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Indoleacetic acid concentration and metabolism changes during bud development in tubers of two potato (*Solanum tuberosum*) cultivars

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Summary

Plant growth regulators are involved in the control of potato (*Solanum tuberosum*) tuber dormancy. Evidence concerning the role of IAA is controversial; we therefore investigated its role by analyzing two cultivars with varying lengths of dormancy. We examined the time course of free and conjugated IAA in tuber tissue isolates from the final stages of tuber growth to the end of dormancy, the distribution of free IAA in tuber tissues by *in situ* analysis, and the biosynthesis of the hormone by feeding experiments. The time course of free IAA showed marked differences between the examined cultivars, although the concentration of the auxin generally was the highest at the early stages of tuber dormancy. Immunodetection showed a similar pattern of IAA distribution in both genotypes: in dormant buds from freshly harvested tubers, the free hormone accumulated mostly in apical meristem, leaf and lateral bud primordia, and differentiating vascular tissues underlying the apical meristem, while at the end of the storage period only axillary bud primordia from growing buds displayed appreciable auxin levels. Feeding experiments indicated that changes in IAA biosynthesis rate were a major cause of auxin variation in buds. In both cultivars, dormancy apparently ceased when free IAA fell below a threshold value. Despite this, our data led us to conclude that IAA would not be directly responsible for inhibiting sprouting. Instead, auxin might shorten dormancy, in a cultivar-dependent manner, by enhancing early developmental processes in buds, ultimately leading to dormancy termination.
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Introduction

The physiology of potato tuber dormancy has been studied extensively, but much remains unknown about the complex systems underlying its regulation. Bud dormancy is controlled by a number of factors both external and internal to the tuber (Sonnewald, 2001), which act to suppress or promote sprouting through a complex signaling network. The central role of plant growth regulators in the control of tuber dormancy is substantiated by a wealth of research reports (for review, see Suttle, 2004a). Nevertheless, the available data are sometimes difficult to interpret and the signal transduction pathway of these molecules is not known in detail. For instance, it is not clear why exogenous cytokinins are effective in promoting bud outgrowth only for limited periods during tuber dormancy (Turnbull and Hanke, 1985a; Suttle, 2001). Cytokinin concentration in tuber buds markedly increases during dormancy, until sprouting. Although such increase also occurs in buds from tubers stored at 2 °C, at this temperature it is not associated with any significant cellular growth (Turnbull and Hanke, 1985b; Sukhova et al., 1993). Gibberellic acid (GA_3) treatments to harvested tubers usually promote bud outgrowth, but only in tubers whose dormancy is terminated (Madec and Perennec, 1969), whereas they are always effective when administered to the mother plant (Alexopoulos et al., 2007). Endogenous gibberellins do not appear to be clearly and directly related to dormancy release (Suttle, 2004b). Abscisic acid is considered to be primarily responsible for dormancy induction and maintenance (Suttle and Hultstrand, 1994; Destefano-Beltran et al., 2006), but some data do not agree with this conclusion (Ji and Wang, 1988; Sorce et al., 1996). With respect to endogenous ethylene, it is not known why its action on bud growth suppression is apparently restricted to the initial stages of tuber dormancy (Suttle, 1998). Surprisingly, the role of auxin in potato tuber physiology has rarely been investigated. Very few data are available regarding a possible relationship between auxin and tuber bud development. Thus, the function of this class of growth regulators deserves in-depth investigations. It has been demonstrated that, during preharvest development, IAA concentration and tuber growth rate are positively correlated. This would occur because IAA may enhance the sink strength of a tissue, thus increasing the phloem transport of assimilates towards the sites of high auxin concentration (Marschner et al., 1984). Support for this hypothesis is the well-documented stimulating action of IAA on vascular tissue differentiation (Aloni, 1995). Treatments with naphthaleneacetic acid restore growth vigor of aged tubers (Kumar and Knowles, 1993), further suggesting that auxin may play a positive role in tuber development. In previous work (Sorce et al., 2000), we found that the concentration of IAA in buds was positively related to dormancy termination. With the aim of investigating more in depth how auxin may influence tuber bud development, we chose a different approach for the present study. Three major points characterize this research: (a) analyses were performed on tubers from two cultivars, markedly differing for dormancy length; (b) only buds from the apical end (i.e. opposite to the stolon scar) of the tuber were analyzed, because they resume growth earlier; for this reason, we assumed that these buds should have been more suitable to highlight any relationship between sprouting and changes of auxin physiology during the period of study; (c) the localization of IAA was also investigated by immunohistochemical detection, thus yielding more detailed information on the hormone dynamics in tuber tissues. Further experiments were performed to evaluate the physiological importance of IAA biosynthesis in buds. The results of this work strongly suggest that IAA plays a positive role in tuber bud development and provide the basis for revisiting our previous knowledge on the subject.

Materials and methods

Plant material

The potato (*Solanum tuberosum* L., cvs. 'Agata' and 'Merit') plants were cultivated in the farm of the 'CRA-Centro di Ricerca per le Colture Industriali' near Bologna, Italy, about 44°32′N, 11°31′E, where certified seed tubers were sown in mid-April. The two cultivars were chosen on the basis of the length of their post-harvest tuber dormancy, which is markedly shorter in 'Agata' than in 'Merit' (Parisi, Personal Communication). The cultural details, including the fertilizer application, the use of aphicides and fungicides (for late blight control) were standard for potatoes in field conditions. For each cultivar, a representative date for mother plant senescence (T0) was preliminarily estimated, based on growers' experience. The T0 was set when plants showed yellowing and/or browning on 50% of stems and leaves. The date was taken as a reference to plan early tuber digging, with the aim of collecting samples 15 d (T-15) before T0 and at T0. These tubers were

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