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Proteinase and alpha-amylase inhibitors of sweet potato: Changes during growth phase, sprouting, and wound induced alterations

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Abstract. Changes in proteinase (trypsin and chymotrypsin) and α-amylase inhibitors were monitored during the growth phase and sprouting of sweet potato, with a view to establishing the biological role of these secondary plant principles. Although the trypsin inhibitor (TI) activity of tuberising roots of sweet potato was very low in four genotypes at 15 days after planting (DAP), a steep increase was observed during the tuber-bulking phase, indicating a possible role for the inhibitor in regulating plant proteinases. In contrast, the chymotrypsin inhibitor (CI) activity decreased from 40-90 DAP, indicating an inconsequential role in protease regulation. With initiation of tuber bulking, the α-amylase inhibitor (AI) increased sharply in three sweet potato genotypes, and after completion of the bulking phase, the activity came down sharply at 105 DAP. A pronounced role for regulation of endogenous α-amylases by the AI of sweet potato could be postulated from the study. The TI activity increased in the tubers until the sixth day of planting in vermiculite, and it decreased with the emergence of sprouts, indicating the possibility of proteolytic cleavage at the time of sprouting. Amylase inhibitor activity continuously decreased until sprouting, indicating the role of these proteins as N-reserve proteins in sweet potato. A significant increase in proteinase inhibitor levels in all the sweet potato leaves was observed within four hours of artificial wounding. The rise in inhibitor levels was more evident in leaves far off from the wounded leaf. The fact that proteinase and amylase inhibitors of sweet potato are wound-inducible points to a major defensive role for them. This information on the proteinase inhibitor levels in sweet potato leaves and vines and their change with plant growth is of interest, especially due to their use as animal feed.

Keywords: α-Amylase inhibitor; Chymotrypsin inhibitor; Growth phase; *Ipomoea batatas*; Sprouting; Trypsin inhibitor; Wounding.

Abbreviations: AI, α-amylase inhibitor; **TI,** trypsin inhibitor; **CI,** chymotrypsin inhibitor; **DAP,** days after planting.

Introduction

Recent developments in the field of proteinase and amylase inhibitors and the increasing use of these phytochemicals in industry and pharmacy have led researchers to inquire closely into their exact biological functions. Their role as natural plant defense proteins has attracted a lot of attention (Farmer and Ryan, 1990; Gatehouse et al., 1986). These inhibitors are believed to make plants less palatable, even lethal, to insects, thus conferring some selective advantage to the plants. Several workers have reported a decrease in proteinase inhibitor activity with germination (Ambekar et al., 1996; Hobday et al., 1973). The transient nature of potato chymotrypsin inhibitor I was reported by Ryan et al. (1968). The changes in trypsin inhibitor activity in the vegetative tissues of sweet potato plants were studied by Zhang and Corke (2001). Mulimani et al. (1994) reported a decrease in α-amylase inhibitor (AI) activity with germination in 28 varieties of chickpea. Reports on the alpha amylase inhibitor changes in plants are scanty, except those indicating alpha amylase accumulation in the seeds of cereal crops during seed formation (Mundy, 1984; Pace et al., 1978). The wounding of the lower terminal leaflets of tomato plants by Colorado potato beetles has been reported to lead to the accumulation of proteinase inhibitor in both wounded and non-wounded leaves (Green and Ryan, 1972). Artificial wounding by heat or even crushing with a file can mimic the action of an insect bite and stimulate the production of the inhibitor (Green and Ryan, 1972; Hilder et al., 1987). Yeh et al. (1997) found that transgenic tobacco plants carrying sweet potato TI genes were resistant to the tobacco cut worm, *Spodoptera litura*.

We first reported the presence of proteinase and α-amylase inhibitors in a large number of sweet potato genotypes (Sasikiran et al., 1999; Rekha et al., 1999). Our later studies highlighted the inhibitor potential of purified protease and α-amylase inhibitors from five sweet potato genotypes on the digestive enzymes of root crop storage pests (Sasikiran, 2000; Rekha, 2000). In this paper, we report the changes in the trypsin, chymotrypsin, and α-amylase inhibitors in sweet potato during growth phase, sprouting, and wounding.

