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EFFECT OF SUCROSE LEVEL ON RICE GRAIN DEPOSITION IN DETACHED PANICLE SYSTEM

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Key Word Index—Rice; detached panicles; sucrose level; rice grain size; rice grain protein.

Abstract—In detached IR36 rice panicles incubated in liquid medium for 10 days in 1.0–2.0% sucrose and 0.6% glutamine, final panicle weight increased but percentage and weight of grain protein decreased with 1.0–1.75% sucrose. Soluble sugars increased in stem and hull but not in developing grain. With five other rice panicles and IR36 panicles differing in grain size incubated in liquid culture containing 0.075% glutamine for 7 days, percentage grain protein was again lower in 1.5% sucrose than in 1.0% sucrose, with correspondingly heavier grain weight in four cases. Free-sugar levels of developing grains were lower in detached panicles than in the field grain samples in both experiments. Thus, sucrose level has a depressing effect on protein accumulation in the developing rice grain. Lysine content of grain protein decreased with increase in protein content.

INTRODUCTION

In the developing rice grain, nitrogen is derived mainly from leaf nitrogen absorbed by the plant before anthesis, whereas carbohydrate is derived mainly from photosynthesis after flowering and from stored starch in the culm and leaf sheaths [1]. Previous studies on the detached rice panicle system in a complete liquid medium demonstrated that the sucrose level (0–1.5%) in the medium determined the extent of dry matter and starch accumulation and influenced physiological development of the ripening grains [1]. Protein content was readily manipulated by changing both the glutamine and sucrose level in the medium at a constant glutamine:sucrose ratio of 1:10. To follow up our previous study on the detached rice panicle system [2], we studied the effect of sucrose level in the medium and grain size on dry matter and protein deposition in developing rice grain at a constant glutamine level in the medium.

RESULTS AND DISCUSSION

Effect of sucrose level

The first set of results on detached IR36 rice panicles showed a positive correlation between sucrose concentration in the culture media and total grain weight per panicle ($r = 0.64^{**}$; $n = 15$) after 10 days (Table 1). The increase in panicle weight was manifested even though glutamine concentration in the medium was kept constant at 0.06%. Mean caryopsis weight of the 10-day-old grains tended to increase with increasing sucrose level to be comparable to that of the field control. Singh *et al.* [2] found a correspondence between grain weight and sucrose level in the medium. Although in detached panicle culture it was possible to produce grains with a physiological age comparable to that of field samples, the large disparity in caryopsis weight indicated that the *in vitro* conditions employed in this study still needed improvement.

The number of fertile grains per panicle was maximal from 1.25% to 1.75% sucrose (24–25/panicle). A gradual desiccation of the whole panicle toward the end of the culturing period was more pronounced in the higher sucrose treatments as evidenced by the higher percentage dry matter of those samples. The cultured panicles generally exhibited two to three times more empty and very small grains (1.0–1.3 mg/grain) compared to the control samples. Florets that opened in the growth chamber failed to set seed. Thus, samples had to be obtained from the field at about two days after anthesis.

Soluble sugars in the 10-day-old grains did not vary much among treatments, in contrast to those in the hull and stem which closely followed the sucrose level in the culture solution (Table 1). Moreover, soluble sugars in the grain of cultured panicles were about one-third that in the field control although the levels in the stem and hull were already three–seven times more. These data indicate the existence of a restriction or a control mechanism which limits sucrose transport from the stem into the developing grain. Similar observations have been reported by Jenner and Rathjen [3] in their culture of detached wheat ears.

No significant differences in grain free-amino nitrogen levels due to increasing sucrose treatment were obtained (Table 1). Even the field control showed values similar to those of the cultured samples.

