Pak. J. Bot., 42(2): 997-1002, 2010.

INCREASE IN FREQUENCY OF THE *RPG*4 GENE IN SOYBEAN CULTIVARS

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Abstract

The objective of this study was to observe the change in frequency of resistance gene (*Rpg*4) to *Pseudomonas syringae* pv. *Glycinae* (*Psg*) between the ancestors and public soybean cultivars. A comparison of the alleles at the locus for resistance of soybean (*Glycine max,* L.) to bacterial blight in a sample of 51 North American public cultivars released through 1990 with the allele in their ancestors indicated that breeders have concentrated the dominant allele in public cultivars from 69 to 84%. The *Rpg*4 gene may be involved in resistance to *Psg* in soybean, however, it has another useful function that somehow contributes to soybean productivity in modern agroecosystems and thus plant breeders have unintentionally increased its frequency in cultivated germplasm.

Introduction

Soybean (*Glycine max* (L) Merrill) of the family Leguminosae is considered a miracle crop due to its extraordinary qualities. In Pakistan, it is one of the nonconventional oilseed crop that can be successfully grown during spring as well as the summer season. It contains 40 to 42% good quality protein and 18 to 22% oil comprising 85% unsaturated fatty acids and is free from cholesterol along with ample mineral elements, so it is highly desirable in human diet (Arshad et al. 2006).

Bacterial blight disease caused by *Pseudomonas syringae* pv. *glycinea* (*Psg*) in soybean (*Glycine max* [L.]Merr.) provides a model system to elucidate the molecular genetics and biochemical events that determine recognition specificity and the subsequent induction of disease resistance in a plant-pathogen interaction (Firdous et al., 2007; Flor, 1942; Staskawicz *et al.*, 1984; Keen, 1990). The *avr* genes cloned from *Pseudomonas syringae* pv. *tomato* (*Pst*) function in *Psg* and cause it to elicit a hypersensitive response (HR) on some soybean cultivars (Keen & Buzzell, 1991; Staskawicz *et al.*, 1987). One of these avirulence genes, *avr*D (Kobayashi *et al.*, 1990), causes the bacterium to produce a low molecular weight elicitor, called syringolides (Slaymaker & Keen, 2004; Smith *et al.*, 1993; Midland *et al.*, 1993). Syringolides are glycolipids produced by Gram-negative bacteria expressing *Pseudomonas syringae* carrying an active allele of *avr*D.

The syringolides mediate gene-for-gene complementarity, inducing the HR only in only those soybean plants carrying the disease resistance gene-*Rpg*4 (Slaymaker & Keen, 2004; Farhatullah *et al.*, 1996; Smith *et al.*, 1993; Midland *et al.*, 1993; Keen & Buzzell, 1991, Keen *et al.*, 1990) but not *rpg4*. The syringolides elecitors are more active (at least 1000 times) in *Rpg*4 than in *rpg*4 cultivars (Okinaka *et al.*, 2002).

The earliest public cultivars were mostly developed using pedigree selection. Today the original plant introductions and the wild and semi-wild species (*G. soja* and *G. gracilis*) are generally used as donor parents. The objective of this study was to observe the change in frequency of *Rpg*4 between the ancestors and public hybrid-derived cultivars.

Materials and Methods

Experimental materials included a random sample of 51 out of 289 US/Canadian public cultivars of hybrid origin and 14 of their 17 original parental lines (Table 1), as reported by Lohnas & Bernard (1991). These lines were screened for the presence of the *Rpg*4 gene. Except two of the lines viz., Norchief and Acme and used as HR (showing hypersensitive response) and nonHR controls (supplied by Dr. Mark Stayton, Department of Molecular Biology, University of Wyoming). The rest of the material involved in this study were obtained from Dr. Randy Nelson, Curator, Soybean Genetic Collection, Department of Agronomy, University of Illinois, Urbana USA.

Specific elicitor preparation, application and reaction: The specific elicitor (SE) was isolated to a high degree of purity from *Psg* transformed with *avr*D by the method used by Keen *et al*., (1990).

Fully expanded primary leaves of soybean seedlings at the 4-leaf stage were infused with a concentration of 125 units of SE/ml of water. The blunt needle mounting end of a disposable 1ml plastic syringe was pressed on to the lower surface of the leaf and approximately 20-25 µL per site of SE were administered by applying pressure to the plunger. After infusion, seedlings were placed in a controlled environment chamber with an 18h photoperiod at a constant temperature of 25°C and 90% relative humidity for 48h. A standard procedure was used to assess the disease reaction. The inoculated leaves were screened visually for the presence or absence of hypersensitive necrosis in response to the SE after 24-48h (Keen *et al*., (1990).

