

Genetic Linkage Between *Mex2*, a Specific Resistance Gene to Anthracnose, and *Anp*, a Gene Involved in Pod Anthocyanin Accumulation in Bean

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ABSTRACT

Gantet, P., Bettini, P., Grisvard, J., and Dron, M. 1991. Genetic linkage between *Mex2*, a specific resistance gene to anthracnose, and *Anp*, a gene involved in pod anthocyanin accumulation in bean. *Plant Dis.* 75:941-942.

Breeding tests in bean (*Phaseolus vulgaris*) with *Mex2*, a single dominant resistance gene to bean anthracnose, suggested a linkage between this gene and pod anthocyanin accumulation. The cross between an inbred, purple-pod, anthracnose-resistant cultivar with *Mex2* (AFN) and an inbred, green-pod, susceptible cultivar (La Victoire) was performed to study the segregation of the two characters and to recover green pod progeny resistant to *Mex2*, because purple pods are commercially unacceptable. Pod anthocyanin accumulation proved, at least in this cross, to be under the control of a single, dominant Mendelian gene, which has been designated *Anp* (anthocyanins in the pod). Among 548 plants of F₂ progenies, 13 recombinant phenotypes were identified. The genetic distance between *Mex2* and *Anp* was estimated by maximum likelihood analysis to be 2.3 ± 1.3 cM (95% CI).

In bean (*Phaseolus vulgaris* L.), resistance to anthracnose caused by *Colletotrichum lindemuthianum* (Sacc. & Magnus) Lams.-Scrib. is controlled by a series of simple dominant genes (11). In Europe, because of limited pathogen variability, a single resistance gene (*ARE*) introduced into most inbred lines since 1960 is still effective against this disease (1). Other single dominant genes for specific resistance have been characterized in Latin America, protecting against *Colletotrichum* races present there (11). Compared to the *ARE* gene, which provides resistance against the so-called "ancestral" races of *Colletotrichum* (α , β , γ , δ , and ϵ types) (3), these genes provide protection against additional races that normally attack *ARE*, such as α Brazil 54, "Ebnet" strain of kappa, Yotta, and λ mutant (6). Breeding programs are currently underway to introduce some of these genes into bean in combination with the *ARE* gene to increase protection against possible introductions of new *Colletotrichum* races (6). Bannerot et al (2) described a new resistance gene, *Mex2*, which provides very efficient resistance against a wide range of *Colletotrichum* races, and incorporated it into the *P. vulgaris* cultivar AFN. The combination of *ARE* and *Mex2* resistances should provide very effective control against anthracnose, at least in Europe.

During the breeding program of AFN, cosegregation of the *Mex2* gene and the accumulation of anthocyanin pigments in the pod has been observed. Purple color, attributable to anthocyanins, has often been correlated with, or involved in, disease resistance in plants (4,7,8,13,17). However, in many cases, resistance and pigment accumulation have been dissociated by genetic analysis. Linkages between genes involved in specific resistance and genes for anthocyanin metabolism have been reported for several host-pathogen interactions, such as tobacco-TMV (*Tm2* gene) (12) and bean-BCMV (*I* gene) (15).

In this paper, we report a segregation analysis of *Mex2* and the purple pod phenotype. The results show that anthocyanin accumulation in the pod is determined by a single locus (*Anp*) and that *Anp* is linked but not identical to *Mex2*.

MATERIALS AND METHODS

Plant material. The inbred susceptible (La Victoire) and resistant (AFN) *P. vulgaris* genotypes were obtained from H. Bannerot and G. Fouilloux (INRA, Versailles, France). Upon exposure to light, La Victoire pods remain green, whereas under the same conditions, AFN pods show a purple-mottled phenotype. On the other hand, both La Victoire and AFN display anthocyanin pigmentation in different plant organs—flowers, cotyledons, and hypocotyls are violet, whereas seed coats are black in La Victoire and mottled black-gray in AFN. Plants were grown in a greenhouse under controlled conditions (8-hr dark/16-hr light photoperiod under fluorescent bulbs [$250 \mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ at the plant level], temperature at 24 C, and relative humidity

at 70%) in pots filled with vermiculite and watered with the nutrient solution of de Bildering and Lourtioux (5).

Five independent crosses between La Victoire (green pods, susceptible) and AFN (purple pods, resistant) were performed. Because of the autogamous behavior of *P. vulgaris* plants (selfing rate >98%), all crosses were performed manually. Young flowers in which the ovules are mature but pollen is not were hand-pollinated by delicately opening floral buds and fertilizing them with pollen obtained from mature flowers. La Victoire was used as a female parent to avoid artificial data in the progeny as a result of selfing. Genetic analysis was performed on F₁, F₂, and F₃ progenies. The purple and resistant (*Mex2*) F₁ plants were allowed to self to obtain F₂ progenies.