One very significant correlation was between percentage protein in the developing grains (mid-milky stage) and sucrose concentration in the media. As sucrose level increased, percent protein decreased ($r = -0.93^{**}$; $n = 15$). This decrease in grain protein may be associated with the higher panicle weight at higher levels of sucrose. In contrast, protein per grain was relatively constant except in 1.0% sucrose culture (Table 1). The negative correlation between yield and percent protein in the grain has been repeatedly established under greenhouse and

Table 1. Effect of sucrose level in a complete liquid medium containing 0.06% glutamine on properties of developing grains and hull plus stem of IR36 rice panicles cultured for 10 days

Property*	Sucrose level in medium (%)					Field control	LSD (5%)
	1.00	1.25	1.50	1.75	2.00		
Developing grain							
Grain dry matter (%)	47.7	52.3	51.8	59.7	64.1	59.0	6.7
Rough rice wt (mg/panicle)	295	367	413	379	492	930	180
Caryopsis wt (mg/panicle)	203	282	306	298	316	745	10
(mg/grain)	11.4	11.4	12.0	12.3	14.2	14.1	1.0
Crude protein (%)	11.9	10.3	9.6	8.3	8.0	10.7	1.1
Crude protein (mg/grain)	1.36	1.17	1.15	1.02	1.14	1.51	0.15
Soluble sugars (mg glc/grain)	0.15	0.12	0.16	0.14	0.17	0.51	0.05
Soluble sugars (% glc)	1.34	1.09	1.29	1.17	1.22	3.66	0.30
Free-amino acids (g leu/grain)	37	41	41	36	34	45	NS
Free-amino acids (% leu)	0.32	0.36	0.34	0.29	0.24	0.34	NS
Weight of grain protein/ panicle (mg)	24.1	29.0	29.2	24.7	26.4	79.3	1.5
Hull plus stem							
Soluble sugars (% glc)	5.4	6.6	8.4	10.7	12.8	1.9	1.5
Free-amino acids (% leu)	1.1	1.4	1.0	0.7	0.8	0.3	0.3

* Percentage all on freeze-dried (6% moisture) basis except grain dry matter which was on fresh weight basis. Loss of weight during freeze-drying was considered as moisture content.

field conditions for rice above the threshold level for yield and protein content [4]. Kjeldahl N was considered mainly crude protein N since RNA is only a small fraction of protein in developing and mature rice grain [5].

The decrease of percent protein in the grain with higher concentration of sucrose supply followed the decrease of free-amino nitrogen level in the stem and hull ($r = 0.60$*; $n = 15$). However, there was no significant correlation with grain free-amino nitrogen, which was similar for all the treatments. Thus, it seems apparent that when the yield is greater, amino acids are exhausted faster in the supply route outside the grain, but the pool within the grain tends to remain at a constant level regardless of protein content. An earlier study of developing rice grains showed that higher-protein varieties or lines tended to have higher levels of free-amino nitrogen [6]; however, that study involved varieties under field conditions. Maximal levels of soluble amino acids have been found to occur between eight to twelve days after flowering [5].

A gradual desiccation of the panicle starting from the hull tips was observed toward the end of the culturing period. This was more pronounced in the 2.00% sucrose treatment as manifested in the higher percentage dry matter (lower moisture content) of the sample (Table 1). Consequently, 1.5% was used as the optimal sucrose concentration in the subsequent culture of several other varieties differing in grain size and protein content.

Detached panicles of different varieties

The improved panicle weight associated with an increase in sucrose concentration in the media was again verified in both total and developing grains of the six varieties used (Table 2). However, the weights were still much lower than those obtained from panicles grown in the field. The smaller-grained varieties, Bomdia and Kalajira, adapted better in 1.5% sucrose than in 1.0% sucrose. This was reflected in the substantial increases in panicle weight, which were due to the fact more grains developed progressively in the higher sucrose concentration. Kalajira specifically produced only early milky grains at 1.0% sucrose and two out of three panicles dried up during culture. This sensitivity to lower sucrose concentration was not observed in the bigger-grained varieties. Moreover, Bomdia and Kalajira also showed the least incidence of hull tip desiccation even in 1.5% sucrose, which was sometimes observed toward the end of the culturing period. It could be that the bigger stems and hence greater area of conducting tissues of the heavier-grained varieties gave them on inherent advantage in *in vitro* cultures where the balance between transpiration and solution uptake is essential. Most of the water that goes to the developing grains transpires through the lemma and palea [7, 8].

