Calathea Bicajoux® 'Gekko Pink'

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How to help heliconia have seeds: Pollen viability and stigma receptivity in *Heliconia* spp.

Vivian Loges[1]; Simone Santos Lira Silva[2]; Ana Cecilia Ribeiro de Castro[3]; Charleston Gonçalves[4]; and Carlos Eduardo Ferreira de Castro[4]

[1]Docente, Universidade Federal Rural de Pernambuco - UFRPE, Laboratório de Floricultura - LAFLOR, Departamento de Agronomia - UFRPE, Av. Dom Manoel Medeiros s/n, Recife, PE, CEP: 52171-900, Brazil, vloges@yahoo.com; [2]Pesquisadora, Bolsista CAPES, LAFLOR, UFRPE; [3]Pesquisadora Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA) – Agroindústria Tropical, Fortaleza, CE, Brazil, cecilia@cnpat.embrapa.br; [4]Pesquisador Instituto Agronômico de Campinas (IAC), charleston@iac.sp.gov.br; ccastro@iac.sp.gov.br.

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Introduction

Heliconia species and natural hybrids are commercialized as ornamental plants, mainly as cut flowers in many parts of the world. Nevertheless, it is necessary to conduct breeding experiments and artificial pollination procedures to control the crosses in Heliconiaceae family. It will permit the production of new hybrids with desired characteristics as: longer production period through the year; novelty aspect and market acceptance; long lasting postharvest durability; packaged inflorescence suitability considering size and weight.

Nevertheless, it is difficult to cross or control the pollination procedures in breeding programs. In nature, pollination depends on the specificity of the pollinators (hummingbirds, bats and melaphagid birds) that fly from plant to plant probing the flowers for nectar and transferring the pollen from flower to flower in the same inflorescence or different inflorescences in the same clump or other clumps of the same heliconia species and even between different heliconia species.

Normally, heliconia flowers open for only one day. Nevertheless, there are many flowers per bract, the inflorescence could have many bracts and a single clump could have many flowers stems. Thus, the blooming period could be very long and permit artificial pollination procedures.

In heliconia breeding programs to cross or control the pollination procedures, the first steps are to identify the best receptivity period of the stigma and check the pollen viability in the anthers.

Stigma receptivity can be determined by quantifying the presence of peroxidase enzyme using hydrogen peroxide. This relatively simple methodology identifies the reaction of the peroxidase enzyme produced by the stigma with

hydrogen peroxide, resulting in release of bubbles (Souza, 2013). Some authors advise that it might not be the best methodology and should be tested with other substances as indicators, mainly for research purposes. Nevertheless, low cost and the possibility to buy hydrogen peroxide in drugstores, permits the use of this methodology by any one.

The pollen viability can be directly determined observing in vitro pollen germination or indirectly based on cytological parameters by the colorimetric method. It is the most used procedures in breeding programs because it is low cost and quick results can be observed. Among the dyes used in the assessment of the viability of pollen, the most common is acetic carmine. These evaluations are usually performed in the laboratory using an optical microscope. However, the use of a portable digital microscope coupled to the computer allows the capture of the images and realization of several samples for later analysis.

Complementing the interesting discovery of Dr. D. G. Gannon presented in the last HSI bulletin (Experimental hiccups sometimes lead to discovery) and Gannon et al. (2017) article about distinguishing old and new flowers of *Heliconia wagneriana*, this paper describes a simple techniqu that could easily check the receptivity of heliconia species stigma and pollen viability. That information might be used to facilitate the choice of the best flower stage to cross and could permit anyone to try to cross heliconia species.

Methodology

The flower stems of thirteen heliconia species (Table 1) were harvested from clumps at the Instituto Agronômico (IAC) collection, located in Ubatuba, São Paulo State, Brasil (latitude 23°26'02" S, longitude 45°04'16" W, 6 meters above sea level) and 2700 mm average annual rainfall. Three flowers in pre-anthesis, anthesis and post-anthesis stages (Figure 1) were removed from the inflorescences bracts of each species. The flower was carefully opened to avoid damage, the petals removed, and the stigma and stamens were cut from the base of the flower.

To evaluate the stigma receptivity, the stigmas of each open stage were placed side by side on a glass slide. Immediately two droplet of hydrogen peroxide solution (3%) was placed directly on the stigmas and inspected for two minutes under a portable digital microscope (Plugable®, with lens adapter M50 x 200) connected to a computer. Photos were capture every 15 seconds for later observations. The stigma receptivity was measured by the quantity of bubbles released and were classified as: without response (-); weak positive response (+); strong positive response (++); very strong positive response (+++) (using the system of Dafni and Maués, 1998).

