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SEROLOGICAL EVIDENCE CONFIRMING THE ASSIGNMENT OF *PHASEOLUS AUREUS* AND *P. MUNGO* TO THE GENUS *VIGNA*

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Key Word Index—*Phaseolus*; *Vigna*; Leguminosae; mung beans; protease; cotyledons; serotaxonomy.

Abstract—Endopeptidase activity was detected in extracts of cotyledons of 11 species of *Vigna* and *Phaseolus* Antibodies against the purified protease isolated from the cotyledons of 5-day-old *P. aureus* seedlings inhibited the activity of that enzyme in crude extracts of cotyledons. A similar inhibition was obtained with *P. mungo*, *P. adenanthus* and 4 species of *Vigna*, while there was no inhibition of endopeptidase activity in extracts of cotyledons of 4 species of *Phaseolus*. Immunodiffusion tests proved that the protease of *Vigna* is distinct from that of *Phaseolus*. The evidence supports the reassignment of *P. aureus* and *P. mungo* to the genus *Vigna* and indicates that the names *Vigna radiata* and *Vigna mungo* are more appropriate than *P. aureus* and *P. mungo* for green gram and black gram respectively.

INTRODUCTION

Two species of cultivated bean—green gram (mung bean) and black gram (urd)—were originally described by Linnaeus as belonging to the genus *Phaseolus*, and are now commonly referred to as *Phaseolus aureus* and *P. mungo* L. respectively, [1]. On the basis of morphological evidence, Wilczek [2] reassigned both green gram and black gram to the genus *Vigna* and the two species are now known to the taxonomists [3, 4] as *Vigna radiata* (L.) Wilczek and *V. mungo* (L.) Hepper, respectively. While this reassignment has not been contested, most biochemists, including ourselves, have continued to use the old nomenclature [5–7]. Chemical evidence confirming the assignment of both species of gram to the genus *Vigna* was obtained independently by Casimir and Le Marchand [8] and by Bell [9]. They examined the pipecolic acid content of the seeds of many Leguminosae and found high levels of this amino acid in most species of *Phaseolus*, but none in the species of *Vigna* they examined or in the two species of gram. Using antisera against the albumins and the globulins (reserve proteins) of *P. aureus*, Kloz and coworkers [10, 11] showed that this species is closely related to *P. mungo* and *V. sinensis*, but more distantly related to other species of *Phaseolus*.

RESULTS AND DISCUSSION

The cotyledons of 5- to 6-day-old green gram contain high levels of a protease capable of hydrolyzing the storage globulins [12]. Protease activity has also been observed in the cotyledons of pea (*Pisum sativum*) seedlings [13] and cow pea (*V. unguiculata*) seedlings [14] and was found in all other species of Leguminosae examined here. The protease from green gram cotyledons has recently been purified to homogeneity and some of its physicochemical characteristics have been determined [15]. The purified protease was used to immunize rabbits, and cross reactivity between the antibodies against the green gram enzyme and enzymes from the other species was examined in two ways: by the formation of precipitin lines in double diffusion plates, and by the inhibition of protease activity due to antibody–antigen interaction. Similar results were obtained with both methods.

Fig. 1 shows the effect of increasing amounts of the immunoglobulin fraction, obtained from the serum of rabbits immunized with green gram protease, on the proteolytic activity of that enzyme measured by our standard viscometric technique [16]. The results show that the proteolytic activity of the enzyme was totally inhibited by the immunoglobulins and that the inhibition was linear with increasing amounts of immunoglobulins. Table 1 shows the effect of a standard amount of the immunoglobulin fraction—enough to inhibit 0.5 units of mung

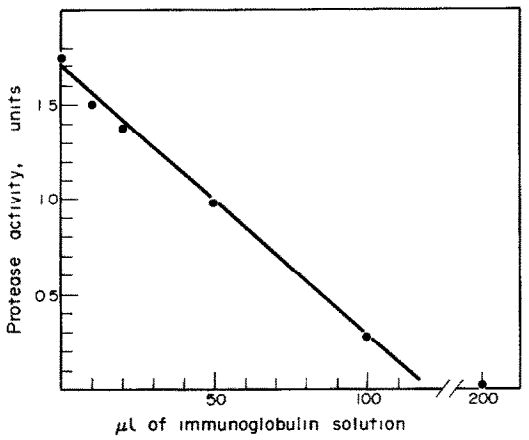

Fig. 1. A standard amount of endopeptidase (1.75 units of activity) obtained from mung beans (green gram) was mixed with increasing amounts of the immunoglobulin fraction obtained from rabbits immunized with the purified enzyme. Three min later the remaining enzyme activity was measured by viscometry using gelatin as substrate. The immunoglobulin fraction contained 18 mg/ml of protein. Similar amounts of the immunoglobulin fraction from pre-immune serum did not affect enzyme activity.