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Materials and Methods

Plant Materials and Chemicals

The planting material for the studies *viz*. four genotypes of sweet potato were procured from the farm of Central Tuber Crops Research Institute, Thiruvananthapuram, India. Trypsin and chymotrypsin (3X crystallized; from bovine pancreas) and Bovine serum albumin (BSA, Fraction V) were obtained from Sigma Chemical Co. (St Louis, MO). Alpha-amylase, was procured from EMerck Inc., Germany. All other chemicals of reagent grade were obtained from Sisco Research Laboratories (Mumbai, India).

Changes in Inhibitor Content During Growth Phase

Four genotypes of sweet potato, RS III-2, Kannukaruppan (KK), Kanhangad, and S 1195 were planted under identical management conditions, and the package of practices prescribed for the crop was followed. They were grown for 105 days on the Institute farm. Soil type in the field was loam. Fertilizers were applied to the subsoil before planting as per the package of practices, and no subsequent fertilizer was applied. A randomized block design was employed in the field plan. Each plot was 1.5 m wide with double rows 2.0 m long, and plants were spaced at 0.25 m within rows on ridges. The changes in the inhibitor content were monitored during the growth phase (105 days). Three whole plants were uprooted and cleaned to remove the dirt. The leaves, stem, and root/tubers were separately collected. Each plant part was chopped and mixed thoroughly to obtain representative samples from each tissue. Three representative samples of 2.0 g each were taken for the extraction of proteinase and amylase inhibitors. As the total growth period for sweet potato is about 105 days, sampling was done up to harvest at 15 days intervals, and the mean value from two growth seasons was taken.

Changes During Sprouting

Fresh undamaged tubers of two genotypes of sweet potato (Kanhangad and S 1195) were surface sterilized with 0.05% sodium hypochlorite and rinsed with distilled water. The proximal tips of the sweet potato were cut and planted in plastic cups containing sterilized vermiculite. Destructive sampling of the tubers was done at two-day intervals until the sprouts attained a height of about five cm. The sprouting study was repeated for a second time (second experiment), and the mean changes in trypsin, chymotrypsin, and α-amylase inhibitor content were calculated. Three replications were maintained per experiment for each accession of sweet potato. Statistical comparison was made with the initial value using the Student's 't' test.

Wound Induced Alterations

The change in the inhibitor levels upon stress was studied by artificially wounding the leaves of sweet potato (genotype-RS-III-2). The plants were grown in a specially constructed net enclosure to prevent damage from natural pests and insects. The plant architecture of the sweet potato variety was such that each branch contained many leaflets. One leaflet in the middle branch was wounded using a cigarette lighter by exposing the under surface of the leaf to the flame for 30 sec. Samples were collected after 4, 8, 16 and 24 h of wounding. Sampling was done as follows: Leaflet above the wounded leaflet from the same branch (herein after designated as L1), leaflet below the wounded leaflet (L2), other leaflets from the wounded branch (L3), leaves from the branch immediately below the wounded branch, (L4) and leaves from the bottom-most branch of the plant (L5). Duplicate samples were collected at each sampling frequency and the inhibitor levels were quantified. The initial level of inhibitors in the intact plant was assayed before inflicting the wounds. Three replications were conducted throughout the experiment.