The pollen viability analysis was conducted by staining with 2% acetic carmine. The pollen of three anthers of each open stage from each species was collected and distributed on a glass slide. After that, one drop of the 2% acetic carmine was placed onto the glass slide and closed with a coverslip and inspected after few seconds under a portable digital microscope (Avantscope®, with lens adapter M200 x 200) connected to a computer. To obtain a random sample of the stained pollen grains, photos were taken from four different parts of the glass slide for later observation. All the grains of pollen in each four photos were counted. The viable pollen grains absorb the acetic carmine and stained a reddish color. Pollen grains which did not absorb the acetic carmine stain became translucent and were classified as nonviable.

Results

Different intensity of stigma receptivity was observed in the three open stages of the species (Table 1). In general, strong or very strong positive response was observed in anthesis or post-anthesis stages or both. Only *H. rauliniana* 'Citra' and *H. rostrata* presented very strong positive stigma receptivity response in the three open stages. *H. stricta, H. pogonantha, H. chartacea, H. bihai* ′Aurea′ and *H. caribea x bihai* presented very strong positive responses at anthesis and post-anthesis stages. In *H. magnifica* and *H. latispatha* very strong positive response was observed at the anthesis stage and *H. orthotricha* and in *H. psittacorum* ′Alan Carle′ at post-anthesis stage. *H. bihai* ′Chocolate Dancer′ and *H. pendula* presented a low level of response.

The hydrogen peroxide test has several advantages: the solution is widely available for purchase; the solution preparation and use are very simple; the results can be observed simultaneously with the application; and the bubbles release only in the apical region of the stigma reducing the possibility of false positive.

The number and percentage of pollen grain viability observed using acetic carmine were very different among species and flower open stage. Most of the species presented higher numbers of pollen grains and higher viability percentage mainly at anthesis stage. Anthesis open stage was the best to collect pollen from *H. bihai* 'Aurea', *H. bihai* 'Chocolate Dancer', *H. chartacea* and *H. pendula* with 1055 to 3308 pollen grains and more than 91% of viability. The same stage was suggested to *H. magnifica, H. pogonantha* and *H. stricta*; nevertheless the pollen grains number was lower (824 to 190).

Pollen could be collected in anthesis and post-anthesis from *H. caribaea x bihai* and *H. orthotricha* since similar numbers of pollen and viability values were observed. In *H. rostrata* the highest percentage of viable pollen was observed in pre-anthesis (384 with 80% of viability) and intermediate number of pollens in anthesis stage (476 with 46% of viability). *H. latispatha* and *H. rauliniana* 'Citra' presented very low numbers of pollen grains in all flower stages and the few pollen grains collected from *H. psittacorum x H. spathocircinata* 'Alan Carle' probably were contamination.

Usually those types of experiment were performed in the laboratory using stereoscopic microscope with real time observation. It limited the number of analyses and, after a great number of samples could lead to error. The technique of image capture every 15 seconds for stigma receptivity

and of many samples with different photos from pollen viability with the portable digital microscope connected to a computer permitted examination of many samples per day and later analysis of the experiment with better accuracy of the research.

It is very important to collect the pollen at the optimum stage of maturation to maintain the viability and cross it at the best stage of stigma receptivity for the heliconia species. Nevertheless, it is still necessary, for other research, to determine the best time and compatibility for successful cross rate fertilization and generation of viable seeds.

