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Gibberellic acid and the inhibition of aerial tuberisation in *Solanum tuberosum* L.

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Abstract. The sensitivity of aerial and subterranean tuberisation to photoperiod was studied in potato (*Solanum tuberosum* cv. Aracy). Although photoperiodic sensitivity varied with the position along the stem, all buds could be induced to develop tubers under SD. Gibberellic acid (GA_3) applied to induced (30 short days) cuttings inhibited the photoperiodic effect. No tubers were formed and orthotropic shoots developed instead. The GA_3 caused a reduction in starch content in induced buds, lowering it to the same level as found in long-day treated plants. However, α-amylase activity of buds of induced plants was not affected by GA_3, suggesting that GA_3 does not inhibit tuberisation by promotion of starch hydrolysis.

Introduction

Tuberisation is a complex process and in most tuberous species is influenced by environmental conditions. Photoperiod is one of the most important factors, tuberisation being a response to short days (SD) [8, 10].

Aerial tuberisation is a natural process in some species such as *Begonia evansiana* [5]. This is not the case in *Solanum tuberosum*, although all the axillary buds are able to develop tubers under certain conditions [6].

The involvement of growth regulators in tuberisation has been the subject of much published work. Gibberellins (GAs) are frequently associated with the inhibition of this process [8] and the promotion of stolon elongation [22]. Aerial tuberisation is also inhibited by GA_3 treatment [12]. It has been shown that the level of endogenous GAs is high in non-induced plants and much lower in plants kept under inductive conditions [19].

There is evidence that starch accumulation occurs before any morphological modifications become visible in a tuberising organ [4]. Gibberellins are associated with starch hydrolysis by inducing α-amylase synthesis and release in cereal grains [11]. A similar effect has been found in other systems such as leaves of tobacco [13] and *Digitaria decumbens* [2] where a reduction of starch levels, together with an increase in starch degrading enzymes, occurs as a result of GA_3 treatment.

In this study, we investigated the possibility that high levels of endogenous GAs may be inhibiting aerial tuberisation in the potato plant, perhaps through

Table 1. Effect of photoperiod on aerial and subterranean tuberisation in *Solanum tuberosum*

Number of short days (8 h)	% of cuttings with aerial tubers	% of plants with subterranean tubers
0	0	0
10	18	62
15	17	70
25	54	100

the inhibition of starch deposition in the buds as a result of increased α-amylase activity.

Material and methods

Tubers of *Solanum tuberosum* cv. Aracy were planted in pots and kept under natural conditions (daylength never less than 12 h) for 10 days (when plants were about 20 cm high). They were then divided in two groups of at least 10 plants each. One group of plants was kept under SD photoperiods of 8 h (natural light) and the other under LD photoperiods of 18 h (natural day-length supplemented with an incandescent bulb ($180\,\mu W \cdot cm^{-2}$)) for a period of 30 days, unless stated otherwise. When the number of SD was less than 30, the plants were returned to LD after the SD treatment. Upon completion of the 40-day period the formation of subterranean tubers was evaluated.

Aerial tuberisation was studied with cuttings taken from 40-day-old induced (SD) and non-induced (LD) plants. The cuttings (10 per treatment), consisting of a leaf with its axillary bud and a stem portion of 5 cm, were kept in vials with distilled water under continuous light for 7 days.

The application of GA_3 (10 µl of a 0.3 mM solution) to the cuttings was made by means of a glass capillary tube inserted into the stem next to the axillary bud. The application was made immediately after the cuttings were taken from the intact plant.

Starch was extracted from the buds following the method described by Shannon [20] and quantified, relative to a standard curve on aliquots of 9.9 ml to which 0.1 ml of a solution containing 2% I_2 + 0, 2% KI was added. After 15 min., the absorbance was read in a spectrophotmeter at 660 nm.

The activity of α-amylase in the buds was assayed by measuring the amount of maltose produced by starch hydrolysis following the method of Bernfeld [1], as modified by Metivier and Paulilo [14]. In this method, 3,5-dinitrosalicylic acid is used as the reagent. The colour formed was measured at 520 nm and maltose determined from a standard curve.

All experiments were repeated at least three times.

Table 2. Tuberisation of the bud in response to SD treatment in relation to its position along the stem of *Solanum tuberosum*

Number of short days (8 h)	% of cuttings with aerial tubers		
	Apical bud	Median bud	Basal bud
0	0	0	0
10	0	24	12
15	0	24	12
25	50	64	0

Results

Initially, the sensitivity of the cultivar Aracy to photoperiodic induction of aerial and subterranean tubers was established (Table 1). The application of 10 SD was sufficient to induce subterranean tubers in about 60% of the plants, while 25 SD were required to produce a similar effect on aerial tuberisation. This indicates that, although axillary buds can be induced to tuberise, subterranean organs are much more sensitive to this environmental stimulus.

The sensitivity to SD differed along the stem (Table 2). Cuttings taken from the apical and basal regions of the stem were less sensitive than those taken from the median portion. In fact, cuttings taken from the basal portion of the stem rarely produced a tuber. Tuberisation in the apical cuttings could be obtained only after 25 SD, whereas about 25% of the cuttings from the median region of the stem developed a tuber after 10 SD, and about 65% developed a tuber after 25 SD.

In all subsequent experiments only median cuttings were used, since they provided more consistent results. A further advantage is that median cuttings bear a single bud, whereas apical cuttings had a variable number of buds.

