Chemistry of Natural Compounds, Vol. 44, No. 1, 2008

RAPID METHOD FOR IDENTIFYING HIGHLY PRODUCTIVE VARIETIES OF COTTON BY A SELECTIVE PROCESS

L. G. Mejlumyan

UDC 577.156:634.651

Screening of genetically enhanced cotton varieties with a high content of protein markers, proteinase inhibitors, was carried out for the first time. It was found that only 5 of 68 studied cotton selection lines were resistant to wilt, black root rot, and gummosis.

Key words: cotton seeds, proteinase inhibitors, inhibitor activity, immunochemistry, titre, screening, selection lines.

Inhibition of enzymes that form a resistant complex has become of great interest in the last decade mainly because of the possibility of using proteinase inhibitors as chemotherapeutic agents and biological protectors since they specifically and irreversibly inhibit enzymes of the target.

Our research on cotton revealed a linear dependence between proteinase inhibitor concentrations and the wilt-resistant variety (Fig. 1) and the accumulation of proteinase inhibitors in response to infection (Fig. 2) [1, 2].

Based on the established relationship, it was possible to screen genetically enhanced cotton varieties with a high content of protein, proteinase inhibitors. For this we isolated and processed inhibitors of serine (A, C) and thiol (B) proteinases from 68 selection lines [3-5] and also isolated proteinase inhibitors from wild cotton species 02672 *Gossypium hirsutum punctatum*, 05152 *G. hirsutum punctatum*, 02800 *G. hirsutum papuracus*, and 06422 *G. hirsutum mexcicanum*, which are grandparents of the highly inhibiting lines found by us (Fig. 3a and b).

The activity of proteinase inhibitors in the studied cotton lines was determined using the Izotova—Stepanov method modified by us [6]. Table 1 gives the analytical results for nine highly inhibiting lines.

The regionalized varieties Namangan-77, Omad, C6530, and C6524 were used as controls because they are indirect or direct parents of the studied lines whereas wild species 02672 *G. hirsutum punctatum*, 05152 *G. hirsutum punctatum*, 02800 *G. hirsutum papuracus*, and 06422 *G. hirsutum mexcicanum* are grandparents.

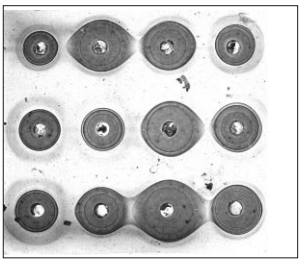


Fig. 1.

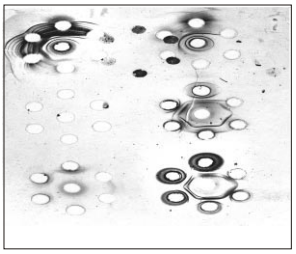


Fig. 2.

Fig. 1. Circular precipitation and precipitation in agar gel containing antibody.

Fig. 2. Immunochemical analysis of marker proteins of damaged and healthy cotton seed.

S. Yu. Yunusov Institute of the Chemistry of Plant Substances, Academy of Sciences of the Republic of Uzbekistan, Tashkent, fax (99871) 120 64 75. Translated from Khimiya Prirodnykh Soedinenii, No. 1, pp. 43-45, January-February, 2008. Original article submitted June 8, 2007.

0009-3130/08/4401-0055 ©2008 Springer Science+Business Media, Inc.

TABLE 1. Inhibitor Activity in Wild and Cultivated Varieties and Highly Inhibiting Selection Lines of Cotton Seeds

Name	Inhibitor activity, %		
	2003 harvest	2004 harvest	2005 harvest
02672 *G. hirsutum punctatum*	71	–	–
05152 *G. hirsutum punctatum*	75	–	–
02800 *G. hirsutum papuracus*	53	–	–
06422 *G. hirsutum mexcicanum*	68	–	–
Namangan-77	79	79	78
Omad	**81**	**81**	**81**
C6524	75	75	75
C6530	76	76	76
Line 0.52	89	87	87
Line 842	**97**	**97**	**98**
Line 218	**96**	**96**	**97**
Line 408	**99**	**99**	**98**
Line 162	93	95	95
Line 215	**95**	**95**	**96**
Line 65/2000	93	93	93
Line 1708/860	**97**	**97**	**98**
Line 178	93	93	93