Fungal material. *C. lindemuthianum* (race α Brazil 54) was supplied by F. Legendre and J. Tailler (Laboratoire Cryptogamie, Orsay, France), and was grown on malt (20 g/L) agar (20 g/L) plates at 23 C in the darkness. Conidia were obtained from 10-day-old cultures by gentle scraping with a spatula, suspended in sterile water, filtered through a sintered glass filter (20–40 μm), and used directly for inoculation (10^7 conidia per milliliter).

Pathogenicity test. The test was adapted from Tu (16). Isolated cotyledonary leaves were inoculated on the abaxial surface by applying a suspension of conidia with a paintbrush. Control leaves were treated with sterile water. Infected and control leaves were then incubated on Whatman No. 3 paper moistened with sterile water in petri dishes for a 10-hr dark/14-hr light photoperiod under fluorescent light (Philips P.L. 24W 183) with the temperature at 20 C and saturated humidity. Ten days after inoculation, the presence or absence of visible symptoms (+/–) was recorded.

Statistical analysis. Theoretical and observed F₂ segregations were compared by the chi-square test. Genetic distance was estimated by maximum likelihood analysis (10).

RESULTS AND DISCUSSION

To score for both resistance and pod color on the same F₂ plant, it was necessary to use a reliable, nondestructive in vitro pathogenicity test. This also per-

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mitted later crossing or selfing of the same plant. For this reason, the detached leaf inoculation technique described by Tu (16) was ideal for segregation test. Preliminary experiments with hypocotyl inoculation in vivo demonstrated that detached leaves show the same differential specificity toward the *Colletotrichum* races as the whole plant (*data not shown*). Symptoms on susceptible leaves, as in planta, involve a general browning that spreads from the vascular bundles.

Resistance segregation analysis of F₂ progenies from the crosses between La Victoire and AFN was done by inoculation with *C. lindemuthianum* race α Brazil 54, a race that attacks La Victoire but not AFN. The observed segregation was an expected 3:1 (nonsignificant at $\alpha = 0.05$), confirming that the resistance is under the control of a single, dominant Mendelian locus present in AFN.

Plants of the F₂ progeny were also examined for pod color phenotype. In the field or greenhouse without additional light, the purple pod phenotype of AFN is barely visible. Therefore, the use of fluorescent bulbs was necessary to obtain a reproducible and intense purple-mottled phenotype on the exposed pods of AFN, whereas La Victoire pods maintained under the same conditions remained green. F₁ plants had purple pods, and the segregation ratio of pod color in 548 plants was 3:1 (nonsignificant at $\alpha = 0.05$), thus demonstrating that purple pod color of AFN is under the control of a single, dominant Mendelian locus. This locus has been designated *Anp* (anthocyanins in the pod). The *Anp* gene seems to be involved in a specific step of the anthocyanin metabolism in the pod, because anthocyanins, which are absent from La Victoire pods, are normally accumulated in the hypocotyls, cotyledons, flowers, and seed coats of both genotypes.

Among 548 F₂ plants, 396 were resistant with red pods, 139 susceptible with green pods, and 13 have been identified as harboring a recombinant phenotype, i.e., green pod-resistant (six plants) or purple pod-susceptible (seven plants). These recombinant phenotypes were confirmed by analysis of F₃ progeny from each plant (a minimum of 20 plants for each assay). Thus, the two genetic loci involved in anthracnose resistance and pod color, respectively, appeared to be

strongly linked but cannot be at the same locus. The genetic distance between *Anp* and *Mex2* was estimated by the maximum likelihood test to be 2.3 ± 1.3 cM. This linkage supports the original observations of Bannerot and Fouilloux (*personal communication*) in the course of *Mex2* breeding.

The green pod-resistant recombinants show the same level of resistance as AFN, whereas the purple pod-susceptible ones display the same symptoms as La Victoire. In this case, anthocyanins appear not to have fungistatic or fungitoxic effect, which is in contrast to previous reports concerning the effect of delphinidin on conidial germination of *Fusarium oxysporum* Schlechtend.:Fr. f. sp. *pisi* (J. C. Hall) W. C. Snyder & Hanna (9) or the biostatic activity of pelargonidin-3-glucoside from bean seeds (13).

