Apical abortion in calabrese is induced by periods of low temperature and results in premature differentiation of apical meristem cells

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Abstract

Apical abortion in calabrese (Brassica oleracea var. italica), a highly destructive disorder which occurs in overwintered transplants, has been investigated using a model system in which blindness (abortion of the apical meristem) can be reproducibly and predictably induced. An initial experiment examined the susceptibility of 12 cultivars to apical abortion when grown throughout a winter period under commercial conditions. Three of those varieties showed very high levels of blindness (100%). Subsequently, plants of the susceptible cultivar PETO 7204 were subjected to an inductive period of low light intensity (30 μmol m⁻² s⁻¹) and low temperature (4 °C). Apical meristematic cells of all plants ceased mitotic activity within 3 d of being transferred to a regime comprising higher light intensity (100 μmol m⁻² s⁻¹) and temperature (15 °C). Using this system the structures of normal apices were compared with those which became blind. Blindness was characterized by a cessation of leaf primordium production by the vegetative apex, the last formed primordium growing on in some cases to form a mature normal leaf, or in others, a deformed structure known as a whip-tail. The inactive apical bud became embedded in the tissues of this last-formed structure. The cells of the inactivated apical bud remained alive, but lost their meristematic capability, becoming enlarged, highly vacuolated parenchyma cells with amyloplasts.

Key words: Apical abortion, apical meristem, blindness, calabrese.

Introduction

Demand from retailers for fresh produce early in the year has led to the practice among growers of Brassica oleracea groups such as calabrese, kohlrabi, brussels sprouts, and cauliflower, of raising autumn-sown seedlings under glass for transplanting outside in late winter. In certain years considerable financial loss has been suffered as a result of a failure of the apical meristem; a condition referred to as ‘blindness’ (Wurr et al., 1994). This is thought to be associated with either apical abortion involving cessation of leaf production by the vegetative apical meristem, or with its failure to change to a reproductive apex.

Previous investigations of this phenomenon (Mounsey-Wood, 1957) suggested that short periods of frost led to blindness in cauliflower transplants, while Nieuwhof (1969) concluded that periods of low temperature (above freezing) caused this phenomenon. In a recent investigation of blindness in calabrese, Wurr et al. (1996) examined the effects of sowing date, freezing, light level, and temperature and concluded that the amount of blindness (between 0 and 80% of plants) was affected by sowing date.

A major problem facing workers investigating the development of blindness in these plants has always been the uncertainty of inducing this syndrome. This is because, hitherto, the factors responsible for the induction of blindness were unknown, and the condition could not be induced reliably and predictably. One aim of the work described in this paper was therefore to develop a model system comprising a susceptible cultivar and a set of inductive conditions, which could be used for systematic studies of the phenomenon.

The term ‘apical abortion’ is often applied to the condition of blindness and has connotations of death.

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Wurr et al. (1996) in their introduction suggested that blindness was caused by death of the apical bud, although their results indicated that this was not necessarily the case. The exact state of the bud in blind plants has never been examined microscopically and it is self-evident that without this information the nature and causes of blindness cannot be fully understood. Consequently, a second aim of this work was to carry out a structural examination of the process of normal apical development in calabrese, and to compare this with the process occurring in apices known to be in the process of going blind.

**Materials and methods**

**Identification of susceptible varieties**

To investigate varietal differences in susceptibility to blindness an investigation was conducted between November 1995 and February 1996 using 12 cultivars grown under commercial conditions. Seeds of twelve varieties (Table 1) were sown on 1 November 1995 in each of four standard 308 (Hassey) seed trays containing peat-based compost (Levington F1). A commercial propagator near Boston, Lincolnshire, UK, raised the plants in an unheated Venlo type glasshouse. The mean hourly temperatures were recorded with a data logger (Skye Instruments; 400 Series) using screened thermistors. They averaged 8.9°C in November, 8.8 at or below 4°C; 4.2°C in December; 6°C in January, and 5°C in February. Mean daily photon flux incident at plant height was 5.3 mol m⁻² d⁻¹ in November, 2.5 mol m⁻² d⁻¹ in December, 3.0 mol m⁻² d⁻¹ in January, and 7.5 mol m⁻² d⁻¹ in February. The seed trays were placed on inverted pots (9 cm) above the soil surface to prevent rooting and the plants were watered fortnightly until 25 December 1995, after which they were fed fortnightly with a liquid fertilizer (Vitax 103, Vitax Ltd, Skelmersdale, UK, at 65 ppm N, 0 ppm, 215 ppm K). The plants were sprayed with Fubol 58 (12 December 1995; mancozeb + metalaxy, 48:10% w/w), Rovral (4 January 1996; iprodione) and a Bravo/?Filex mix (6 March 1996; chlorothalonil + propamocarb hydrochloride). On the 7 March 1996, 20–30 plants from each cultivar were sampled randomly (from 500 plants per cultivar), dissected and the apices fixed for SEM, TEM and light microscopy as described below.