Extraction and Assay of Inhibitor Proteins

The proteinase inhibitors were extracted from the samples in an Ultra-Turrax homogenizer, using 0.01 M sodium phosphate buffer (pH 8.0; 1:5 w/v) in presence of 1.0% polyvinyl pyrrolidone (PVP). The native proteases were inactivated by heating at 70°C for 10 min, and the precipitated proteins were removed by centrifugation at 1,000 *g* for 10 min. To optimize the recovery of proteinase inhibitor from the leaves and stem the crude extract was treated with 5% TCA. After the removal of the precipitated proteins by centrifugation at 5,000 *g*, the pH was rapidly brought back to 8.0 and dialyzed overnight against sodium phosphate buffer (0.01 M, pH 8.0). The chlorophyll pigments and other native proteases could effectively be removed by this method. The trypsin inhibitor (TI) and chymotrypsin inhibitor (CI) content were assayed according to the procedure described earlier (Sasikiran et al., 1997). One trypsin/chymotrypsin inhibitor unit is the amount of inhibitor that inhibits one unit of trypsin/chymotrypsin. One trypsin/chymotrypsin unit is the mg peptides released per min at 30°C under the assay conditions. The α-amylase inhibitors were extracted from the tissues using sodium phosphate buffer (0.02 M; pH 6.9) containing 0.3 M NaCl. The crude extracts were subjected to TCA treatment similar to the protocol adopted for proteinase inhibitors. The AI was quantified in the extracts by the method described in a previous communication (Rekha et al., 1997). One α-amylase inhibitor unit is the amount of α-amylase units inhibited. One α-amylase unit is the micrograms starch hydrolysed per min at 30°C under the assay conditions.

Results

Growth Phase Studies

The change in proteinase and α-amylase inhibitor content in the different tissues of sweet potato during its life cycle is presented in Figures 1-3. The levels of TI in the tuberising roots were very low in the four genotypes of sweet potato (0.081-0.269 TIU/g dry wt) at 15 days after

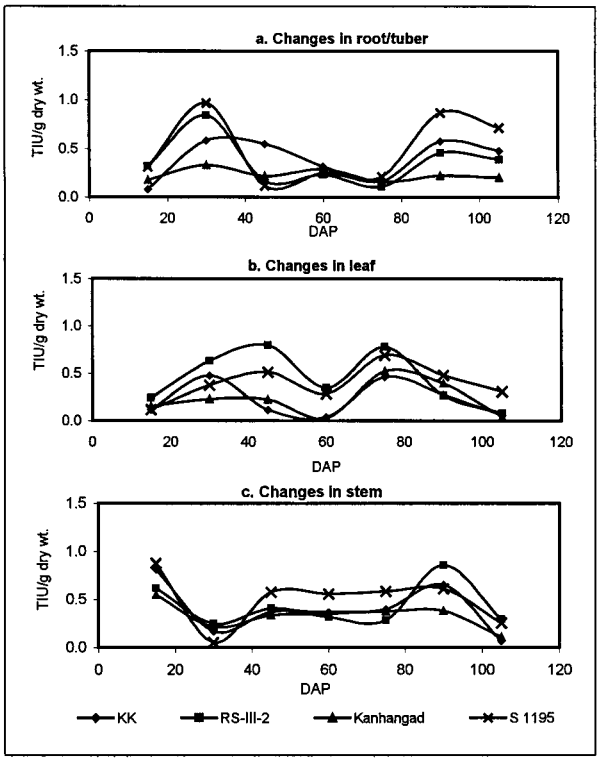

Figure 1. Changes in trypsin inhibitor content in sweet potato tissues during growth phase. Levels expressed as trypsin inhibitor units (TIU) /g dry wt. The values represent mean values from two growth seasons at each sampling interval.

