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The progression of primary bud necrosis in the grapevine cv. Shiraz (*Vitis vinifera* L.): A histological analysis

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Summary

Primary Bud Necrosis (PBN) is a physiological disorder occurring in the compound axillary buds of grapevines. PBN causes the axillary bud to senesce and in some cases secondary buds can also abort. Since PBN is common in the grapevine variety Shiraz the aim of this study was to characterise anatomical changes at different stages of PBN development in this cultivar. Grapevine buds were collected from a vineyard located at Charleston, South Australia, Australia. Buds were dissected, assessed for the presence of PBN and rated on severity of the disorder. Buds at various stages of PBN were fixed for light microscopy. Cell breakdown was observed in all buds where PBN was visible. Collapse and thickening of cell walls was observed in a region of necrotic tissue and severity of PBN appeared to increase over time. The location of cell breakdown due to PBN appeared to be random and was not isolated to one region within the primary bud. PBN appeared to stop primordial growth, with cells differentiating further and maturing more rapidly without forming whole leaves. This cell region then breaks down and the necrosis can extend into the secondary buds.

K e y w o r d s : Primary bud necrosis, buds, light microscopy, plant anatomy, physiological disorder, bud development, *Vitis vinifera* L., grapevine, Shiraz, Vitaceae.

Introduction

Grapevines have compound buds consisting of a primary bud and two or more secondary buds (Pratt 1974). If the primary bud does not survive or is unable to develop, the secondary buds enlarge, and produce shoots to compensate for the loss of the primary bud (Lavee *et al*. 1981; Naito *et al*. 1986). Although little information is available on the fruitfulness of secondary buds, it is widely observed that secondary shoots produce less fruit than primary shoots. On occasion secondary buds have also been affected by bud necrosis (Naito *et al*. 1986; Morrison and Iodi 1990; Wolf and Warren 1995).

Primary bud necrosis (PBN) is a physiological disorder of grapevines whereby the primary bud dies. PBN is characterised by an abortion and subsequent drying of the primary bud within a developing compound bud (Lavee *et al.* 1981; Morrison and Iodi 1990; Dry and Coombe 1994; Wolf and Warren 1995). The extent and location of necrosis in the primary bud is dependent on stage of bud development. Sections taken of Riesling buds under a light microscope (Vasudevan *et al*. 1998 a) revealed zones of distorted misshapen cells immediately beneath the primary bud axis within 60 d after budbreak. Ninety d after budbreak Vasudevan *et al*. (1998 a) observed non-uniform cell compression and cell lysis. Necrosis in some buds occurred at the base of the primary axis (Morrison and Iodi 1990) and in young undifferentiated buds, necrosis developed below the apex causing death of the primary bud (Ziv *et al*. 1981). Scanning electron microscopy revealed a similar pattern of tissue destruction and indicated that cell destruction was not a result of tissue preparation or microtomy (Vasudevan *et al*. 1998 a, b).

The incidence of PBN was found to depend on cultivar and viticultural practices. Susceptible cultivars include Queen of the Vineyard (Ziv *et al*. 1981), Flame Seedless (Morrison and Iodi 1990), Riesling (Wolf and Cook 1992), Viognier (Wolf and Cook 2000), and in Australia the most susceptible variety is Shiraz (Dry and Coombe 1994, Collins and Rawnsley 2004). A number of different stresses were assumed to be responsible for the occurrence of PBN, e.g. excessive shoot vigour (Lavee *et al*. 1981, Dry and Coombe 1994), canopy shading (May 1961, Perez and Kliewer 1990, Wolf and Cook 1992, Wolf and Warren 1995), high levels of gibberellins (Ziv *et al*. 1981), and low carbohydrate levels associated with shading (Vasudevan *et al*. 1998 a, b). This study was performed to investigate severity and the development of PBN in cv. Shiraz.

Material and Methods

Dormant grapevine buds from 8-year-old Shiraz vines in a vineyard located at Charleston, South Australia, were collected during March 2004. Healthy and necrotic primary buds were removed from shoots in the laboratory using

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a Binocular microscope at 10-40x magnification. Thirty buds were examined prior to embedding and scored using a severity rating of PBN (Table).