Several linkages between specific resistance genes to plant disease and anthocyanin gene markers have been described. Two examples of this are the linkage between a gene controlling bean seed color and a dominant gene involved in hypersensitive response to bean common mosaic virus (15), and between a gene for tomato anthocyanin pigmentation and a resistance gene to tobacco mosaic virus (12). All of the observed linkages between anthocyanin color and specific resistance genes to pathogens are in the range of 1–4 cM.

The *Mex2-Anp* recombinants obtained will be used to introduce the *Mex2* resistance gene in breeding programs to obtain *Mex2* resistant plants with green pods, because purple pods are commercially unacceptable. The pod color character is currently being used as a tag to construct nearly isogenic lines differing for the presence or absence of *Mex2* (18). These nearly isogenic lines will be suitable to unravel molecular aspects of *Mex2* resistance specificity. RFLP markers will be searched for in the *Anp-Mex2* area to allow *Mex2* gene cloning by chromosome walking strategies (14).

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LITERATURE CITED

- Bannerot, H. 1965. Résultats de l'infection d'une collection de haricots par six races physiologiques d'antracnose. *Ann. Amélior. Plant.* 15:201-222.
- Bannerot, H., Derieux M., and Fouilloux, G. 1971. Mise en évidence d'un second gène de résistance totale à l'antracnose chez le haricot. *Ann. Amélior. Plant.* 21:83-85.
- Charrier, A., and Bannerot, H. 1970. Contribution à l'étude des races physiologiques de l'antracnose du haricot. *Ann. Phytopathol.* 2:489-506.
- Chiang, H. S., and Norris, D. M. 1984. "Purple stem", a new indicator of soybean stem resistance to bean flies (Diptera: Agromyzidae). *J. Econ. Entomol.* 77:121-125.
- de Bilderling, N., and Lourtiaux, A. 1976. Quinze années de phytotronique. Pages 331-341 in: *Etudes de Biologie Végétale. Hommage au professeur Chouard*. R. Jacques, ed., Paris.
- Fouilloux, G. 1979. New races of bean anthracnose and consequences on our breeding programs. Pages 221-235 in: *Diseases of Tropical Food Crops*. H. Maraite and J. A. Meyer, eds. Université Catholique de Louvain-la-Neuve, Belgium.
- Hammerschmidt, R., and Nicholson, R. L. 1977. Resistance of maize to anthracnose: Effect of light intensity on lesion development. *Phytopathology* 67:247-250.
- Hammerschmidt, R., and Nicholson, R. L. 1977. Resistance of maize to anthracnose: Changes in host phenols and pigments. *Phytopathology* 67:251-258.
- Kraft, J. M. 1977. The role of delphinidin and sugars in the resistance of pea seedlings to *Fusarium* root rot. *Phytopathology* 67:1057-1061.
- Mather, K. 1951. *The Measurement of Linkage in Heredity*. 2nd ed. Methuen and Co., London. 169 pp.
- Pastor-Corrales, M. A., and Tu, J. C. 1989. Anthracnose. Pages 77-104 in: *Bean Production Problems in the Tropics*. H. F. Schwartz and M. A. Pastor-Corrales, eds. CIAT, Cali, Colombia.
- Robinson, R. W., Provvidenti R., and Schroeder, W. T. 1970. A marker for tobacco mosaic resistance. *Rep. Tomato Genet. Coop.* 20:55-56.
- Stanton, W. R., and Francis, B. J. 1966. Ecological significance of anthocyanins in the seed coats of Phaseolae. *Nature* 211:970-971.
- Tanksley, S. D., Young, N. D., Paterson, A. H., and Bonierbale, M. W. 1989. RFLP mapping in plant breeding: New tools for an old science. *Biotechnol. Adv.* 7:257-264.
- Temple, S. R., and Morales, F. J. 1986. Linkage of dominant hypersensitive resistance to bean common mosaic virus to seed color in *Phaseolus vulgaris* L. *Euphytica* 35:331-333.
- Tu, J. C. 1986. A detached leaf technique for screening beans (*Phaseolus vulgaris* L.) in vitro against anthracnose (*Colletotrichum lindemuthianum*). *Can. J. Plant Sci.* 66:805-809.
- van Loon, J. J. A. 1990. Chemoreception of phenolic acids and flavonoids in larvae of two species of Pieris. *J. Comp. Physiol.* 166:889-899.
- Young, N. D., Zamir, D., Ganai, M. W., and Tanksley, S. D. 1988. Use of isogenic lines and simultaneous probing to identify DNA markers tightly linked to the *Tm-2a* gene in tomato. *Genetics* 120:579-585.