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Utilization of Amygdalin during Seedling Development of *Prunus serotina*[1]

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Cotyledons of mature black cherry (*Prunus serotina* Ehrh.) seeds contain the cyanogenic diglucoside (*R*)-amygdalin. The levels of amygdalin, its corresponding monoglucoside (*R*)-prunasin, and the enzymes that metabolize these cyanoglycosides were measured during the course of seedling development. During the first 3 weeks following imbibition, cotyledonary amygdalin levels declined by more than 80%, but free hydrogen cyanide was not released to the atmosphere. Concomitantly, prunasin, which was not present in mature, ungerminated seeds, accumulated in the seedling epicotyls, hypocotyls, and cotyledons to levels approaching 4 μmol per seedling. Whether this prunasin resulted from amygdalin hydrolysis remains unclear, however, because these organs also possess UDPG:mandelonitrile glucosyltransferase, which catalyzes de novo prunasin biosynthesis. The reduction in amygdalin levels was paralleled by declines in the levels of amygdalin hydrolase (AH), prunasin hydrolase (PH), mandelonitrile lyase (MDL), and β-cyanoalanine synthase. At all stages of seedling development, AH and PH were localized by immunocytochemistry within the vascular tissues. In contrast, MDL occurred mostly in the cotyledonary parenchyma cells but was also present in the vascular tissues. Soon after imbibition, AH, PH, and MDL were found within protein bodies but were later detected in vacuoles derived from these organelles.

Cyanoglycosides are β-glycosylated α-hydroxynitriles that occur in several thousand plant species and a few insect species (Nahrstedt, 1985). When tissues are disrupted, these glycosides are degraded to HCN, sugars, and carbonyl compounds by the action of endogenous enzymes. Because HCN is toxic to aerobic organisms, and because its large-scale production coincides with tissue damage, it is likely that these cyanogenic compounds act in concert with the enzymes that catabolize them to defend cyanophoric plants against herbivores and pathogens (Nahrstedt, 1985; Jones, 1988). In addition, there is increasing evidence that the cyanogenic compounds that accumulate in certain angiosperm seeds may be catabolized upon germination to provide nitrogen and carbonyl compounds for the developing seedling (Lieberei et al., 1985; Selmar et al., 1988, 1990).

Among the most highly cyanogenic plant tissues known are the seeds of rosaceous stone fruits (e.g. cherries, peaches, plums), which contain the diglucoside (*R*)-amygdalin and its catabolic enzymes AH, PH, and MDL. Their cyanogenicity has caused numerous cases of acute cyanide poisoning in humans and livestock (Poulton, 1983). In previous studies, our laboratory monitored the development of the potential for cyanogenesis of maturing black cherry (*Prunus serotina*) fruits (Swain et al., 1992a). Levels of amygdalin, its corresponding monoglucoside (*R*)-prunasin, and AH, PH, and MDL were determined from soon after flowering until maturity. At maturity, each seed contained approximately 3 μmol of amygdalin, which is located within the cotyledonary parenchyma cells (Poulton and Li, 1994). By contrast, AH and PH are restricted to the procambium (Swain et al., 1992b). Thus, premature, large-scale cyanogenesis is prevented in undamaged seeds by tissue level compartmentalization.

The present study was undertaken to learn the metabolic fate of amygdalin on seed germination and to evaluate whether this glycoside might serve as a nitrogen storage compound in rosaceous stone fruits. The levels of amygdalin and prunasin were measured in the epicotyl, hypocotyl, and cotyledonary tissues of *P. serotina* seedlings at several developmental stages. In addition, several potential routes for amygdalin and prunasin metabolism and HCN reassimilation, as shown in Figure 1, were evaluated by measuring the following enzyme activities: AH, PH, and MDL; amygdalin diglucosidase, a putative enzyme catalyzing catabolism of amygdalin to mandelonitrile and β-gentiobiose; β-gentiobiosidase; β-CAS, which detoxifies HCN to β-cyanoalanine, an Asn precursor; and GT-I, which catalyzes the final step in de novo prunasin biosynthesis. Furthermore, AH, PH, and MDL were immunolocalized at several stages of seedling development to determine the effect of development on the cellular and intracellular distributions of these enzymes and to assess any implications for the developmental fate of amygdalin.

MATERIALS AND METHODS

Chemicals and Antisera

All chemicals were obtained from Sigma with the following exceptions: UDP-D-[U-^{14}C]Glc (ICN, Costa Mesa, CA), LR White embedding medium (Polysciences Inc., Warrington, PA), basic fuchsin (Fischer Scientific, Pittsburgh, PA), DEAE-

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Abbreviations: AH, amygdalin hydrolase; β-CAS, β-cyanoalanine synthase; GT-I, UDPG:mandelonitrile glucosyltransferase; HCN, hydrogen cyanide; MDL, mandelonitrile lyase; PH, prunasin hydrolase.

cellulose (Whatman Chemical Separation Ltd., Kent, UK). Monospecific polyclonal anti-AH, anti-PH, and anti-MDL antisera were produced and characterized in our laboratory (Wu and Poulton, 1991; Li et al., 1992).

