

BREEDING AND GENETICS

Joint Segregation of High Glanding with Nectariless and Frego Bract in Cotton

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ABSTRACT

There are mutant alleles in the genus *Gossypium* that increase its tolerance to insect pests. Among the traits that are effective against different insects, high glanding and nectariless increase cotton tolerance to lepidopteran insects, whereas frego bract suppresses the oviposition of the boll weevil, *Anthonomus grandis* Boheman. The objectives of this research were to transfer the high-glanding trait to frego-bract and nectariless plants. The genotypes used belonged to *G. hirsutum*: Guazuncho 3 INTA nectariless ne, SP 26 high glanding HG, and SP 47304 frego bract BF. Crosses between SP 26 HG/Guazuncho 3 INTA ne and SP 26 HG/SP 47304 BF and the F₂ and F₃ generations were obtained. For the combination of high glanding with frego bract, the frequency of individuals with both features varied from 6.4% in the F₂ generations to 24.6% in the F₃. For the other combination, high glanding with nectariless, the frequency of recombination was of 7.3% in the F₂ and 11.8% in the F₃. High glanding, nectariless, and frego bract are independently inherited traits in *G. hirsutum*. Therefore, it is possible to combine the high-glanding trait with frego bracts or nectariless in the same line.

Cotton (*Gossypium hirsutum* L.) cultivation is affected by different species of insect pests. Among the most important are heliothines, myriads, aphids, thrips, and arachnids (Jenkins, 1994). The use of Bt cotton cultivars reduces the number of treatments with insecticides needed. However, not all insect pests are controlled by

this technology. It is possible that some groups increase their pressure on the crop (Meredith, 1998). In this context, it is necessary to incorporate other sources of resistance to maintain yield increases and continue producing with less use of insecticides. The creation of cotton cultivars with multiple mechanisms of resistance to insect pests could maintain high yields under increasing pressure of insect pests (Scheffler et al., 2003). At present, several sources of variation are available for the selection of pest-resistant cultivars. Among the most important are morphological traits, biochemical products of the plants, wild genetic resources, and transgenic plants (Jenkins, 1994).

Within morphological traits, modified bracts stand out because they protect the flower buds and capsules. A special form known as frego bract is determined by a single gene in its homozygous recessive allelic form *fg fg* (Green, 1955). Rahman et al. (2008) observed that frego bracts segregated in a 3:1 ratio, normal and frego bracts respectively. Bracts with these characteristics are smaller in area than normal ones. Jenkins and Parrott (1971) evaluated the effect of frego lines against the attack of *Anthonomus grandis* Boheman, demonstrating a 50% decrease of damage in flower buds and 46% reduction in the use of insecticides to control this insect, compared to plants with normal bracts. The effect was attributed to the inhibition of feeding and oviposition, and to the elimination of the refuge effect for insecticides.

Another outstanding morphological feature is the absence of extra-floral nectaries, known as nectariless. Nectaries are structures that release substances rich in carbohydrates. These products are a source of food for adult insects. Therefore, the absence of nectaries decreases the insect population (Benedict et al., 1981). Jenkins (1994) indicated that this trait generates resistance to hemiptera and lepidoptera that affect reproductive organs. Meyer and Meyer (1961) evaluated the inheritance of the character and determined that it was controlled by two genes, *ne*¹ and *ne*², the genotype being *ne*¹*ne*¹ *ne*² *ne*², the homozygous double recessive did not have extra-floral nectaries.

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Finally, within the traits that present potential to develop cultivars with better tolerance to insect pests, are the phenolic compounds present in the tissues of cotton plants. Among the most important is gossypol. This compound is a pigment produced in sub-epidermal structures that is found in all organs of the plant except for roots, stamen, and petals (Arturi, 1984). Shaver et al. (1980) estimated the correlation between the larval weight of *Heliothis virescens* Fab. and the content of gossypol in the flowers, finding a coefficient $R^2 = -0.90$. Calhoun (1997) found a special form of the GI^3 allele, which is characterized by having gossypol glands in the margin of the flower's calyx. This phenotype was called high glanding (HG), being a source of resistance to insects, due to its higher content of gossypol in reproductive organs. This trait is dominant and segregates 3:1 (high glanding:normal glanding) in the F_2 (Calhoun, 1997). Parrott et al. (1989) and Hedin et al. (1992) determined that the presence of this trait in flower buds was an antibiotic factor for *H. virescens*.

The objective of this research was to evaluate the feasibility of introducing the high-glanding trait to genotypes with frego bract and nectariless.

