Hormone levels and protein patterns in dormant and non-dormant buds of strawberry, and induction of bud break by gibberellic acid

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ABSTRACT

Strawberry runners are characterized by the occurrence of a dormant bud followed by a consequent non-dormant bud. Dormant buds were found to contain relatively low levels of endogenous gibberellic acid (GA$_3$) and enhanced levels of abscisic acid (ABA), as compared with non-dormant buds. The lower contents of GA$_3$ in the dormant buds were also accompanied by attenuated levels of auxin (IAA) and cytokinins (zeatin and benzyladenine). Exogenous application of GA$_3$ (50 ppm) to the latent buds could achieve 97% break of their onset of dormancy. Histological examination showed that the initial stage of bud formation was similar in the dormant and non-dormant buds. Thus, triggering the burst of a dormant bud by GA$_3$ treatment is assumed to occur in the second developmental stage. Comparison of the protein-banding patterns in the dormant and non-dormant buds showed a slight difference in the number of protein bands, but a wide variation in their types. The results suggest differences in the regulation of gene expression, perhaps controlled mainly by ABA in the dormant buds and GA$_3$ in the active state.

KEYWORDS: Abscisic acid, auxin, cytokinin, zeatin, benzyladenine, histology, PAGE-electrophoresis

INTRODUCTION

The production of strawberry transplants has become an important industry in Egypt (Ragab 1996). Increasing the production of good-quality transplants and consequently the yield are desirable for the nurseryman. The strawberry plant is characterized by nodes carried on runners that are produced all summer from buds in the axils of new leaves and in succession as the leaves develop (Darrow 1966). These runners are mostly two nodes and two internodes in length, where the bud at the first node is usually dormant.

In recent years, attention has been directed mainly towards the control mechanisms of dormancy in buds (Erez 2000). However, despite different ideas about unravelling this physiological phenomenon, the regulation of dormancy certainly involves interference with growth hormones (Grabbé 1994; Erez 2000; Shimizu-Sato & Mori 2001; Rosin et al. 2003).

In general, the regulation of bud (as well as seed) activity may be considered to result from a concerted interaction between the inhibitory influence of abscisic acid (ABA) and an enhancement effect by gibberellins (GAs) (Debeaujon & Koornneef 2000). Scattered lines of evidence, however, indicate that other plant hormones may also help to regulate dormancy. In this connection, ABA content is elevated in dormant Phaseolus (Gocal et al. 1991) and Elytrigia (Pearce et al. 1995) buds. It is generally stated that ABA is synthesized within the bud or in its vicinity, perhaps in response to indoleacetic acid (IAA) within the stem (Stafstrom 2000). For GAs, on the other hand, the data are very suggestive of enhanced levels with bud-break. Thus, Ozguven & Kaska (1992) showed that GA$_3$ application increased the levels of growth promoters with particular dominance in plant runners and leaves. Similarly, application of GA$_3$ (50 ppm) to mother plants of strawberry significantly enhanced the number of runners (Turemis & Kaska 1997). A
significant rise in GA level was also evident during the release of bud dormancy (Khattab et al. 2000).

According to general theory, bud dormancy results from high concentration of IAA within buds (Stafstrom 2000). The author added that this model is difficult to reconcile with the fact that terminal buds contain high levels of IAA, and yet are able to grow. Gocal et al. (1991) showed that growing buds contain more IAA than dormant buds. Furthermore, auxin-stimulated genes are expressed at low level in dormant buds (Stafstrom 1993). Cytokininns are also considered as good candidates for the promotion of bud growth (Stafstrom 2000).

The main objective of this study is to investigate why half the number of strawberry nodes fails to grow. As this is a reflection of the dormancy of lateral buds, the causative factors would first be investigated from a hormonal point of view. The possible changes in gene expression are also studied, as reflected by concomitant modulation of protein-banding patterns.

MATERIALS AND METHODS

The cultivar of strawberry used is Camarosa (Fragaria x ananassa cv. Camarosa). Homogenous transplants were obtained from the Strawberry Development Center, Faculty of Agriculture, Ain Shams University, Cairo, Egypt. The growth hormones (IAA, GA₃, ABA, zeatin and benzyladenine) used as authentic materials, and protein markers (molecular weights ranging from 17.5 to 209 kDa) were purchased from Bio-Rad Laboratories Headquarters 1000 Alfred Nobel Drive Hercules, CA 94547.