Plant Materials

Mature black cherry (*Prunus serotina*) fruits were harvested in 1991 from a single tree. In 1992, fruits were obtained from a different tree located approximately 50 m from the tree used previously. Their seeds were processed and stored for at least 3 months at 4°C as described by Li et al. (1992). Seeds were germinated by soaking in aerated distilled H_2O at 22°C before planting in Jiffy Mix potting soil. Seedlings were grown at 22°C under fluorescent lights (12-h photoperiod) before harvesting. To facilitate biochemical analyses, seedling development was arbitrarily divided into four stages based on the following criteria: stage 1, poststorage mature seeds with intact endocarps; stage 2, mature seeds whose endocarps are cracked due to imbibition in aerated distilled H_2O for 1 to 2 d; stage 3, seedlings approximately 1 week after imbibition in which the hypocotyl (radicle), but not the epicotyl, has emerged from the seed and is approximately 2 cm in length and in which the cotyledons are yellow; stage 4, seedlings approximately 2 weeks after imbibition in which the epicotyl has emerged from the seed and the first pair of true leaves has expanded and in which the cotyledons appear green. Where possible, epicotyls, hypocotyls, and cotyledons were analyzed separately in the following determinations.

Determination of Cyanoglycoside Content

A known number (usually $n = 10$) of stage 1 to 4 seeds or seedling parts was extracted in methanol and chromatographed by HPLC to resolve amygdalin and prunasin (Swain et al., 1992a), which were quantitated as described by Brinker and Seigler (1989).

Determination of AH, PH, and MDL Levels by Enzymatic Assay and Immunoblotting

A known number of stage 1 to 4 seeds or seedling parts (usually $n = 10$) was weighed and homogenized at 4°C with mortar and pestle. For each gram of tissue, the following were added: 10 mL of 0.1 M His-HCl, pH 6.0, containing 5 mM EDTA, 1 mM PMSF, and 1 μM *N*-α-*p*-tosyl-L-Lys chloromethyl ketone; 0.01 g of polyvinylpolypyrrolidone; and 0.1 g of sand. The homogenate was centrifuged in a Beckman microfuge for 25 min, and the resulting supernatant was dialyzed against eight × 1-L changes (30 min each) of 20 mM sodium acetate, pH 5.0. The dialyzed preparation was assayed for PH and MDL activities as previously described (Yemm and Poulton, 1986; Kuroki and Poulton, 1987). For analysis of AH levels, AH and PH were resolved by DEAE-cellulose chromatography. An aliquot (0.6 mL) of the dialyzed enzyme was gently rotated (Fisher nutator) for 30 min at 4°C with an equal volume of a DEAE-cellulose slurry (65% [v/v] DEAE-cellulose in 20 mM sodium acetate, pH 5.0). After the sample was centrifuged for 5 min at 13,000*g* to sediment the ion exchanger with attached PH, the resulting supernatant was assayed for AH activity (Kuroki and Poulton, 1986). To confirm the successful removal of PH, the supernatant was also routinely assayed for PH activity.

The levels of AH, PH, and MDL protein in stage 1 to 4 seeds or seedling parts were also analyzed by western immunoblotting as described by Swain et al. (1992a).

Determination of β-CAS Activity

Homogenates of stage 1 to 4 seeds or seedling parts were prepared as described above, except that 0.1 M potassium phosphate, pH 8.0, replaced His-HCl as buffer. After homogenates were microfuged for 25 min, the resulting supernatants were dialyzed against eight × 1-L changes (30 min each) of 0.1 M potassium phosphate, pH 8.0, before assaying for β-CAS activity essentially as described by Hendrickson and Conn (1969).

Determination of GT-I Activity

Homogenates of stage 1 to 4 seeds or seedling parts were prepared as described for AH except that 0.2 M Tris-HCl, pH 8.63, containing 14 mM β-mercaptoethanol, replaced His-HCl as buffer. Homogenates were centrifuged for 25 min, and the resulting supernatants were dialyzed against eight × 1-L changes (30 min each) of 20 mM Tris-HCl, pH 8.0. To avoid potential interference by PH, this glycoprotein was removed by incubating the homogenates with Con A-Sepharose 4B. An aliquot (0.5 mL) of the dialyzed enzyme was gently rotated for 25 min at 4°C with an equal volume of a Con A-Sepharose 4B slurry (60% [v/v] affinity matrix in 20 mM Tris-HCl, pH 8.0). After the sample was centrifuged for 5 min at 13,000*g* to pellet the PH-Con A-Sepharose 4B complexes, the resulting supernatants were assayed for GT-I essentially as described by Poulton and Shin (1983).

