

THE RELEASE OF PROTEIN PROTEINASE INHIBITORS (PPIS) FROM SOYBEAN GENERA DURING GERMINATION

Le Trong Quang, Pham Tran Chau

Center of Biotechnology - VNU

In a previous study [1] we have dealt with the role of trypsin inhibitors (TI) in term of resistance to virus in various soybean genera. Some previous studies ([4] for example about the release of protein proteinase inhibitors (PPIs) of certain Papillionaceae family plants into the soaking media have raised a hypothesis on the role of PPIs in protection and resistance against pest invasion into the germs. This paper presents some results about the release of PPIs from seeds of 7 soybean genera with different resistance properties into the soaking media.

I. METHOD OF STUDY

Dormant seeds were provided by the Institute of Agricultural Sciences. Preparation of seeds for study: Dormant soybean seeds from 7 samples with same mass were soaked in the same volume of water in small trays, after the determined time (12, 24, 48 and 72 hours), a certain volume of soaking solution was taken for trypsin inhibitory activity (TIA), chymotrypsin inhibitory activity (ChIA) and proteolytic activity (PA) analysis. In order to eliminate the effect might be caused by the growth of microorganisms during seed soaking period, in a parallel experiment sample was carried out in the same way but with an addition of 0,02% sodium azide antibacterial.

TI, ChI bands were detected by Hanspal method [2]. Electrophoretic mobility was represented by the R_m value, defined by:

$$R_m = \frac{\text{distance between the starting point to PPI - band}}{\text{distance between the starting point and the bromophenol blue}}$$

Proteinase band was detected by Heussen and Dowle method [3]. Proteolytic activity (PA) was determined by the modified Anson method [5]. Proteinase inhibitory activity was determined by the difference of the enzyme activity before and after treatment with inhibitor solution for 10 min. One inhibitory unit (IU) is the amount of inhibitor that decrease the activity of 2 mg by 50%.

Chemicals used in the study reach the PA level.

II. RESULTS AND DISCUSSION

The release of TI from soybean seeds into the media during the soaking process

The results of the study (Fig.1) showed that the changing of the TI release from soybean sample seeds into the soaking media have relatively similar manner: after 12 and 24 hours soaking, the level of TI release of seed samples 0.7-1 IU/g of dry matter, representing about 1.3-2% of the total TIA existing in the seed. As soaking continues into 48 hours, the total TIA in the soaking solution reached 2.3-4.3 IU/g of dry matter representing about 6-13% the total of TIA present in the seed. On the third day soaking (72 hours), TIA level in the soaking solution of two virus resistant genera samples (CV and Cuc) decreased. Fig.1 also indicated that the release of TI into soaking solution was highest in the case of two virus resistance genera samples (CV and Cuc), representing more than 13% of the total TIA present in the seed (Table 1); on the contrary, the virus infected genera sample (V74), had lowest TI release level (6.2%) (Table 1).

Samples	Soaking times (h)	TIA	KIA	Soaking times (h)	TIA	KIA
IV ₁	12	0.79	0	48	9.50	11.80
IV ₂		0.81	0		8.61	10.5
IV ₃		0.77	0		8.96	13.00
X ₇₄		1.17	0		8.07	13.90
V ₇₁ (infected virus)		0.84	0		5.47	13.20
V (anti virus)		1.38	0		10.02	15.3
UC (anti virus)		1.26	0		12.35	13
IV ₁	24	3.40	0.92	72	4.50	13.80
IV ₂		3.20	0.62		3.94	16.80
IV ₃		2.86	1.36		5.52	18.90
X ₇₄		3.32	0.97		5.12	17.10
V ₇₁ (infected virus)		2.46	0.61		3.03	15.71
V (anti virus)		3.25	1.04		6.74	20.00
UC (anti virus)		3.64	1.08		9.48	20.00

Table 1. PPI released from seeds into the soaking solution (% of the initial PPI in dry seeds before soaking)

For better understanding of the release mechanism, eliminating other eventual impacts, the experiment was reproduced in totally identical conditions except the addition of antibacterial (sodium azide 0.02%) into the media. The obtained results showed also a release of TIs from the seeds into the media, however with a lower level, reaching about 57-76% in comparison with TIA of the soaking solution without antibacterial. Maybe in the conditions of media without sodium azide, the activity of microorganisms has enhanced the strong release of TIs from the seeds into the media, as protecting factors. In both conditions-with and without antibacterial, the TI release level of two genera samples CV and Cuc were the highest.