Percentage protein of the midmilky grains also showed a lower value at the higher sucrose treatment (Table 2) as was observed in the earlier IR36 experiment. It approached that of the field controls especially for the bigger grained varieties. Only in the two higher protein rices IR480-5-9 (1.66 and 1.48 vs 1.36 mg) and IR2153-338-3 (0.92 and 0.93 vs 0.81 mg) were the protein levels per grain higher in detached-panicle samples than in the field samples. Percent protein in the grain and soluble amino nitrogen level in the stem plus hull were not significantly correlated for the test samples, but they were significantly highly correlated in the control samples ($r = 0.75$**; $n = 15$).

The soluble sugar percentage and content per grain of the test samples tended to be lower than those of the control (Table 2), except for Bomdia where the levels were almost similar for the two treatments and the control. Kalajira had 8.0% soluble sugars (0.24 mg/grain) in 1% sucrose culture. However, the final grain of Khao Lo had the highest sugar level in the grain (3.5–4.8%,

Table 2. Mean properties of developing grains and hull plus stem of two rices each with small, medium and large grains cultured as detached panicle for seven days with 0.075% glutamine and 1.0 or 1.5% sucrose as compared to field samples*

Property	1.0% sucrose			1.5% sucrose			Field control			LSD (5%)
	Small	Medium	Large	Small	Medium	Large	Small	Medium	Large	
Developing grains										
Rough rice wt (mg/panicle)	145	263	338	235	328	418	527	658	697	143
Caryopsis wt (mg/panicle)	78	174	206	124	236	244	340	576	454	157
(mg/grain)	4.3	6.4	11.8	5.2	7.9	12.0	6.2	10.0	14.0	1.1
Moisture content (% wet basis)	52	53	58	48	50	60	54	50	56	4
Crude protein (%)	14.4	13.7	14.0	11.6	11.0	11.9	10.1	9.8	11.5	1.0
Crude protein (mg/grain)	0.62	0.88	1.64	0.62	0.87	1.43	0.63	0.98	1.62	0.16
Free-sugars (% glc)	5.2	1.9	2.6	3.1	2.2	3.2	3.5	3.2	3.8	0.8
Free-sugars (mg glc/grain)	0.19	0.14	0.32	0.14	0.20	0.38	0.22	0.29	0.54	0.17
Free-amino acids (% leu)	1.7	0.60	0.53	0.50	0.66	0.52	0.65	0.52	0.47	0.20
Free-amino acids (mg leu/grain)	0.06	0.04	0.10	0.03	0.05	0.10	0.04	0.04	0.10	0.02
Hull plus stem										
Free-sugars (% glc)	10.2	5.0	3.3	11.1	7.2	6.0	4.5	2.2	2.4	1.7
Free-amino acids (% leu)	0.6	0.6	0.6	0.4	0.6	0.5	0.2	0.2	0.4	0.2

*Small: Bomdia and Kalajira; medium: IR2153-338-3 and IR36, and large: IR480-5-9 and Khao Lo.
All percentages on freeze-dried basis except moisture.

0.4–0.8 mg/grain). In the stem plus hull, the two smaller grained varieties accumulated more soluble sugars (11.7–12.1% vs 4.7–9.8% in 1.5% sucrose) which were about two to three times that in the controls (3.6–5.4% vs 1.6–3.2% sugars). The differences were not as dramatic in the larger grained rices. These findings suggest that there is a relationship between total sink capacity and the influx of sucrose from the stem. Apparently, the smaller grains with smaller sink size attained their saturation levels for sucrose earlier and hence further transport into the grain diminishes. A gradual build-up of the metabolite thus occurred in the hull and stem tissues as transpiration and solution uptake progressed. The high sucrose level in the hull of Kalajira may have caused hull desiccation. Free-amino acids concentration was also highest in Kalajira grain in 1% sucrose culture than in the other developing grains. Although amino acid levels in the hull and stem of the test samples were higher than in the control, they did not show any definite trend with grain size.