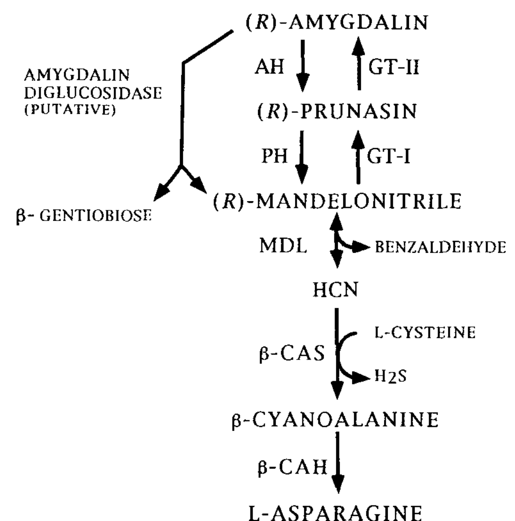

Figure 1. Potential pathways for amygdalin metabolism and HCN reassimilation in developing *P. serotina* seedlings. GT-II, UDPG: prunasin glucosyltransferase; β-CAH, β-cyanoalanine hydrolase.

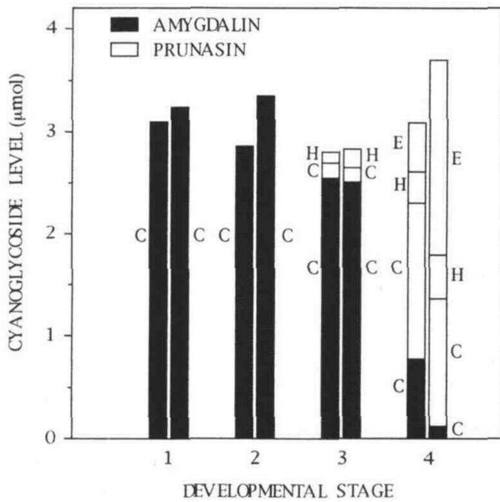

Figure 2. Utilization of amygdalin during the development of *P. serotina* seedlings. The levels of amygdalin and prunasin within the cotyledons (C), epicotyls (E), and hypocotyls (H) of seedlings at four specific stages (stages 1–4) of development were estimated as described in "Materials and Methods." At each stage, the left and right bars represent data obtained with seedlings grown from seeds collected in 1991 and 1992, respectively.

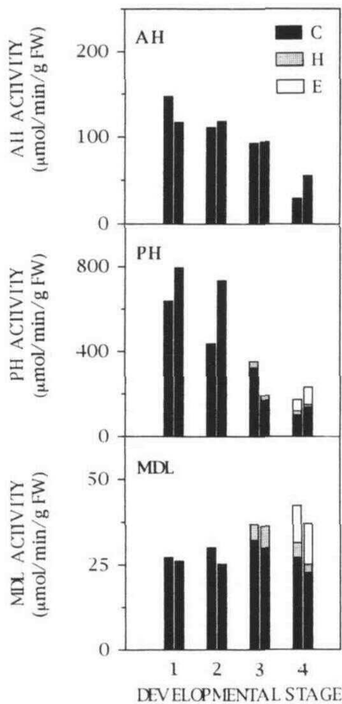

Figure 3. Levels of AH, PH, and MDL in developing *P. serotina* seedlings. Extracts of developing seedlings were partially purified as described in "Materials and Methods." Where possible, enzyme levels within cotyledons (C), epicotyls (E), and hypocotyls (H) are indicated. At each stage, the left and right bars represent data obtained with seedlings grown from seeds collected in 1991 and 1992, respectively. Because AH occurs only in cotyledons, the AH contents of the other seedling organs are not indicated. FW, Fresh weight.

Determination of Potential Amygdalin Diglucosidase and β-Gentiobiosidase Activities

Attempts to detect amygdalin diglucosidase and β-gentiobiosidase activities in cherry homogenates were made as follows. A known number (usually $n = 10$) of stage 1 to 4 seeds or seedling parts was weighed and homogenized at 4°C with mortar and pestle in one of the following buffers: 0.1 M potassium phosphate, pH 8.0; 0.1 M His-HCl pH 6.0; or 0.1 M citrate-phosphate, pH 5.0. For each gram of tissue, 10 mL of buffer, 0.01 g of polyvinylpolypyrrolidone, and 0.1 g of sand were added. The homogenates were centrifuged in a Beckman microfuge for 25 min, and the resulting supernatants were dialyzed against eight × 1-L changes (30 min each) of a 1:20 dilution of the respective homogenization