TABLE 2. Resistance of Cotton Varieties and Lines to Wilt, Black Root Rot, and Gummosis as a Function of Proteinase Inhibitor Content

Variety and line	Degree of wilt damage vs. nat. infect. bkgd., %		Laboratory estimate of degree of damage				Proteinase inhibitor content, %
			black root rot, %		gummosis, %		
	total	incl. high degree	total	degree of resistance	total	degree of resistance	
C-6524	40.0	18.0	28.9	Tolerant	33.4	Tolerant	75.0
Namangan-77	36.6	11.8	20.5	Tolerant	23.5	Tolerant	79.0
Omad	**27.6**	**4.2**	**18.7**	**Highly resist.**	**6.6**	**Highly resist.**	**81.0**
L-162	25.7	3.8	14.5	Resistant	16.8	Resistant	93.0
L-408	**18.5**	**2.5**	**10.5**	**Resistant**	**6.5**	**Resistant**	**99.0**
L-842	**18.3**	**2.6**	**8.4**	**Highly resist.**	**12.4**	**Resistant**	**97.0**
L-215	**21.4**	**3.9**	**16.5**	**Resistant**	**10.8**	**Resistant**	**95.0**
L-218	**22.8**	**4.6**	**14.2**	**Resistant**	**10.2**	**Resistant**	**96.0**
L-866	23.2	4.3	23.5	Tolerant	18.6	Resistant	93.0
L-1708	**24.0**	**4.9**	**13.1**	**Resistant**	**11.8**	**Resistant**	**97.0**
L-052	24.3	7.8	21.0	Tolerant	25.4	Tolerant	89.0
L-178	18.7	5.2	12.8	Resistant	13.6	Resistant	93.0
C-6530	36.6	15.6	16.4	Resistant	18.9	Resistant	76.0

Table 1 shows that lines L-408, L-842, L-218, L-215, and L-1708 had the highest inhibiting activity during three-year field tests. Variety Omad had the highest content of proteinase inhibitors among the studied regionalized cotton varieties. Furthermore, seed material was identified from the morphological traits [7, 8]. Table 2 shows that variety Omad and highly inhibiting lines L-408, L-842, L-218, L-215, and L-1708 typically had not only wilt resistance but also resistance to black root rot and gummosis.

Furthermore, variety Omad and lines L-408, L-1708, L-842, L-215, and L-218 had selection value according to several morphological traits (early ripening, yield, quantity and length of fiber, boll size, mass of 1000 seeds, oil content) [9].