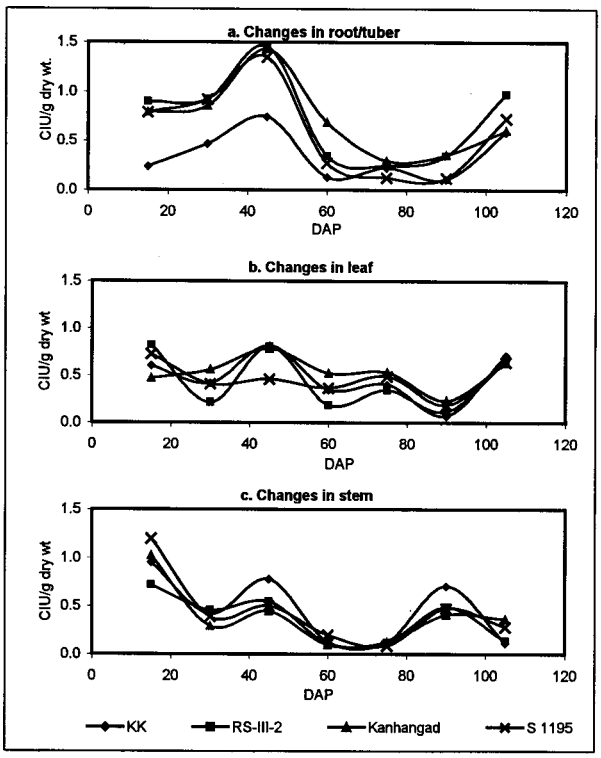

Figure 2. Changes in chymotrypsin inhibitor content in sweet potato tissues over growth stages. Levels expressed as chymotrypsin inhibitor units (CIU) /g dry wt. The values represent mean values from two growth seasons at each sampling interval.

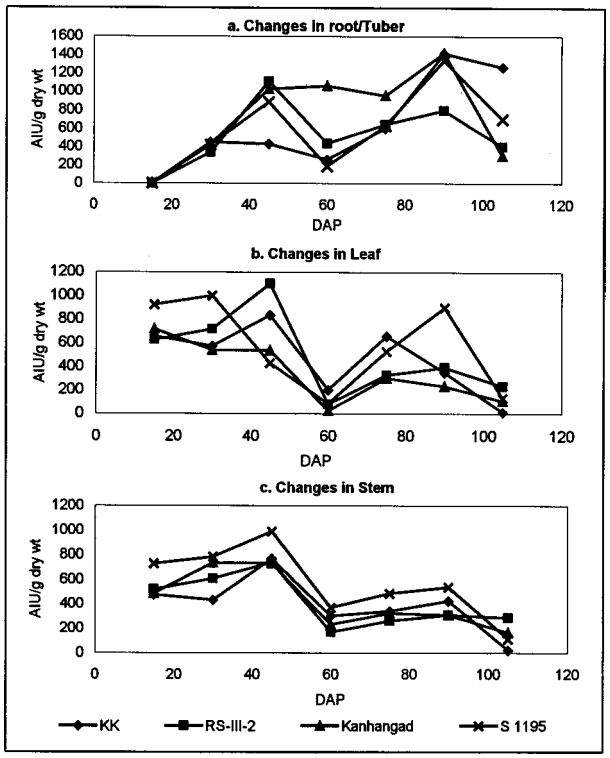

Figure 3. Changes in α-amylase inhibitor content in sweet potato tissues during growth phase. Levels expressed as α-amylase inhibitor units (AIU) /g dry wt. The values represent mean values from two growth seasons at each sampling interval.

planting (DAP), but they increased to 0.328-0.965 TIU by 30 days. In contrast, TI content in the stem of sweet potato was high, in the range of 0.549-0.876 units at 15 DAP. In the leaves, TI activity was low, but it increased to 0.218-0.74-97 units by 30-45 days after planting (DAP) and then decreased at 60 DAP. A surge in TI activity was observed in the leaves during 60-75 DAP, but it then continuously decreased until harvest (Figure 1b). In the stems and storage root, an increase in TI content was observed from 75 to 90 days (Figure 1a, c), but it decreased sharply in stem at the time of harvest (105 days).

The CI changes during the growth phase of sweet potato were quite different from the pattern observed for TI. CI activity was found to decrease in the leaves and stem up to 30 DAP, followed by an increase up to 45 DAP (Figure 2b, c). In contrast, CI activity in roots of sweet potato continuously increased from 0.239-0.897 CI units at 15 DAP to 0.746-1.43 CI units by 40 DAP in all four genotypes and then sharply decreased until 90 DAP (0.124-0.355 units). An interesting finding was the very low CI activities in the leaves of sweet potato at 90 DAP, which however soared to 0.639-0.712 CI units at 105 DAP (Figure 2b). As in the roots and leaves, increased CI activity was observed at 45 DAP in the stem (Figure 2c). However, the activity which decreased, again increased at 90 DAP, which was very specific to the stem.