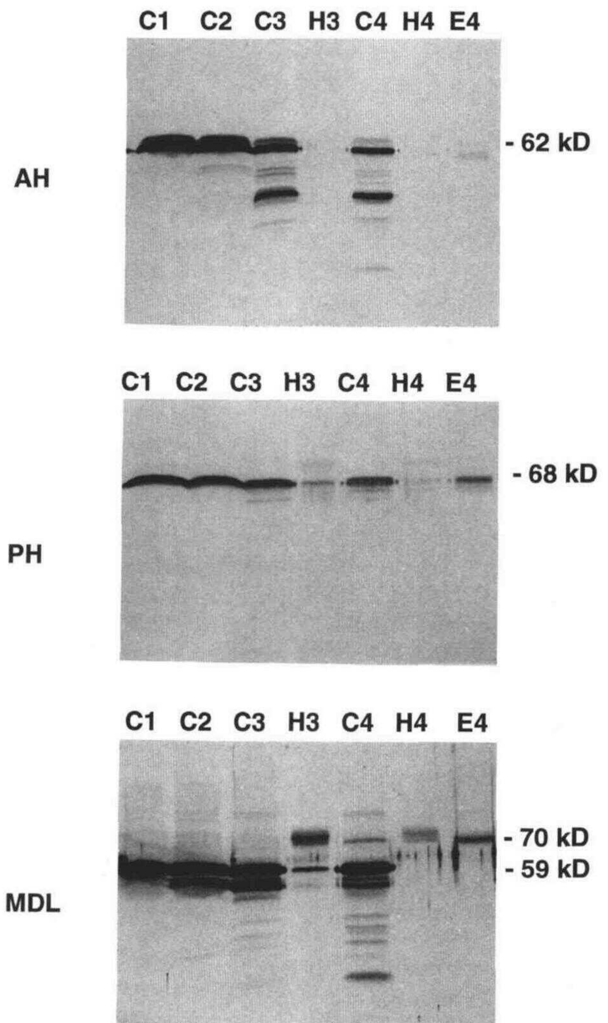

Figure 4. Immunoblot analysis of AH, PH, and MDL in developing *P. serotina* seedlings (stages 1–4). Crude seedling homogenates (100 μg of total protein) were prepared as described in "Materials and Methods" and subjected to SDS-PAGE and immunoblot analysis using polyclonal antibodies monospecific for each protein. At stages at which sufficient differentiation had occurred, cotyledons (C1–C4), hypocotyls (H3–H4), and epicotyls (E4) were analyzed separately.

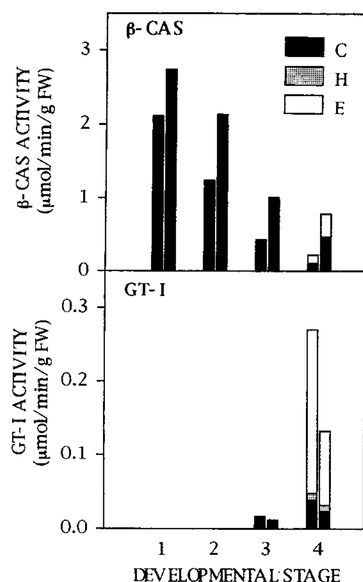

Figure 5. Levels of β-CAS and GT-I in developing *P. serotina* seedlings. Extracts of developing seedlings were prepared and assayed for β-CAS and GT-I activity as described in "Materials and Methods." Where possible, enzyme levels within cotyledons (C), epicotyls (E), and hypocotyls (H) are indicated. At each stage, the left and right bars represent data obtained with seedlings grown from seeds collected in 1991 and 1992, respectively. FW, Fresh weight.

buffer. The assay mixtures (total volume of 2.5 mL) contained 37 μmol of amygdalin (or 37 μmol of β-gentiobiose), 0.25 mg of BSA, 10 mM buffer, and up to 10 mg of *P. serotina* protein. Control reactions, which lacked either glycosidic substrate or enzyme, were routinely included. Reactions were initiated by adding substrate and were terminated after 1 h at 30°C by heating at 95°C for 5 min. Amygdalin diglucosidase activity was assessed by chromatographing 20 μL of each terminated assay on SigmaCell type 100 TLC plates (250-μm thickness) alongside authentic samples of β-gentiobiose, Glc, amygdalin, and prunasin. Carbohydrates were detected by staining with analine phthalate (Ribereau-Gayon, 1972). Potential β-gentiobiosidase activity was monitored by Glc production (Kuroki and Poulton, 1986).

Assessment of HCN Release by Developing Seedlings

Approximately 50 seeds were soaked overnight in aerated H_2O to initiate germination. These seeds were then placed in a 1-L glass chamber equipped with an inlet tube through which air was continuously supplied. A second tube that vented the chamber was submerged in 1 M NaOH (20 mL) to trap any HCN present in the outgoing air. For 3 weeks (throughout stages 1 to 4), the NaOH solution was collected daily, replaced by fresh NaOH, and assayed for HCN by the method of Brinker and Seigler (1989). As a positive control, 4 μmol of amygdalin, approximating the levels in a single, mature black cherry seed, were catabolized by almond emulsin in the seedling chamber, and the respective NaOH trap was assayed for cyanide as described above.

Immunolocalization of AH, PH, and MDL in Maturing Cotyledons

Immunolocalization was undertaken essentially as described by Swain et al. (1992b). In brief, stage 1 to 4 cotyledons were fixed, embedded in LR White medium, sectioned, and collected on glass microscope slides. Sections were probed with undiluted preparations of either anti-AH, anti-PH, or anti-MDL antisera or with preimmune serum in solutions containing 5% (w/v) nonfat dry milk and 0.05% (v/v) Tween 20. The resulting primary immune complexes were visualized by probing these sections with colloidal gold-conjugated goat anti-rabbit IgG (diluted 1:10 in blocking buffer) followed by silver enhancement.