The study was carried out in 2002 in the Strawberry and Non-Traditional Horticulture Crops-Research Station, South Tahreer, Behaira Governorate, Egypt. Fresh transplants ready for cultivation were soaked in Benlate solution (1.5 g/l) for protection against root-rot diseases. Preparation of the cleaned silty soil was carried out according to the agriculture extension by the strawberry commission, Horticulture Institute, Ministry of Agriculture and Soil Reclamation, Egypt. After 21 days, both dormant and non-dormant buds were taken from uniform transplants and kept separately in cold extraction solution.

The extraction, methylation and estimation of IAA, GA₃, and ABA were done according to the method adopted by Guinn et al. (1986) and that of Muller & Hilgenberg (1986). For cytokinins, estimation was carried out using HPLC. One-dimensional SDS-PAGE (polyacrylamide gel electrophoresis) was used according to Hames (1981).

Exogenous application of GA₃ (50 ppm), was done by mixing with a paste of lanolin. The paste was then stacked against the dormant buds. The process was carried out after two months from transplanting (until the runners reached their maximum growth). The paste was renewed after two weeks for once.

Microtome transverse and longitudinal sections of the runners at the node regions were prepared following the embedding method (Johansen 1940). Double staining of the sections was carried out using safranin-light green (Johansen 1940).

RESULTS AND DISCUSSION

Vegetative growth in strawberry plants is characterized by a particular branching architecture. The runners are mostly two nodes and two internodes in length, where the bud at the first node is usually dormant and the following is non-dormant (Figure 1). Recent work on branching mutants from several species provides important insights into lateral bud development. Napoli et al. (1999) stated that buds usually perceive their position along the plant axis, and as a consequence grow or remain dormant, based on the relative levels of hormones in adjacent stem tissue. The tremendous diversity in vegetative pattern
formation between plant species arises from general basic processes (Sussex & Kerk 2001). These are determined genetically by interactions between the shoot apical meristem, the axillary meristems, and signalling compounds transported from the roots. Hormone levels mediate the development of axillary meristems (Rosin et al. 2003). Consequently, we looked at the concentration of growth hormones concomitant with dormancy and activity of non-dormant buds, the effect of exogenous application of a hormone on dormant buds, and variation in protein patterns.

Figure 1: [A] Strawberry runner showing a dormant (D) and non-dormant (ND) bud. An induced dormant (ID) bud by GA$_3$ treatment is shown in [B].

Based on the results of a large number of workers it seems likely that ABA might be the main hormonal factor responsible of the bud dormancy because ABA accumulates in the dormant buds of many plants and decreases afterwards with the release of dormancy and occurs at low levels in non-dormant buds (Gocal et al. 1991; Pearce et al. 1995; Stafstrom, 2000; Shimizu-Sato & Mori 2001). Hopkins (1999) stated that the increase of ABA (originally called ‘dormin’) is thought to be the principle causative factor in the onset of bud dormancy. The results obtained in the present work (Table 1) are in alliance with the above mentioned bases. Table 1 shows the levels of endogenous IAA, GA$_3$, ABA, and cytokinins (zeatin and benzyladenine) as well as their total values in dormant and non-dormant strawberry buds. It is obvious that the dormant buds contain much lower levels of IAA, GA$_3$, and cytokinins than the non-dormant buds. On the other hand, ABA concentration is markedly higher in dormant as opposed to non-dormant buds.

In the present work, supplementation of GA$_3$ (50ppm) to the dormant buds of strawberry induced 97% breaking of dormancy and onset of bud outgrowth. Rebers et al. (1994) confirmed that exogenously applied gibberellins partially substitute for the cold treatment of dormant tulip bulbs, and stimulate shoot growth and flowering, and the authors concluded that gibberellins seemed to act as a dormancy-breaking agent.

