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双向电泳分析鸢尾绿白嵌合叶片的蛋白质*

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摘要: 利用双向聚丙烯酰胺凝胶电泳对鸢尾 (*Iris japonica*) 绿白嵌合叶片的蛋白质进行分离, 并初步鉴定了蛋白质的相对分子量和等电点。每个电泳图谱共检测到 400 余个蛋白点, 其中至少 13 个蛋白的表达变化明显; 结果表明, 嵌合叶片的绿色与白色叶组织具有明显不同的蛋白质双向电泳图谱。与数据库中拟南芥双向电泳图谱相比较, 发现 Rubisco 大亚基、标记为 W 和 T 蛋白的表达变化与产生绿白嵌合叶片的表型密切相关。

关键词: 双向聚丙烯酰胺凝胶电泳; 绿白嵌合叶片; 鸢尾
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Two-Dimensional Polyacrylamide Gel Electrophoresis Analysis of Proteins from Albino-Green Chimeric Leaves of *Iris japonica*

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Abstract: Proteins extracted from albino-green chimeric leaves of *Iris japonica* were separated by two-dimensional polyacrylamide gel electrophoresis and relative molecular weights and isoelectric points were determined. Approximately 400 protein spots were identified in each 2 - D PAGE gel. Altered expression was apparent for at least 13 proteins. The results showed that protein expression patterns changed obviously in the green and white leaf strips. In comparison with the 2 - D PAGE map from *Arabidopsis thaliana* in SWISS - 2DPAGE database, it revealed that the expression pattern of large subunit of RuBisCO and the proteins labeled W and T was closely related to the mechanism involved in chimeric variations of *Iris japonic*.

Key words: Two-dimensional polyacrylamide gel electrophoresis; Albino-green chimeric leaf; *Iris japonica*

High-resolution two-dimensional polyacrylamide gel electrophoresis (2 - D PAGE) is useful for separating complex protein mixtures (O' Farrel, 1975). Due to its high-resolving power, the technique has been employed to study alterations in cellular protein expression in response to various stimuli or as a result of differentiation and development (Celis *et al*, 1984). Proteins separated by 2 - D PAGE can be characterized by mass spectrometry and amino acid sequencing, which provides useful information to our

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understanding of function of the unknown proteins. Combined with automated gel scanning and computer-assisted analysis, 2 – D PAGE has made significant contributions to protein database. 2 – D PAGE is still a key technique of proteome approach. As Thiellement (1998) deduced, proteomics will be a relevant field for the study of the relationship between genotype and phenotype.

Two cultivars of *Iris japonica* were grown in Kunming Botanical Garden. Leaves are green in one cultivar which flourishes under the shade of Camellia trees, whereas another cultivar has white and green strips in a same leaf which predominates in the area of strong sunlight. The latter cultivar is an albino-green chimera. The aim of this work is to study protein variations between the white and green leaf tissues. Our approach has consisted mainly in characterizing specific proteins by means of two-dimensional electrophoresis.

1 Material and Methods

Sample Preparation Fresh leaves were collected from two cultivars of *Iris japonica* grown in Kunming Botanical Garden. The green and white leaf strips were separately ground into fine powder in liquid nitrogen using a pre-chilled mortar. The following procedure was performed according to Wang (1999). The powder was homogenized in ten volumes of cold (– 20℃) acetone containing 10% w/v trichloroacetic acid (TCA) and 0.07% v/v 2-mercaptoethanol. The mixture was kept at – 20℃ for 2 h, then centrifuged at 12 000 r/min at 4℃ for 20 min. The supernatant was discarded and the pellet was washed with cold acetone containing 0.07% v/v 2-mercaptoethanol to obtain the white pellet. Then the white pellet was lyophilized and stored at – 70℃.

50 mg powder of each sample was extracted at 30℃ for 30 min with 1 mL of a solution containing 9.5 mol/L super-pure urea, 2% v/v Triton X – 100, 1.6% w/v Ampholine pH 5 – 7 (Amersham Pharmacia Biotech), 0.4% w/v Phymalyte pH 3 – 10 (Amersham Pharmacia Biotech) and 5 mg/mL DTT. After centrifuged at 15 000 r/m for 20 min at 20℃, the supernatant was loaded onto the tube gel for the first dimensional electrophoresis.