Further sub-fractionation of the stem and hull tissues of IR36 after seven days of culture revealed that the sugars actually accumulated more in the hull than in the stem (Table 3). Sugar content expressed on tissue moisture basis was three to four times higher in the hull than in the grain. In contrast, field control samples gave comparable sugar values of 39.0 mg/ml water in hull and 48.4 mg/ml water in developing grain. Sugar content of grain in the field sample was higher than in the detached panicle system, but the opposite was true of the hull and stem sugars. In contrast, free amino acids in the test panicles tended to distribute more evenly among the developing grain, hull and stem tissues. In the field sample, however, the developing grain and hull had higher free amino acid levels than the stem. The results suggest that there was little barrier nor restriction to amino acid transport to the grain. In fact, the overall data from the field samples supported the operation of active transport [8] for both sucrose and amino acids because the concentrations in the

Table 3. Moisture content and concentration of soluble sugars and free-amino acids in IR36 detached panicles cultured for seven days in a complete liquid medium containing 0.075% glutamine and 1.0 or 1.5% sucrose as compared to field samples

Property*	Tissue	Detached panicle		Field control	LSD (5%)
		1.0% sucrose	1.5% sucrose		
Moisture (%)	Grain	51.1	50.6	44.5	NS
	Hull	29.6	31.7	34.1	3.1
	Stem	61.4	63.1	65.8	2.5
Soluble sugars (% glc)	Grain	0.87	1.40	2.15	0.48
	Hull	2.20	2.92	1.33	0.41
	Stem	1.75	2.05	0.81	0.19
Free-amino acids (% leu)	Grain	0.30	0.35	0.26	NS
	Hull	0.32	0.27	0.18	0.04
	Stem	0.40	0.41	0.08	0.09

*All recalculated on fresh weight basis.

developing grain were higher than those in the hull and stem.

However, the detached-panicle system is probably not directly comparable to the intact panicle, because the whole vascular bundle, not just the phloem, is exposed to the nutrient medium. Hulls and stems showed abnormally high nutrient levels, although the developing grain tended to have lower free sugars than that of the field samples in both experiments.

Assimilate translocation has been shown to involve the single vascular bundle at the dorsal region of the pericarp through the long distance pathway of the phloem [8], then a short-distance pathway between the terminal sieve elements of the pericarp vascular bundle, moving to the pigment strand cells and on to the nucellus. The rate limiting step is probably active transport from nucellus to the aleurone and sub-aleurone layers [9] or through the unusual cells with uneven walls at the dorsal aleurone layer [10]. The relative efficiency of active transport in the sucrose translocation system may explain the differences in response to the sucrose level in the culture medium in the detached panicle system and to variety relative to the field samples.

The amino acid composition of seven-day-old caryopses from two varieties grown in detached-panicle culture gave normal patterns quite similar to those of the control (Table 4). Lysine tended to be higher in the lower protein samples that were produced at higher sucrose concentrations. Tyrosine in protein of IR36 decreased with higher sucrose level. A negative correlation between percent protein and lysine content [11] and a positive correlation for tyrosine content [12] had been observed in field-grown grains. SDS-PAGE patterns of SDS-β-mercaptoethanol extracts of cultured grains revealed no differences with field samples [2]. Wheat heads cultured *in vitro* showed patterns of protein accumulation that closely resembled that observed in intact plants [13, 14].

Differences in composition among samples can be ascribed mainly to the endosperm fraction since the outer layers, pericarp, seedcoat, nucellus, aleurone layer and embryo are already fully developed in the milky stage grain samples [15]. Actual period of dry matter accumulation is 14 days after flowering for most varieties and 18 days after flowering for Khao Lo.

EXPERIMENTAL

Rice panicles were obtained at random from the IRRI experimental fields 2 days after anthesis. They were cut under distilled H_2O and immediately transferred to sterile H_2O for transport to the laboratory. The outside leaf sheaths were peeled off and the panicle stems were further cut under sterile H_2O to 12 cm long. They were transferred inside an UV chamber and the stems surface sterilized by wiping with cotton soaked in 0.5% NaOCl, then thoroughly rinsed in sterile H_2O again. After blotting the stems dry with sterile cotton, the panicles were inserted into cotton-plugged sterile Erlenmeyer flasks containing 40–75 ml of the culture medium. Preparation of the culture media and conditions for subsequent incubation were essentially those of ref. [2] except that the glutamine concentration was reduced to 0.06% and 0.075%, instead of 0.1%.