No α-amylase inhibitor activity in the roots was detected on the 15th DAP (Figure 3a). However, 30 DAP, alpha

Table 1. Changes in trypsin inhibitor content (expressed as TIU/g dry wt) during sprouting of sweet potato tubers.

Days	Leaf		Sprouts	
	S 1195	Kanhangad	S 1195	Kanhangad
0	0.784 ± 0.021	0.484 ± 0.013	-	-
2	0.843 ± 0.020	0.634 ± 0.017	-	-
4	1.475 ± 0.031	1.142 ± 0.020	-	-
6	1.555 ± 0.034	1.200 ± 0.032	-	-
8	1.324 ± 0.032*	0.871 ± 0.004	-	-
10	1.180 ± 0.029	0.648 ± 0.011	0.061 ± 0.002	0.093 ± 0.016
12	0.897 ± 0.016	0.387 ± 0.012	0.031 ± 0.004	0.053 ± 0.001
14	0.764 ± 0.014*	0.399 ± 0.006	0.047 ± 0.001*	0.031 ± 0.004
16	0.915 ± 0.040^{NS}	0.791 ± 0.099	0.174 ± 0.024	0.157 ± 0.010*
18	1.160 ± 0.037^{NS}	0.947 ± 0.032	0.199 ± 0.019	0.207 ± 0.006

All values represent Mean ± SD from six observations.
^{NS}Nonsignificance; *Significant at $p<0.05$. All other values are significant at $p<0.01$. TIU = Trypsin inhibitor unit is equal to the number of trypsin units inhibited. One trypsin unit = mg peptides released per min at 30°C under the assay conditions.

amylase inhibitor activity in the range of 333-448 AI units was detected in the roots of the four genotypes. The AI activity increased sharply until 45 DAP (426-1110 AI units). With initiation of tuber bulking (45 DAP), inhibitor activity decreased sharply in the three genotypes *viz*., KK, RS-III-2 and S 1195, while in Kanhangad, a slight decrease was observed only on the 75th day after planting. In all genotypes the amylase inhibitor activity further increased from 75-90 days and then decreased (Figure 3a). Unlike in the roots, alpha amylase inhibitor activity in the range of 625-922 AI units was present in the leaf and stem from 15 DAP. The inhibitory activity was low in the leaves and stems of the genotypes like KK, RS III-2, and S 1195 30 DAP. During the tuber bulking phase (60-90 DAP), low levels of AI were observed in the leaves and stems in almost all the genotypes, except for an increase in the stem of S 1195 (990 AI units). The levels of AI were almost insignificant in the leaves and stem of Kannukaruppan (10 and 21 AI units respectively) at 105 DAP, but it had the highest level of AI (1267 AI units) in the tuber during this period, indicating the possibility for translocation to tubers. After 105 DAP, when full maturity was attained and the bulking phase was almost completed, the levels of α-amylase inhibitor plunged again.

Changes During Sprouting

The level of TI increased significantly in the sweet potato tubers quantitatively until the sixth day. It then decreased gradually until 14 DAP and then increased (Table 1). With the emergence of sprouts in sweet potato (10 days), TI activity decreased sharply in both genotypes. Inhibitor levels in the sprouts increased remarkably by the 16th day. The pattern of changes in the CI level of sweet potato tubers was also similar (Table 2). After an initial increase in the first few days (2-4 days), the level decreased and then increased again on the eighth day.

The initial AI activity was found to significantly decrease in the tubers of S 1195 up to the tenth day after planting (DAP), when the sprouts started appearing. However in tubers of Kanhangad, the AI values increased significantly on the second day and decreased further on the

Table 2. Changes in chymotrypsin inhibitor content (expressed as CIU/g dry wt) during sprouting of sweet potato tubers.