RESULTS

Cyanoglycoside Levels during Seed Development

Both ungerminated (stage 1) and hydrated (stage 2) black cherry seeds contained approximately 3.2 μmol of amygdalin per seed but lacked the monoglucoside prunasin (Fig. 2). During seedling development, amygdalin levels declined sharply, and by stage 4, levels were only 5 to 25% of that observed in ungerminated seeds. Nevertheless, the cyanogenic potential of the germinating seedling remained virtually constant during this entire period because of the concomitant appearance of prunasin in the cotyledons, epicotyls, and, to a lesser extent, hypocotyls.

Levels of Amygdalin-Catabolizing Enzymes during Seed Development

In theory, this observed decline in amygdalin levels might result from the activity of AH and/or the putative enzyme amygdalin diglucosidase (analogous to the *Hevea* linustatinase [Selmar et al., 1988]). To evaluate these possibilities, the levels of these enzymes, as well as those of PH and MDL, were determined during early seedling development. As shown in Figure 3, cotyledonary levels of AH and PH decreased during this period and by stage 4 were approximately 30 and 20%, respectively, of levels observed in ungerminated seeds. Nonetheless, this residual activity still exceeded 25 μmol glycoside hydrolyzed min^{-1} g^{-1} fresh weight. Because of its prevalence and protein body location, MDL is suspected to play an additional role as a storage protein and as such might be expected to be mobilized during germination (Swain et al., 1992b). However, this mobilization apparently occurs

Figure 6. (On facing page). Tissue localization of AH, PH, and MDL in stage 1 cotyledons by silver-enhanced immunogold labeling. Longitudinal serial sections of cotyledons embedded in LR White resin were challenged with either anti-AH (A), anti-PH (B), anti-MDL (C), or preimmune serum (D). Immunogold-labeled antigens appear as black areas after silver enhancement. Poststaining was undertaken with basic fuchsin. Scale bar equals 25 μm.

