Indonesian Journal of Agricultural Science 8(2), 2007: 48-52

DIALLEL ANALYSIS OF RESISTANCE TO BACTERIAL STALK ROT (*Pectobacterium chrysanthemi* pv. *zeae* Burk., McFad. and Dim.) IN CORN (*Zea mays* L.)

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ABSTRACT

One of the major disease problems affecting maize farming in the Philippines is bacterial stalk rot (BSR) caused by *Pectobacterium chrysanthemi* pv. *zeae* Burk., McFad. and Dim., which is formerly known as *Erwinia chrysanthemi*. The annual loss due to BSR is estimated at PhP. 20 million equivalent to Rp3.5 billion. At present, there is no effective control method against BSR and, therefore, varietal improvement through breeding resistant germplasms is needed. The present study aimed to determine the combining ability and the extent of additive and non-additive genetic effects in corn inbred lines with a range of reaction to BSR. Four resistant lines (S3YB 137-1-1-B, TUPI (S3) 5-1-B, TUPI (S3) 15-2-B, and 97-835) and two susceptible lines (CML 295 and 97-733) were used as genetic materials. Generation of test entries and evaluation of disease resistance were conducted at the experimental farm station of University of the Philippines Los Banos and Institute of Plant Breeding Los Banos, respectively, during 2002 to 2003 wet seasons. Griffing's diallel mating system Model 1, Method 1 was followed in generating the test entries to make a total of 36 entries (six selfed parental lines and 15 each of F_1 crosses and their reciprocal crosses). The entries were then evaluated for disease resistance in a yield trial following a randomized complete block design (RCBD) with two replications. Results of diallel analysis showed two lines, S3YB 137-1-1-B and TUPI (S3) 5-1-B, exhibited the best general combining ability (GCA) for resistance to BSR, while the crosses S3YB 137-1-1-B x TUPI (S3) 5-1-B and TUPI (S3) 5-1-B x 97-835 performed the best specific combining ability (SCA) for the resistance. GCA effect was greater than that of SCA. This indicated that additive gene effects were found to be more important than non-additive gene effects in the expression of resistance to BSR in the six corn lines used. Therefore, breeding programs towards recurrent selection that emphasize GCA would be more appropriate for BSR resistance improvement involving those six lines.

[*Keywords*: *Zea mays*, diallel analysis, disease resistance, bacterial stalk rot]

INTRODUCTION

Bacterial stalk rot (BSR) caused by *Pectobacterium chrysanthemi* pv. *zeae* Burk., McFad. and Dim. is considered a serious problem affecting corn production in the Philippines. In Indonesia, BSR is not that prevalent compared to fungal stalk rot, but stalk rot in general is considered one of the most important disease in corn after downy mildew and leaf blight.

Bacterial stalk rot in corn usually appears as a tan to dark brown, water-soaked, soft or slimy disintegration of pith tissues at a single internode. Affected stalks suddenly collapse and are usually twisted. The tip of the uppermost leaves often wilt, followed by the appearance of a slimy soft rot at the base of the whorl. The decay spreads rapidly downward until the affected plants collapse. Diseased plants often have a foul odor. Occurrence of stalk rot before physiological maturity results in yield loss due to poorly filled kernels or premature death of plants. Cultural management to control the disease has been ineffective or very expensive using fungicides. Host plant resistance offers a more practical solution to this problem.

There are very few studies conducted on the resistance to BSR in the Philippines. Dionio and Raymundo (1990) tested 14 isolates of *Erwinia chrysanthemi* collected from various localities in Central and Southern Mindanao. Their results showed that both hybrids and open-pollinated varieties appeared to possess genes for resistance that can be accumulated through appropriate selection techniques. Lozano (2000) used generation mean analysis (GMA) to study the genetics of resistance to BSR, which he found to be due to additive and additive by additive gene effects.

Further study of the inheritance of resistance to BSR using different genetic materials and genetic models is still needed. Appropriate experimental design that includes the BSR resistant lines previously screened should provide additional information on the gene action involved in the expression of resistance. The results of the study should serve as guide in breeding varieties resistant to BSR.

