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Role of Calonyctin on Free Sugars in Relation to Starch Accumulation in Developing Sweet Potatoes

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Abstract. Calonyctin, a natural plant growth regulator extracted from the leaves of *Calonyction aculeatum* (L.) House, can promote crop growth and increase crop yield. The specific reasons for this response are unknown. This study was conducted to determine the effect of calonyctin treatment on the free sugars of sweet potato [*Ipomoea batatas* (L.) Lam.] as related to starch accumulation. The sweet potatoes were grown in the field in 1992, treated by foliar spray with calonyctin concentrations of 0 (control) and 0.1 activity unit (CTSP) at 20 days after planting (DAP) at the rate of 190 liters of diluted solution/ha., and sampled periodically to determine free sugars. The response of sweet potato to calonyctin was first detected at 40 days after treatment (on 60 DAP). Data indicated that calonyctin treatment significantly increased starch synthesis in storage roots, decreased the fluctuation tendency of total sugar level during the growth period, and kept the sugar level relatively constant with a gradual rise regardless of variations in weather. The level of the reducing sugars in CTSP leaves was higher at 60 and 160 DAP and lower at 100, 120, and 140 DAP. During rainy days (100 DAP), the reducing sugars in CTSP storage roots remained at a lower level when those in controls reached high levels. The sucrose content in CTSP leaves was 40–138% greater than that in controls except at 80 and 120 DAP, and the ratio of sucrose to total nonreducing sugars remained at 100% in CTSP leaves even on rainy and cool days and above 96% in CTSP storage roots except on cool days (140 and 160 DAP), suggesting that calonyctin treatment promoted the synthesis and transfer of sucrose and supplied abundant sugar precursors for starch accumulation in storage roots.

Key Words. *Ipomoea batatas* (L.) Lam.—*Calonyction aculeatum* (L.) House—Growth regulation

Calonyctin is a natural plant growth regulator extracted from leaves of *Calonyction aculeatum* (L.) House (Wang 1975). Its study originated from the grafting test of *C. aculeatum* on sweet potato [*Ipomoea batatas* (L.) Lam.], after grafting storage root fresh weight increased from 2 to 10–15 kg per plant (Ding et al. 1952). The chemical structure of calonyctin has been determined and found to be a tetrasaccharide containing two hydroxy fatty acid residues (3-hydroxy-2-methylbutanoic acid and 11-hydroxytetradecanoic acid) and four 6-deoxyhexose units (three of quinovose and one rhamnose) (Fang et al. 1993, Guo et al. 1980). The tetrasaccharide moiety of the calonyctin molecule has been synthesized (Jiang and Schmidt 1994). Its physiologic activity has been tested on the flowering of peanut, growth of rice seedlings and sweet potato plants (Wang 1975), and plant regeneration of rice callus tissues (Wang and Xu 1980). Previous studies showed the promoting effect of calonyctin on agronomic characters and yield (Shen et al. 1994) and metabolism of sweet potato (Shen et al., submitted for publication) and found an average increase of 14% in sweet potato storage roots (Shen et al. 1994) and about an 18% increase in potato (Shen et al., in press). In this report the effect of calonyctin, with a concentration suitable for yield increase, on the free sugars as related to starch accumulation in developing sweet potato under field condition was studied.

Materials and Methods

Calonyctin was extracted by the method of Fang et al. (1993). The leaves of *C. aculeatum* were collected from the university farm. Dried leaves (200 g) were ground and extracted with 95% ethanol under

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Abbreviations: DAP, days after planting; CTSP, calonyctin-treated sweet potato with 0.1 activity unit.

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Table 1. Variation in weather conditions during sweet potato development period.

Index	Days after planting					
	41–60	61–80	81–100	101–120	121–140	141–160
Average air temperature (°C)	30	28	27	23	18	15
Average relative humidity (%)	71	76	86	81	72	68
Total rainy days	6	10	16	8	4	3
Total rainfall (mm)	12	90	154	118	3	3
Total water evaporation (mm)	166	100	79	63	73	66
Total sunshine time (h)	207	98	90	71	111	146
Weather category	Sunny	Warm	Rainy	Wet	Cool	Cool

reflux, then decolorized by passage through a charcoal column that had been previously treated with acetic acid. The glycosides were eluted with 95% ethanol, and the ethanol was evaporated. The residue was taken up in water. The calonyctin concentration of the extract was measured as activity unit by a biologic method (Wang 1975); rice (*Oryza sativa*) cultivar Xiushui 37 was used as an indicator.

