Content differences of glycoprotein and amino acids between self-compatible and self-incompatible lines in Yunjie (*Eruca Sativa* Mill.)

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Abstract

The glycoprotein and the components of amino acids in the stigmas of self-compatible and self-incompatible lines of Yunjie were extracted using hexane and ninhydrin and separated by SDS-PAGE. A protein band with MW of 50~55KDa was specific for self-compatible lines, but absent in the self-incompatible lines.[0] Seventeen types of amino acids (Gly, Met, Asp, Cys, Tyr, Lys, Thr, Val, Ser, Pro, Ile, Leu, Phe, Glu, Arg, Ala and His) were identified in the stigmas of Yunjie. The amount of amino acids in self-incompatible lines was higher than that in self-compatible lines in bud stage stigma, but in mature stigma, that was opposite. In self-compatible lines, Arg was 0.4758 mg and 2.7071 mg in both bud and mature stigma, respectively, which was comprised of about 54.1% and 25.8% of the total acids. In self-incompatible lines, Arg amount in both bud and mature stigma was 4.0733 mg and 0.1925 mg, respectively, and comprised of 60.8% and 17.5% of total acids. The amount of amino acid in bud stage stigma in self-incompatible lines was higher than that in self-compatible lines, but the amount of self-compatible was higher than self-incompatible lines in mature stigma, which suggested that the content of Arg might be related to self-compatible.

Key words: YunJie, glycoprotein, amino acid, self-incompatible

Introduction

Self-incompatibility formed in the evolution of plant (Goring1992; Nishio, 1992). It is a physiological reflection which aroused by reciprocity between farina and rumples cell of chapter. Self-incompatibility accelerates genetic habitability species and has important effects on polarization of species, especially on forepart evolution of angiosperm. It has been one of the hottest research areas in plant reproductive biology (Xue, 2002) for its importance in crop heterosis utilization.

Amino acids connect life activity and has special physiological function. It is one of the indispensable nourishment components in plants' body and is important in accelerating on plants growth (Schopfer, 2000). It is studied that self-compatible is connect closely with amino acid in chapter. The difference of kind and amount of amino acid can affect on protein synthesis, which will affect on all kinds of functions in cell (Wang, 2000). This paper studied the protein and amino acid of chapter of self-incompatible ESI1 and its self-compatible mutation ESC1.

Material and methods

This research chosen the chapter of Yunjie self-compatible (SC)and self-incompatible lines(SI) in former a blossom 2-3 days and after blossom1-2 days, The fresh material of chapter was conserved in refrigerator with -70°C for use. The chemistry reagents in this experimentation were as following: acryl amide, sodium laurel sulfate, glycogen, glycerol, phosphoric acid, Tris, acetone, acetic acid atrium et all. The apparatus in the experiment included amino acid automatism instrument, high speed refrigerated centrifuge, oven, electrophoresis chamber and so on.

0.2g material was quantified respectively and was placed in 2ml Eppendorf tube. and 1ml extracting solution(0.5MTris-HCl buffer, and pH=6.8) was added to the tube followed by distilling for 1h in ice bath and then centrifuge for10 minutes at 10000 rpm and 4°C. The supernatant fluid was transferred to another Eppendorf tube, -20°C chilled 10%TCA trichloroacetic acid was added according to 1:2.5(v / v) and the tube was shaken evenly at -20°C and then placed at -20°C for 2h for sedimentation protein followed by centrifuge for 15 min at 12000 rpm and 4°C. Then the supernatant luid was get rid of and the deposition was washed for 2-3 times with hypothermal acetone. The washed deposition was placed for 20 minutes to volatilize fully of acetone at-20°C. After that added protein lytic solution 200ul (62.5mmol/L Tris-HCl；2% sodium laurel sulfate；10% glycerol；5%β-sulfhedryl alcohol), and then boiled it for 2-3 minutes followed by SDS-PAGE electrophoresis to separate protein (Ji,1991).

Yunjie's fresh chapter of bud and maturation stage of SI and SC were chosen respectively. The material was placed into oven to dry and then grinded to powder. 100mg sample powder was measured precisely and put into 20ml test tube. Then added 3mol/L HCL to form 5ml solution, The tube was vacuum zed and sealed followed by being put in 110°C phosphoric acid bath for 24h and filtrated, The filtrate was transferred to capacitance bottle to suitable cubage (25ml), and then 1 ml was precisely extracted. Make it to be dry at 60°C by decompression, then 2ml ddH2O was added and braised to dry for two times. Finally, the residue was dissolved with 2 ml ddH2O, and the supernatant fluid was took to measure amino acid on the auto analyzer of amino acid (chromatogram condition: runoff: 0.22ml/min; the temperature of stove: 30°C; adopting the methods of grads washing and drafting) Measuration(Ai, 2005)