Towards the development of resistant but agronomically adapted germplasm, diallel mating design has been widely used. It could determine the presence of heterosis and combining ability in the materials being used. Depending on the outcome of the study,

appropriate selection method could be proposed. If additive gene effects with partial to complete dominance are important, recurrent selection methods that emphasize general combining ability (GCA) should be used (Jenkins 1940). If overdominance is of primary importance, recurrent selection methods that emphasize specific combining ability (SCA) would be appropriate (Hull 1945). The objective of this study was to determine the combining ability and the extent of additive and non-additive genetic effects in corn inbred lines with a range of reaction to BSR.

MATERIALS AND METHODS

Six inbred lines of corn derived from Institute of Plant Breeding Los Baños and CIMMYT germplasms collection, consisted of four resistant (S3YB 137-1-1-B, TUPI (S3) 5-1-B, TUPI (S3) 15-2-B, 97-835) and two susceptible (CML 295 and 97-733) lines, were planted at the experimental farm station of University of Philippines Los Baños during two planting seasons (2002 wet season and 2003 dry season) for parental inbred seed increase and generation of crosses, respectively. Each inbred line was crossed in all possible combinations using diallel mating design method 1 of Griffing's to make a total of 15 F_1 crosses and 15 F_1 reciprocal crosses. At maturity, ears were harvested, sun-dried and individually shelled. Seeds of the same cross were bulked and labeled properly.

The 30 crosses and their parents were evaluated for their reaction to BSR at the experimental farm station of Institute of Plant Breeding Los Baños during 2003 wet season using randomized complete block design (RCBD) with two replications. Seeds were germinated on Petri dish for one week before planting to assure their germination. The seedlings were transferred into polyethylene planting bags. A plot consisted of 25 polyethylene planting bags with one plant per bag. Appropriate management practices for the care of the trials were followed.

BSR inoculation was done following the methods developed by Pascual (2001). Plants were inoculated with BSR by pouring 20 ml bacterial cell suspension (10^6-10^7 cells ml^{-1} at 50% transmittance) at the basal portion of 5-week-old plants under wet soil condition, followed by stab-inoculation to optimize the disease reaction. The stab-inoculation was done by using a locally made stab-inoculator, dipped in approximately 2 ml bacterial cell suspension (10^6-10^7 cells ml^{-1} at 50% transmittance), punctured at the basal portion of plants before flowering time (approximately 8-week-old plants), in nearly water saturated soil.

The number of infected plants was observed one week after stab-inoculation and the observation was repeated every week until 3 weeks before harvest. Disease incidence was measured by counting the number of infected plants over the total number of plants per plot.

The data obtained were subjected to analysis of variance (ANOVA) in RCBD. The disease incidence was transformed using *arcsine square root of percentage* to fulfill the assumption of normal distribution, since the data are in percentage and largely ranged from 0 to 100%. Single factor ANOVA was conducted on the transformed data for genotype difference followed by ANOVA of Griffing's Model 1, Method I diallel to determine the relative importance of GCA and SCA and their effects. Mid-parent heterosis between the crossed inbreds was also computed.

RESULTS AND DISCUSSION

During the first week after inoculation, necrotic appearance was observed on the basal portion of the plant. After entering the second and third week after inoculation, the necrotic appearance spread over a wider area of the stalk followed by rotting and foul odor. At the fifth week after inoculation, the whole plant dried and lodged. These results are almost similar to those observed by Karganilla and Exconde (1972), Laysa (1980), and Lozano (2000).

Observation on disease development of the parental lines at 1-5 weeks after inoculation showed that CML 295 was the most rapidly infected by BSR, while TUPI (S3) 5-1-B was the one with the lowest disease incidence and slow disease development (Fig. 1). Two inbreds, S3YB 137-1-1-B and 97-733, expressed susceptibility to BSR since the earlier week after inoculation, but the development of the disease increased slowly, while TUPI (S3) 15-2-B showed low disease incidence at first week after inoculation, but it increased rapidly after the second week after inoculation. Inbred 97-835 showed low disease development at 1-3 weeks after inoculation, but entering the fourth week after inoculation it showed high disease incidence.