T a b l e

Severity ratings for incidence of primary bud necrosis (PBN) expressed as the percentage of necrosis within the primary bud

Severity rating	Primary bud necrosis (%)
0	0 (healthy primary bud)
1	1-25
2	25-49
3	50-75
4	76-99
5	100 (completely necrotic)

L i g h t m i c r o s c o p y : Bud samples were fixed overnight in 3 % glutaraldehyde buffered with 0.025M phosphate buffer to pH 7.2, for a minimum of 48 h at 0-4 ºC. After fixation, samples were put through an alcohol dehydration series: methoxy-ethanol, ethanol, propanol and butanol. Samples were left for a minimum of 2 h in each alcohol, then infiltrated overnight in a 1:1 mixture of butanol: glycol methacrylate (GMA). Samples were then infiltrated over 4 d with two changes of 100 % GMA and embedded in GMA in gelatine capsules and polymerised at 60 ºC.

Embedded buds were trimmed and filed to expose the longitudinal sections (LS) for assessment of different PBN severity ratings. Sections 3-4 µm thick were made with an ultra-microtome (Reichert-Jung 2050 supercut) and stained with periodic acid-Schiff's reagent (PAS) and 0.5 % Toluidine blue O (TBO) in 50 mM sodium acetate, pH 4.5 (O'Brien and McCully 1981). Sections were mounted using microscope slide media (Surgipath, Sub-X mounting medium) and examined using an Olympus BH2 light microscope and micrographs taken using a Nikon TE300 inverted microscope at magnifications from 40x to 600x.

Results

Due to visual similarities, grapevine buds were assigned to one of 4 developmental stages of PBN: healthy (severity rating 0), early (severity rating 1 and 2), medial (severity rating 3) and advanced (severity rating 4 and 5). Assessments of healthy buds and developmental levels of primary bud necrosis (PBN) are reported.

H e a l t h y p r i m a r y b u d : Healthy primary and secondary buds displayed little cell breakdown (Fig. 1). Microscopic examination of healthy primary buds revealed dividing rectangular parenchyma cells forming the shoot apex (Fig. 2 A). This area of the bud is known as the apical meristem, indicating the region of undifferentiated cells that divide in an organised manner. The apical meristem develops into three types of primordia, namely leaf, tendril and inflorescence primordia, which are protected by overlapping scales and hairs (Fig. 2 A). The prophylls contain storage cells with starch granules. During leaf development these starch granules are replaced with chloroplasts when photosynthesis commences (Fig. 2 B and C). Individual cells within the leaf meristem produce raphides that are long, slender, needle-like crystals of calcium oxalate (Fig. 2 B).

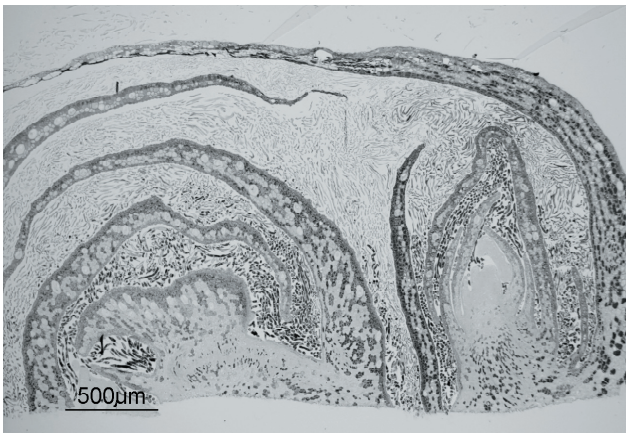

Fig. 1: Longitudinal section of a healthy primary (pb) and secondary grapevine buds (sb), cv. Shiraz.