MATERIALS AND METHODS

Plant Material. The genotypes used belonged to *G. hirsutum*; the first was 'Guazuncho 3 INTA nectariless ne', a commercial cultivar characterized by the absence of extra-floral nectaries. The second was 'SP 47304 frego bract BF', characterized by possessing the modified bracts called frego. The third genotype used was a selected line named 'SP 26 high glanding HG' that presents gossypol glands in the margin (crown) of the calyx of the flowers, a feature described by Calhoun (1997). Figure 1 shows the different traits analyzed in this research: high and normal glanding, nectary, nectariless, frego bract, and normal bract.

Generation and Processing of the F_1 and F_2 Seed. To advance generations in seed production, greenhouse and field plantings were used (Fig. 2). The crosses between SP 26 HG X Guazuncho 3 INTA ne and SP 26 HG X SP 47304 BF were performed in July 2008. The F_1 generation seeds were planted in November 2008, at the experimental station INTA Roque Sáenz Peña, Chaco, Argentina. These F_1 plants were self-pollinated under greenhouse conditions to obtain the F_2 . The morphological features were registered and analyzed by means of

a Chi-square test (χ^2). The F_2 progenies derived from both types of crosses were classified into four families: high glanding-nectariless, high glanding-nectaried, high glanding-frego bract and high glanding-normal bract (Fig. 1). For classification related to the glanding phenotype, the number of glands in the upper third of the calyx was recorded, with three being considered the lower limit to refer to the high-glanding phenotype (Calhoun, 1997). During 2010 to 2011, the F_3 generation resulting from each family was produced and the phenotypic frequencies were recorded.

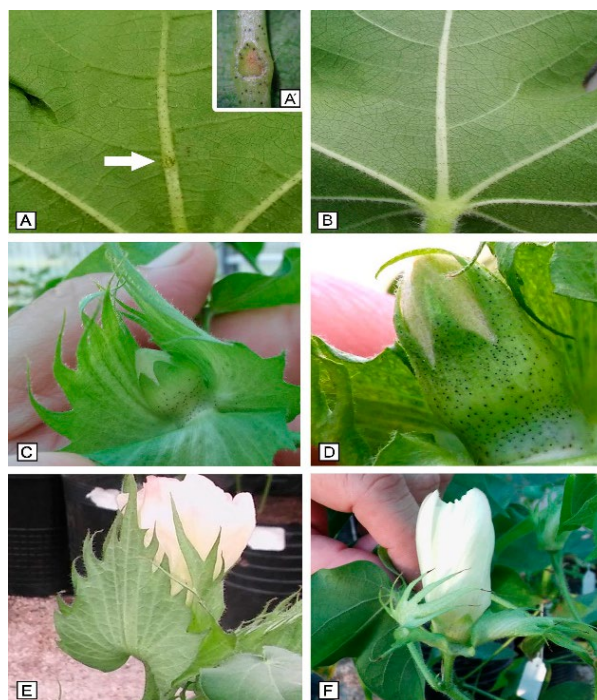


Figure 1. Several morphological features of cotton related to pest tolerance. A: nectaried, A' nectary B: nectariless, C: normal glanding, D: high glanding, E: normal bract, F: frego bract.

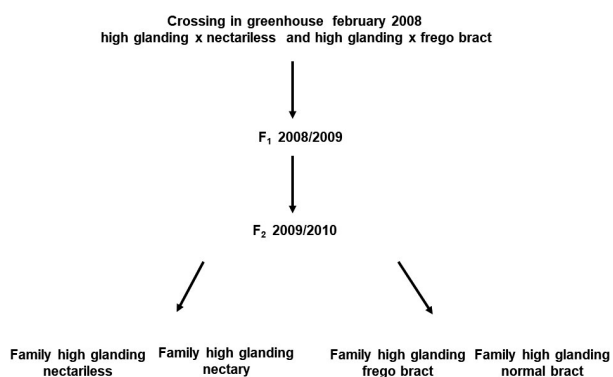


Figure 2. Flowchart of breeding procedure for analyzing the joint segregation of high glanding with nectariless and frego bract.

RESULTS AND DISCUSSION

Characterization of the F₂ Generation.

For the combination between SP 26 HG x Guazuncho 3 INTA ne, 136 plants were obtained in the F₂ generation. In this, the following phenotypes were observed: high glanding-nectaried (HG-NE), high glanding-nectariless (HG-ne), normal glanding-nectaried (NG-NE), and normal glanding-nectariless (NG-ne). The phenotypic frequencies are presented in Table 1. Considering only high glanding, the relative frequency of high glanding:normal glanding was 3.8:1, which was not statistically different from those observed by Calhoun (1997), of 3:1 high glanding:normal glanding ($\chi^2 = 1.41, p = 0.275$).

