. The induction of symplastic phloem unloading in tuberizing stolons appears spatially confined to the subapical stolon region and the apical, meristematic region becomes symplastically isolated from the rest of the tuber. Here, sucrose hydrolysis may occur in the free space before retrieval of hexoses, as suggested by the localization of cell wall

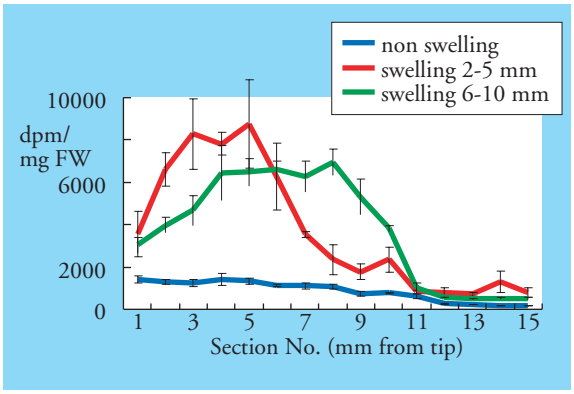

Figure 4 Concentration of radioactivity in 1-mm sections excised along the axis of non-swelling or tuberising stolon tips. Data collected 4h after labelling of foliage with $^{14}CO_2$.

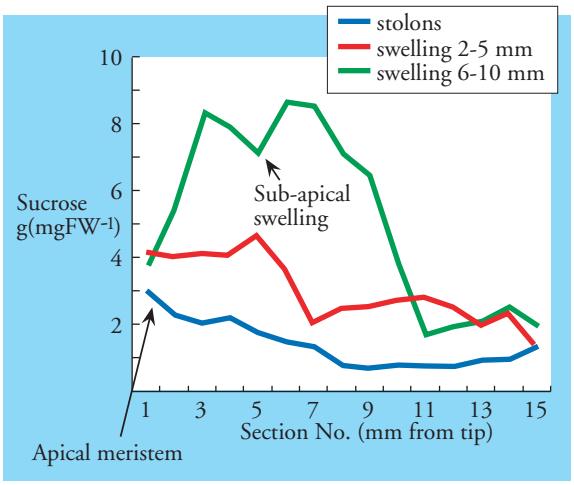

Figure 5 Distribution of sucrose along the axis of non-swelling and tuberising stolon tips.

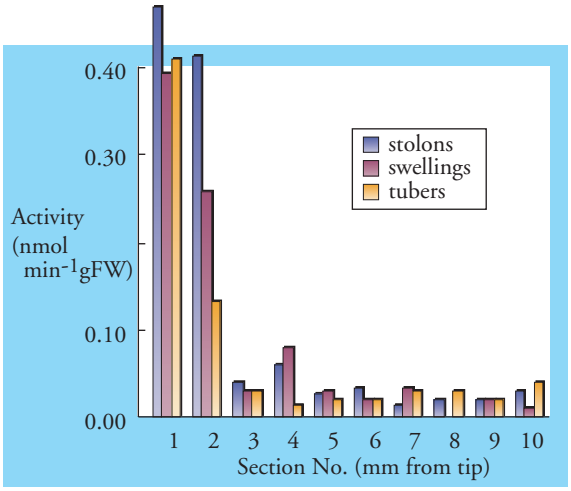

Figure 6 Distribution of cell wall-bound acid invertase activity along the axis of stolon and tuberising tips.

invertase activity (Fig. 6). This hypothesis is also supported by the highly localized expression from the *invGE* promoter in the apical region of stolons and tubers (Fig. 7). The symplastic isolation of the apical meristem from the rest of the tuber coincides with the cessation of cell division in the apical meristem and the induction of dormancy.

Figure 7 Expression of an apoplastic invertase (*invGE*) in stolons and tubers revealed by GUS staining.