Days	Leaf		Sprouts	
	S 1195	Kanhangad	S 1195	Kanhangad
0	0.184 ± 0.013	0.114 ± 0.004	-	-
2	0.233 ± 0.010	0.286 ± 0.013	-	-
4	0.257 ± 0.006	0.219 ± 0.016	-	-
6	0.086 ± 0.019	0.070 ± 0.015	-	-
8	0.149 ± 0.011	0.116 ± 0.009^{NS}	-	-
10	0.142 ± 0.024	0.089 ± 0.020	0.061 ± 0.002	0.093 ± 0.016
12	0.118 ± 0.001*	0.073 ± 0.024	0.031 ± 0.004	0.053 ± 0.001
14	0.075 ± 0.014	0.061 ± 0.006	0.047 ± 0.001*	0.031 ± 0.004
16	0.112 ± 0.011	0.097 ± 0.014	0.174 ± 0.024	0.157 ± 0.010*
18	0.124 ± 0.008^{NS}	0.104 ± 0.023^{NS}	0.199 ± 0.019	0.207 ± 0.006

All values represent Mean ± SD from six observations.
^{NS}Nonsignificance; *Significant at $p<0.05$. All other values are significant at $p<0.01$. CIU = Chymotrypsin inhibitor unit is equal to the number of chymotrypsin units inhibited. One chymotrypsin unit = mg peptides released per min at 30°C under the assay conditions.

Table 3. Changes in α-amylase inhibitor content (expressed as AIU/g dry wt) during sprouting of sweet potato tubers.

Days	Leaf		Sprouts	
	S 1195	Kanhangad	S 1195	Kanhangad
0	3403 ± 12	3415 ± 1	–	–
2	2749 ± 8	3607 ± 10	–	–
4	2429 ± 7	2931 ± 15	–	–
6	2251 ± 9	305 ± 9	–	–
8	1840 ± 9	2854 ± 6	–	–
10	1776 ± 6	1927 ± 8	4095 ± 26	4231 ± 31
12	2102 ± 10	2028 ± 11	4791 ± 15	4452 ± 34
14	2429 ± 12	2132 ± 7	4443 ± 18	4262 ± 34
16	2551 ± 11	2351 ± 12	4228 ± 17*	3995 ± 27
18	2265 ± 7	2813 ± 13	4184 ± 21*	4071 ± 19

All values represent Mean ± SD from six observations.
[NS]Nonsignificance; *Significant at $p<0.05$. All other values are significant at $p<0.01$. AIU = One α-amylase inhibitor unit is equal to the number of α-amylase units inhibited. One α-amylase unit = mg starch hydrolysed per min at 30°C under the assay conditions.

fourth day. AI activity in the tuber increased from the 12th DAP to 18th DAP in Kanhangad and up to the 16th DAP in S 1195 (Table 3).

Inhibitor Changes Upon Wounding

Artificial wounding of the leaf of sweet potato induced considerable synthesis of inhibitor protein. The rise in the inhibitor protein was more evident in the leaves far off from the wounded leaf, ie. L3, L4 and L5 (Figure 4). The inhibitor concentration shot up rapidly in the leaves within 4 h, then remained at almost the same levels up to 16 h. However, in the leaflets very close to the wounded leaflet, *viz*. L1 and L2, a sharp increase in TI was observed from 8-16 h, which decreased further until 48 h. A similar pattern in chymotrypsin inhibitor content was also observed in the sweet potato leaves upon wounding (Figure 4). After an initial increase of CI after 4 h of wounding, the levels were almost static in most leaflets. A surge in AI activity was observed after 8 h and 16 h in the sweet potato leaflets above the wounded leaflet (L2) from the same branch, which then decreased after 24 h. However, only a slight increase in AI activity was observed after 8 h of wounding in the leaflet below the wounded leaflet from the same branch (L1). An increase in AI level was also observed in the other leaflets from the wounded branch. In other leaves, no noticeable change in AI content was observed (Figure 4).