Sucrose concentration. IR36 panicles were cultured for 10 days in solutions containing from 1.0 to 2.0% at a constant concen-

Table 4. Amino acid composition of a developing caryopsis in detached panicle culture with 0.06% or 0.075% glutamine and 1.0 and 1.5% or 2.0% sucrose as compared to fields samples (g/16.8 g N)

	IR36 (10 days)				Khao Lo (7 days)			
	Detached panicle				Detached panicle			
Amino acid	1.0% sucrose	1.5% sucrose	2.0% sucrose	Field control	1.0% sucrose	1.5% sucrose	Field control	LSD (5%)
Caryopsis wt (mg)	11.4	12.0	14.2	14.1	12.1	11.8	15.6	1.1
Crude protein (%)	11.3	8.4	7.8	10.1	12.6	11.2	11.3	1.1
Lys	4.2	4.6	5.0	4.2	4.6	5.0	5.3	0.4
His	2.4	2.5	2.6	2.7	2.6	2.6	3.0	NS
NH_3	2.0	2.2	2.3	1.7	1.7	1.6	2.2	0.5
Arg	9.3	7.7	9.3	8.7	8.0	8.6	8.9	0.8
Asp	11.2	12.5	10.9	11.2	14.0	12.8	12.0	1.0
Thr	3.9	4.2	3.9	3.7	4.8	4.2	3.6	0.4
Ser	5.6	5.5	5.1	5.7	6.2	5.6	5.6	NS
Glu	20.5	20.3	18.8	22.7	20.2	19.2	18.6	1.4
Pro	6.1	6.1	5.8	5.9	5.5	6.1	5.7	NS
Cys	(1.0)*	(0.7)*	(1.1)*	(0.8)*	(0.9)*	(0.9)*	(0.7)*	NS
Gly	5.1	5.5	5.2	5.1	5.6	5.5	4.9	NS
Ala	6.4	7.2	6.5	6.8	7.2	6.9	7.0	0.5
Val	6.1	6.4	5.8	6.3	6.2	6.0	5.6	0.5
Met	(2.0)*	(1.3)*	(2.1)*	(1.4)*	(1.9)*	(2.3)*	(1.0)*	NS
Ile	4.2	4.4	4.0	4.4	4.4	4.2	4.1	NS
Leu	9.2	9.3	8.6	9.4	9.0	9.2	8.7	NS
Tyr	5.7	4.4	3.9	5.6	5.0	5.4	4.8	1.1
Phe	6.0	6.0	5.7	6.2	5.8	6.9	5.8	0.6

*Minimal values obtained by normal hydrolysis procedure.

tration of glutamine (0.06%). Comparable field controls were tagged at sampling time and were harvested 10 days after. All panicles were weighed, frozen, freeze-dried for further processing and reweighed. There were three replicates per treatment.

Varietal differences. Six rice varieties differing in grain size and protein content were similarly grown under detached panicle culture conditions for 7 days at 1.0 and 1.5% sucrose levels. Corresponding field controls for each variety were also harvested and treated similarly as described above. For IR36, panicles were subfractionated over ice into stems, hulls and grains before weighing, freeze-drying and reweighing.

Processing. Freeze-dried grains were separated from the stem and hull by manual dehulling. They were counted, weighed and sorted out according to age groups. Only grains at age of 6–7 days and 9–10 days were ground for chemical analysis. The 6- to 7-day-old grains were at the mid-milky stage, while the 9- to 10-day-old grains were at the late-milky dough stages. The grains were ground in a Wig-L-Bug Amalgamator and the stem and hull tissues in a Wiley mill with a 40-mesh screen.

Chemical analyses. Powdered, freeze dried materials were extracted with 80% EtOH at 70°. Clarified extracts were analysed for total soluble-sugars [16] and free-amino nitrogen [17]. Kjeldahl protein [16] was determined on all the grain samples using the factor 5.95 while amino acid analysis [16] was done on only two varieties, Khao Lo and IR36. Grain flours of Khao Lo and IR36 were extracted with 0.5% SDS–0.6% β-mercaptoethanol and subjected to SDS-PAGE electrophoresis according to ref. [16].

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