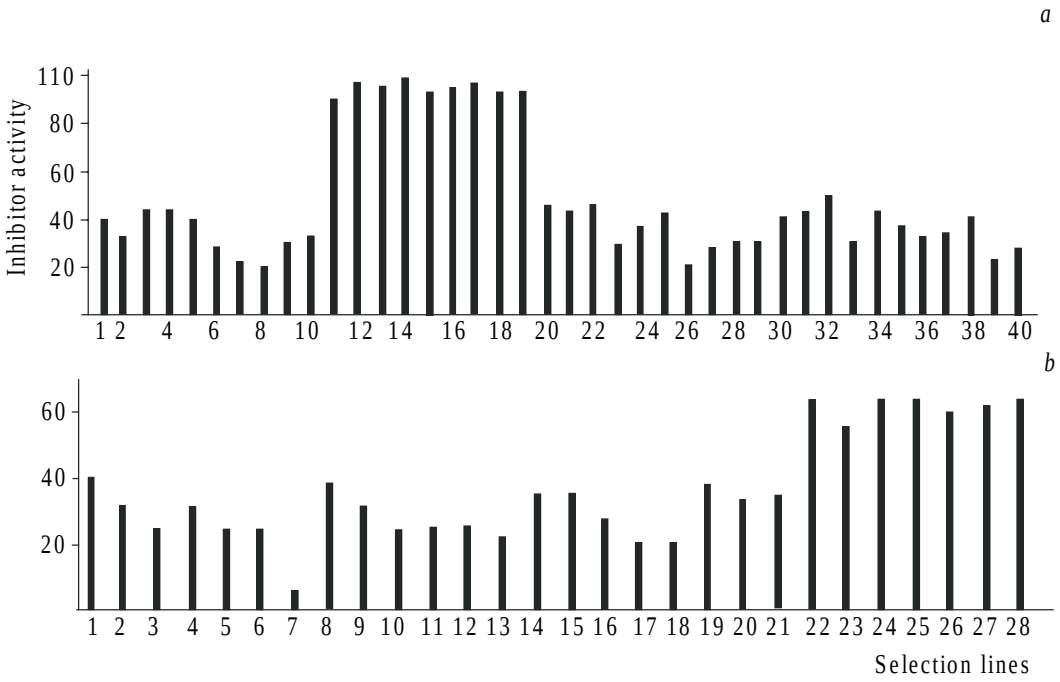


Fig. 3. Inhibitor activity in studied new selection lines of cotton seeds.

Protein preparations of plant orgin were studied using highly sensitive and specific methods such as immunochemical analysis by double diffusion in a gel [10]. Antigens and antibodies are necessary to use this analytical method. It relies on the formation of precipitation bands if similar or identical antigenic epitopes are present on two organisms and no reaction if similar epitopes are absent. Proteinase inhibitors that were isolated from control varieties Namangan-77, Omad, C6530, and C6524 were used as the antigen for immunization of rabbits. The resulting antibodies were used to carry out the immunoprecipitation reaction by the Ouchterloni method. The results showed that the control varieties were related by immunochemistry to four of their wild grandparents (Fig. 4a) and nine highly inhibiting lines (Fig. 4b).

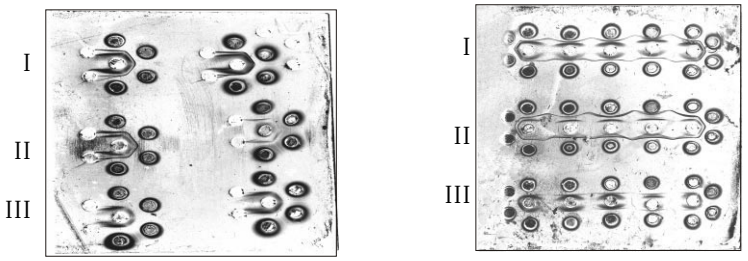
The degree of immunological relation to the parent forms was established using the resulting sera to carry out screening of the cotton lines studied by us using an antibody concentration gradient according to Feinberg (highest cultivation of antigen at which the precipitation reaction is still observed) [11].

Titration results of proteinase inhibitors from the 9 highly inhibiting lines of the 40 studied showed a different degree of immunochemical similarity to the control varieties. All 9 varieties were most related to Namangan-77 and C6524. The titre in Namangan-77 was 1/512; in C6524, 1/128. According to the immunological analysis, lines L-842, L-1708 L-218, L-215, and L-408 were related to Omad. The titre in Omad was 1/16.

Regarding the other 28 lines (Fig. 3b), L-1, L-2, L-8, and L-9 of the first ten lines were highly inhibiting; L-14, L-15, L-19, and L-21, of the second set of ten. The inhibition analysis showed that practically each of lines L-22—L-28 was inhibiting. The 28 lines studied by us were inferior in inhibiting activity (less than 60%) to lines L-408, L-842, L-1708, L-218, and L-215 (greater than 90%) of cotton seeds.

Feinberg immunochemical analysis was used to determine the degree of immunochemical relation of the highly inhibiting lines L-1, L-8, L-14, L-19, and L-22 to Omad. Furthermore, L-1, L-14, and L-19 were related to Namangan-77; line L-22, to C6524.