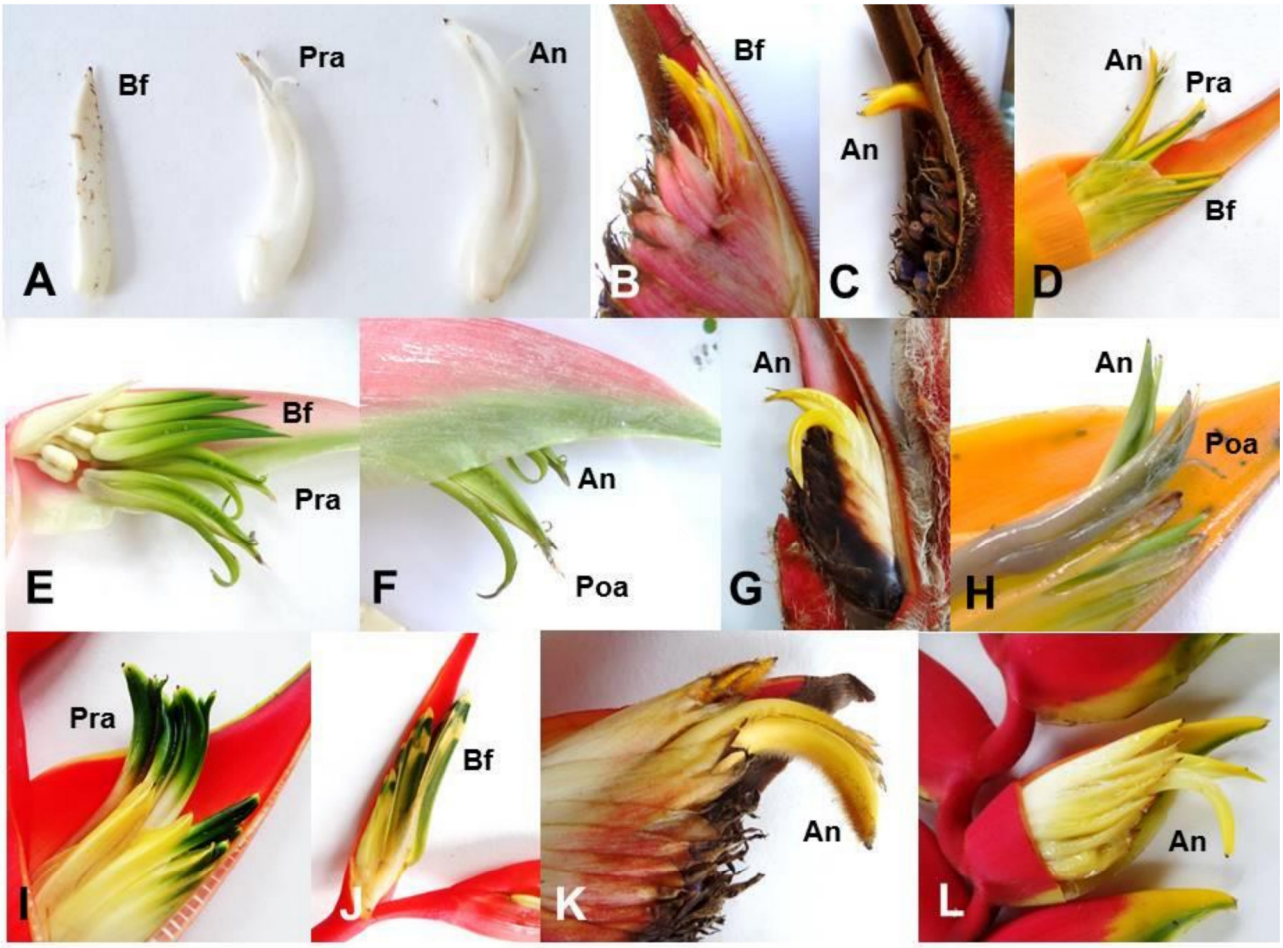

Figure 1. Open flower stages in inflorescences of *Heliconia pendula* (A), *H. magnifica* (B and C), *H. latispatha* (D), *H. chartacea* (E and F), *H. vellerigera* (G), *H. Jacquinii* (H), *H. stricta* (I), *H. acuminate* (J), *H. pogonantha* (K) and *H. rostrata* (L): Floral bud (Bf); Pre-anthesis (Pra) the anthers could not be seen; Anthesis (An) the anthers just could be partially seen above the perianth and are fresh and close each other; and Post-anthesis (Poa) the anthers were completely visible above the perianth. Ubatuba-SP, Brazil, December 2014.