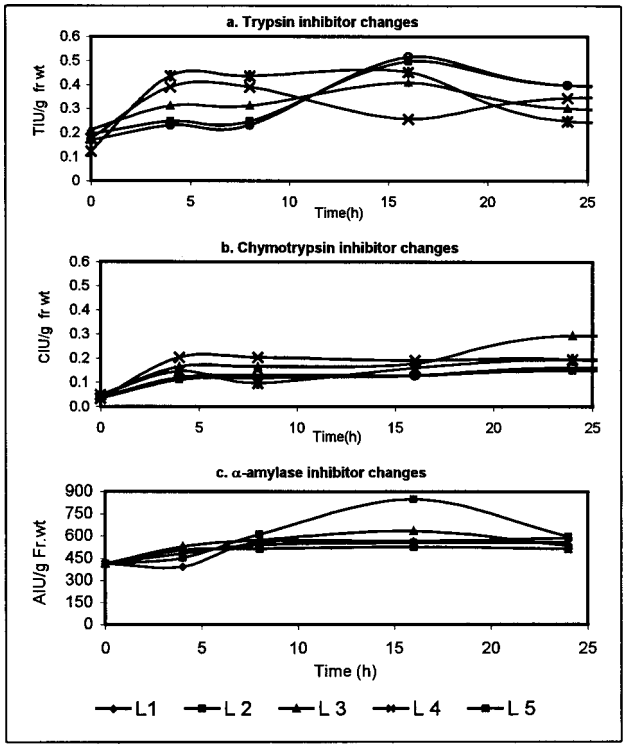

Figure 4. Changes in proteinase and α-amylase inhibitor activity in sweet potato tissues upon artificial wounding. Levels expressed as inhibitor units/g fresh wt. The values represent mean values from three replications.

Discussion

A steep increase in TI activity was observed in the tuberous roots of sweet potato during the tuber bulking phase (75-90 days) in three genotypes Kannukaruppan, RS III-2 and S 1195 indicating a possible role of the inhibitor in regulating the activity of plant proteases during tuber bulking. Ryan et al. (1968) reported a transitory existence for the chymotrypsin inhibitor in potatoes. The inhibitor completely disappeared during sprouting of the potato tuber and accumulated in the stem and aerial parts quickly. They found that its concentration was influenced by the meristematic growth and was highest at a time just preceding new growth, indicating that these inhibitors might be closely associated with internal changes in the plant, which are necessary for the initiation of new growth. In this study, no definite correlation was observed between the TI content in the leaf, stem, or tuber of a particular accession at any stage of growth. Also, considerable variation in the inhibitor levels was observed among the different genotypes. Zhang and Corke (2001) observed that the qualitative differences in the inhibitor proteins could be responsible for variation among genotypes.

The relatively low amount of CI in the sweet potato tissues during 70-90 DAP indicate that these proteins played only a trivial role in the regulation of plant proteases during tuber bulking. However, the high AI activity in the storage roots of most sweet potato genotypes is an indication of a regulatory role for the α-amylase inhibitor in sweet potato.

The trypsin and chymotrypsin inhibitor content was reported to decline gradually in the endosperm of sorghum, and by the sixth day it had disappeared in sorghum seeds (Mulimani and Vadiraj, 1993). This decrease in the inhibitor content of seeds after germination has been reported in many cereals and legumes also (Hartl et al., 1986; Mundy, 1984; Kamalakannan et al., 1981). TI and CI of sweet potato tubers seemed to be mobilized and degraded at the time of sprouting in this study, and the decrease in TI activity coincided with the emergence of sprouts. High TI activity in the sprouts indicates the possibility of its synthesis there and could have a protective/defensive role of safeguarding the newly emerged sprouts from pathogens. Biosynthesis of TI was reported to be increased by exogenous plant regulators and water deficiency (Lin and Tsai, 1992), indicating a physiological role for them. This study suggests that sweet potato TI might also have a role as reserve protein. The trypsin inhibitor purified from sweet potato sprouts by Hou and Lin (1997) was reported to exist as a complex with polyamines, and the polyamine-bound TI may play a vital regulatory role in sweet potato.

In comparison to the tubers, the newly emerged sprouts had very high alpha amylase inhibitor activity, indicating that the inhibitor may exert a protective mechanism in the new sprouts against invading pathogens/pests. Mulimani et al. (1994) reported that the AI activity of chick pea decreased as the days of germination increased and after sixth day, only negligible activity was detected in the seeds. Pace et al. (1978) established a clear regulatory role for the AI in wheat. The amylase inhibitor started accumulating after 8 days of fertilization, rapidly increasing with maturation and reaching a maximum when the seed was fully mature. Ambekar et al. (1996) found that the TI and AI slowly degraded during the germination of pigeon pea.