In relation to the presence of nectaries, the frequency observed for nectaried:nectariless was 10.3:1, similar to that found by Amir et al. (2002) of 16:1 in the F₂ generation ($\chi^2 = 2.13, p = 0.201$). These results agree with those observed by Meyer and Meyer (1961) for inheritance of nectariless. When both phenotypes high glanding-nectaried:high glanding-nectariless were considered, the expected frequency was 14:1; this ratio results from considering three loci, two for nectariless ($ne^1ne^1 ne^2ne^2$), which segregate independently, and one for high glanding (HG^zHG). Thus, the high glanding-nectariless phenotype corresponds to HG^zHG $ne^1ne^1 ne^2ne^2$. The expected theoretical ratio did not differ statistically from the one observed in this work (Table 2).

For the combination SP 26 HG x SP 47304 BF, 315 plants were obtained in the F₂ genera-

tion. The following phenotypes were observed: high glanding-normal bract, high glanding-frego bract, normal glanding-normal bract, and normal glanding-frego bract, whose frequency is presented in Table 1. Considering only the high and normal glanding phenotypes, the number of plants obtained was 186 high glanding and 129 normal glanding, with a phenotypic ratio of 1.4:1. These data differed statistically ($\chi^2 = 42.24, p < 0.001$), from the distribution of 3:1, observed by Calhoun (1997). Calhoun (1997), reported homozygous populations for high glanding with variation from 3 to 50 glands in the calyx crown. This variability could explain the distortions found in the observed frequency because it is not easy to distinguish between the homozygous and heterozygous phenotypes, even analyzing several generations.

With respect to the frego-bract phenotype, the number of plants with this trait was 56 in the F₂ generation. The calculated ratio between both phenotypes was 4.6:1 normal bract:frego bract. These data generate a different frequency ($\chi^2 = 8.94, p < 0.0034$) to that published by Green (1955) and Rahman et al. (2008), where both found the distribution 3:1 normal bract:frego bract. Again, the differences found between the expected and the observed proportion can be explained by the presence of phenotypically intermediate plants for frego bract. The presence of intermediate bracts was observed in progenies from crosses involving plants with normal bracts of larger size. The presence of these phenotypes could cause distortions in the interpretation of the inheritance.

Table 1. Number of F₂ plants for two combinations of parents: high glanding with nectariless and high glanding with frego bract

Phenotype	Number of F ₂ plants	Phenotype	Number of F ₂ plants
high glanding-frego bract	19	high glanding nectaried	98
high glanding-normal bract	167	high glanding-nectariless	10
normal glanding-frego bract	37	normal glanding nectaried	26
normal glanding- normal bract	92	normal glanding-nectariless	2
Total	315	Total	136

Table 2. Chi-Squared values and probabilities of goodness of fit of F₂ generation phenotypes high glanding-nectary and high glanding-nectariless

Generation	Expected ratio to be tested	Observed value		X ² value	P
		HG ^z /NE ^y	HG/ne ^x		
F ₂	14:1	98	10	2.71	0.151

^z HG: high glanding

^y NE: nectaried

^x ne: nectariless

Considering both characteristics, only 19 plants were high glanding-frego bract (Table 1), being the relationship high glanding-normal bract:high glanding-frego bract of 8.7:1. The observed proportion differed statistically from the theoretical one (Table 3). The differences between the phenotypic distributions can be explained by the previous frequencies, which are also different from the theoretical frequencies. For this, two loci were considerate (HG^z fgfg), for high glanding-frego bract.

Characterization of the F₃ Generations. For the high glanding-nectariless combination it should be noted that the high glanding-nectariless plants obtained in the F₃ generation come from two F₂ families described in Fig. 2, high glanding-nectary and high glanding-nectariless. For the first family (high glanding-nectariless, the number of recombinant plants F₃ was 18 out of 57, representing 31.5%. In the second high glanding-nectary, from 343 plants only 29 had both features (high glanding nectariless); the latter corresponded to 8.45% of the total. Although a large percentage of plants with both features was obtained from the high glanding-nectariless family. Calhoun and Bowman (1999) advised to select mainly dominant characteristics like high glanding in early generations, because those are recessive features.