Results

1.Measurement sugar protein of chapiter in bud stage in SC and SI lines

The proteins of Yunjie in bud stage in self-compatible and self-incompatible lines were analyzed by the SDS-PAGE electrophoresis (Fig.1). There was a special band (about 50KD) in self-compatible (1,2 lines) between 66 KD and 45 KD on the gel electrophoresis map; but this band was absent in self-incompatible (3,4 lines). The electrophoresis band of self-compatible was stronger apparently than self-incompatible lines at about 18.0 KD. The electrophoresis bands of protein in bud stage chapter of self-compatible and self-incompatible lines were also analyzed statistically. The results showed that the self-compatible had 17 clearer strips. However, the self-incompatible had 16 strips. As analyzed above, a principium estimate that the difference strips of SC lines are connected with self- compatibility can be drawn.

2.The protein expression of autumn chapter in SC and SI lines

The results of the protein of Yunjie's autumn chapter in self-compatible and self-incompatible by the SDS-PAGE gel electrophoresis were shown in Fig.2. The results showed that the self-compatible had 19 clearer strips. However, the self-incompatible had 18 strips. The self-compatible lines were obviously brighter than self-incompatible lines at about 66 KD. A special strip (1, 2 lines) at about 50.5 KD was found in the self-compatible lines' strips. Comparing the gel electrophoresis maps of chapter in bud stage and autumn, the self-compatible lines had a strong different strip at about 66KD, but there is no change at about 50.0 KD. The expression quantity of every protein strip was different.

3. Amino acid of bud chapter and autumn of Yunjie SC

The content of amino acid of bud chipper and autumn were measured with amino acid automatism analysis. 17 amino acids in bud and autumn of Yunjie SC were found, including Gly, Met, Asp, Cys, Tyr, Lys, Thr, Val, Ser, Pro, Ile, Leu, Phe, Glu, Arg, Ala and His (table.1). The contents of Arg were the highest in both bud phase and autumn and arrived to 0.47580 and 2.70714 respectively. Among all these amino acids, the contents of Arg, Ala, Thr, Gly, Val, Pro, Leu, Met, His, Glu, Cys took on the trend of ascending from bud phase to autumn. Among them, Arg, Ala, Gly, Val, Leu, Met, His, Glu and Cys extended to significant difference level. The contents of Lys, Ser, Ile, Phe, Asp and Tyr took on the trend of descending. Among them, Lys, Ile, Phe, Asp and Tyr extended to significant difference level. The results indicated that the contents of most of amino acid in Yunjie SC took on the trend of going up from bud phase to autumn phase, and four kinds of amino acid were distinguished for their most increasing in content: Arg, Ala, Val, Cys, reaching 468.97%, 336.2%, 4014.27% and 104.27% respectively.

4. Amino acid of bud chapter and autumn of Yunjie SI

17 kinds of amino acids in the chapter of bud phase and autumn of Yunjie in SI lines were measured. Comparing all amino acids, it could be found that the content of Arg was the highest in bus phases (table.1), reached to 4.0733 and accounted for 60.8% of the whole amino acids. The content of Arg in autumn phase was 0.19252 and accounted for 17.5% of the whole amino acids. From the Fig.4,it could also be seen that the contents of some amino acids in bud phase, such as Arg, Lys, Ala, Gly, Val, Ser, Pro, Ile, His, Phe, Asp, Tyr ect, were far higher than that of autumn phase. Only the contents of Thr, Glu and Cys in autumn were higher than that of

bud phase. On the whole, the content of amino acid in chapter of SI takes on the descend trend.

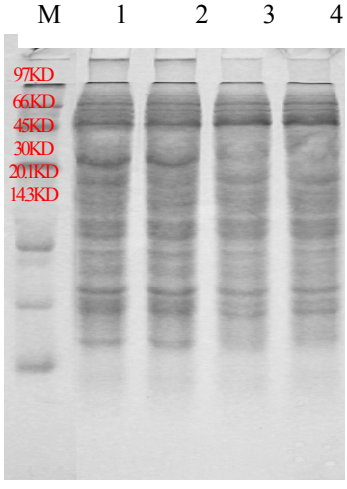

Fig.1 SDS-PAGE electrophoresis graphs of protein in bud stage stigma of self-compatible line and self- incompatible line in Yunjie Lines1,2 is self-compatible; Lines3,4 is self- incompatible