**Table 1.** The seed varieties, their sources and results from the dissections of the commercially grown plants (total leaf numbers are true leaves plus primordia); samples are from 20–30 replicate plants; standard errors shown where they were greater than zero

<table>
<thead>
<tr>
<th>Seed cultivar</th>
<th>Source</th>
<th>Total leaf number</th>
<th>Percentage of blind plants</th>
</tr>
</thead>
<tbody>
<tr>
<td>S 210</td>
<td>Sakata</td>
<td>9.1 ± 1.1</td>
<td>3</td>
</tr>
<tr>
<td>TEX 322</td>
<td>Takia</td>
<td>8.4 ± 0.86</td>
<td>10</td>
</tr>
<tr>
<td>TEX 307</td>
<td>Takia</td>
<td>9.2 ± 0.63</td>
<td>0</td>
</tr>
<tr>
<td>PETO 286</td>
<td>Royal Sluis</td>
<td>8.2 ± 1.2</td>
<td>0</td>
</tr>
<tr>
<td>PETO 7204</td>
<td>Royal Sluis</td>
<td>4.0</td>
<td>100</td>
</tr>
<tr>
<td>Rainbow</td>
<td>Royal Sluis</td>
<td>8.6 ± 0.42</td>
<td>3</td>
</tr>
<tr>
<td>SG4459</td>
<td>Sluis and Groot</td>
<td>9.8 ± 0.33</td>
<td>0</td>
</tr>
<tr>
<td>90-839</td>
<td>Yates Seed</td>
<td>4.6</td>
<td>100</td>
</tr>
<tr>
<td>SC15</td>
<td>Sakata</td>
<td>8.1 ± 0.44</td>
<td>4</td>
</tr>
<tr>
<td>BEJO 1626</td>
<td>Elsoms</td>
<td>7.1 ± 0.75</td>
<td>76</td>
</tr>
<tr>
<td>TEX 320</td>
<td>Royal Sluis</td>
<td>8.2 ± 0.91</td>
<td>0</td>
</tr>
<tr>
<td>Fiesta</td>
<td>Takia</td>
<td>7.0</td>
<td>100</td>
</tr>
</tbody>
</table>

**Effects of temperature on blindness**

From the results of the above experiment, four cultivars were selected for study in a second experiment. Peto 7204 and Bejo 1626 were chosen because they both exhibited high levels of apical abortion (100% and 76%, respectively) and SG 4459 and Peto 286 because neither exhibited apical abortion when grown under commercial conditions. Seeds of each cultivar were sown in standard 308 seed trays containing peat-based compost (Levington F1) and germinated in a glasshouse at 15°C. At the cotyledon stage, one tray of each cultivar was placed in one of three controlled environment rooms set at 4°C, 8°C, or 12°C. The light level in each of the rooms was set at photon flux density of 30 μmol m⁻² s⁻¹ (cool white fluorescent tubes, 10 h day), approximately equivalent to the light transmitted through a greenhouse on an overcast winter’s day. The plants were watered as required. After 12 weeks of induction, 20 plants of each cultivar from each temperature were sampled randomly at weekly intervals and this was repeated during each of a further 7 weeks. The sampled plants were transplanted into 9 cm pots (containing Levington F1 and Vermiculite at a 70:30 ratio by volume) and placed in a growth room at 15°C, photon flux density 100 μmol m⁻² s⁻¹ (cool white fluorescent tubes). Following transplanting, the plants were monitored continuously for 9 weeks after the last sampling and incidence of blindness was recorded following apical dissections. One hundred further plants remained in each of the three temperature compartments for 25 weeks as controls. In each experiment, a minimum of 300 plants was observed for each treatment.