Our results also showed the occurrence of a comparatively low concentration of auxin (IAA) in dormant buds and high levels in non-dormant buds (Table 1). However, exogenous application of auxin in our laboratory (unpublished work) to dormant strawberry buds was not effective, confirming the conclusions of many others who either did not assign a specific role for auxin in the regulation of dormancy (Gocal et al. 1991; Stafstrom 1993, 2000; Saniewski et al. 2000) or suggested an inhibitory effect (Shimizu-Sato & Mori 2001; Rosin et al. 2003).

Table 1 also shows an obviously higher concentration of cytokinins (zeatin and benzyladenine) in non-dormant over dormant buds. Elevation of cytokinin levels was found to be a concomitant of breaking dormancy (Taiz & Zeiger 1998), or preceded bud growth resumption (Suttle 1998). Dormancy of axillary buds does not correlate with the absolute concentrations of IAA or cytokinins, or their relative concentrations in the arrest
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stage (Shimizu-Sato et al. 2001), but a good correlation was observed since the earlier developmental stages of the bud burst.

Table 1: Contents (mg/100g dry wt equivalents) of endogenous phytohormones in dormant and non-dormant strawberry buds.

<table>
<thead>
<tr>
<th>Cytokinins</th>
<th>Zeatin</th>
<th>Benzyladenine</th>
<th>Total cytokinin</th>
<th>GA3</th>
<th>ABA</th>
<th>IAA</th>
<th>Hormone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dormant</td>
<td>0.26</td>
<td>223.8</td>
<td>224.06</td>
<td>9.4</td>
<td>309</td>
<td>34.03</td>
<td>Dormant</td>
</tr>
<tr>
<td>Non-Dormant</td>
<td>14.8</td>
<td>333.4</td>
<td>348.2</td>
<td>5.3</td>
<td>540</td>
<td>253.4</td>
<td>Non-Dormant</td>
</tr>
</tbody>
</table>

Thus, endogenous ABA appears to antagonize the effect of gibberellins in the dormant buds, since one can overcome its effects completely by adding a sufficient concentration of GA₃. The work of Viemont & Grabbé (1999) could further support this assumption. In general, ABA counteracts the effect of GA₃ in a number of physiological processes, including the induction of α-amylase in barley aleurone cells, control of cell elongation, and the dormancy of seeds and apical buds (Cleland 1999). Another possibility suggested here is that exogenously supplemented GA₃ to dormant buds might stimulate auxin availability on the basis that gibberellins are well known to increase auxin transport and enhance auxin biosynthesis (Saniewski et al. 2000).

Potential of GA₃ treatment: Axillary meristems are typically located on the leaf axils, where they undergo immediate development to form an axillary shoot, or initiate a few leaves (bud-scale complex) and then become developmentally arrested or dormant (Shimizu-Sato & Mori 2001). These dormant axillary buds can resume development at a later time, depending on their signal transduction programme, mostly in response to environmental cues. Axillary meristem developments are known to include two stages: initial formation and subsequent growth. Thus, in our work, serial microtome sections of the zone adjacent to the lateral buds of strawberry were compared in the dormant, non-dormant and induced (treated with GA₃) states (Figure 2). Examination of the stained sections indicated no differences between the dormant and non-dormant buds, when the latter were not induced to grow. Consequently, it was assumed that the initial formation of the axillary buds is comparable in both cases. Serial sections showed that the central stele is protruding and ready to supply axillary dormant buds as well as non-dormant buds at early stages of bud burst. At different levels of the runner, the first signs of differences appeared as a divergence of the central stele to form the bud vascular trace in both the non-dormant buds or those induced to develop by GA₃. Thus, this hormone might stimulate growth and induce the development of vascular traces responsible for supplying the active bud. However, the evoked vasculature in response to GA₃ might be a consequence of a hormonal effect on activation of cell division in the meristematic tissue, and on orientation of the cell microtubules in the direction of growth (Cleland 1999).

