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Studies on Starch and Protein Contents of HPGMR during Pollen Abortion

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Abstract

The results have shown that: (1) During the development of fertile anthers, soluble sugar in function leaves is translocated to anthers and the starch content increases significantly; the content of soluble sugar decreases continuously in both anthers and spikelets. The physiological function of leaves doesn't decline under long day condition, and soluble sugar can also be transported to lemma and palea but seems to accumulate therein. The starch content increases only a little in sterile anthers, and the content of soluble sugar decreases slightly. (2) The content of soluble protein in fertile anthers decreases from the differentiating stage of pollen mother cells to the late uninucleate stage, but increases markedly after the binucleate stage; in the sterile anthers, however, the protein content almost decreases continuously. The total content of free amino acids coincides well with that of protein under either LD or SD induction but there is no obvious difference in any individual amino acid between sterile and fertile anthers. It can be considered that starch and protein synthesis inhibition is a basic cause in male sterility of HPGMR under long day induction, and only indirect relation may exist between free amino acid and pollen degeneration.

Keywords HPGMR, starch, protein, LD induction, pollen abortion

1 Introduction

Hubei Photoperiodic sensitive Genic Male-sterile Rice(i.e.HPGMR), as a typical example of physiological genetics, is male-sterile under long day (LD, 13.5～14 hrs/day) after the secondary branch differentiation, but fertile under short day (SD) condition[1～3]. Pollens are mainly composed of starch and protein[4], and HPGMR sterile anthers have iodine-aborted pollens with no contents[5]. Furthermore, the function leaves play very important roles in pollen development by supplying most of the assimilates, we use the contents of chlorophyll, soluble sugar and protein to indicate the physiological function of leaves[6].

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The relations between free amino acid (FAA) and male sterility is hitherto an unsolved problem. Some researchers think that the dificiency of free amino acids is a cause of male sterility[7,8], but others think that FAA dificiency is the result of pollen abortion[9,10]. Our paper will put FAA together with protein to discuss this.

2 Materials and Methods

2.1 Materials The HPGMR(Nong ken 58A)(*Oryza sativa* L., *subsp. Sinica*) seeds were sown in the greenhouse. After short day (9h/day) treatment at the 5-leaf stage, some rice plants were induced under long day(14h/day) from the secondary branch differentiation until heading, the compensational light intensity is about 1500 Lux. Those growing continuously under short day condition were used as control.

2.2 Sampling Samples were taken according to the methods of Ding Ying[11] and Yoshida[12] at the differentiating stage of pollen mother cells (Ⅰ), early uninucleate stage (Ⅱ), late uninucleate stage (Ⅲ), binucleate stage (Ⅳ), and trinucleate stage (Ⅴ) of microsporogenesis.

2.3 Determination of soluble sugar and starch content We refer to literature[13] and Ling[14].

2.4 Determination of chlorophyll content employed the method of Chen Fuming[15].

2.5 Determination of soluble protein We extracted soluble protein according to Wang shuiping et al.[16] and refer to Layne[17] for measurement.

2.6 Determination of free amino acids contents Refer to the method of Kern and Atikin[7], which was modified in some points. The anthers from each sample were vaccum dried and then pulverized in water in a ground glass homogenizer. A solution of 4% sulfosalicylic acid (protein precipitant) and a little 0.01 mol/L hydrochloric acid were added to the mixture, and the materials were centrifuged at 10 000 rpm for 20 minutes. The samples were then kept frozen until they were analyzed on a HITACHI (Type 835～50) amino acid analyzer.

3 Results

3.1 Variance of starch and soluble sugar contents in the anthers and spikelets

During the development of fertile anthers under short day induction, the starch content increases continuously(Fig.1), from 0.46mg/gFW at the differentiating stage of pollen mother cells up to 1.43 at the late uninucleate stage; Especially when pollens begin to be filled, the starch

content increases very markedly. At the trinucleate stage as microsporogenesis proceeds to the mature pollen stage, the starch content is as high as 16.54 mg/gFW, which is nearly 36-fold that of stage I. In the sterile anthers under long day induction, however, the starch content is 1.23mg/gFW at stage V, only an increase of 1.6-fold compared with that of stage I.