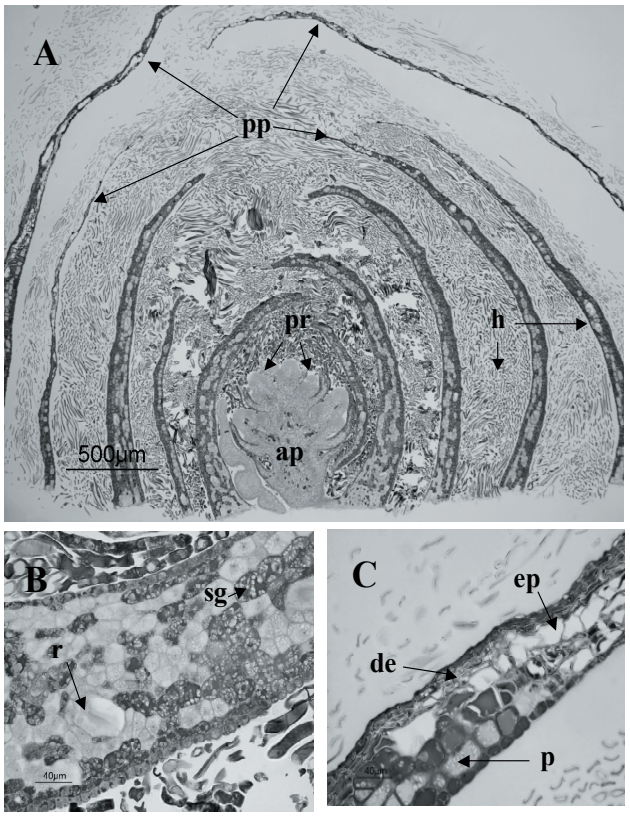

Fig. 2: Longitudinal section of a healthy primary grapevine bud, cv. Shiraz. (**A**): Primary bud showing the apical meristem (ap), leaf primordia, inflorescence primordia and tendril primordia (pr), prophylls with some cell degeneration (pp), bud hairs (h). (**B**): Leaf primordia with storage cells containing starch granules (sg), single cell containing a raphide (r). (**C**): Prophylls near apex with cellular damage caused by dehydration, distorted epithelial cells (de), empty parenchyma cells with thickened walls (ep), parenchyma cell with starch granules (p).

Healthy primary buds displayed slight cell degeneration on the prophylls. Degeneration was observed at the second and third leaf layer tips. This damage was confined to the outer epidermal cell layer and was observed by cell walls buckling and folding leading to distortion of epithelial cells (Fig. 2 C). Parenchyma cells with thickened cell walls showed breakdown of the cellular contents. Cellular damage was confined to the epidermal cell layers of prophylls and leaf primordia tips (Fig. 2 C) and was typical of a reaction caused by dehydration that was not related to PBN.

Early development of primary bud necrosis: Small differences in the number of necrotic cells were observed between healthy buds and those with PBN severity ratings of 1-2 (1-49 % PBN). As shown in healthy buds, cellular breakdown and loss of cell contents occurred towards the apex of the first, second and third leaves. Most cell damage occurred in the prophyll and leaf primordia cells while the apical meristem was still intact with little cellular damage (Fig. 3 A). Below the meristem, in an area where vascular tissue was developing, multiple layers of collapsed parenchymatous cells were also observed (Fig. 3 B). Associated with this area were several layers of cells with thickened walls. These collenchyma cells had non-staining walls and dense-staining cytoplasm. The nearby parenchymatous cells displayed cellular abnormalities with irregular and distorted cell walls many being smaller than the surrounding cells (Fig. 3 C) compared to healthy parenchyma cells (Fig. 3 B). The meristem cells that normally differentiate into the vascular system showed cellular compaction and less organization than those of healthy primary bud sections. Normally this cellular region would differentiate into healthy xylem and phloem parenchyma cells, but instead, large **lacunae** (empty spaces) cells formed in the regions where parenchyma cells would have occurred. Raphides were also observed.

Medial development of primary bud necrosis: Breakdown of the epidermis was also observed near the leaf apex in the second and third primordial cellular layers (Fig. 4 A and B). This resulted in the production of **lacunae** where cells collapsed and displayed further breakdown of the cellular contents (Fig. 4 A and B). Cell lysis occurred progressively towards the apex of the leaves producing lunular (crescent-shaped) areas between the cell layers. Epidermal cells showed signs of breakdown and raphides were formed between two or more cells instead of one cell (Fig. 4 C). In comparison to healthy buds, cell breakdown was obvious, especially in leaf cells.