**Changes in mitotic divisions and cell structure during blindness**

Following analysis of the results, Peto 7204 was chosen to study changes in cellular structure and cell division during blindness, but under a narrower range of conditions. The cultivar Marathon was also subjected to these conditions in order to assess the induction of apical abortion in this commercially important cultivar. The system used involved subjecting the plants to a 16 week inductive period (post cotyledon expansion) at either 4°C or 8°C at 30 μmol m⁻² s⁻¹ followed by transfer to 15°C and 100 μmol m⁻² s⁻¹. Samples (five plants) were taken daily from each cultivar and each inductive regime, 7 d prior to transfer to 20 d after transfer. The samples were prepared for TEM and light microscopy as described below.

**Microscopy**

Apices were dissected under a dissecting microscope and processed in one or more of the following ways as required.

**Scanning electron microscopy (SEM):** The dissected apices were fixed in glutaraldehyde 4% (v/v) in 0.1 M phosphate buffer (pH 7) for 2 h in sealed bottles at room temperature. The fixative was decanted off and the material washed with three changes of glass distilled water. The material was then fixed in 1% (w/v) aqueous osmium tetroxide (OsO₄) for 2 h and washed with three changes of glass-distilled water before being dehydrated through a graded acetone series (30%, 50%, 70%, 90%, 95%, 100%, (×2) followed by dry 100%, 30 min per change. The material for SEM was dried by critical point drying, mounted on stubs using silver paint and coated with gold using a sputter coater.

**Transmission electron microscopy (TEM) and light microscopy:** Material for TEM and light microscopy was fixed with glutaraldehyde and osmium tetroxide as described above and embedded in Epon 812 following the use of propylene oxide as an intermediate between the acetone and the resin. Sections
Mitotic activity: Peto 7204 plants became visibly blind less than 10 d after transfer from inductive conditions. Therefore daily samples of each cultivar (Peto 7204 and Marathon) from each temperature regime were taken from 7 d before and up to 20 d after transfer to the higher light and temperature level. The sampled apices were fixed overnight in ethanol:glacial acetic acid (3:1, v/v) at 4 °C. The samples were washed with three changes of glass distilled water and stained with Schiff’s reagent for 2–3 h at room temperature and subsequently overnight at 4 °C. The material was washed with three changes of chilled glass distilled water and dehydrated with ethanol. The apices were dissected further using a high-power dissecting microscope to ensure that only apical tissues were present at the final stage.

The apex was placed on a clean microscope slide, a drop of propionic orcein applied to cover the sample and a squash prepared. Cells undergoing mitosis were counted and the number averaged for three apices per cultivar per inductive regime. This was repeated three times to give an overall average score.

Results

Susceptible varieties

The results of subjecting the twelve different calabrese varieties to commercial conditions are summarized in Table 1. The varieties PETO 7204, 90-839 and Fiesta exhibited 100% apical abortion at the 4, 4 and 7 leaf stages, respectively. In four varieties, no apical abortion was recorded and there was a range of blindness in the remaining varieties (3–76%), although the majority of these varieties only had low levels of blindness (less than 5%).

The varieties PETO 7204, BEJO 1626, SG4459, and PETO 286 were selected for more detailed study, with plants grown at 4, 8 and 12 °C prior to transfer to higher temperatures and higher irradiances. In this experiment, SG4459 and PETO 286 did not exhibit apical abortion in any of the three temperature treatments and transfer dates. Also there was no blindness for either PETO 7204 or BEJO 1626 when grown at either 8 °C or 12 °C. In the 4 °C treatment, the cultivar PETO 7204 exhibited 100% blindness from each sampling (12–18 weeks of cold), following transfer to higher temperatures. However, the control plants, which remained at 4 °C for 25 weeks continued to grow normally. This indicates that blindness is an induced phenomenon. The length of the inductive period at 4 °C affected the period at 15 °C for the plants to become visibly blind (i.e. without employing dissection). For PETO 7204, short inductive periods (at 4 °C) required longer duration for plants to become visibly blind following transfer to 15 °C (28 d for visible blindness to occur if the plants were exposed to 4 °C for 12 weeks compared to 7 d after 18 weeks; Fig. 1). Table 2 shows the leaf numbers of the plants following potting up and transfer. Previous observations of seed showed that calabrese had two leaves in the embryo prior to germination (data not shown), thus all plants in the chilling treatment increased leaf number during the experiment (relative to the number at sowing). By sample 4 (15 weeks, and for subsequent samples) leaf initiation had ceased and the last leaf primordium formed prior to transfer grew to enclose the apex. However, this also indicated that the phenomenon is inducible, since final leaf number increased (by between 1 to 2) following transfer to the warmer environment if the plants had only received 12, 13 or 14 weeks of cold. No new leaves developed when the duration of the cold exposure increased from 12 to 18 weeks; on transfer to warmer conditions all treatments had produced five leaves (including primordia). The cultivar BEJO 1626 exhibited a lower rate of apical abortion (30–40%) at each sampling following the 4 °C induction period.