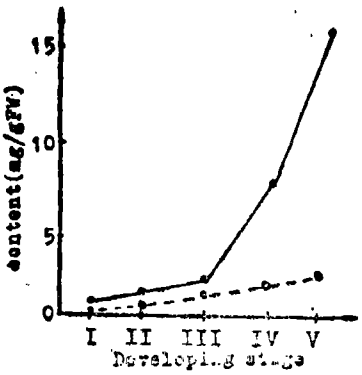

Fig.1 Variance of starch content in the anthers of HPGMR during different stages of microsporogenesis under LD(•---•) and SD (●——●) inductions (Data are means of 3 replicates)

But in the fertile anthers, along with the pollen maturation, the soluble sugar content reduces continuously (Fig.2A), which is 138 mg/gFW at stage I, and only 72 at stage V. This occurs just because soluble sugar is utilized to synthesize starch. But in the sterile anthers, the soluble sugar content is lower, and the variance is not obivious, which coincides well with the small increase of its starch content. Moreover, the soluble sugar accumulates in the lemma and palea of sterile anthers under long day induction but it is not the case under short day, the soluble sugar content in the spikelets of fertile anthers reduces continuously(Fig.2B)

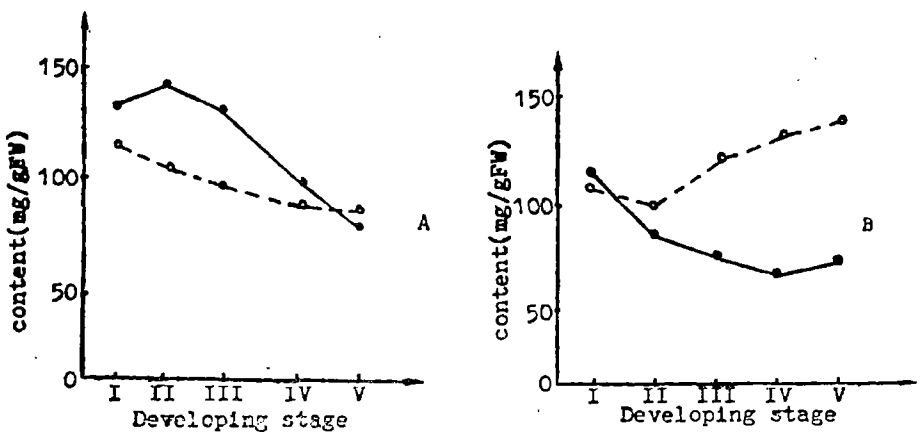

Fig.2 Variance of the soluble sugar content in the anthers (A) and lemma and palea (B) of HPGMR during different stages of microsporogenesis under LD(◦ --- ◦) and SD(●——●) inductions (Data are means of 3 replicates)

3.2 Variance of soluble protein and free amino acid contents

Fig.3 shows the variance of soluble protein content in the fertile and sterile anthers. At every stage of pollen development, the soluble protein content in the sterile anthers is always lower than that of fertile anthers. At stage I, the content in sterile anthers is only 49.7% that of fertile ones. Furthermore, the soluble protein content in fertile anthers reduces markedly (to 51.4%) from stage I to II, and so is that of sterile ones but the extent of reduction is smaller. To explain this, we think the division of pollen cells is so rapid that the protein synthesis can't catch up with the cell growth. But after the stage IV, the protein content in fertile anthers increases very rapidly, up to 12.48 mg/FW at stage V because pollens begin to be filled at this stage, and the protein synthesis is very active. Otherwise the protein content in sterile anthers still reduces, indicating that most of their pollens can't be filled.