Table 1. Inhibition of endopeptidase activity from different species by antibodies against the green gram enzyme

Species	Enzyme activity		
	Control	With Antibodies	Inhibition
green gram	1.08	0.56	0.52
black gram	1.20	0.60	0.60
V. unguiculata	1 12	0.56	0.56
V. caracalla	0.32	nd	0.32
V. umbellata	0.88	0 44	0.44
V. luteola	1.00	0.40	0.60
P. vulgaris	1.48	1.44	0.04
P. lunatus	1.12	1.12	0.00
P. coccineus	1.20	1.20	0.00
P. adenanthus	1.08	0.78	0.30
P acutifolius	0.68	0.72	−0.04

An aliquot of cotyledon extract containing *ca* one unit of enzymy activity was assayed first in the absence and then in the presence of the antibodies. In each case the same amount of the immunoglobulin was added, enough to inhibit 0.5 units of green gram enzyme activity.

nd = not detectable.

bean enzyme—on the activity of the proteases from other species. The antibodies inhibited the activity of the proteases from all other species of *Vigna* to a similar extent, with the possible exception of *V. caracalla*, which had too low an enzyme titer for an accurate determination of the extent of the inhibition. The antibodies did not inhibit the proteolytic activity of the enzymes present in any of the species of *Phaseolus* with the single exception of *P. adenanthus*, which was inhibited to *ca* 60% of the extent of the green gram enzyme.

When the crude homogenates of cotyledons were allowed to react with antibodies against green gram protease in double diffusion plates, precipitin lines were observed with all species of *Vigna* and with *P. adenanthus*, but not with any of the other species of *Phaseolus*. When extracts of green gram and *P. adenanthus* were in adjacent wells the precipitin lines merged with one another rather than forming spurs. These results provide clear evidence that the green and black grams belong in the genus *Vigna* rather than in the genus *Phaseolus*, and supports the earlier reassignment of green and black gram to the genus *Vigna* [2]. The cross-reaction between antibodies against green gram protease and *P. adenanthus* protease indicates that the latter may also be more closely related to the genus *Vigna* than to the genus *Phaseolus*. A similar conclusion has recently been reached by Maréchal [private communication] on the basis of a study of the *Phaseoleae* by numerical taxonomy.

EXPERIMENTAL

Seeds of the following species were germinated in the dark in moist vermiculite at room temp.: *Phaseolus vulgaris* L. (kidney or French bean), *P. lunatus* L. (lima bean), *P coccineus* L. (scarlet runner bean), *P. acutifolius* A. Gray (tepary bean), *P. adenanthus* G. Mey, *Vigna unguiculata* (L.) Walp, aggreg (black-eyed or cow pea), *V umbellata* (Thunb.) Ohwi and Ohasi (rice bean), *V. caracalla* (L.) Verdc., *V. luteola* (Jacq.) Benth., *P. mungo* L. (black gram, urd) and *P. aureus* Roxb. (green gram, mung bean). Green and black gram, runner beans, kidney beans, lima beans and black-eyed peas were obtained from commercial dealers. All other seeds were a generous gift from Drs R Maréchal and E. Otoul from the Faculté des Sciences Agronomiques in Gembloux, Belgium. Growth was allowed to continue until the cotyledons started to shrivel (5 to 15 days depending on the species; lima beans and scarlet runner beans were moved into the light after one week). The cotyledons were harvested and homogenized in 25 mM citrate–Pi buffer containing 10 mM β-mercaptoethanol. The homogenates were centrifuged at 20 000 *g* for 15 min and the clear supernatants used as a source of protease. The enzyme activity was assayed viscometrically using gelatin as a substrate [16]. The antiserum was prepared by injecting rabbits with endopeptidase, purified to homogeneity from the cotyledons of 5-day-old mung bean seedlings [15]. The immunoglobulin fraction was purified by precipitation with 50% $(NH_4)_2SO_4$ followed by chromatography on DEAE cellulose.

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