These results give an indication that resistance to BSR was possibly manifested as ability to delay the onset of the disease on plants. The ability ranged from a short-period delay, as what was observed in S3YB 137-1-1-B and TUPI (S3) 15-2-B, to longer-period delay as shown by TUPI (S3) 5-1-B and 97-835.

Analysis of variance for disease incidence showed significant variation among genotypes (Table 1).

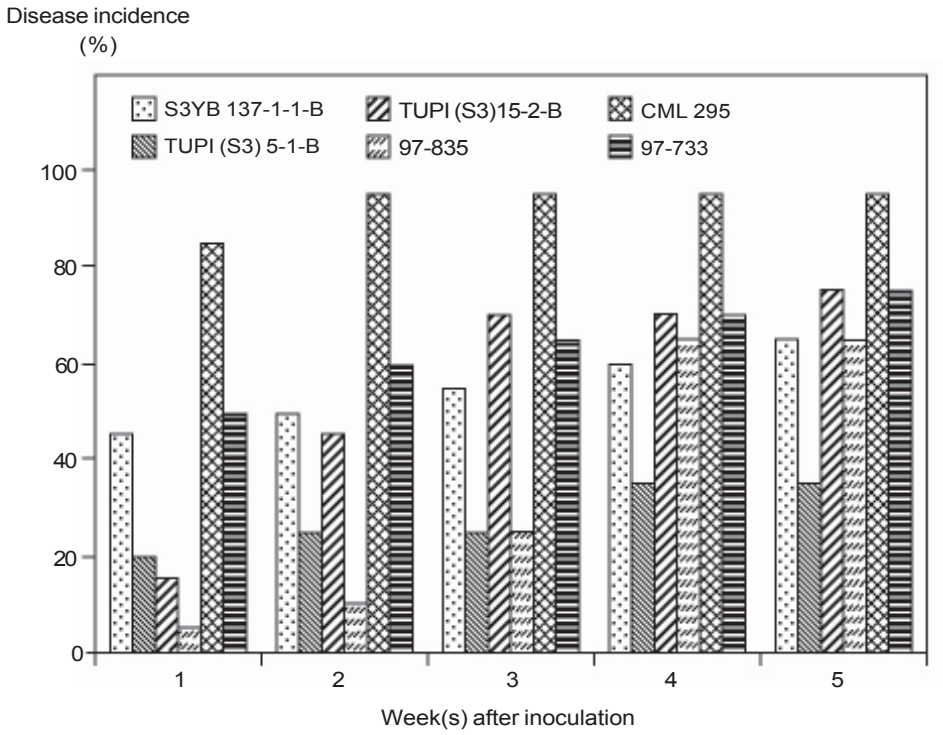

Fig. 1. Bacterial stalk rot disease incidence of six parents of corn observed at 1-5 weeks after inoculation (6-10 days after planting).

Disease incidence ranged from 20 to 100% with an average of 65.97% (Table 2).

The enhanced inoculation technique (pouring followed by stab-inoculation) could be one factor that triggered susceptibility shown by almost all the genotypes tested. Inoculation through bacterial cell suspension poured into the soil provided a sustaining environment for the pathogen. The succeeding stab-inoculation into the plant's stalk further enhanced the infection by the pathogen. The combination of the two techniques allowed disease establishment to be more rapid and effective.

Furthermore, the cool weather and wet conditions during infection might have contributed to the susceptible reactions of even the supposedly resistant genotypes. As observed by Reifschneider and Lopes (1982), Saxena and Lal (1984), and Sah (1991), BSR infection was highest at relative humidity of about 100% and temperature between 28-32°C. Resistant genotypes may have limited value in disease management when pathogen is abundant and weather is favorable for infection to occur. The present study was conducted in only one location. It would have been more desirable to sample the range of conditions under which the pathogen could infect.

Table 1. ANOVA for bacterial stalk rot incidence on corn.