Meristem activity in mature tubers Following maturation of potato tubers, there is an obligate period of rest, e.g. lack of bud growth. It is not known whether the repression of bud growth in this phase is controlled by factors within the buds themselves or by factors within the tuber. In preliminary experiments, we observed that dormant tubers appear to have the competence to produce substrates for bud growth (e.g. sucrose) and to transfer these substrates from the storage parenchyma into the transport system. We also found evidence that the apical bud of dormant tubers is metabolically competent to metabolise exogenously labelled substrates (not shown). Compositional analyses of apical buds during storage of mature tubers revealed a marked increase in carbohydrates (Fig. 8) in advance of visible bud growth. The pattern of CF

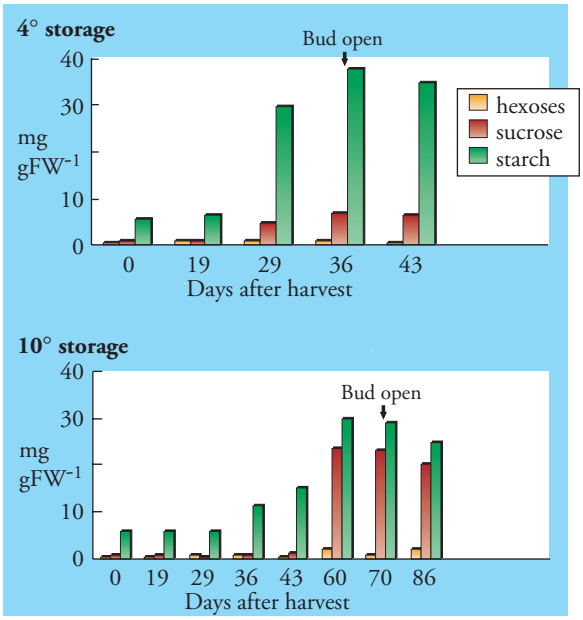

Figure 8 Change in carbohydrate content of apical buds during tuber storage.

unloading in the bud at various developmental stages (Fig. 9) shows that dormancy release is correlated with the activation of phloem unloading. Thus it appears

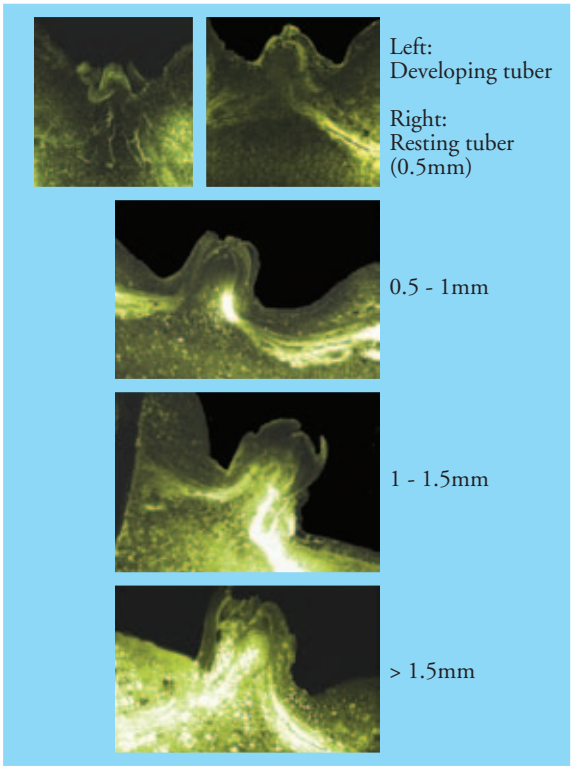

Figure 9 Unloading of CF in the apical buds at different developmental stages after application of CFDA through a well at the stolon-end of the tuber.

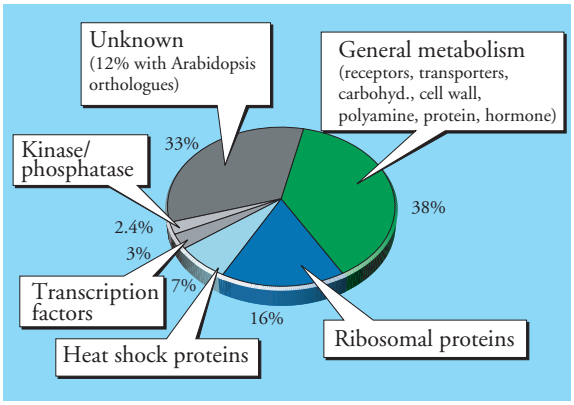

Figure 10 Genes up-regulated in the very early stages of dormancy release isolated by subtractive hybridisation methodology.

that the re-activation of metabolic processes and of mitotic activity occurring in the apical meristem upon release from dormancy is a direct result of activation of phloem unloading and ensuing carbohydrates accumulation in the tissue.