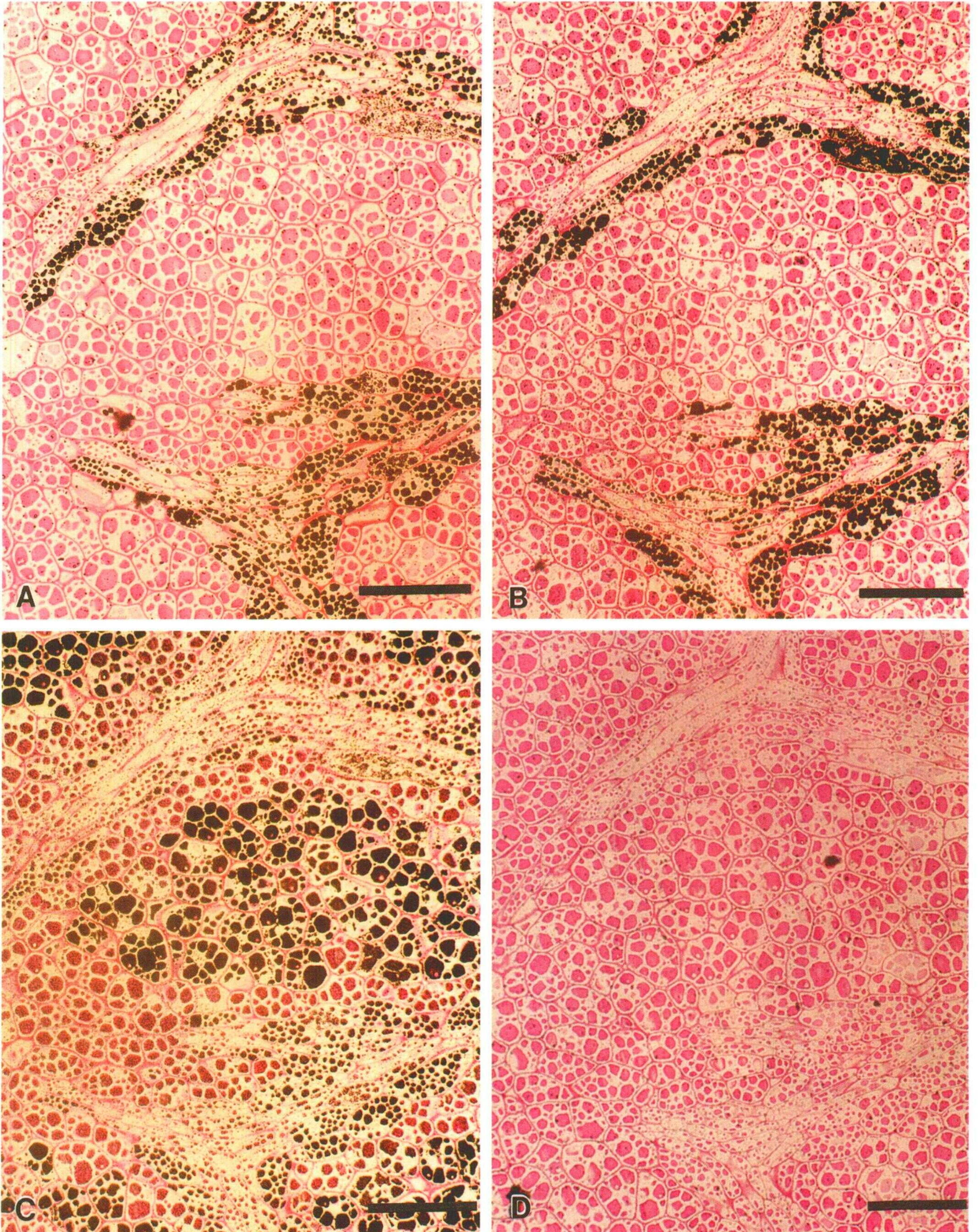

Figure 6. Legend appears on facing page.

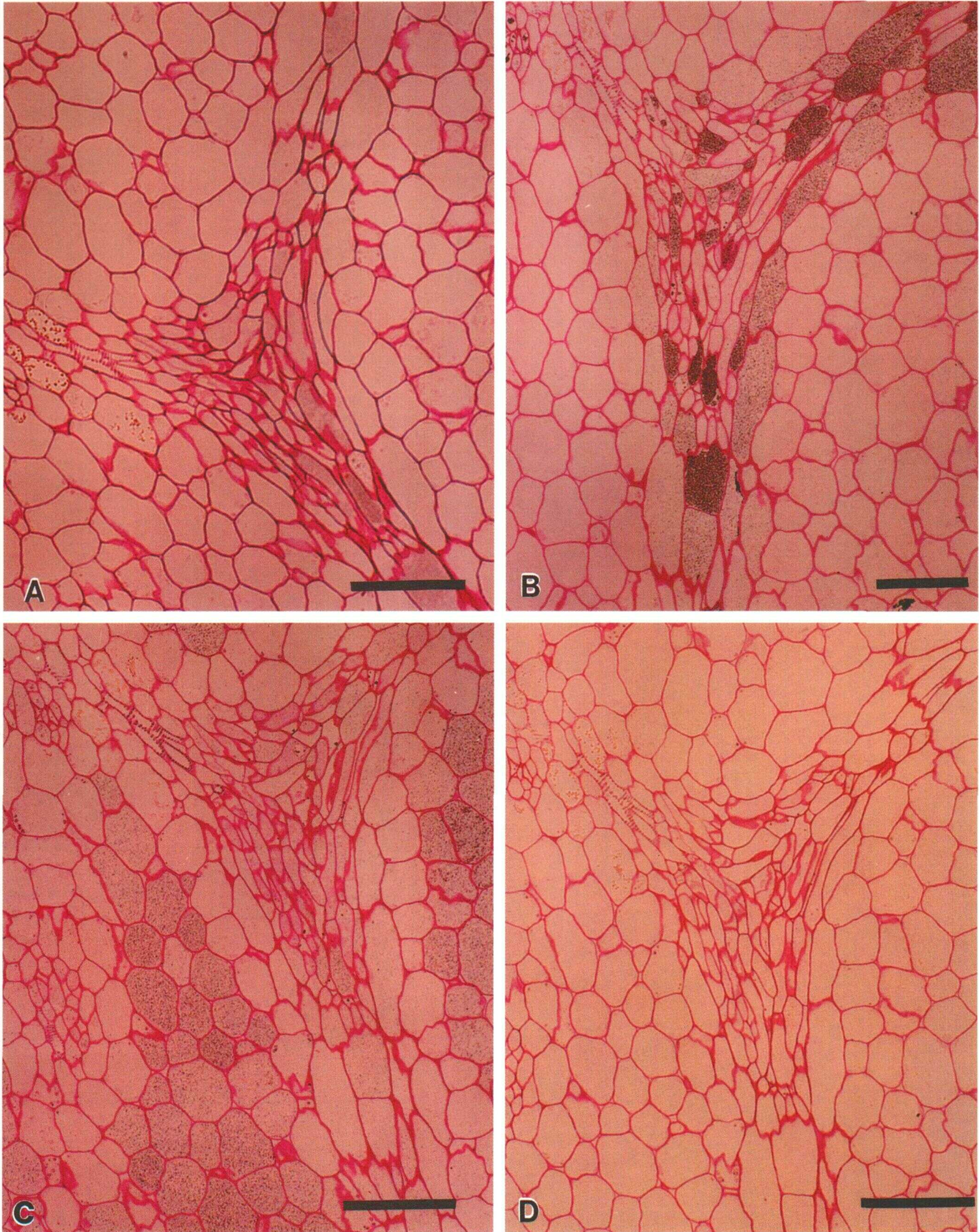

Figure 7. Legend appears on facing page.

after the period surveyed here since cotyledonary MDL levels, unlike AH and PH levels, remained essentially unchanged throughout stages 1 to 4 (Fig. 3). The distinct behavior shown by MDL may reflect additional constraints imposed on this enzyme by its multifunctionality. Although epicotyls and hypocotyl preparations exhibited PH and MDL activity, they lacked AH activity (Fig. 3).