Results and Discussions

Analysis of *Rpg*4 locus ancestry of public cultivars: Seven each of the 14 important ancestral lines produced hypersensitive response (HR) to elicitor application which means that these carry disease resistant genes (*Rpg*4/*Rpg*4); while seven were nonHR (*rpg*4/*rpg*4). The HR lines have contributed 63.4% more germplasm to the public hybrid-derived cultivars (Table 1). Evaluation of the random 51 public cultivars provided further evidence for concentration of the dominant *Rpg*4 allele (Table 1). Forty-three lines were HR (84.3%) and 8 lines were nonHR (15.7%). The high proportion of HR lines may partly be attributed to sampling error. For the sample of 51 cultivars, the contribution of alleles from the 14 important ancestors was 92.6% (63.4% HR and 29.2% nonHR) (Table 1). Therefore, the sample of public cultivars has a higher proportion of HR ancestry than the population as a whole. The remaining 7.4% is unknown, if this is in the same proportion as for the known ancestors, the overall contributions of HR and nonHR germplasm would have been 69% and 31%, respectively. The data suggested that the ancestral germplasm contributed 68.6% *Rpg*4 and 31.4% *rpg*4 alleles in the crosses that gave rise to the sample of 51 US/Canadian public cultivars as shown in Table 1. However, the proportion of HR lines observed among the 51 public cultivars (84.3%) was much higher than the proportion of HR germplasm among the ancestors of these cultivars (68.6%). Analysis of the proportion by chi-square test indicates that the distribution of HR and nonHR lines observed in the sample is significantly different from what would be expected on the basis of a 69.62% HR: 30.38% nonHR ancestral contribution of germplasm ($X^2_{1df} = 5.85$; $p<0.025$).

Evolution at the *Rpg*4 locus: The germplasm survey (Farhatullah *et al.*, 1996) indicated that the dominant *Rpg*4 allele was concentrated at high frequency in public hybrid-derived cultivars (more than 80% HR lines) compared to wild and semi-wild lines, plant introductions, and ancestral lines (approximately 50% HR lines). The pedigree analysis and evaluation of *Rpg*4 locus genotypes for 51 public cultivars demonstrates that the frequency of *Rpg*4 alleles has increased in the public cultivars released out of breeding. One possibility for this increase in gene frequency is that *Rpg*4 has another function that somehow contributes to productivity in modern agroecosystems, and as soybean breeders have consciously selected for the best new agronomic lines among segregating populations produced in crossing programs, they have incidentally selected for the *Rpg*4 allele. Similarly increase in frequency of the *Rpg*4 allele is that selection was made against soybean lines which are highly susceptible to bacterial blight.

It is interesting to note the dimorphism at the *Rpg*4 locus was observed in wild and semi-wild soybean germplasm, landraces of soybean, and the ancestral lines (Farhatullah *et al.*, 1996). Whatever advantage *Rpg*4 alleles might provide in modern agroecosystems, this dimorphism suggests that *Rpg*4 and *rpg*4 alleles are of relatively neutral fitness in natural and primitive agricultural ecosystems, otherwise natural selection in the wild or unconscious selection during domestication would have fixed the more favorable allele. Perhaps a mechanism such as frequency-dependent selection had maintained the relatively equal frequencies of both alleles in the wild and in primitive agriculture; or perhaps genotype X environment interactions which served to maintain both alleles in wild and primitive soybean populations growing in different habitats. What so ever the relative advantages and disadvantages of the two alleles at the *Rpg*4 locus in wild and primitive soybean, it appears that plant breeders might have selected the *Rpg*4 allele towards fixation in public hybrid-derived cultivars.

Association of maturity groups with *Rpg*4 allele: The sample of 51 cultivars (Table 1) is earlier maturing (mmg-mean maturity group II.92) than their parents and includes more Mandarin (mmg 0.5) germplasm (24.9%). It appears that earliness is associated with HR but that this association is relatively weak. Some public HR cultivars are late (eg., Gregg and Semmes are both maturity group VII) while some public nonHR cultivars are early (Morsoy is group 00). If the *Rpg*4 allele itself somehow contributes to earliness, the contribution must be a small one. The maturity group for the 14 important ancestors range from 0 to VII. In contrast, the maturity group of all 289 public cultivars ranges from 000 to IX.