The large carbohydrate accumulation observed in apical tuber buds prior to any visible signs of bud growth, has proved a useful tool to identify buds in the very early stages of dormancy release. Using a suppression subtractive hybridisation approach, a library of cDNA clones, enriched for genes up-regulated at the very early stages of dormancy release, has been produced. Approximately 400 different cDNA sequences (ESTs) have been obtained (Fig. 10). *In situ* hybridisation methodology has confirmed tissue-specific and developmental stage-specific expression of some of the genes isolated via the SSH approach (Fig. 11). Further functional characterisation of these genes through DNA arrays and transgenic approaches is in progress.

Conclusion We have established the crucial role of meristem activity in the life cycle of the potato tuber. Inactivation of the apical meristem is accompanied by symplastic isolation during tuberisation of stolons and this status is maintained throughout tuber development, maturation and rest. The resumption of cell-to-cell communication between the apical bud and the rest of the tuber coincides with dormancy release and results in carbohydrate accumulation in the bud. Specific events at the interface between the meristem

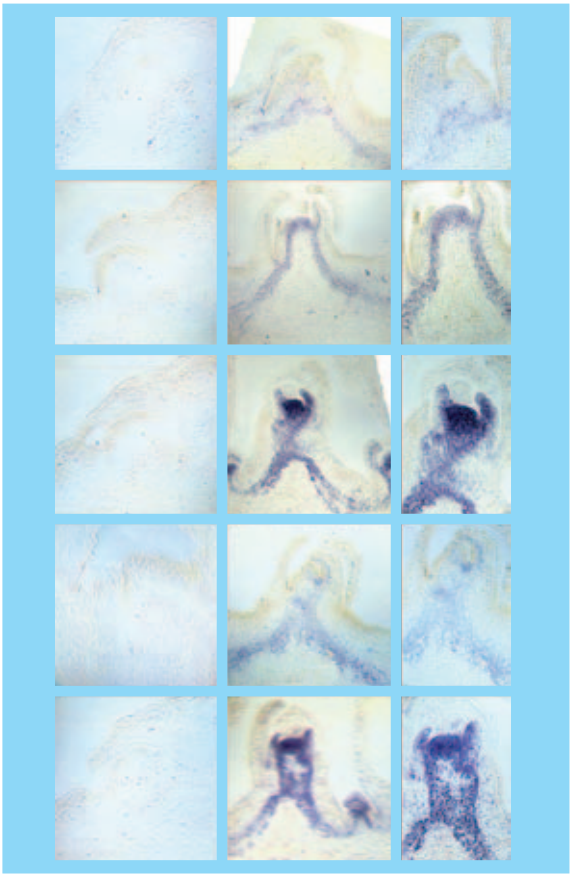

Figure 11 Tissue-specific expression patterns of selected genes isolated by the SSH approach.

and the plant transport system have been identified as control points for meristem activation and deactivation. In addition, biochemical markers and genes specifically expressed at these stages have been discovered making possible, for the first time, the dissection of the mechanisms responsible for meristem activity at the cellular and molecular level. These mechanisms may well be widespread and form the basis for improved understanding of phenomena such as bud dormancy and apical dominance in plants.

Acknowledgements

Many other colleagues contributed to this work, in particular Alison Roberts and Sophie Haupt (Unit of Cell Biology) and Rob Hancock (Unit of Plant Biochemistry). Their input has been greatly appreciated. Support for this work has been provided by EU (Framework IV) and by SEERAD (SCR/550/00).

Reference

[1] Viola, R., Haupt, S., Roberts, A., Hancock, R.D., Grazzani, S. & Oparka, K. (2001). *Plant Cell* 13, 385-398.