Advanced development of primary bud necrosis: Extensive damage to leaf cells and other cellular disintegration was observed in buds scored for advanced development of PBN (Fig. 5 A). Total breakdown of the leaf structure occurred with degeneration in the mesophyll as well as in the primary axis and the apical meristem (Fig. 5 B and C). However, complete death of the primary bud was not observed in all cases, as some cell breakdown had not fully occurred. PBN cells in the primordia began to differentiate into xylem or phloem pa-

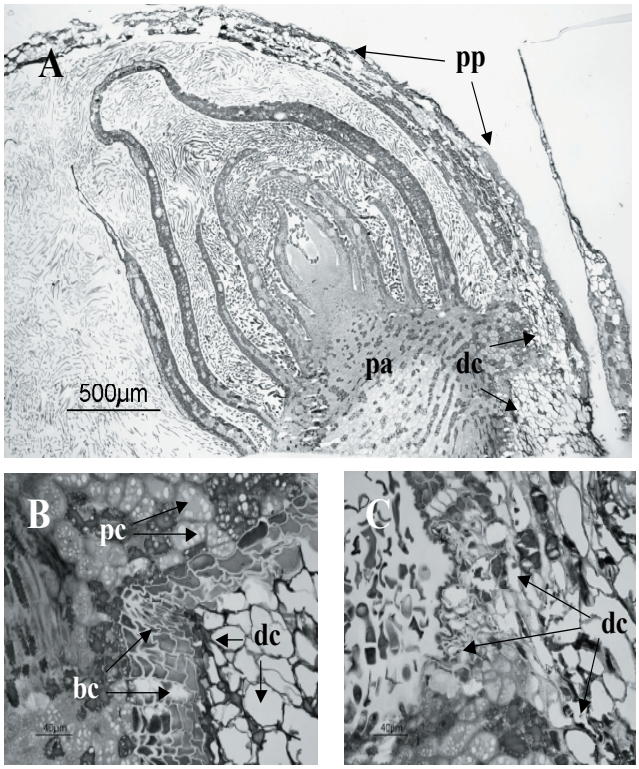

Fig. 3: Early development of primary bud necrosis in cv. Shiraz. (**A**): The prophyll cellular breakdown (pp), formation of a distorted cellular zone (dc) to the right of the primary axis (pa). (**B**): Healthy parenchyma cells with starch granules (pc), 3-5 buckled cell layer (bc) and deformed cells (dc). (**C**): Deformed cells (dc) to the right of the shoot apex.

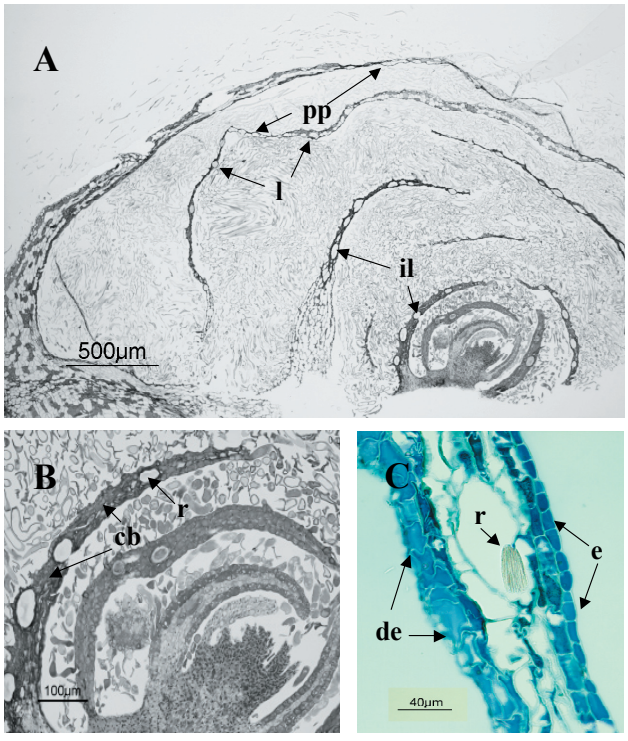

Fig. 4: Medial development of primary bud necrosis in cv. Shiraz. (**A**): Prophylls showing further cell damage (pp) and formation of *lacunae* (l), leaf primordia cell damage and formation of *lacunae* (il). (**B**): Medial stages of PBN showing raphide cells (r) and close up of leaf primordia cell breakdown (cb). (**C**): Leaf epidermal cells (e), damaged epidermal cells (de), raphide between two cells (r).