The study of TI changes during the germination process [1] as well as the release of TI from seeds of the soybean genera samples with different viral resistance showed dif-

ferences between soybean genera with different viral resistance. Should important role of TI into the soaking media be linked with their high resistance properties? This is an extremely interesting issue needing deeper studies in order to find out the mechanism.

Electrophoresis technique for the detection of TIs, proteiunase released from the seed into the soaking media has cleared up in a larger extent the relationship between the components as well as the relationship between their changes and the viral resistance of soybean.

Electrophoresis technique on SDS-PAGE 12.5% with case in (Fig.2) showed spectrum of the soaking solution of genera samples, similarly to TI of the extract from soaked seeds, had 4 bands with Rm value respectively 0.54; 0.66; 0.8 and 0.91. Out of which bands with Rm value 0.66 and 0.8 were the main ones. Similarly, results in terms of the number of the TI bands of the virus resistant genera samples CV and CUC were the strongest. On the other hand, in comparison with other samples, these two genera samples had one more rather sharp TI band with Rm= 0.46. In the meantime for the virus infected sample V74, band with Rm=0.91 was not detected. Perhaps this was also one of the causes making TIA of the soaking solution of V74 lower than other samples especially CV and CUC.

Since in soybean there was also chymotrypsin inhibitor, we have carried out the analysis of ChIA in the soaking solution.