Yield tests of sweet potato were carried out with a total of six different cultivars during 1992, 1993, and 1994, at north and south Zhejiang Province and at high and low altitudes. Calonyctin concentrations of 0.05, 0.1, 0.2, and 0.5 activity unit were used to foliar spray the sweet potato plants. Consistently increased yields from 10.2 to 21.3% were obtained with 0.1 and 0.2 activity unit calonyctin treatments (Shen et al. 1994). In the present experiment sweet potato cultivar Xushu 18 was used to show the developmental progress under calonyctin regulation. The experiment was conducted at the university farm in 1992 and was a randomized block design using three replicates. Each plot was 10 m long and 2 m wide, with a total of 90 plants raised. Conventional cultivation methods were followed. Because this was the first year of yield tests and it was yet unknown what concentration would increase the yield, the following four concentrations of calonyctin were made: 0 (distilled water, controls), 0.05, 0.1, and 0.5 activity unit. All treatments were applied as foliar sprays at 20 days after planting (DAP) at the rate of 190 liters of diluted solution/ha. The sweet potatoes were planted on May 30, sprayed June 20, and harvested December 7.

To demonstrate how calonyctin with a suitable concentration functions to increase sweet potato yield, only samples treated with calonyctin concentration 0 (controls) and 0.1 activity unit (CTSP) were used to determine free sugars and starch because only the storage roots of CTSP were found significantly increased previously (Shen et al. 1994). The fourth unfolded leaves at vine tips and well growing storage roots of ten random plants of each plot were used for analysis of free sugars and starch at 20-day intervals after treatment. The free sugars were extracted with 80% ethanol by the method of Ozbun et al. (1973). The total sugar content of the extract was assayed by the anthrone method with glucose as the standard (Handel 1968). Reducing sugars were estimated by the method of dinitrosalicylic acid reagent (Miller 1959), and nonreducing sugars were calculated by difference. Sucrose was analyzed by the method of direct microdetermination (Handel 1968). Starch was extracted by $HClO_4$ hydrolysis from the remains after free sugar extraction and determined using the anthrone method according to Yoshida et al. (1971).

Results

Weather Condition during Sweet Potato Development Period

Because carbohydrate metabolism is greatly affected by weather conditions, it is important to give meteorologic data when starch and free sugar data are presented. According to the degree of affecting sweet potato growth, the main meteorologic factor was used to refer to sunny, warm, rainy, wet, and cool days (Table 1).

Effect of Calonyctin on Storage Roots and Starch

Data indicated that calonyctin treatment significantly promoted storage root growth and starch accumulation (Table 2). Storage root fresh weight of CTSP remained 15–17% higher than controls from 60 through 160 DAP. Starch collected in storage roots in 20 days was 13, 52, 8, and 27% higher in CTSP than in controls at 60, 80, 140, and 160 DAP, respectively. Starch did not accumulate on rainy days (100 DAP) in either CTSP or controls. During 101–120 DAP, although storage root fresh weight increased 30% for both CTSP and controls, starch was not increased in controls because of the higher water content in storage roots, but starch in CTSP increased 10 g.

Effect of Calonyctin on Total Sugars

The total amount of sugars in sweet potato leaves increased by about 80% following calonyctin treatment at 60 DAP and 27% at 160 DAP. Total sugars were higher for control than CTSP at 100 and 120 DAP but were similar for calonyctin-treated and untreated leaves at 80 and 140 DAP (Table 3). The general fluctuation tendency of the sugar level was not changed by calonyctin treatment during the whole growth period.

The total sugars in CTSP and control storage roots were similar at 40 DAP but 30% higher for CTSP than control at 60 DAP (Table 3). From 80 DAP, the sugar level increased progressively from 2.41 to 3.44% in CTSP storage roots regardless of the change of weather; however, in control storage roots wide fluctuations were observed.

Effect of Calonyctin on Reducing Sugars

The change in the level of reducing sugars in sweet potato leaves was similar to that of total sugars (Table 4).

Table 2. Effect of calonyctin on fresh weight and starch synthesis of sweet potato storage roots at different stages of development.