For the high glanding-frego bract combination, the number of plants F₃ with both features, obtained from the family F₂ high glanding-frego bract (Fig. 2) was 57 out of a total of 188, representing 30.3%. The F₃ plant with high glanding-frego bract, obtained on the other F₂ family high glanding-normal bract was 137, representing 20.9% of the total. For this combination, the increase in frequency from one family to another was similar to that observed in high

glanding-nectariless. The higher frequency of high glanding-frego bract in relation to high glanding-nectariless could be due to the genetic nature of frego bracts, controlled by a single recessive gene (Green, 1955) unlike nectariless, controlled by two allelic recessive alleles ne¹ne² (Meyer and Meyer, 1961). For the two combinations, the number of plants with both characteristics increased from F₂ to F₃, for the four evaluated families (Table 4).

CONCLUSIONS

It was possible to combine and transfer the high-glanding feature to both frego bract and nectariless lines. In addition, it was observed that recessive characteristics such as nectariless and frego bract can be selected during F₃ generation. The knowledge generated will contribute to the incorporation of these traits to different genotypes of cotton and could improve the resistance to certain pests.

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REFERENCES

- Amir, A.H. 2002. Genetics of leaf nectarines in upland cotton. *Asian J. Pl. Sci.* 4:423–442.
- Arturi, M.J. 1984. Especies silvestres y cultivadas. p. 7–12. In M.J. Arturi (ed.), *El Algodón. Mejoramiento genético y técnica de su cultivo*. Buenos Aires. Editorial Hemisferio Sur S.A.

Table 3. Chi-Squared values and probabilities of goodness of fit of F₂ generation phenotypes high glanding-normal bract and high glanding-frego bract

Generations	Expected ratio to be tested	Observed value		X ² value	P
		HG ^z -BN ^y	HG-BF ^x		
F ₂	4.1	167	19	11.13	0.001

^z HG: high glanding

^y BN: normal bract

^x BF: frego bract

Table 4. Number of plants with both features high glanding-nectariless and high glanding-frego bract for F₂ and F₃ generations

Generation	high glanding-nectariless	high glanding- frego bract
F ₂	10	19
F ₃	47	194

- Benedict, J.H., T.F. Leigh, A.H. Hyer, and P.F. Wynholds. 1981. Nectariless cotton effect on growth survival and fecundity of lygus bugs. *Crop Sci.* 21:28–30.
- Calhoun, D.S. 1997. Inheritance of high glanding, an insect resistance trait in cotton. *Crop Sci.* 37:1181–1186.
- Calhoun, S.D., and D.T. Bowman. 1999. Techniques for development of new cultivars. p. 377–378. *In* C.W. Smith and J.T. Cothrem (eds.), *Cotton: Origin, History, Technology, and Production*. John Wiley & Sons, New York, NY.
- Green, J.M. 1955. Frego bract, a genetic marker in upland cotton. *J. Hered.* 46:232.
- Hedin, P.A., W.L. Parrott, and J.N. Jenkins. 1992. Relationship of glands cotton square terpenoid aldehydes and other allelochemicals to larval growth of *Heliothis virescens* Lepidoptera: Noctuidae. *J. Econ. Ent.* 85:359–364.
- Jenkins, J.N. 1994. Host plant resistance to insect in cotton. *In* *Proceeding of 1st World Cotton Conference Research*, Brisbane Australia. 1:359–372.
- Jenkins, J.N., and W.L. Parrott. 1971. Effectiveness of frego bract as a boll weevil resistance character in cotton. *Crop Sci.* 11:739–743.
- Meyer, J.R., and V.G. Meyer. 1961. Origin and heritance of *nectariless* cotton. *Crop Sci.* 1:167–169.
- Meredith, W.R. 1998. The role of host plant resistance in *Lygus* management. p. 940–945 *In* *Proc. Beltwide Cotton Conf.*, San Diego, CA. 5-9 Jan. 1998. *Natl. Cotton Counc. Am.*, Memphis, TN.
- Parrott, W.L., J.N. Jenkins, J.E. Mulrooney, J.C. McCarty, and R.L. Shepherd. 1989. Relationship between gossypol gland density on cotton squares and resistance to tobacco budworm larvae. *J. Econ. Ent.* 82:589–592.
- Rahman, S.U., T.A. Malik, M. Ashraf, and S. Malik. 2008. Inheritance of frego bract and its linkage with fiber and seed traits in cotton. *Pak. J. Bot.* 40:1621–1626.
- Scheffler, J.A., M.K. Dowd, W.R. Meredith, and B.E. Scheffer. 2003. New possibilities for some old genes: improved host plant resistance. *In* *Proceeding of the 3rd World Cotton Conference Research*, South Africa 1:175–182.
- Shaver, T.N., R.H. Dilday, and F.D. Wilson. 1980. Use of glandless breeding stocks to evaluate unknown *Heliothis* growth inhibitors (X-Factors) in cotton. *Crop Sci.* 20:545–548.