Immunoblotting analysis using antisera monospecific for each catabolic enzyme confirmed these enzymatic data and provided additional information (Fig. 4). Interestingly, the polypeptide in hypocotyl and epicotyl homogenates from older seedlings that was recognized most strongly by the anti-MDL antiserum was significantly larger (70 kD) than cotyledonary MDL (59 kD). The possibility that MDL indeed exists in axillary tissues as a higher molecular mass isoform is under investigation. The low molecular mass bands recognized by anti-AH and anti-MDL antisera within cotyledon homogenates of older seedlings are presumably the respective breakdown products of AH and MDL (Fig. 4).

Despite repeated efforts, an amygdalin diglucosidase capable of hydrolyzing amygdalin to mandelonitrile and β-gentiobiose was not detectable in homogenates of any seedling organ at any developmental stage. Likewise, seedling homogenates lacked detectable β-gentiobiosidase activity.

Assessment of HCN Release by Developing Seedlings

To determine whether cyanogenesis occurs concomitantly with declining amygdalin levels during seedling development, 50 seeds were allowed to germinate in a closed glass chamber vented through an NaOH trap. HCN was not detected in any of the trap samples taken daily during this 3-week period. As a positive control, 4 μmol of amygdalin (i.e. the approximate level in a single cherry seed) were added to almond emulsin in the glass chamber under identical conditions. In this case, HCN was quantitatively recovered in the NaOH trap, attesting to the likelihood of detecting any HCN released by 50 developing seedlings.

Measurement of β-CAS

To assess the availability of β-CAS for assimilation of any HCN generated during germination, the levels of this detoxification enzyme were assayed in homogenates of developing seedlings. Stage 1 seeds exhibited high β-CAS activity (2–3 $\mu mol\ min^{-1}\ g^{-1}$ fresh weight). However, as noted with AH and PH (Fig. 3), cotyledonary β-CAS levels declined greatly during seedling development (Fig. 5). Stage 4 epicotyls, which contain both prunasin and PH and are, therefore, potentially cyanogenic, exhibited significant β-CAS activity. By contrast, β-CAS was undetectable in hypocotyls, which possess only approximately 6% of the PH and 20% of the prunasin found in epicotyls.

Measurement of GT-I

Realizing that the appearance of prunasin in developing seedlings might have resulted from its de novo biosynthesis rather than by amygdalin hydrolysis, we also assayed GT-I levels during germination. As Figure 5 shows, the time course of GT-I appearance closely paralleled that observed for prunasin in these seeds or seedling parts (Fig. 2). Both GT-I and prunasin were first detected in stage 3 cotyledons, and their levels increased markedly in this organ between stages 3 and 4. Stage 4 epicotyls exhibited high GT-I activity, consistent with their elevated prunasin levels. By contrast, stage 4 hypocotyls had much lower GT-I and prunasin levels.

Immunolocalization of AH, PH, and MDL in Developing Seedlings

As reported previously (Swain et al., 1992b), AH and PH were immunolocalized in mature, ungerminated seeds (i.e. stage 1 seeds) within the protein bodies of procambial cells. AH occurred within the majority of procambial cells, whereas PH was confined to the peripheral layers of this tissue. Highest levels of MDL were observed in the protein bodies of the cotyledonary parenchymal cells with lesser amounts in the procambial protein bodies. All three enzymes were absent from the bundle sheath layer, which encircles the procambium, and the endosperm.

As illustrated in Figures 6 and 7, AH, PH, and MDL retained these same tissue- and cell-specific distributions throughout seedling development. Although xylem- and phloem-conducting elements could be distinguished beginning in stage 3, none of these catabolic enzymes was detected in any type of conducting element. Concomitantly with, and perhaps resulting from, the increasing differentiation, we observed a decline in the intensity of immunolabel for AH and PH. This corresponds well with the declines in enzyme protein and activity noted earlier. Also apparent from this study is the well-established ontogeny of vacuoles from protein bodies (Pernollet, 1978; Brown et al., 1982; Bollini et al., 1983). Between stages 2 and 3, the numerous small protein bodies within a given cotyledonary cell coalesced to form larger protein bodies. Between stages 3 and 4, these large protein bodies enlarged and became less dense in the process of becoming vacuolar.