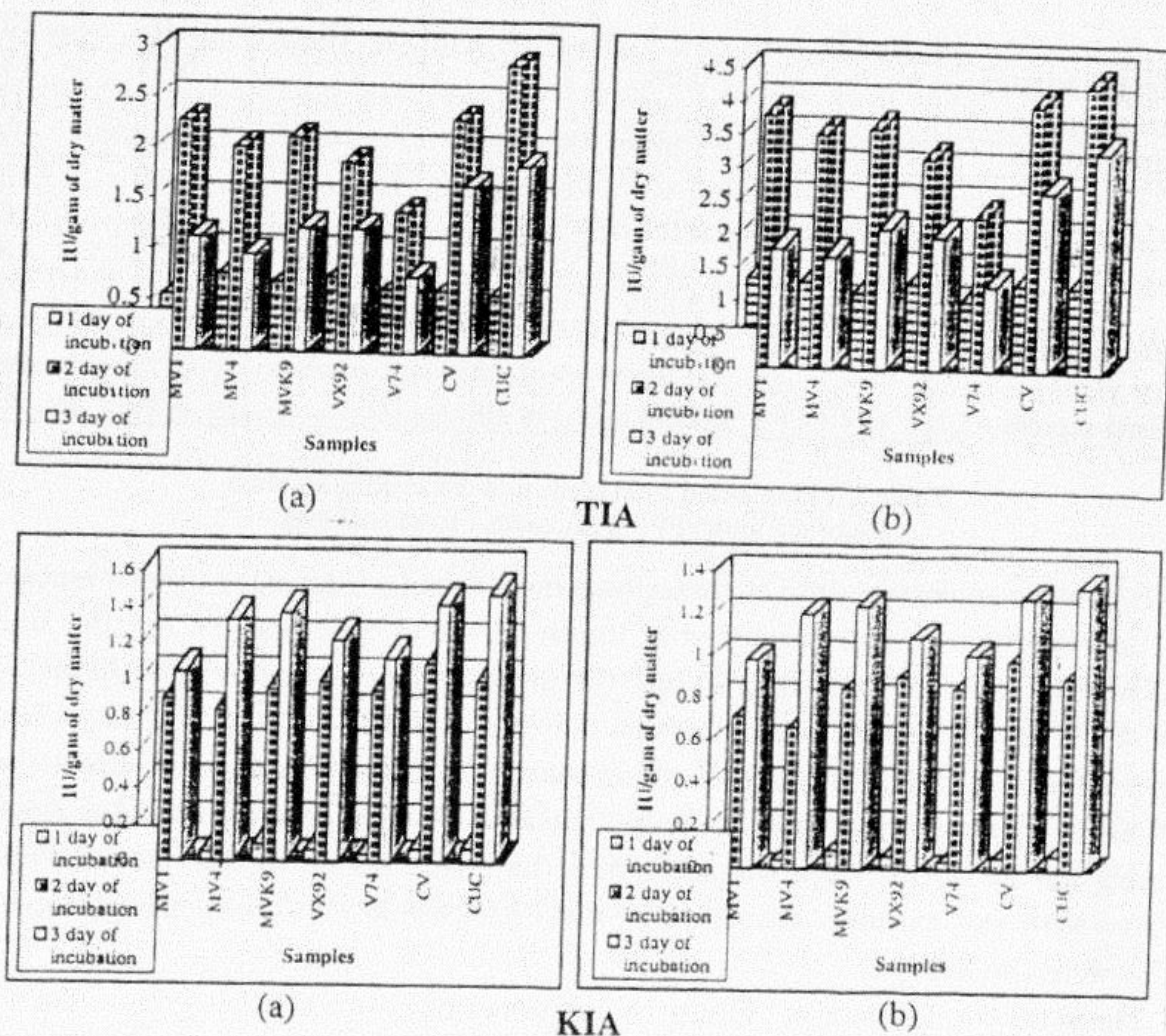


Fig 1 : PIA in the soaking solution of seeds (IU per gram of dry matter)
a/ Without sodium azide - b/ In the presence of 0,02% of sodium azide (used as antibacterial substance)

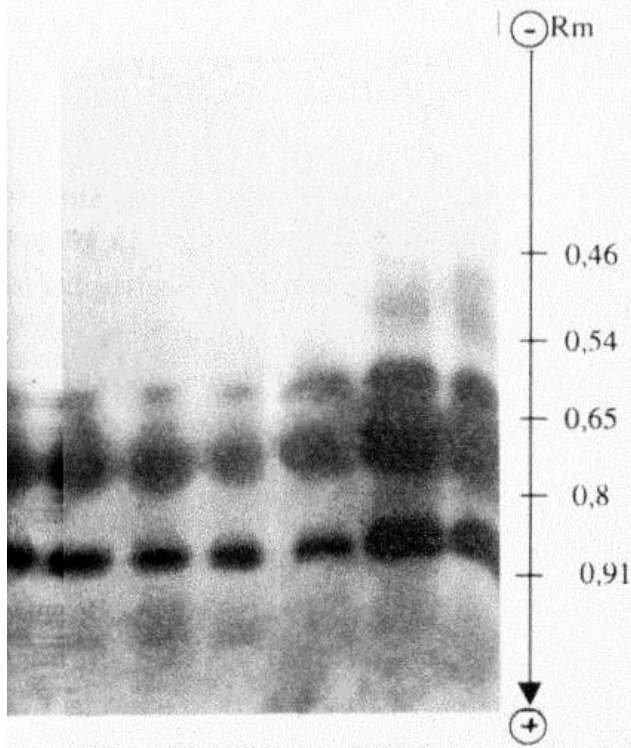


Fig. 2 Electrophoretic of trypsin inhibitors released into soaking solution.

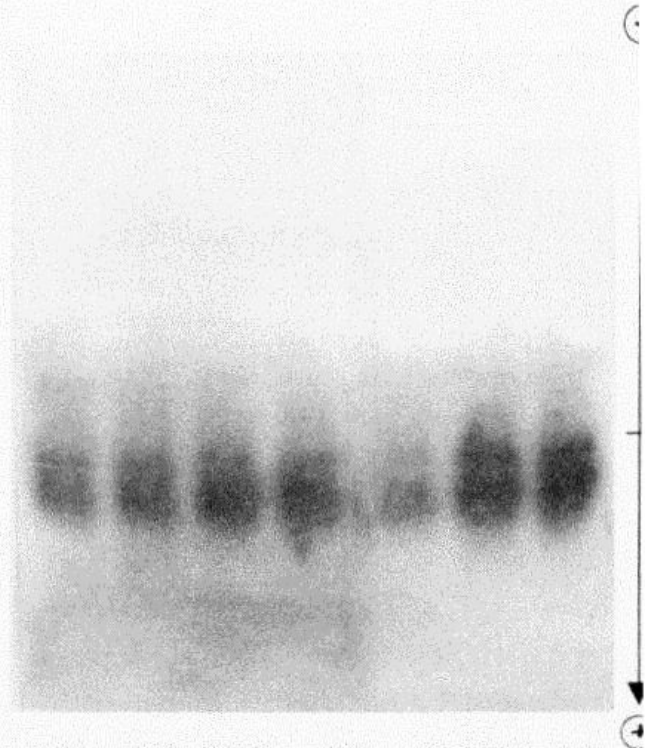


Fig 3 : Electrophoretic pattern of chymotrypsin inhibitors released into soaking solution



Fig 4 : Proteinase electrophoretic pattern of soaking solution and extract from imbibited seeds.