Source of variation	Degree of freedom	Mean squares	F calc.
Replication	1	162.75	0.92ns
Genotype	35	730.40	4.16*
CGA	5	1939.26	22.07*
SCA	15	1333.34	15.17*
Reciprocal	15	72.38	0.82ns
Error	35	175.73	
Total	71		

*Significantly different at 0.05 probability level.
ns = Not significantly different at 0.05 probability level.

Table 2. Bacterial stalk rot disease incidence on parental lines, F_1 crosses, and F_1 reciprocals of corn (%).

♀/♂	P1	P2	P3	P4	P5	P6
P1	65	20	45	65	80	45
P2	40	35	45	35	70	45
P3	50	75	80	85	100	85
P4	60	20	100	65	100	75
P5	80	70	95	90	95	85
P6	35	30	90	50	95	75

P1 = S3YB 137-1-1-B, P2 = TUPI (S3) 5-1-B, P3 = TUPI (S3) 15-2-B, P4 = 97-835, P5 = CML 295, P6 = 97-733

GCA and SCA estimates were significant for disease incidence (Table 3). Additive gene effects were found to be more important than non-additive gene effects in the inheritance of BSR resistance, shown by the value of MS GCA which is much greater than that of SCA. This result is consistent with the study of Lozano (2000), who showed that additive gene effect is highly significant in the inheritance of BSR resistance in all cross combinations using generation mean analysis (GMA).

GCA estimates ranged from -17.187 to 18.844. The highest negative-valued GCA was exhibited by TUPI (S3) 5-1-B followed by S3YB 137-1-1-B, while the highest positive-valued one was shown by CML 295. GCA with negative value is desirable in terms of BSR infection. The negative-valued GCA for TUPI (S3) 5-1-B and S3YB 137-1-1-B imparts their ability to transmit resistance to their progenies.

The cross of S3YB 137-1-1-B and TUPI (S3) 5-1-B resulted in a good specific combiner for BSR resistance. Another good specific combiner for BSR resistance resulted from the cross of TUPI (S3) 5-1-B to 97-835, whose GCA value is positive. This result could be due to the effects of resistance gene in TUPI (S3) 5-1-B, which were able to mask the effects of susceptible gene in 97-835. Another possible explanation is that the resistance to BSR showed by 97-835 could be controlled by partial dominance.

It was also found from this study that reciprocal effects do not play a role in the inheritance of resistance to BSR. This implies that neither maternal effects nor cytoplasmic effects direct the inheritance of resistance to BSR.

Some crosses exhibited lesser disease incidence compared to their parents. This phenomenon is very common observed in breeding for resistance in particular and in quantitative traits in general. Several hypotheses have been proposed and discussed by several authors to account for it, including the hypothesis of heterosis that might operate in some crosses (Hallauer and Miranda 1981; Bernardo 2002).

The cross TUPI (S3) 5-1-B x 97-835 exhibited the greatest superiority in resistance to BSR over the mid-parent. This was shown by its heterosis value for disease incidence of -45.00 (Table 4). Another best specific combination for resistance to BSR (S3YB 137-1-1-B x TUPI (S3) 5-1-B) exhibited good heterosis value as well (-40.00). The negative value of heterosis for disease incidence is desirable because it reflects the higher degree of resistance of the hybrids to BSR, in comparison with their mid-parents' performance.

Almost all the crosses involving S3YB 137-1-1-B, except in combination with CML 295, resulted in better resistance to BSR than the mid-parent with a range of -42.86% to -3.85%. One could therefore expect to obtain more resistant hybrids by crossing inbred lines to S3YB 137-1-1-B. On the other hand, all crosses involving CML 295 showed no heterosis (indicated by positive or zero value of heterosis) for resistance to BSR. The hybrids produced from crossing with CML 295 exhibited poorer reaction to the disease, in comparison to their mid-parent.

CONCLUSION

The GCA estimates were found to be significant and much higher than the SCA estimates for BSR incidence. Therefore, additive gene effects were more important than non-additive gene effects in the expression of resistance to BSR in corn. Two resistant parents, S3YB 137-1-1-B and TUPI (S3) 5-1-B, were found to be the best general combiners for BSR resistance. The cross between these two parents and the cross TUPI (S3) 5-1-B x 97-835 resulted in the best specific combiners for BSR resistance.