DISCUSSION

In addition to their likely role as allelochemicals, there is mounting evidence that cyanoglycosides and cyanolipids may serve as storage forms for reduced nitrogen in plants (Clegg et al., 1979; Selmar et al., 1988, 1990). The best documented case involves the mobilization of linamarin from the endosperm of *Hevea brasiliensis* seeds (Selmar et al.,

Figure 7. (On facing page). Tissue localization of AH, PH, and MDL in stage 4 cotyledons by silver-enhanced immunogold labeling. Longitudinal serial sections of cotyledons embedded in LR White resin were challenged with either anti-AH (A), anti-PH (B), anti-MDL (C), or preimmune serum (D). Immunogold-labeled antigens appear as black areas after silver enhancement. Poststaining was undertaken with basic fuchsin. Scale bar equals 25 μm.

1988). During seedling development, this cyanogenic monoglucoside is glycosylated to the diglucoside linustatin for transport to the seedling leaves. The subsequent degradation of linustatin by a diglucosidase yields HCN, which is detoxified to L-Asn by β-CAS and β-cyanoalanine hydrolase. Thus, instead of being lost to the environment as HCN, the nitrogen content of linamarin is returned to the general amino acid pool.

Recently, we described the accumulation of the cyanogenic diglucoside amygdalin by developing black cherry seeds (Swain et al., 1992a). Extending these studies, our goal was to ascertain whether amygdalin is catabolized upon germination to furnish the developing seedlings with reduced nitrogen and carbonyl compounds. The dramatic decline in amygdalin levels observed here during early seedling development supports this notion. Furthermore, similar to the mobilization of cyanoglycosides and cyanolipids in germinating seedlings of *H. brasiliensis* and *Ungnadia speciosa*, respectively (Selmar et al., 1988, 1990), this event occurred without loss of HCN to the atmosphere.

At first glance, the simplest explanation for the appearance of prunasin within the cotyledons, epicotyls, and hypocotyls during stages 3 and 4 is the AH-mediated hydrolysis of amygdalin to prunasin within the cotyledons. This monoglucoside might subsequently undergo translocation to the growing axes. However, our immunocytochemical localization studies might argue against the partial hydrolysis of amygdalin. As shown by previous studies with mature seeds, amygdalin is restricted to the ground parenchymal cells of the cotyledons, whereas AH and PH are procambial enzymes (Poulton and Li, 1994). Because the current immunolocalization studies failed to detect AH (or PH) within the ground parenchyma at any time during stages 1 to 4, it seems probable that amygdalin must pass upon germination to the procambium for catabolism. Given the co-occurrence of AH, PH, and MDL within the peripheral procambial cells flanking the developing xylem and phloem, the partial hydrolysis of amygdalin to prunasin appears unlikely. More likely, in our opinion, is its complete degradation by the procambial catabolic enzymes to HCN, which occurs concomitantly with the de novo biosynthesis of prunasin from L-Phe involving GT-I. This interpretation is supported by the observation that the activity profile of GT-I precisely parallels prunasin levels in all seedling organs.

An alternative mechanism for amygdalin degradation that was entertained here is its hydrolysis to mandelonitrile and β-gentiobiose by a diglucosidase analogous to the *Hevea* linustatin diglucosidase (Selmar et al., 1988). However, this catabolic route was excluded from further consideration after numerous unsuccessful attempts to demonstrate this enzyme in extracts from all seedling organs.

The catabolism of amygdalin to HCN, benzaldehyde, and Glc during normal seedling development would demand some mechanism for HCN detoxification. Most plants are capable of assimilating the extremely low levels of HCN produced during ethylene biosynthesis using β-CAS. This enzyme catalyzes the reaction between HCN and L-Cys to form β-cyanoalanine and H_2S. Consistent with the observation that cyanogenic plants generally have significantly higher β-CAS levels than acyanogenic species (Miller and Conn, 1980), developing *P. serotina* seedlings exhibited high β-CAS activities. Moreover, the excellent correlation between the time courses of PH and β-CAS levels suggests that the latter enzyme plays a significant role in detoxifying HCN derived from cyanoglycoside degradation. It is interesting to note that L-Asn, which is derivable from β-cyanoalanine by hydrolysis, is a major nitrogen transport form in a related rosaceous species (Tromp and Ovaa, 1979).

Benzaldehyde and Glc resulting from amygdalin catabolism could also be advantageous to the developing seedling. Horsley (1979) reported that benzoic acid, derived from benzaldehyde during prunasin catabolism in senescing black cherry leaves, was allelopathic to *Acer rubrum* seedlings, which have habitat requirements similar to those of *P. serotina.* Whether benzoic acid is released from germinating seedlings as an allelopathic agent following amygdalin mobilization remains to be investigated. Glc resulting from cyanoglycoside catabolism could clearly serve as a carbon or energy source.

This study, although providing substantial evidence that amygdalin decomposition might furnish the developing seedling with nitrogen and carbonyl compounds, does not support the possibility that amygdalin itself might be transported to the growing axes. Not only are the vascular strands encompassed by cells containing AH, PH, and MDL but also the axillary seedling organs appear incapable of amygdalin catabolism since they lack both AH and amygdalin diglucosidase activities.

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