2. The release of ChI from soybean seeds outwards during soaking process

The obtained results (Fig. 3) showed that like TIA, after 24 hours soaking, the ChIA release increased. At 72 hours, ChIA in soaking solution of the samples reached

about 1.05 to 1.54 IU/g of dry matter equal to 15-18% the total amount of ChIA in the seeds (Table 1). Both CV and Cuc samples had ChI release level stronger than other samples.

The electrophoresis spectrum on PAGE 12.5% with SDS substrate showed that the genera samples had 2 ChI bands, out of which the 0.8 band was the main one. In genera between the genera samples there were no significant difference in terms of electrophoresis spectrum.

By using the method of proteinase detection on gel according to Heussen and Dowling [3], we have identified from both seed soaking solution and soaked seed extract a proteinase band with $R_m = 0.03$ like in the dry seed extract. Observation of electrophoresis spectrum of soaking solutions showed also clear differences between virus infected genera sample and other genera samples. On electrophoresis spectrum of genera sample V74, there was an appearance of another rather sharp new proteinase band with $R_m = 0.4$. Maybe there is a correlation between the appearance of this proteinase band with the non abundant secretion of TI and the non appearance of a new TI band with $R_m = 0.91$ in sample V74.

In the past, while studying the release of TIs from Leguminosae seeds into the soaking media, Hwang [4], Wilson [6] thought that it was a particular mechanism of Papilionaceae plants, and they might have a role in protecting the germinated plant against the invasion of Papilionaceae plants. However, these studies usually focused on the quantity, no author touched upon, identified TIs in soaking media by electrophoresis with SDS and substrate, confirming that these TI belong in fact to the seed.

At the same time no study compared, dealt with PPI released from soybean genera samples with different virus resistant properties. Therefore the results that we have obtained contribute to complement and clarify the role of PPIs in terms of resistance in vegetal, namely in our case, the soybean.

CONCLUSION

From the obtained results we have drawn the following conclusions:

1. In the conditions of media with and without antibacterial sodium azide 0.02% there was a release of TI, ChI from the seeds of 7 soybean genera samples into the soaking media. The amount of TI, ChI in the seed soaking solution reached maximum after 48 hrs and 72hrs, respectively.

2. Out of the 7 studied genera samples, 2 virus resistant genera samples CV and Cuc had highest TIA and ChIA levels in the soaking solution.

3. PPI electrophoresis pattern of the seed soaking solution had about 4 TI bands and 2 ChI bands. Unlike other samples, in the virus infected genera samples the TI band with $R_m = 0.91$ did not appear. On the contrary, in TI electrophoresis pattern of virus resistant genera samples CV and Cuc, this band appeared the most sharply. Besides, these two samples had a small band with $R_m = 0.46$.

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NGHIÊN CỨU SỰ THOÁT CÁC PPI TỪ HẠT ĐẬU TƯƠNG VÀO MÔI TRƯỜNG TRONG QUÁ TRÌNH NẤY MẦM

Lê Trọng Quang, Phạm Trần Châu

Trung tâm Công nghệ Sinh học

Đại học Quốc gia Hà Nội

Công trình này giới thiệu kết quả nghiên cứu quá trình tiết PPI của 7 mẫu giống đậu tương vào môi trường ngâm nhằm làm sáng tỏ thêm vai trò, chức năng bảo vệ của PPI ở thực vật. Từ các kết quả thu được chúng tôi rút ra một số kết luận:

1. Trong điều kiện môi trường có và không có chất kháng khuẩn natri azit 0.02% sự tiết các TI, KI từ hạt 7 mẫu giống đậu tương vào môi trường ngâm. Lượng TI trong dịch ngâm đạt cực đại sau 48 giờ ngâm, còn đối với KI là sau 72 giờ.

2. Trong số các mẫu giống, nghiên cứu 2 mẫu giống CV và Cúc (giống kháng vi rút) có mức tiết TI, KI vào môi trường cao nhất.

3. Phổ điện di PPI trong dịch ngâm hạt phát hiện thấy 4 băng TI, 2 băng KI, trong giống như các mẫu giống khác, băng TI có $R_m = 0.91$ ở mẫu nhiễm vi rút (V 74) thì hầu như không xuất hiện. Ngược lại băng này ở các mẫu kháng vi rút (CV và Cúc) khá cao. Mặt khác ở 2 mẫu giống kháng vi rút còn xuất hiện thêm một băng nhỏ có $R_m =$