[A]
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Protein Banding Patterns: Table 2 and Figure 3 show that the number of protein bands is slightly different and the protein type is obviously variable in dormant and non-dormant buds. The plant extracts show the occurrence of four protein bands specific for dormant buds (M.Wt: 42, 35, 10, and 9 KDa). On the other hand, five different protein bands are characteristic of the non-dormant buds (M.Wt: 49.7, 46, 31, 20, and 18 KDa), this means that different patterns of gene expression exist in the dormant and non-dormant states. It seems likely that high levels of ABA in the dormant buds and gibberellins in the non-dormant buds dominate cell functions. Thus, ABA may control the maintenance of cells in a dormant state, as it does in apical buds (Cleland 1999) and seeds (Crozier et al. 2000). In this respect, ABA-responsive elements (ABREs) of the DNA, including a G-box (ACGT) and an additional promoter sequence called the coupling element (CE), have been proved to bind directly transcription factors in order to activate the transcription of each ABA-responsive gene independently (Shen & Ho 1999; Bray et al. 2000). According to Trewavas (2000), gibberellin signal-response pathways also indicate the involvement of transcription factors responsible for inducing the formation of GA receptors and/or biosynthetic enzymes. Therefore, the switch to the non-dormant state by GA$_3$ treatment, suggests that the GA-dependent transduction pathways are constitutively activated in dormant strawberry buds.

Table 2: Protein band concentration (% protein) of dormant and non-dormant buds of strawberry runners. Molecular weights are determined on the bases of comparison with those of the listed molecular markers.

<table>
<thead>
<tr>
<th>Mol. Wt.</th>
<th>Dormant</th>
<th>Non-Dormant</th>
</tr>
</thead>
<tbody>
<tr>
<td>49.7</td>
<td>6.94</td>
<td></td>
</tr>
<tr>
<td>46</td>
<td></td>
<td>14.8</td>
</tr>
<tr>
<td>42</td>
<td>12.81</td>
<td></td>
</tr>
<tr>
<td>35</td>
<td>12.34</td>
<td></td>
</tr>
<tr>
<td>31</td>
<td></td>
<td>17.04</td>
</tr>
<tr>
<td>25</td>
<td>17.41</td>
<td>16.55</td>
</tr>
<tr>
<td>20</td>
<td></td>
<td>16.34</td>
</tr>
<tr>
<td>18</td>
<td></td>
<td>12.87</td>
</tr>
<tr>
<td>17</td>
<td>24.81</td>
<td>15.46</td>
</tr>
<tr>
<td>10</td>
<td>20.34</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>12.3</td>
<td></td>
</tr>
<tr>
<td>Sum</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>
Figure 3: [A] Protein-banding patterns of non-dormant (ND) and dormant (D) buds of strawberry runners. Molecular markers (M) are shown on the left, and the molecular weights from top to bottom are: 209, 112, 83.1, 49.7, 31.7, 25.3 & 17.5 KDa. [B] Same pattern using Computer scan

The exogenously applied GA$_3$ might also have a direct effect on DNA-responsive elements, inducing the expression of non-dormant protein patterns (Table 2). In this connection, Rogers & Rogers (1992) and Rogers et al. (1994) demonstrated the occurrence of consensus GA-responsive elements (GAREs) in the promoters of barley GA-responsive $\alpha$-amylase genes, which required a nearby coupling element to function. Guilfoyle & Hagen (1999) also referred to the occurrence of GAREs in other plant tissues. Exogenous application of GA$_3$ might also be predicted to affect some enzymes within the GAs pool. The most intriguing recent insight is the feedback regulation by GAs of some of the enzymes included within their biosynthesis (Crozier et al. 2000). Phillips et al. (1995) and Silverstone et al. (1997), for example showed that transcription expression of each of the three Arabidopsis GA-20-oxidase genes (At 2301, At 2353, and YAP169) is much higher in GA-deficient ga-1-2 than in wild-type plants. They showed that GA$_3$ treatment of the mutants resulted in a substantial decrease in GA-20-oxidase mRNA within one to three hours of GA$_3$ application, long before a growth response was discernible. A similar feedback regulation by GA$_3$ application is also suggested for 3-\(\beta\) hydroxylases, which are responsible for creating active forms of GAs (Crozier et al. 2000).

Thus, we conclude that triggering the activity of dormant lateral buds in strawberry by GA$_3$ may result mainly from crosstalk between GA and ABA. This conclusion may be reinforced by recent approaches showing that many inducible plant promoters contain composite response elements specific for more than one hormone (Guilfoyle 1997; Guilfoyle & Hagen 1999).

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