First Dimensional Electrophoresis According to Rosenberg (1996) and Wang (1999), the gel mixture was 4% w/v acrylamide, 9.5 mol/L super-pure urea, 2% v/v Triton X – 1 00, 1.6% w/v Ampholine pH 5 – 7 and 0.4% w/v Phymalyte pH 3 – 10. Pre-run the gel for 15 min at 200V, 30 min at 300 V and 30 min at 400 V. Then 50 μL of the above supernatant was loaded on the basic end of the gels. IEF was performed at 16℃, 200 V for 60 mm, 400 V for 14 h and then 800 V for 90 min. The tube gels were extruded into 5 mL equilibration buffer and stored at – 20℃.

Second Dimensional Electrophoresis The tube gels were equilibrated at 30℃ for 10 min in 5 mL equilibration buffer containing 10% v/v glycerol, 2% w/v SDS, 0.0625 mol/L Tris – HCl (pH6.8), 1.5 mg/mL DTT. Then the gels were deposited on 12.5% separating gels with the agarose M (Amersham Pharmacia Biotech) melted in equilibration buffer without DTT. Proteins were separated according to the conventional SDS polyacrylamide gel procedure for about 3 h at a constant current of 20 mA each gel.

Silver Staining and Data Analysis The gels were silver-stained according to Rosenberg (1996) with some modification. The relative molecular weight and isoelectric points of proteins were estimated using Bio-Rad 2 – D SDS – PAGE standards. At least two reproductive gels were obtained for each sample. Gels were digitized with an UMAX PowerLook III scanner and spots were detected with ImageMaster 2 – D Elite software (Amersham Pharmacia Biotech).

2 Results and Discussion

Approximately 400 protein spots were identified by silver staining in each 2 – D PAGE pattern of

the green and white leaf proteins (Fig. 1). The green cultivar has a very similar electrophoretic pattern compared to green leaf strips from the albino-green chimeric cultivar (data not shown). The spots showed a symmetric shape indicating a good resolution in both electrophoresis dimensions. Replicate gels for the same sample showed a good reproducibility as the same amount of proteins was loaded on each IEF gel. For each gel, the investigated zone was restricted between p*I* 5.9 – 6.0 and between

Table 1 Protein spots in 2 – D gels of green and white leaf strips

Spot number	Relative molecular weight (kDa)	*pI*	Comparison with green leaf gel
A	31.4	5.74	eq
B	31.8	5.69	eq
C	32	5.65	eq
D	30.9	5.6	eq
d	28.2	5.0	dec
E	30.9	5 2	eq
F	30.9	5 1	eq
G	28.7	5.15	dec
H	26	5.12	dec
I	26	5.76	dec
J	49.8	5.71	inc
K	42 7	5.44	eq
M	46.2	5.18	eq
N	32.4	5.2	eq
O	56.9	5 12	dec
P	33.9	5.22	dec
R	54	5.8	dec
S	28.1	5.25	dec
W	47.0	5.8	absent
Y	59.2	5.2	dec
Z	40.8	5.69	inc
T	35.6	4.95	inc

Notes: dec/inc. protein amount decreased /increased in white leaf tissue in comparison with green leaf tissue; absent. the protein spot is absent or not detectable in white leaf gel.

20 and 76 kDa for molecular weight. More than seventy percent of the spots were found to be common in both gels. Twenty-two major spots were selected from green and white leaf gels for further analysis (table 1). They were labeled alphabetically.

The R protein amount was the highest in green leaf proteins. Its relative molecular weight was about 54 kDa and the isoelectric point about 5.8. When compared with reference map from *Arabidopsis thaliana* in SWISS – 2DPAGE database (*http: //www.expasy.ch/ch2d/*), it was found that the relative molecular weight of RuBisCO large chain of *Arabidopsis thaliana* was 51 kDa, and isoelectric point about 5.7. As RuBisCO devotes about 50% to total soluble mesophyll proteins (Li *et al*, 1999), it was deduced that the R protein was the large subunit of RuBisCO. About 4 – fold difference was found in the amounts of R protein between green and white leaf tissues. This is coincident with the fact that in *Anjibaicha* (*Camellia sinensis*) both large and small subunits of RuBisCO decreased at the albescent stage and became normal after leaves recovered to green (Li *et al*, 1999).