In the experiment involving cvs PETO 7204 and Marathon, in which the plants were exposed to a 16 week induction period at 4 °C or 8 °C (control) under the lighting conditions described above, followed by exposure...
to 15°C and 100 μmol m⁻² s⁻¹, PETO 7204 exhibited 100% apical abortion with the induction temperature set at 4°C. All plants in the tray were visibly blind 10 d after transfer and no new leaves were produced. The induction of apical abortion in PETO 7204 using this system was successfully repeated in six separate experiments in which a minimum of 300 plants per treatment was observed. No apical abortion was recorded in trays of 300 plants subjected to the 8°C induction regime. This system did not induce significant levels of blindness in Marathon where similar numbers of plants were examined, when 8°C was used as the induction temperature, although at 4°C, 12% apical abortion was observed after 5 weeks at 15°C with the addition of six new leaves.

It is worth noting that prolonged periods of lower temperatures do occur during commercial cultivation, as shown by the temperature data recorded during the experiment, where for 3 months temperature averaged 4.2, 6 and 5°C.

The development and structure of flowering and blind apices

Blind calabrese plants were readily identifiable after dissection by their lack of an apical shoot. In some cases the apical bud became overgrown by the development of the petiole of the last formed leaf (Fig. 2A) with the position of the bud represented by a navel-like indentation on the adaxial surface of the petiole base. Plants which had been blind for some time show activation of axillary buds as a result of loss of apical dominance. The last-formed leaf was often normal in appearance, but in other cases, the leaf primordium did not develop properly, producing effectively a petiole without a leaf (Fig. 2B). This could be confused with the so-called whip-tail condition which is brought about by molybdenum deficiency (Nieuwhof, 1969). Splitting the petiole in the region of the navel-like structure revealed that it represented the outer end of a tube which extends through the petiole to the presumed site of the apical meristem which produced it (Fig. 2C).

There was no evidence of any reproductive structures in any of the 100 blind plants sampled. Instead, the pointed dome of the apical meristem continued to elongate without producing any more leaf primordia, whilst at the same time it became enveloped by the developing petiole of the last formed leaf (Fig. 2D). Small structures were sometimes seen which probably represented leaf bracts and primordia whose development was checked when blindness was induced. Eventually the primordium became completely buried by the petiole, giving rise to the navel-like structure (Fig. 2E).

Light microscopy of an apex in a recently blind plant showed the tunica-corpus structure normally found in an apical meristem had been largely lost, although a layer of smaller, vacuolated cells marked the site of the original tunica (Fig. 3A). Examination of sections through normally developing apices of PETO 7204 and Marathon showed that the cellular ultra-structure in non-blind plants was similar between the two varieties. PETO 7204 has been used to provide the illustrations here since blindness could be reliably induced in individual plants of this cultivar. Figure 3A shows a section through a normal apex (control, i.e. grown at 8°C) 7 d before transplanting. The cells were typical of those of the apical meristematic region of an angiosperm shoot, being thin-walled, with a prominent nucleous occupying much of the cell volume. Vacuolation comprised a few small- to medium-sized vacuoles, while the cytoplasm contained the usual range of organelles, including proplastids, which in cells slightly removed from the active meristem, were beginning to accumulate small amounts of starch. Cells of plants subjected to the inductive regime (4°C) also showed a similar structure at this time (Fig. 3C). In the case of normal plants (control) the appearance of cells in the region of the apical meristem did not change throughout the course of the experiment. However, cells at the apex of plants subjected to the inductive regime showed a significant change in their appearance within 2 d of transfer to higher temperatures. There was a marked increase in cell size, in the amount of starch present in the plastids, and in the proportion of the cell volume occupied by the vacuole (Fig. 3D). The cells had lost the appearance of being meristematic and now looked like normal parenchyma cells. By 16 d after transplanting this change was more or less complete (Fig. 3E). An additional feature observed at this time was the differentiation of primary xylem elements within the region which had formerly been meristematic (Fig. 3F), indicating that differentiation of cells was continuing although the meristem was no longer producing new cells and maintaining its distance from developing vascular cells.

