BIOCHEMISTRY

Pitomba versus pests

Protein extracted from the fruit eliminates fungi and borers

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The pitomba is not just the yellowish and sweet fruit of the pitomba tree (*Talisia esculenta*), a tree of up to 10 meters in height found in the northern and northeastern regions of Brazil. The word pitomba comes from Tupi (one the languages spoken by native Brazilian indians), and it means a slap, blow or strong kick. In certain way, it was precisely this pitomba that the biochemist Maria Lígia Macedo, from the Federal University of Mato Grosso do Sul (UFMS), in Três Lagoas, gave to the fungi and beetles known as "borers" that attack the varieties of beans most consumed in Brazil and in sub-Saharan Africa, and which at times of intense infestation can cause heavy loss of grains of beans, soy and corn under storage.

From the seed of this olive-sized fruit, Maria Lígia extracted a protein – to be precise, lectin – that reduced by 60% the growth of two species of fungi and killed most of the beetles that damage both the plants and the stored grain. If the field tests prove the effectiveness achieved in the laboratory, this molecule may become an option in the fight against these pests and replace agrochemicals, which are toxic for animals and human beings.

Pitomba lectin, also known by the acronym TEL, from *Talisia esculenta lectin*, has proven to be versatile in inhibiting the development of fungi of broad action: it acts against *Fusarium oxysporum*, which also attacks the leaves of the sugarcane and coffee, and against *Colletotrichum lindemuthianum*, which causes anthracnose, a disease common in agriculture which is, for example, responsible for the dark blotches on mangoes. It was also effective against the larvae of two species of beetles that leave the grains of beans with more holes in them than a Swiss cheese: *Callosobruchus maculatus* and *Zabrotes subfasciatus*. Both attack not only peas, a legume with a modest consumption in Brazil, but beans as well, one of the main sources of proteins and carbohydrates in the country.

Maria Lígia and the researchers from the Campinas State University (Unicamp), and the North Rio de Janeiro State University (UENF) and the Oswaldo Cruz Foundation (Fiocruz)

do not yet know for certain how lectin works against the fungi or borers. They suspect that this protein prevents the growth of these organisms by combining itself with another molecule called chitin, the main component of some fungi cell walls. By reacting with chitin, the TEL could affect the growth of hyphae – the ramifications found in the majority of fungi. In the case of the insects, it seems to hinder the action of digestive enzymes that contain sugar in its composition and binds to a structure similar to a membrane – known as a peritrophic structure, which covers the inside of the intestines of these insects and is rich in chitin –, causing an imbalance in the absorption of nutrients.

Distant plants - TEL shows a sequence of some 20 amino acids (the blocks that makes up proteins) similar to the one shown by a compound extracted from rice, the inhibitor of alpha-amylase, which plays a role on the defense mechanism of plants by acting on one of the insects' digestive enzymes, alpha-amylase. Following this clue, Maria Lígia decided to assess the action of pitomba lectin on borers, because other known lectins – extracted from peas, wheat germ, nettles and potato – work against *Callosobruchus*, but show no toxic effect on *Zabrotes*.

TEL represented a promising alternative, since it belongs to a plant family (the Sapindacea),which is distant from the leguminosae. "As the borers had not had any contact with the pitomba protein, there was the possibility that lectin would work", comments Maria Lígia, who has known of the seeds of the pitomba tree since her childhood in Fortaleza in the state of Ceará, when she would frequently hear people claim that hens that ate the stone of the pitomba would always die. The popular saying did not inspire any more deeper investigation in this area, but it did open another path: in collaboration with the team led by Sérgio Marangoni, from the Biology Institute at Unicamp, the researcher purified and characterized the pitomba lectin, in work carried out with funds from the Foundation for the Support and Development of Teaching, Science and Technology of the State of Mato Grosso do Sul (Fundect).

PHOTOGRAPHS BY MIGUEL BOYAYAN

Reduced harvest: beans devoured by *Zabrotes* larvae. On the left, the adult insect

At the same time, Maria Lígia tested the *in vitro* interaction between the new lectin and the digestive enzymes of the larvae of these borers and found that TEL does not suffer any action from them: for not being digested, it appears to build up in the insects' intestines, causing a sort of indigestion. She also assessed the effectiveness of pitomba lectin in fighting the larvae that feed on grain – the adult beetles merely reproduce on the beans. The researcher's team put females from the two species of borer to lay eggs on artificial seeds, made with a gelatinous capsule with a mixture of paste of yard-long beans (*Vigna unguiculata*) and pitomba lectin in variable concentrations (from 0.5% to 2%). After the eggs hatched, 90% of the larvae that had consumed the false beans containing a 2% concentration of TEL died, as is attested by a study published in August's *Biochimica et Biophysica Acta*.

What was most surprising was that the pitomba lectin eliminated not only the larvae of *Callosobruchus maculatus*, the main pest for yard-long beans, much consumed in the northeast of Brazil and in sub-Saharan Africa. The protein also showed an unprecedented effect for a plant lectin, which normally serves as a nutrient in the process of the seed germination: it killed the larvae of *Zabrotes subfasciatus*, which attacks, besides the yard-long bean, another much more common kind of bean, the common bean (*Phaseolus vulgaris*).

The team from Mato Grosso do Sul intends to experiment shortly with dusting pitomba lectin extracts on corn and soy crops, a more accessible alternative for small plantations, and to assess the action of TEL against another bean borer, the *Acanthoscelides obtectus*, and the caterpillars of three moths: *Spodoptera frugiperda*, a predator of corn; *Diatrea saccharalis*, which attacks sugarcane; and *Anticarsia gemmatalis*, which damages soy leaves.

Maria Lígia is also considering the possibility of producing genetically modified plants capable of expressing pitomba lectin at levels that combat insects – not much would be needed, since the protein has already worked in a concentration (2%) regarded as lower than the one produced naturally by the plants (from 2% to 10%). "This would be an alternative for the large plantations", is the biochemist's comment. •