	Days after planting						
Treatment	40	60	80	100	120	140	160
Fresh weight of storage roots (g/plant)							
Control	12	114	212	214	278	517	672
CTSP	12	131**	244**	248**	325**	607**	779**
Starch increased (g/plant/20 days)							
Control	1	23	27	1	0	38	30
CTSP	1	26*	41**	0	10**	41*	38**

* $p < 0.05$; ** $p < 0.01$.

Table 3. Effect of calonyctin on total sugars in sweet potato leaves and storage roots at different stages of development.

	Days after planting						
Treatment	40	60	80	100	120	140	160
Leaves							
Control		0.17	0.28	0.12	0.26	0.78	0.29
CTSP		0.28**	0.24	0.07*	0.18*	0.74	0.37**
Storage roots							
Control	1.93	2.12	2.47	3.71	2.80	2.21	3.29
CTSP	1.92	2.75**	2.41	2.54**	2.76	2.96**	3.44

Data listed are expressed as percent of fresh weight. * $p < 0.05$; ** $p < 0.01$.

Table 4. Effect of calonyctin on reducing sugars in sweet potato leaves at different stages of development.

	Days after planting					
Treatment	60	80	100	120	140	160
Percent of fresh weight						
Control	0.16	0.26	0.09	0.22	0.74	0.25
CTSP	0.25**	0.22	0.05*	0.17*	0.65**	0.32**
Percent in total sugars						
Control	94	93	74	84	94	84
CTSP	91	91	64	92	87	86

* $p < 0.05$; ** $p < 0.01$.

The reducing sugars attained the highest level at 140 DAP and reached the lowest level on rainy days (100 DAP) in both CTSP and controls. CTSP leaves generally had reducing sugar levels 12–44% lower than those of controls but had a 56 and 28% higher level at 60 and 160 DAP, respectively.

The ratio of reducing sugars to total sugars in leaves was above 90% during early growth up to 80 DAP and about 85% at the last harvest in both controls and CTSP (Table 4). It dropped sharply to 74% in controls and 64% in CTSP on rainy days (100 DAP). After rain (120 DAP) it rose again, immediately in CTSP, but with a much lower rate in controls.

Storage root reducing sugars decreased to a minimum in both controls and CTSP during 40–80 DAP, but there was a 50% increase at 60 DAP in CTSP (Table 5). On rainy days (100 DAP) its level was low in CTSP but 2.5 times higher in controls. After 120 DAP, its level showed a consistent increase in both CTSP and controls but was 38 and 29% higher in CTSP than in control at 120 and 140 DAP, respectively. The ratio of reducing sugars to total sugars was below 50% in both controls and CTSP storage roots, and its change paralleled that of reducing sugars (Table 5).

Table 5. Effect of calonyctin on reducing sugars in sweet potato storage roots at different stages of development.

	Days after planting						
Treatment	40	60	80	100	120	140	160
Percent of fresh weight							
Control	0.82	0.63	0.28	1.02	0.55	1.12	1.64
CTSP	0.78	1.17**	0.23	0.28**	0.76*	1.44*	1.56
Percent in total sugars							
Control	42.5	29.7	11.7	27.5	19.6	50.0	49.8
CTSP	40.6	42.5	11.6	11.0	27.5	48.6	45.3

* $p < 0.05$; ** $p < 0.01$.

Effect of Calonyctin on Sucrose

Sucrose level in sweet potato leaves reached the maximum at 140 DAP, decreased on rainy days (100 DAP, for controls) or after rain (120 DAP, for CTSP) and during maturing stage (160 DAP). It kept constant up to 100 DAP in CTSP and was 81, 40, 102, and 138% higher than those in controls at 60, 100, 140, and 160 DAP, respectively (Table 6).

The ratio of sucrose to total sugars in leaves ranged from 5 to 13% in controls and from 10 to 13% in CTSP at most stages of development; however, it increased to 17% in controls and 40% in CTSP on rainy days (100 DAP) (Table 6). The ratio of sucrose to total nonreducing sugars remained at 100% at all growth stages in CTSP leaves, but it showed wide fluctuations in the leaves of controls.

The level of sucrose in storage roots increased with time up to 100 DAP and then declined thereafter in both CTSP and controls, and it was higher in CTSP than in controls at 60 and 140 DAP (Table 7).