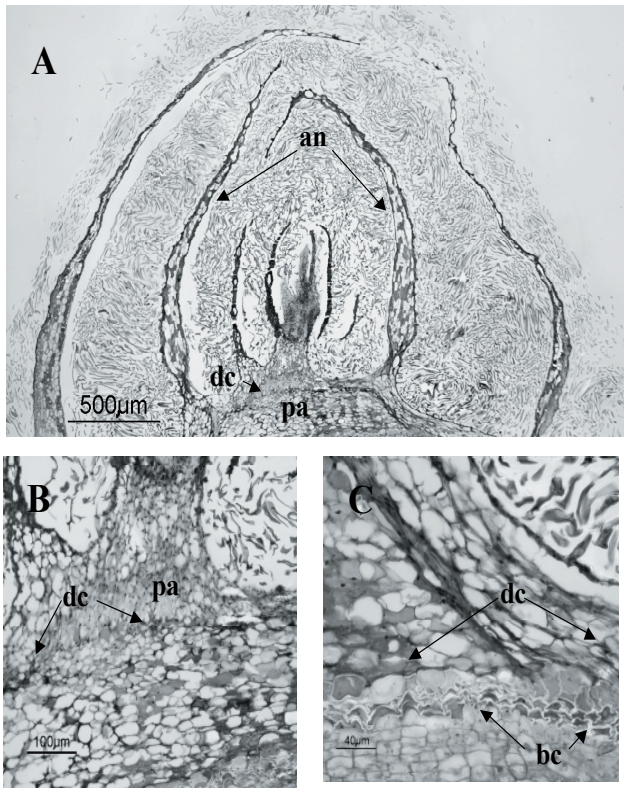

Fig. 5: Advanced development of primary bud necrosis. (**A**): Longitudinal section of primary bud, with advanced necrosis of leaf primordia (an), and a damaged cell zone (dc) near the primary axis (pa). (**B**): Damaged cell zone (dc) near the primary axis (pa). (**C**): Distorted cell layers (dc) and buckled cells below (bc).

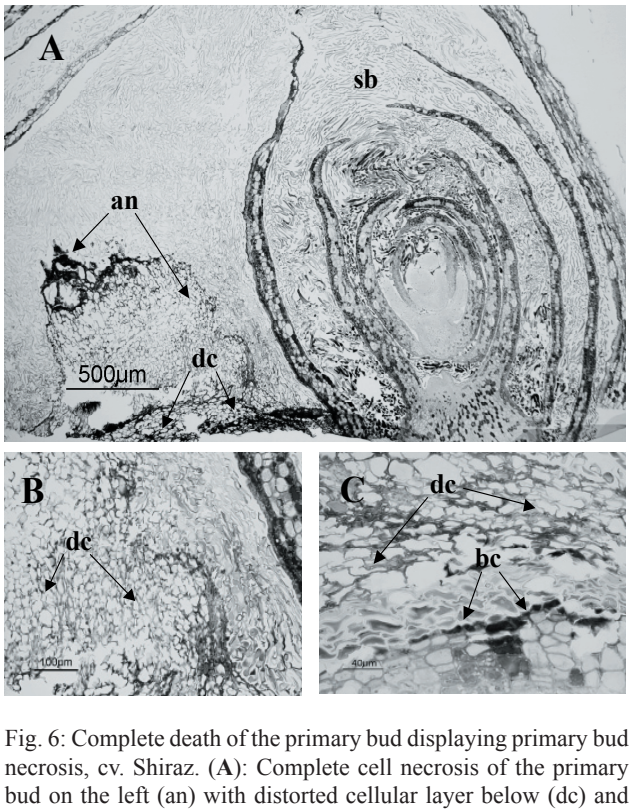

Fig. 6: Complete death of the primary bud displaying primary bud necrosis, cv. Shiraz. (**A**): Complete cell necrosis of the primary bud on the left (an) with distorted cellular layer below (dc) and secondary bud remaining intact on right (sb). (**B**) and (**C**): Breakdown of primary bud cells displaying little cellular contents (dc) and a buckled cell layer below (bc).

renchyma. PBN then appeared to stop primordial growth, so cells differentiated further and matured more rapidly without forming whole leaves. The cell region then broke down and necrosis, in some cases, progressed into the secondary buds.

Some dissected buds displayed complete breakdown of the primary bud (Fig. 6 A-C). When loss of the primary bud occurred, it was also not uncommon to observe secondary buds developing and secondary bud cells were healthy and intact (Fig. 6 A). Cells in the primary bud were distorted and buckled with numerous empty cells suggesting that complete breakdown of the cellular contents had occurred (Fig. 6 B and C).