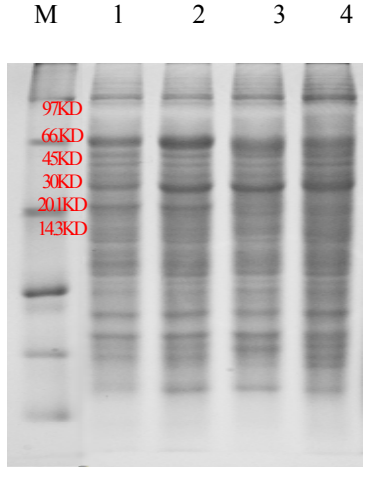

Fig.2 SDS-PAGE electrophoresis graphs of protein in mature stigma of self-compatible line and self-incompatible line in Yunjie. **Lines 1,2** is self-compatible**Lines 3,4** is self-incompatible

Discussion

The differences between SC lines and SI lines were obvious at about 50.5KD, 18.0KD and 66.0KD. The differences at

50.5 KD accorded with the former research conclusion: the main component of Brassica chapter rumbles cell is 45-55KD alkalescency sugar protein (chapter sugar protein is connected with SC property); Yunjie SC lines bud chapter map was different from SI lines; and expression quantity of protein was also different. In Yunjie SI lines, there was no difference in the map of bud phase and autumn. Based on the above analysis, the maps of chapter protein electrophoresis of Yunjie SI lines and SC lines were different significantly, which explained some protein expression were different significantly. Because special protein expression was connected closely with plants' hereditary information themselves, the special strips of SC lines may be relate to the property of its self-compatible, and the growth of plants anaphase chapter is based on the protein difference of bud phase in SC lines. From above results (Table.1), A conclusion can be drawn that In both SC lines and SI lines increasing and decreasing of Arg are both great in both bud phase and autumn, which is accordant with others research. It is thought that change of Arg is connected with SC property, Thus it can be seen that content of Arg has strong connections with self-compatibility.

Conclusions

The Yunjie chapter protein was measured and analyzed by technology of SDS-PAGE gel electrophoresis. From the Fig of electrophoresis maps, a special strips could be seen in Yunjie SC lines at about 50.5 KD in bud phase chapter. The strips of SC lines were much stronger than that of SI at about 18.0 KD and 15.5KD. SC lines bud chapter protein electrophoresis map had 17 strips, while SI lines had 16 strips; As shown in the mature chapter protein electrophoresis map(Fig 2), SI lines had 18 strips.

The amino acids of chapter in Yunjie SC and SI were measured and analyzed, the results indicated there were 17 kinds of amino acids identified. Arg distinguished itself for its highest content and highest change in SC lines and SI. In SC lines Content of Arg in bud phase arrive to 0.47580 and 2.70714 respectively and account for 25.8% and 54.1% of the whole content of amino acids respectively. In SI lines Content of Arg in bud phase and autumn arrive to 4.0733 and 0.19252 respectively, and account for 60.8% and 17.5% of the whole content of amino acids respectively.

Table.1Variance analysis of amino acid composition in stigma of self-compatible and self-incompatible line in Yunjie

	Amino acid	Material					
		Self-compatible line in Yunjie			Self-incompatible line in Yunjie		
		Bud stigma	Mature stigma	(±%)	Bud stigma	Mature stigma	(±%)
Amino acid composition (%)	Are	0.47580	2.70714**	468.97	4.07330**	0.19252	-95.27
	Lys	0.15480**	0.01422	-90.82	0.63247**	0.06809	-89.23
	Ala	0.05365	0.23404**	336.20	0.23400**	0.13235	-43.44
	Thr	0.01412	0.02688**	90.38	0.01315	0.03407*	159.19
	Gly	0.12327	0.28465**	130.91	0.02871**	0.01583	-44.87
	Val	0.01757	0.72287**	4014.27	0.61687**	0.33992	-44.89
	Ser	0.08548	0.07172	-16.09	0.05692**	0.02247	-60.52
	Pro	0.04734	0.04888	3.25	0.04889**	0.01378	-71.82
	Ile	0.08844**	0.02328	-73.68	0.34435**	0.01956	-94.32
	Leu	0.32237	0.37475**	16.25	0.09461	0.11437	20.89
	Met	0.01213	0.01829*	50.81	0.03397	0.01073	-68.43
	His	0.03087	0.08475**	174.53	0.05413*	0.03513	-35.09
	Phe	0.03402**	0.01260	-62.95	0.09526**	0.01508	-84.17
	Glu	0.10985	0.16279**	48.20	0.05443	0.09346*	71.706
	Asp	0.11489**	0.04366	-62.00	0.05001**	0.00579	-88.41
	Cys	0.07227	0.14763**	104.27	0.01640	0.04571**	178.69
	Tyr	0.10711**	0.02132	-80.094	0.24845**	0.00449	-98.19

Note: * mean significant difference at 0.05 level; ** mean significant difference at

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