The well-known inhibitory effect of GA_3 on subterranean tuberisation [18] was also found for aerial tuberisation here. Treatment with GA_3 completely eliminated the formation of tubers in induced cuttings, whereas 89% of control cuttings produced tubers. The GA_3-treated buds developed as orthotropic elongated shoots instead of tubers.

Analysis of starch accumulation in the buds showed that from the third day after GA_3 treatment onwards the amount of starch present in the buds of cuttings taken from induced plants was much reduced (Figure 1). This level of starch was very similar to that found for buds of non-induced (LD) cuttings. In buds of control (SD) cuttings, starch accumulation increased steadily up to day 4, followed by a more rapid increase to day 5.

The activity of α-amylase was determined in the buds three days after GA_3 treatment, since differences in starch levels were detected from this day onwards. However, treatment with GA_3 did not alter the extractable levels of this enzyme since identical values were obtained for cuttings of SD-induced

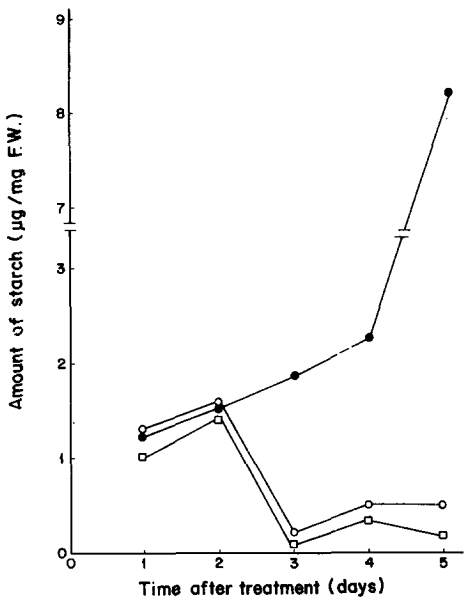

Figure 1. Effect of photoperiod and GA_3 treatment on starch accumulation in axillary buds of stem cuttings.
• SD ○ SD + GA_3 □ LD

Table 3. Effect of photoperiod given to intact plants and GA_3 applied to the cuttings on α-amylase levels in axillary buds of stem cuttings of *Solanum tuberosum*

Treatments given to		α-amylase activity
intact plant	cutting	(µg maltose/mgFW)
30 SD	H_2O	7.0
30 SD	GA_3	7.0
30 LD	H_2O	5.0

plants with or without GA_3 treatment (Table 3). The level of α-amylase was only slightly lower in buds of non-induced (LD) plants.

Discussion

Tuberisation in the potato plant has been studied mainly in subterranean organs using the whole plant [8, 10]. The study of a complex process such as tuberisation should be less problematic in a simpler system in which the interaction between organs can be reduced. This was achieved in this study by the use of cuttings taken from the median portion of the stem. Such cuttings, which bear only one bud and one leaf, have only one growing point to develop as a tuber, and only a single leaf for photoperiodic induction. Leafless cuttings, it should be noted, do not tuberise [23]. In spite of the lower sensitivity of aerial buds to photoperiod (relative to subterranean stolons), all of the aerial buds can be induced to develop tubers (see Table 1 above), the system is thus appropriate for the study of tuberisation.

One limitation of our system, however, is that the sensitivity is not the same along the stem. For example, basal cuttings rarely tuberise. A possible explanation for this lack of response is that the basal leaf may already be entering senescence and can no longer be induced. Also, apical cuttings did not develop tubers unless they received the longest photoperiodic treatment (25 SD; 50% of the cuttings tuberised). Several possibilities may be considered to explain this low sensitivity. First, high GA levels in the rapidly expanding leaves of the apical region may be inhibitory to tuberisation. Second, the leaf of the apical cutting is not fully expanded at the beginning of the SD treatment. Since there is a minimum number of SD needed for induction, the leaf may not have received a sufficient number of SD. In favour of this idea is the fact that the highest percentage of tuberisation was obtained with the more prolonged SD treatment. Third, the leaf may not be induced because it is immature. Although there is still some controversy as to whether mature leaves [7] or developing leaves and shoot tips [3] are responsible for the formation of the tuberisation stimulus, Hamnes and Beyers [9] have shown that both young developing leaves and mature leaves can be induced to promote tuberisation in potato plants. They also found that young leaves produce an inhibitor of tuber formation in plants under non-inductive conditions and suggested that this inhibitor may be a GA.

Exogenous GA application usually causes an inhibition of tuberisation in the potato plant [17, 21, 22]. Studies of endogenous GA levels have shown that they are higher in plants under LD and lower in plants under SD. Furthermore, the high level of GAs in plants under LD can be reduced after transferring the plants to SD [19]. In our system, aerial tuberisation of induced cuttings was inhibited by GA_3 treatment, thereby nullifying the effect of SD.

This inhibition could be explained by an involvement of GAs in starch accumulation, since this is the first event in tuber development [4], and GA_3 treatment reduces the starch level from the third day onwards (see Figure 1 above).

The interesting question that arises is how GA_3 could act to reduce starch levels. Essentially, this must involve either starch synthesis or degradation. Although it is known in other systems that GA_3 induces α-amylase synthesis [11] our data suggest that this does not explain the inhibition of the tuberisation. Alternatively, GA_3 may be affecting enzymes involved in starch synthesis. One possibility is that GA_3 may modify phosphorylase levels, since it has been shown that the activity of phosphorylase increases with the rate of starch deposition in potato stolons cultured "in vitro" [15]. Furthermore, a decrease in GA level and an increase in phosphorylase activity was demonstrated in potato stolon tips during tuber initiation [16]. Soluble starch synthetase on the other hand was not found at initial stages of the process, although its level did increase later [16].

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