Mitotic divisions during the onset of blindness

Figure 4 shows the average number of mitotic divisions recorded in plants subjected to the inductive regime at 4°C and 8°C for 16 weeks. This shows that for PETO 7204 after the 16 week (4°C) induction period mitotic division slowed 1 d after transfer to 15°C, and ceased totally after 3 d. PETO 7204 after the 8°C induction period and Marathon (data not shown) after 4°C and 8°C induction periods all continued to produce leaves, mitotic divisions and remained vegetative throughout the experiment.

Discussion

This investigation has led to a reproducible and reliable system for producing blind plants. This is the first report...
Fig. 2. (A) A blind calabrese plant. The navel-like aperture marking the location of the original apical meristem is visible near the base of the petiole of the last-formed leaf (arrow). Note the activation of axillary buds of the basal leaves (× 0.5). (B) Part of a blind calabrese plant showing a whiptail deformity (arrow) produced by abnormal development of the last-formed leaf primordium (× 0.75). (C) Photomacrograph of a split surface of the last-formed petiole in a blind plant. The tube-like structure formed when the growth of the petiole enveloped the quiescent apical meristem is clearly visible (arrows) (× 6.5). (D) Scanning electron micrograph showing the petiole of the last-formed leaf primordium beginning to envelop the apical meristem (× 70). (E) A petiole almost entirely enclosing the apical meristem in a blind plant. The leaf bracts are clearly visible (arrow) (× 130).
of a reliable strategy to induce blindness in calabrese under controlled conditions, despite many previous attempts (Wurr et al., 1996; Forsyth et al., unpublished results). The observations described above provide important new basic information regarding the phenomenon of blindness in calabrese. Firstly, they show that blindness is not a result of the death of the cells of the apical meristem. Instead, the state of the meristem in blind plants appears to be one in which previously meristematic cells have lost their meristematic character and instead entered on a course of differentiation to parenchyma cells. This conclusion is based on the structural observations presented here, in which the cells show no features normally associated with death and senescence, but instead have features typical of ground parenchyma. The cells were clearly still alive when they were sampled for microscopic examination. A better way to describe the state of the meristem would therefore be to say it had terminally differentiated, although it is impossible to say at this time whether the change could be reversed, and meristematic activity resumed. Clearly, the fact that blindness is associated with plants sown at a particular time of the year (Wurr et al., 1996) indicates that the phenomenon is under the control of environmental or edaphic factors.

Secondly, it appears that blindness is a condition which sets in while the apex is still in a vegetative condition. Microscopy of apices at early stages of blindness revealed that the apex had the pointed dome structure characteristic of the vegetative state with leaf bracts occasionally being the last produced structures. It also suggests that once the development of new leaf primordia had ceased, extension growth of the apex continued before meristematic activity was lost. Similarly, the growth of the last-formed leaf primordium continued, showing that the meristematic cells of the developing leaf were not affected in the same way by the factors which had stopped the primordium-forming activity of the apical meristem. In some cases, however, normal development was affected, giving rise to the whip-tail deformity. It may be that the process of leaf development from the last-formed primordium was influenced by the stage of development reached by the primordium at the moment of cessation of meristematic activity at the apex.

The factors responsible for the cessation of meristematic activity in the apex do not affect quiescent axillary buds, which develop into lateral shoots in a way which indicates that apical dominance has been lost. Thus, loss of meristematic activity seems to be restricted to the cells of the apical bud.

These facts taken together imply that blindness involves a turning off of the genes which maintain the meristematic activity in the vegetative apex. Perhaps this is triggered when the apex, under the stimulus of environmental factors, progresses towards a floral state at a time when the plant is not in a suitable physiological condition to sustain a floral apex. Thus, blindness may represent a state into which the meristem has been forced by loss of vegetative meristematic activity coupled with a failure to meet the requirements for successful transition to the floral state.

This study has also identified a method using sensitive cultivars to produce blind and normal plants reliably. The use of such a system has made it possible to calculate, through mitotic index studies, the precise time at which meristem activity stops. This will facilitate examination of gene expression within the apex, leading to a better understanding of the way environmental factors influence meristematic activity per se and perhaps the developmental processes leading to flowering.
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References