Discussion

The first visible symptom of PBN was indicated by the presence of distorted and compressed cells with irregular cell walls. Cell compression was followed by cell breakage that was consistent with observations of Morrison and Iodi (1990) and Vasudevan *et al*. (1998 a). Compressed cells lacked structural integrity and were subject to external pressures, such as the expansion of adjacent cells.

Raphides were observed in cells in both 'healthy' buds and in buds displaying the early to medial development of PBN. Many higher plants form these acicular crystals of calcium oxalate in bundles of several hundred within the vacuoles of specialised cells (Webb 1999). Previous research has indicated that raphides act as a feeding deterrent against predators (Sakai *et al*. 1972, Perera *et al*. 1990, Ward *et al*. 1997, Bradbury and Nixon 1998). Arnott and Webb (2000) suggest that the synthesis of a strong and stable structure such as the acicular morphology of raphides is essential for this function. Other studies have shown that the crystallization of calcium oxalate occurs as a means for the plant to sequester oxalates, as well as removing surplus calcium (Frank 1972, Franceschi and Horner 1979, Borchert 1985, 1986, Franceschi 1989, Fink 1991). This may provide a mechanism for regulating calcium in the cytosol, preventing excess calcium that would be toxic to cells at high concentrations (Webb *et al*. 1995). The transport of calcium may vary depending on the source of calcium and the location of the cells containing crystals (Webb 1999). For example, calcium was transported from internal reorganization through the degradation of cell walls (Horner and Wagner 1980) and by the reabsorption of crystals elsewhere in the plant (Franceschi 1989). Another suggestion for the potential roles of controlled crystallization is that the crystals may provide structural reinforcement in protective tissue (Webb 1999). As raphides were not observed in advanced development of PBN, further investigation into the function of raphides in grapevines may help to indicate if the formation of these crystals is influenced by cell breakdown. In cv. Thompson Seedless, PBN was defined by the formation of a distinct necrotic zone most commonly located in the 4th leaf primordium (Perez and Kliewer 1990). In the present study, similar observations were made of necrotic cells in the primary bud causing a rupture or separation between the basal part of

the bud and the apex. This resulted in eventual death of the primary bud. Primordial growth was restricted in buds with PBN by cells maturing too rapidly, preventing formation of whole leaves. The entire primary bud displayed signs of cell breakdown and, if severe, degeneration extended into the secondary buds. Determination of severity of PBN with a dissection microscope was not as accurate as light microscopy. Buds initially categorised as severity rating 5 appeared completely dead under a dissecting microscope, however, upon closer examination, it was evident that cells in the lower primary shoot apex were intact. Nevertheless, it is likely that cell breakdown would have progressed leading to complete death of the primary bud, as over 75 % of bud tissue was affected.

Vasudevan *et al*. (1998 a) found that the zone of compressed cells began at the base of the primary bud and advanced to the leaf primordia; however our observations indicated that PBN could start in the prophylls and progress towards the apical meristem in some primary buds, not just at the base. Morrison and Iodi (1990) also observed the random distribution of PBN in the early stages of development.

The shoot apical meristem goes through "maximal" and "minimal" phases between the initiation of one leaf and the next. Axillary buds are under the control of the shoot apex, and their further development is usually suppressed by hormonal control (Lavee *et al*. 1981). If the shoot apex is damaged, a secondary bud may form due to the lack of hormonal suppression (Lavee *et al*. 1981).

The abscission or abortion of plant parts is a normal part of the growth and development of many species (Addicott and Lyon 1973), however, adverse conditions such as drought, flooding, frost, heat, mineral deficiencies and insect infestation can promote abortion (Wittwer 1954). Plants can respond to a variety of environmental changes, even to a small degree, and may react with the abscission of one or more organs (Addicott and Lyon 1973). The supply of products of photosynthesis, particularly carbohydrates, is central to the metabolism of cell walls - if ample carbohydrates are available, cell wall deposition will be more significant and the walls more difficult to hydrolyse. Lower carbohydrate levels can lead to weaker cell walls and abscission or abortion of different plants parts (Biggs and Leopold 1957). Carbohydrates tend to be transported to sinks like developing shoots, roots and bunches. This may weaken the less vigorous parts and encourage hydrolysis of cell walls that may lead to abortion or death of the primary bud. Further knowledge on the role of carbohydrate and hormone levels during this period of development would be valuable for a better understanding of the physiological processes leading to this disorder.

Acknowledgements

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