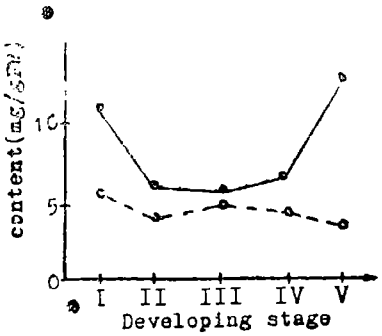

Fig.3 Variance of soluble protein content in the anthers of HPGMR during different stages of microsporogenesis under LD(∘---∘) and SD (●——●) inductions (Data are means of 3 replicates)

Before stage III, the total content of free amino acids in fertile anthers increases continuously (Fig.4). This accumulation of FAA may prepare for the protein synthesis after stage IV. Thereafter, along with the increase of protein content, the total content of FAA begins to reduce. In the sterile anthers, the variance of total FAA content is very small, like the variance of protein. Therefore we think that the variance of FAA can, to some extent, reflect the level of protein content, i.e. when the FAA content reduces, the protein content may increase, and vice versa. On the other hand, there is almost no difference in any individual amino acid between sterile and fertile anthers (Tab.1). We take proline as an example, the difference is very small after stage II, which is determinant to male sterility or fertility.

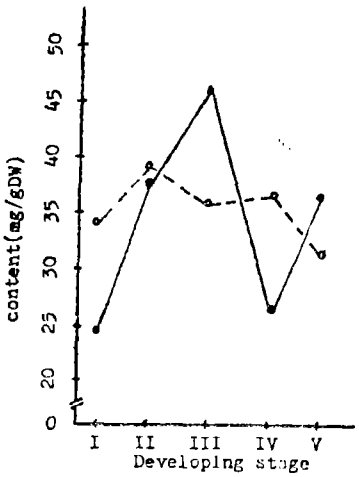

Fig.4 Variance of the content of free amino acids in the anthers of HPGMR during different stages of microsporogenesis under LD (∘---∘) and SD (●—●) inductions

Tab.1 Variance of free amino acids in the anthers of HPGMR during different stages of microsporogenesis under LD and SD inductions (mg/gDW)

	I		II		III		IV		V	
	LD	SD	LD	SD	LD	SD	LD	SD	LD	SD
Asp	2.271	2.178	2.157	1.644	1.588	2.713	1.521	1.400	1.156	1.578
Thr	1.771	1.142	1.900	1.911	1.658	2.444	1.688	1.835	1.195	1.827
Ser	3.390	2.254	3.626	2.535	3.109	3.762	3.117	2.363	2.123	2.484
Glu	2.837	1.241	3.855	1.999	2.221	0.812	1.386	0.901	0.903	0.535
Pro	0.165	0.833	0.994	1.145	1.774	3.895	1.677	2.564	2.335	2.940
Gly	0.756	0.699	0.766	1.535	0.706	1.002	0.745	0.637	0.497	0.896
Ala	2.825	1.626	2.988	3.608	2.781	2.991	2.866	2.238	2.521	4.042
Cys	0.389	0.492	0.498	1.045	0.482	0.617	0.545	0.446	0.426	0.629
Val	1.901	1.521	2.054	2.523	1.992	2.615	2.137	1.947	1.528	2.141
Met	1.124	0.811	1.291	2.451	1.224	1.661	1.297	1.147	0.841	1.229
Ile	1.214	0.742	1.459	1.659	1.235	1.793	1.469	1.237	0.973	1.454
Leu	2.669	1.867	3.042	3.209	2.759	3.746	3.050	2.693	2.100	3.076
Tyr	2.118	1.574	2.185	2.440	1.930	2.138	2.217	1.425	1.325	1.839
Phe	2.724	1.823	2.374	2.744	2.435	3.020	2.742	2.294	1.934	2.677
Lys	3.780	2.581	3.816	3.632	3.621	5.399	4.076	3.440	2.830	3.712
NH_3	0.550	0.514	0.534	0.523	0.471	0.646	0.447	0.567	0.344	0.649
His	0.798	0.522	0.950	0.923	0.710	1.332	0.927	0.849	0.587	0.999
Trp	1.331	0.958	1.026	0.793	0.687	0.081	——	1.245	——	1.638
Arg	3.275	2.277	3.651	3.299	4.369	5.795	4.008	3.845	2.795	2.674
Total	34.583	24.986	39.217	37.767	35.752	46.453	36.802	30.186	26.433	37.039

3.3 Variance of the contents of chlorophyll, soluble sugar and protein in function leaves

During pollen development or degeneration, the difference in the contents of chlorophyll(a, b), soluble sugar and protein in function leaves is not significant between LD and SD conditions (Tab.2 and 3), though the contents are slightly higher under SD induction. After stage IV, the chlorophyll content increases a little compared with the former stage and this may be related to the maturation and opening of flag leaves. The sugar content is also higher at stage V than that at stage IV, which may be caused by the maturation of flag leaves and reduction of photosynthates translocated from leaves to anthers when pollens proceed to be mature.