Graham et al. (1986) reported that the accumulation rate of inhibitor in tomato leaves following wounding varied between batches of young plants, but the time of initial detection was usually about 4-5 h. A similar increase in TI levels was observed in sweet potato leaves during this study. A rise in proteinase and amylase inhibitor levels was observed in leaves far from the wound. Cortes et al. (1988) observed that wounding of either leaves or tubers of potato led to the systemic induction of PI-II expression in both upper and lower leaves as well as in upper parts of the stem whereas no induction was detected in roots or lower segments. The proteinase inhibitor induction in *Nicotiana attenuata* leaves by different wounding treatments feeding by *Manduca sexta* larvae, methyl jasmonate treatment, and mechanical wounding was reported by Van Dam et al. (2001), who observed that the response to methyl jasmonate was stronger and longer lasting than mechanical wounding.

Trypsin inhibitors from sweet potatoes could have a role in regulating the endogenous proteases during tuber bulking in addition to serving as reserve proteins. A clear role as regulatory proteins was indicated for the α-amylase inhibitors of sweet potato, with their concentration increasing during the tuber bulking phase. High inhibitor activity in the newly emerged sprouts offers a protective mechanism to the young shoot. Wound-induced synthesis of secondary metabolites has been reported in many crop plants, as an immediate response. No reports are available on the inhibitor synthesis in field grown plants resulting from insect attack. Such studies can highlight the actual potential of these inhibitors in engineering resistance in plants. The findings open up the possibility for developing insect/pathogen resistant transgenic plants in sweet potato. Sweet potato leaves and vines are widely used as animal feed, either fresh or after ensiling (Lin et al., 1988). The information on the genotypic differences in inhibitor content at various growth stages could also improve the efficiency of its use as animal feed.

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甘藷中的蛋白酶及 α-澱粉酶抑制劑：在生長、發芽期間的變化及損傷所引起的改變

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本研究在甘藷生長及發芽期間，監測蛋白酶（胰蛋白酶及凝乳胰蛋白酶）及 α-澱粉酶抑制劑（AI）的變化，以證實這些植物萃取成分的生物性角色。雖然胰蛋白酶抑制劑（TI）的活性於栽種後 (DAP) 15 天，在四種基因型的甘藷塊根成形期間非常的低，但根據觀察，在塊根體積變大的時期卻急劇上升，這顯示了該抑制劑在調節植物蛋白酶中，可能扮演著重要的角色。相反地，凝乳胰蛋白酶抑制劑 （CI） 活性，從栽種 40-90 天後即顯著降低，表示它在蛋白酶的調節上並不是很重要。在三種基因型甘藷的塊根體積增長起始期， α-澱粉酶抑制劑突然顯著增加，且於種植 105 天後，也就是塊根體積增長完成時期，活性突然明顯下降，所以從本研究的結果，可以闡明 AI 在調節甘藷內生性 α-澱粉酶的角色。將塊根栽種在蛭石中直到第 6 天，TI 活性持續增加，但芽冒出後即下降，顯示發芽期間蛋白質開始進行水解的可能性。AI 活性則持續下降直到發芽，表示 AI 在甘藷中為氮儲存蛋白質。當組織受到損傷時，在四種基因型甘藷的葉中，蛋白酶抑制劑均有顯著的增加。且相較於受損葉片，在沒有受損傷的葉片中增加的更明顯。甘藷的蛋白酶及澱粉酶抑制劑乃由傷害所引發的這個事實，表明了其主要扮演著一個防禦性的角色。因為甘藷常用來當作動物的飼料，所以，蛋白酶抑制劑在甘藷葉、藤蔓中的含量，以及在植物生長各階段的變化等更具意義。

關鍵詞： α-澱粉酶抑制劑；胰凝乳蛋白酶抑制劑；生長期；甘藷（*Ipomoea batatas*）；發芽；胰蛋白酶抑制劑；損傷。