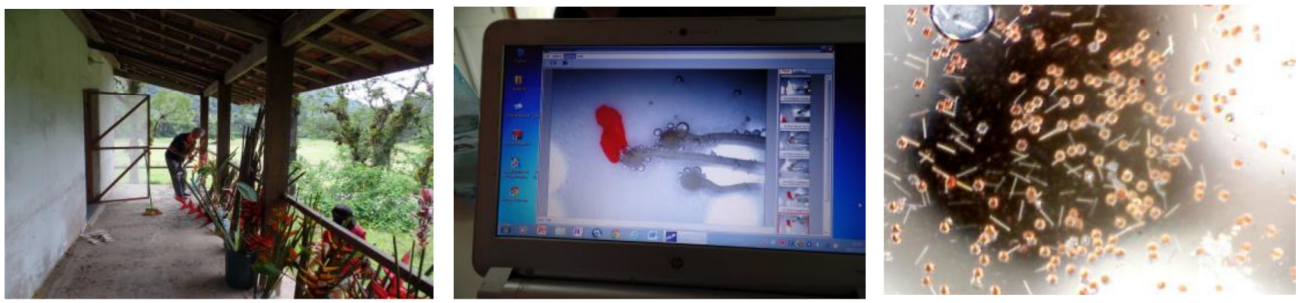

Figure 2. Phases of the experiment: A – Heliconia flower stems harvested to collect the flowers; B – Images of the receptivity of the stigmas just after a droplet of hydrogen peroxide solution (3%); C – Images of pollen viability after a droplet acetic carmine (2%). The images were obtained using a portable digital microscope connected to a computer. Ubatuba-SP, Brazil, December 2014

Table 1 - Stigma receptivity in *Heliconia* spp. in different open flower stages evaluated using hydrogen peroxide immersion (3%) for two minutes and latter analyzed by images from a portable microscope connected to a computer. Ubatuba-SP, Brazil, December 2014.

Genotypes	Pre-anthesis	Anthesis	Post-Anthesis
Heliconia bihai cv. 'Aurea'	+	+++	+++
Heliconia bihai cv. 'Chocolate Dancer'	+	++	++
Heliconia caribaea x bihai	+	+++	++
Heliconia chartacea	+	+++	+++
Heliconia latispatha	+	+++	++
Heliconia magnifica	+	+++	++
Heliconia orthotricha	+	++	+++
Heliconia pendula	+	++	++
Heliconia pogonantha	+	+++	+++
Heliconia psittacorum x H. spathocircinata cv. 'Alan Carle'	+	++	+++
Heliconia rauliniana 'Citra'	+++	+++	+++
Heliconia rostrata	+++	+++	+++
Heliconia stricta	+	+++	+++

(-) without response; (+) weak positive response; (++) strong positive response; (+++) very strong positie response (after Dafni and Maués, 1998).

Table 2 – Number and percentage of viable and nonviable pollen grains of *Heliconia* spp. in different open flower stages evaluated using 2% acetic carmine and later analyzed by digital images from a portable microscope and computer. Ubatuba-SP, Brazil, December 2014.

Genotypes	Pre-anthesis		Anthesis		Post-anthesis	
	viable	nonviable	viable	nonviable	viable	nonviable
H. bihai 'Aurea'	12 (92%)	1 (7,69%)	1121 (91%)	114 (9%)	378 (78%)	108 (22 %)
H. bihai 'Chocolate Dancer'	208 (97%)	6 (3%)	1055 (99%)	9 (1%)	323 (85%)	58 (15%)
H. caribaea x bihai	132 (87%)	20 (13%)	214 (93%)	17 (7%)	260 (89%)	32 (11%)
H. chartacea	5 (83%)	1 (17%)	3308 (99%)	40 (1%)	1260 (99%)	6 (1%)
H. latispatha	17 (89%)	2 (11%)	64 (86%)	10 (14%)	24 (63%)	14 (37%)
H. magnifica	181 (96%)	7 (4%)	824 (97%)	21 (3%)	427 (87%)	63 (13%)
H. orthotricha	77 (96%)	3 (4%)	750 (94%)	51 (6%)	779 (92%)	69 (8%)
H. pendula	199 (99%)	1 (1%)	2853 (99%)	10 (1%)	481 (94%)	32 (6%)
H. pogonantha	7 (87%)	1 (13%)	735 (62%)	445 (38%)	406 (51%)	383 (49%)
H. psittacorum x H. spathocircinata 'Alan Carle'		0 (0%)	5 (100%)	0 (0%)	1 (100%)	0 (0%)
H. rauliniana 'Citra'	4 (22%)	14 (78%)	1 (9%)	10 (91%)	16 (76%)	5 (24%)
H. rostrata	384 (80%)	94 (20%)	476 (46%)	556 (54%)	260 (54%)	225 (46%)
H. stricta	5 (45%)	6 (55%)	190 (60%)	125 (40%)	99 (74%)	35 (26%)

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Dr. David H. Lorence
National Tropical Botanical Garden
3530 Papalina Road
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