Thus, a highly effective method combining inhibitor analysis with immunochemical analysis was developed for the first time for screening studied selection lines of cotton for the most important valuable traits. Our results confirmed that variety Omad and lines L-408, L-1708, L-842, L-216, and L-215 had selection value for early ripening, yield, quantity and length of fiber, mass of 1000 seeds, and oil content. The rapid method for identifying highly productive cotton varieties by a selection process is patented where proteinase inhibitors were used as markers (IAP 20070091).



a *b*

Fig. 4. Analysis of marker proteins from wild cotton varieties (a) and highly inhibiting varieties (b) by Ouchterloni immunoprecipitation: I, II, and III; antibody to Namangan-77, C6524, and Omad.

EXPERIMENTAL

Isolation of total inhibiting fractions was performed as before [4].

Immunization used rabbits six months old weighing 2-3 kg. Animals were immunized as follows. Proteinase inhibitors (10 mg/kg) were administered daily for two weeks. Antigen was administered in parts, one half of the dose intramuscularly and one half subcutaneously. Rabbit blood was collected on the ninth and tenth day after the last injection (8 mL from one animal). Blood serum was prepared by storing for 2 h at room temperature and then in a refrigerator (+4°C) overnight. Then serum was separated by centrifugation at 3000 rpm for 20 min, transferred to sterile tubes, and treated with boric acid (0.05%) as a preservative. Sterile serum was stored in a refrigerator.

Inhibiting activity was determined by the literature method [5].

Precipitation Reaction. The reaction was carried out on agar (1%) prepared by mixing equal volumes of hot agar (2%) and NaCl solution (1.7%). After the agar solidified, wells were made by a stamp of definite shape. The wells were filled with antigen and antiserum. The plates were placed in a moist chamber and stored for 5-7 days, daily checking for the appearance of precipitation lines.

ACKNOWLEDGMENT

We thank Candidate of Agricultural Sciences R. G. Kim, Institute of Selection and Cotton Seed Production, for the material supplied.

REFERENCES

1. L. G. Mejlumyan, E. F. Redina, and P. H. Yuldashev, *Khim. Prir. Soedin.*, 274 (1994).
2. L. G. Mejlumyan, E. F. Redina, and P. H. Yuldashev, *Khim. Prir. Soedin.*, 42 (1997).
3. T. D. Kasymova and P. H. Yuldashev, *Khim. Prir. Soedin.*, 744 (1988).
4. T. D. Kasymova, L. G. Mejlumyan, and P. H. Yuldashev, in: *Vth International Symposium on Verticillium* [in Russian], Leningrad (1990).
5. L. G. Mejlumyan, E. F. Redina, and P. H. Yuldashev, *Khim. Prir. Soedin.*, 533 (1994).
6. T. Devenyi and J. Gergely, *Amino Acids, Peptides, and Proteins: Biochemical and Immunochemical Techniques in Protein Chemistry*, Elsevier-Scientific, New York (1974).
7. L. G. Mejlumyan, R. G. Kim, P. H. Yuldashev, and Ya. Babaev, in: *Vth International Symposium on the Chemistry of Natural Compounds*, Tashkent (2003).
8. L. G. Mejlumyan, P. H. Yuldashev, R. G. Kim, and Sh. Kh. Rakhimova, *Khim. Prir. Soedin.*, 51 (2004).
9. R. G. Kim, L. G. Mejlumyan, Ya. A. Babaev, P. H. Yuldashev, and M. R. Kim, in: *International Conference on the Development of Cotton and Grain* [in Russian], Tashkent (2004).
10. N. N. Guseva, B. B. Gromova, and E. S. Lantas, *Methodical Instruction: Immunochemical and Biochemical Methods in Plant Immunology* [in Russian], Leningrad, 5-12 (1985).
11. B. B. Gromova, Author's Abstract of a Doctoral Dissertation in Biological Sciences, St. Petersburg (1992).