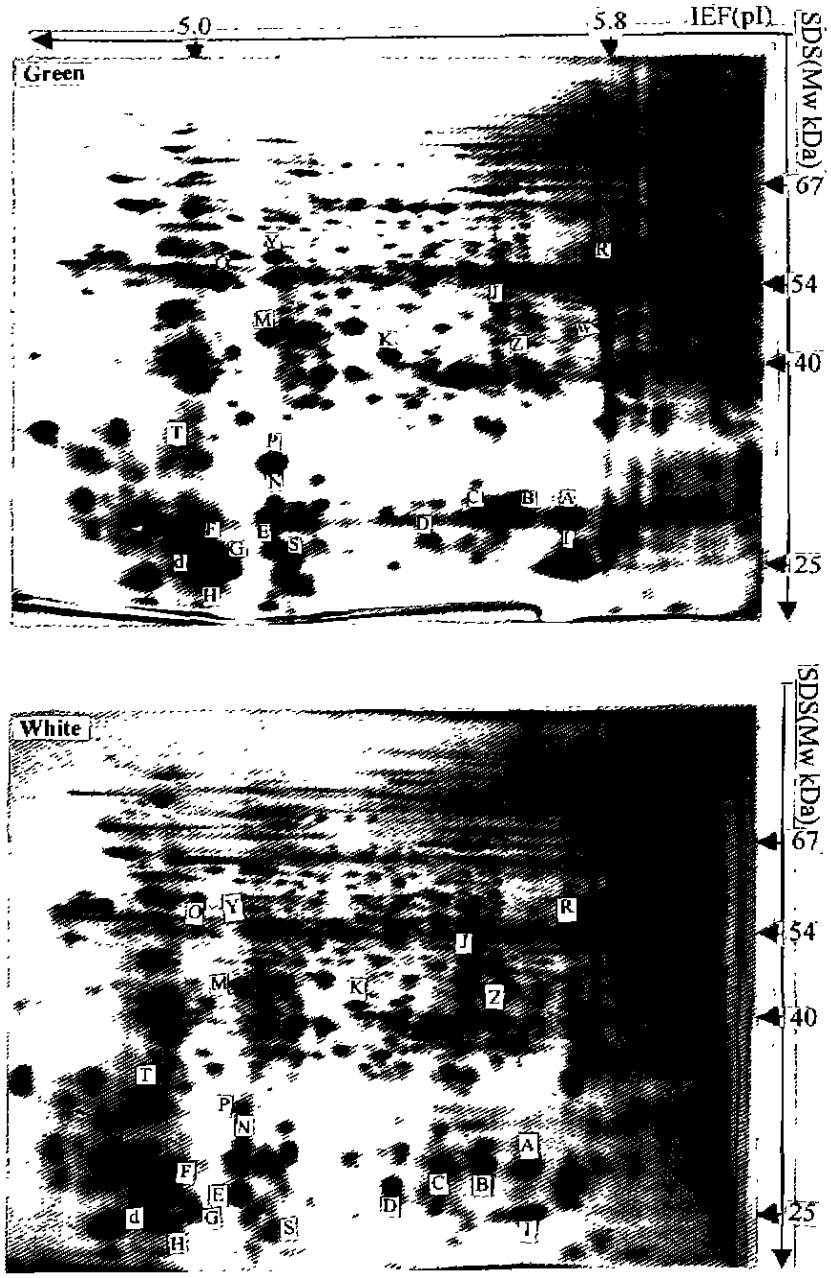

Fig 1 2-D PAGE pattern of green (above) and white (below) leaf proteins. The major protein spots were labeled alphabetically. Relative molecular weight and *pI*, together with the protein amount difference between green and white leaf els., were shown in table 1.

The labeled W protein, with the relative molecular weight about 40 kDa and the same *pI* to RuBiscCO large subunit, was absent or not detectable in the white leaf gel. On the contrary, an increase in protein content was observed for the labeled T spot in the same gel. The disappearance or deep increase of these proteins is very interesting and further characterization is required to clarify the specific

function.

In the region between the pH 4.9 and 6, the protein spots of labeled O, P, Y, I, S, G, H, J and Z were present on both gels. Taking the green leaf gel as a reference gel, the first seven spots presented a significant decrease in white leaf gels whereas the later two showed a little increase. Proteins increasing or decreasing in abundance could be associated with the metabolism of plastids. Other protein spots, such as A, B, C, D, E, F, K, M and N (table 1), whose expression levels vary little or not, were suggested to be necessary for growth and development of *Iris japonica* cultivars.

Our 2 – DE study showed that the green and white leaf tissues presented considerable biochemical changes. The major differences in white leaf strip were a four-fold decrease of RuBisCO large subunit, the disappearance of the labeled W protein and a deep increase of the labeled T protein. Amino acid sequence information and localization of the different proteins may provide a better understanding of their physiological significance.

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