The mmg for the 7 important HR ancestors is IV.00 (range 0 to VII, treating Mandarin as before) whereas the mean maturity group for the 7 important nonHR ancestors is IV.43 (range I-VII). Thus, the sampled HR ancestors as a group were slightly although not statistically earlier than the nonHR ancestors. The relatively weak association of earliness with HR frequency is probably due to chance. Some HR ancestral lines are late (CNS and Palmetto are both VII) while some nonHR ancestral lines are early (PI 54610 is I). The same is true for public cultivars. The possibility that breeding for earlier maturity in soybean might also play a role in the higher frequency of HR germplasm in the ancestry of public hybrid cultivars must also be considered.

Natural selection can cause changes in the characteristics of a bulk population during inbreeding. Visual selection among lines in unreplicated or replicated plots is effective for maturity, height, lodging and most other characters. In some cases, the high yielding lines frequently are not selected visually due to lodging and variable maturity.

References

Arshad, M., Naazar A. and Abdul G. 2006. Character correlation and path coefficient in soybean glycine max (l.) Merrill. Pak. J. Bot., 38(1): 121-130,

Farhatullah, M.M. Stayton and R.W. Groose. 1996. Survey of soybean germplasm for bacterial blight resistance alleles at the *Rpg*4 locus. *Sabrao J*., 28: 57-62.

Firdous, S. S., Rehana A., M.I. Hague and S. N. Afzal. 2007. Development of hypersensitive response by *xanthomonas campestris* pv. *sesami* on *lycopersicon esculentum* l., and solanum tuberosum l., leaves. *Pak. J. Bot*., 39(6): 2135-2139.

Flor, H.M. 1942. Inheritance of pathogenicity in Melampsora lini. *Phytopathol*., 32: 653-669.

Keen, N.T. and R.I. Buzzell. 1991. New disease resistance genes in soybean against *Pseudomonas syringae* pv. *glycinea*: evidence that one of them interacts with a bacterial elicitor. *Theor. Appl. Genet*., 81: 133-138.

Keen, N.T., S. Tamaki, D. Kobayashi, D. Gerhold, M. Stayton, H. Shen, S. Gold, J. Lorang, H. Thordal-Christensen, D. Dahlbeck and B. Staskawicz. 1990. Bacteria expressing avirulence gene D produce a specific elicitor of the soybean hypersensitive reaction. *Mol. Plant-Microbe Interac*., 3: 112-121.

Kobayashi, D.Y., S.J. Tamaki and N.T. Keen. 1990. Molecular characterization of avirulence gene D from *Pseudomonas syringae* pv. *tomato*. *Mol. Plant-Microbe Interact*., 3: 94-102.

Lohnes, D.G. and R.L. Bernard.1991. Ancestry of US/Canadian commercial cultivars developed by public institutions. *Soybean Genet. Newsl*., 18: 243-255.

Midland, S.L., N.T. Keen, J.J. Sims, M.M. Midland, M.M. Stayton, V. Burton, M.J. Smith, E.P. Mazzola, K.J. Graham and J. Clardy. 1993. The structure of syringolides 1 and 2, novel C-glycosidic elicitors from *Pseudomonas syringae* pv. *tomato*. *J. Org. Chem*., 58: 2940-2945.

Okinaka, Y., C.H. Young, E. Herman, A. Kinney and N.T. Keen. 2002. The P34 syringolide elecitor receptor interacts with a soybean photorespiration enzyme, NADH-dependent hydroxypyruvate reductase. *Mol. Plant-Microb Interact*., 15: 1213-1218.

Slaymaker, D.H. and N.T. Keen. 2004. Syringolides elicitor-induced oxidative burst and protein phosphorylation in soybean cells, and tentative identification of two affected phosphoproteins. *Plant Sci*., 166: 387-396.

Smith, M.J., E.P. Mazzola, J.J. Sims, S.L. Midland, N.T. Keen, V. Burton and M.M. Stayton. 1993. The syringolides: Bacterial C-glycosyl lipids that trigger plant disease resistance. *Tetrahedron Lett*., 34: 223-226.

Staskawicz, B.J., D. Dahlbeck and N.T. Keen. 1987. Cloned avirulence gene of *Pseudomonas syringae* pv. *glycinea* determines race-specific incompatibility on *Glycine max*. *Proc. Natl. Acad. Sci*. USA., 81: 6024-6028.

(Received for publication 4 May 2009)