Tab.2 The content of chlorophyll a, b in the function leaves of HPGMR during different stages of microsporogenesis under LD and SD inductions (mg/gFW)

	I		II		III		IV		V	
	LD	SD	LD	SD	LD	SD	LD	SD	LD	SD
a	2.53	2.60	2.41	2.52	2.34	2.54	2.52	2.58	2.61	2.64
b	1.43	1.66	1.22	1.31	1.17	1.39	1.45	1.52	1.82	1.74
(a+b)	3.96	4.26	3.63	3.83	3.51	3.93	3.97	4.10	4.43	4.38

Means of 3 replicates

Tab.3 The contents of soluble sugar (ss) and soluble protein (sp) in the function leaves of HPGMR during different stages of microsporogenesis under LD and SD inductions (mg/gFW)

	I		II		III		IV		V	
	ss	sp	ss	sp	ss	sp	ss	sp	ss	sp
SD	38.1	21.6	41.7	23.5	39.7	20.8	36.3	18.4	38.4	16.7
LD	34.5	21.8	32.9	22.6	30.5	20.1	30.9	18.5	35.7	16.2

Means of 3 replicates

4 Discussion and Conclusion

When pollens develop, photosynthates in function leaves must be translocated to anthers for starch and protein synthesis, and Fukasawa[18] proposed that, in cytoplasmic male-steriles, some nutritive substances necessary for normal development of microspores either are not produced in the vegetative tissue or are not transmitted from the tapetum to the microspores. Our results show that the soluble sugar in leaves can be transported to spikelets and then to anthers continuously under SD in-

duction, starch and protein can be synthsized to ensure the regular development of pollens. But under LD condition, although soluble sugar can also be transmitted to lemma and palea but seems to accumulate there, starch and protein synthesis is very low because of the low supply of the substrates. These results agree with reports from similar studies with three-line sterile rices[19,20].

It must be pointed out that, the physiological function of leaves doesn't decrease under LD induction and assimilates can also be translocated to lemma and palea, which ensures the normal development of pistils and so HPGMR sterile rices can be used as mother plants in crossbreeding[1,3,21].

Sarvella et al.[8] concluded that the total quantity of amino acids seemed more important than any individual amino acid in causing male sterility. Our results indicate that the total FAA content coincides well with that of soluble protein content, and the difference in individual amino acids is small between LD and SD inductions, so it is proposed that only indirect relation may exist between male sterility and FAA, i. e. FAA can reflect the change of protein content during microsporogenesis. But we don't deny that such amino acid as proline may play some important effects on pollen abortion.

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光敏核不育水稻花粉败育中淀粉和蛋白质含量的研究

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摘　要

在可育花粉的发育中，功能叶同化产物运往花药合成淀粉和蛋白质，淀粉含量增加显著，颖壳和花药可溶性糖减少。长日下功能叶生理机能并未下降，糖也可运往颖花，但在颖壳中积累，不育花药中淀粉含量增加不明显，可溶性糖含量略有下降。单核晚期前可育花药蛋白质含量先是下降，但双核期后又迅速上升；不育花药中则基本上一直呈下降趋势。游离氨基酸总量与蛋白质含量变化相吻合，脯氨酸含量在可育和不育花粉之间差异不大。作者认为，淀粉和蛋白质的合成障碍是长日诱导光敏核不育水稻花粉败育的基本原因，这种障碍是由其底物供应不足引起的；游离氨基酸的变化与花粉败育仅有间接关系。

关键词 光敏核不育水稻，长日诱导，淀粉，蛋白质，花粉败育

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