The fluctuation range of the ratio of sucrose to total sugars in storage roots was similar for controls and CTSP (Table 7). During sunny and warm days (40–80 DAP), the ratios increased progressively from 61 to 87% in controls, but in CTSP they remained at 61% during the first 20 days and then increased to 84% in the second 20 days. During rainy days (100 DAP) the ratio reached 89% in CTSP and fell to 61% in controls. The ratio dropped during cool days.

The ratio of sucrose to total nonreducing sugars was above 95% on sunny and warm days (40, 60, 80, and 120 DAP) and fell on cool days (140 and 160 DAP) in both control and CTSP storage roots (Table 7). During rainy days it dropped in controls but kept high in CTSP.

Discussion

All of the data indicated that the differences between controls and CTSP were first detected at 60 DAP (40 days after calonyctin treatment). This meant that sweet potato plants needed at least 20 days to be prepared to respond to the calonyctin treatment.

The level of free sugars in sweet potato depends largely on the amount produced in leaves through photosynthesis and their use in storage roots for starch synthesis. The difference of free sugar levels between CTSP and controls is the difference of sugar production and use between these sweet potatoes. Studies showed that CTSP plants had a much larger leaf area (Shen et al. 1994) and accumulated much more starch in their storage roots than controls, whereas leaf photosynthetic rates were similar for CTSP and controls (Shen et al., submitted for publication). So the levels of reducing sugars and sucrose in both leaves and storage roots were higher in CTSP than in controls when the increase of leaf area was more than the increase of starch accumulation after calonyctin treatment, such as 40–60 and 140–160 DAP. These sugar levels were lower in CTSP than in controls at 60–80 DAP because the enzymes responsible for starch synthesis in CTSP storage roots were greatly activated (Shen et al., submitted for publication), and the increase of starch accumulation was more than the increase of leaf area after calonyctin treatment. The free sugar level in CTSP was also low on rainy days (100 DAP) and the stage just after rain (120 DAP) because of the higher rates of vine growth (Shen et al. 1994) and starch accumulation in storage roots, respectively.

Besides large leaf area and high activities of enzymes for starch synthesis in storage roots, another good explanation for yield improvement by calonyctin treatment seemed to be that the reducing sugars in leaves were more easily transformed into sucrose and supplied abundant sugar precursors for starch synthetase in storage roots, which was supported by the fact that CTSP leaves generally contained much less reducing sugars and much more sucrose than control leaves. Further, CTSP plants kept the ratio of sucrose to total nonreducing sugars constant at 100% during the entire growth period including rainy and cool days.

Calonyctin treatment regulated the change of total sugar level in storage roots at different stages of development. The free sugars in control storage roots were from a relatively small leaf area (Shen et al. 1994) and were not abundant. Accordingly, the total sugar level was low when the starch was synthesized at a high rate on sunny and warm days, but it was at a high level on rainy and cool days when starch synthesis was low and

Table 6. Effect of calonyctin on sucrose in sweet potato leaves at different stages of development.

	Days after planting					
Treatment	60	80	100	120	140	160
Percent of fresh weight						
Control	0.016	0.027	0.020	0.033	0.041	0.021
CTSP	0.029**	0.028	0.028*	0.018**	0.083**	0.050**
Percent in total sugars						
Control	9	10	17	13	5	7
CTSP	10	12	40	10	11	13
Percent in nonreducing sugars						
Control	100	100	80	93	100	53
CTSP	100	100	100	100	100	100

* $p < 0.05$; ** $p < 0.01$.

Table 7. Effect of calonyctin on sucrose in sweet potato storage roots at different stages of development.

	Days after planting						
Treatment	40	60	80	100	120	140	160
Percent of fresh weight							
Control	1.18	1.53	2.15	2.24	2.14	1.02	0.92
CTSP	1.20	1.66*	2.03*	2.25	1.92*	1.15*	0.98
Percent in total sugars							
Control	61	72	87	61	76	45	27
CTSP	62	61	84	89	70	39	28
Percent in nonreducing sugars							
Control	100	100	99	84	95	89	52
CTSP	100	100	96	100	96	76	53

* $p < 0.05$; ** $p < 0.01$.

α-amylase was greatly activated (Shen et al., submitted for publication), and a large amount of reducing sugars was released from starch hydrolysis. However, the free sugars in CTSP were from a large leaf area and were more than enough for starch synthesis on sunny and warm days; α-amylase activity was not raised on rainy and cool days (Shen et al., submitted for publication); therefore the level of the total sugars remained relatively constant with a gradually rising tendency.

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