Based on the results of this study, S3YB 137-1-1-B and TUPI (S3) 5-1-B could be promising materials in a hybrid breeding program. They could also be used in recurrent selection, which mostly exploits additive

Table 3. General combining ability effects (diagonal values), specific combining ability effects (above diagonal), and reciprocal effects (below diagonal) for bacterial stalk rot disease incidence on corn.

♀/♂	P1	P2	P3	P4	P5	P6
P1	-9.196	-12.973	1.481	3.636	0.263	-7.654
P2	-6.640	-17.187	2.575	-13.231	-1.919	-1.692
P3	-1.442	-9.000	8.359	12.273	0.818	7.952
P4	1.662	8.082	-11.250	0.749	6.398	-3.989
P5	3.105	0.000	4.610	6.640	18.844	-0.502
P6	2.727	4.022	-4.610	7.557	-6.640	-1.569

P1 = S3YB 137-1-1-B, P2 = TUPI (S3) 5-1-B, P3 = TUPI (S3) 15-2-B, P4 = 97-835, P5 = CML 295, P6 = 97-733

Table 4. Mid-parent heterosis (MPH) value of some crosses of corn for bacterial stalk rot incidence.

Cross	MPH (%)	Cross	MPH (%)	Cross	MPH (%)
P1 x P2	-40.00	P2 x P3	4.35	P3 x P5	11.43
P1 x P3	-34.48	P2 x P4	-45.00	P3 x P6	12.90
P1 x P4	-3.85	P2 x P5	7.69	P4 x P5	18.75
P1 x P5	0.00	P2 x P6	-31.82	P4 x P6	-10.71
P1 x P6	-42.86	P3 x P4	27.59	P5 x P6	5.88

P1 = S3YB 137-1-1-B, P2 = TUPI (S3) 5-1-B, P3 = TUPI (S3) 15-2-B, P4 = 97-835, P5 = CML 295, P6 = 97-733

gene action. The improved cycles of the population can then be used for varietal release directly or for hybrid development if the heterotic grouping could be kept separately.

REFERENCES

Bernardo, R. 2002. Breeding for Quantitative Traits in Plants. Stemma Press, Woodbury, Minnesota, USA. 369 pp.

Dionio, B.T. and A.D. Raymundo. 1990. Components of pathogenic variability and host resistance to *Erwinia chrysanthemi* Burk., McF. and Dim. in corn. Phillip. Phytopathol. 26: 42-49.

Hallauer, A.R. and J.B. Miranda. 1981. Quantitative Genetics in Maize Breeding. Iowa State Univ. Press, Ames, Iowa, USA. 468 pp.

Hull, H.F. 1945. Recurrent selection for specific combining ability in corn. J. Amer. Soc. Agron. 37: 134-145.

Jenkins, M.T. 1940. The segregation of genes affecting yield of grain in maize. J. Amer. Soc. Agron. 32: 55-63.

Karganilla, A.D. and O.R. Exconde. 1972. Bacterial stalk rot of corn and sorghum. Philipp. Phytophatol. 8: 4.

Laysa, F.D. 1980. Etiology and epidemiology of bacterial stalk rot of corn. Ph.D. Dissertation, University of The Philippines Los Banos, College Laguna, Philippines.

Lozano, G.P. 2000. Inheritance of resistance to bacterial stalk rot of maize. MS Thesis, University of The Philippines Los Banos, College Laguna, Philippines.

Pascual, C.B. 2001. Institute of Plant Breeding Annual Report. Los Banos. Philippines.

Reifschneider, F.J.B. and C.A. Lopes. 1982. Bacterial top and stalk rot of maize in Brazil. Plant Dis. 66: 519-520.

Sah, D.N. 1991. Influence of environmental factors on infection of maize by *Erwinia chrysanthemi* pv. *zeae*. J. Inst. Agric. and Animal Sci. 12 :41-45.

Saxena, S.C. and S. Lal. 1984. Use of meteorological factors in prediction of *Erwinia* stalk rot of maize. Trop. Pest Magt. 30: 82-85.