Uganda Journal of Agricultural Sciences, 2013, 14 (2): 141 - 149 ISSN 1026-0919

Inheritance of resistance to *Pyricularia grisea* in GULU-E finger millet blast resistant variety

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Abstract

Gene action and heritability of blast resistance in GULU-E was determined from crosses between GULU-E as female parent mated to four susceptible genotypes using North Carolina 1 crossing design to determine nature of resistance. Inoculation was done using one potentially most virulent local pathogen isolate (NGR1) identified from Ngora in Odwarat parish, one of the pathogen hotspots of eastern agro-ecology of Uganda. It was identified following isolate screening trial for virulence in Makerere University during 2012 B. The F_1, F_2 and backcrosses were evaluated under controlled conditions and disease reaction indicated that resistance is partially dominant and additive based on mid parent values from crosses. Segregating ratios and chi-square tests of F_2 populations fitted 13R:3S genetic model, indicating presence of duplicate dominant epistasis at probability level of 0.05. Broad-sense heritability estimated by variance components method was high at about 88.8% on entry mean basis. Selection for resistant progeny derived from crosses between GULU-E and DR21 would be most effective in early generations followed by modified backcrossing at F_3 to the adapted recurrent resistant parent leading to diversification of a population and derivation of materials for selection for disease resistance. From the study it is possible to accumulate genes for race specific resistance in host cultivars that might reduce development of disease epidemics in some areas. The genetic control of components of resistance and mechanisms of resistance in the host which affect the rate of development of a disease epidemic need to be determined, since they are important variables for durable resistance.

Key words: Broad sense heritability, durable resistance, gene action, modified backcrossing, virulence

Introduction

Finger millet is basis for food security which directly supports the livelihoods of rural majority living in marginal areas of Uganda. This consists of traditional and new cultivars grown by farmers, however new high yielding cultivars have been characterized by lack of durability of resistance to blast disease. This caused by highly variable fungus *pyricularia grisea*, one of the key biotic constraints to finger millet production. This causes by break down of single gene which confers resistance (Takan *et al*., 2011).

This frequency of breakdown of varieties is predicted to increase following the recent variability in weather and climate. This will affect livelihoods of people who depend on finger millet and other actors of the value chain. The breakdown can occur immediately after

release or sometimes even before breeding lines reach farmers. Durability of resistance is important for crop protection against emergence of new races, therefore it is imperative to breed for varieties that are stable. There are now efforts in ICRISAT and Uganda to breed for durable resistance to pathogen populations in our environment using locally adaptable varieties.

The major components of this control strategy are identification of local genetic sources of resistance, utilization and deployment of resistance genes. GULU-E is local variety released in 1960's and grown in north eastern agro-ecology and has maintained its disease resistance reaction through test seasons and locations. It is used as blast resistant check in many pathological studies in testing for virulence and race identification (Adipala and Wandera, 2001). Therefore for the purpose of initiating breeding for durable resistance, GULU-E has a potential for providing the necessary genetic variability for selection.

However, there is limited information on the nature of genetic inheritance of genes controlling blast resistance in this local variety. This information is important in developing breeding plan for efficient transfer of resistance in to breeding lines. This will lead to development of durable finger millet varieties released and contributing to increasing agricultural productivity (Bio-innovate, 2013).

Therefore, the purpose of this study was to determine gene action in GULU-E as basis of breeding strategy for incorporating blast resistance on to elite breeder's lines from millet programme in Serere Research Institute. The other objective of the study was to develop a mapping population to be used in depth studies of sequencing, for identification Quantitative Trait loci (QTLs) for novel genes for resistance to blast hence facilitating breeding for multiple resistance or pyramiding resistance genes.

Materials and methods

Experimental design

Gene action and heritability of blast resistance in GULU-E was determined from crosses between GULU-E as female parent mated to four susceptible genotypes (Table 1) below, using North Carolina 1 crossing design. The full-sib progenies were selfed and advanced to F_2 generation. Inoculation was done using one potentially most virulent local pathogen isolate (NGR1) identified from Ngora in Odwarat parish, one of the pathogen

Table 1. Description of Parents used in crossing (on the basis of previous study)

S/N	Genotype	Origin	Description under artificial infection
1	Gulu-E	GULU in northern Uganda -landrace accession	Resistant to blast
2	Katfm1	Kenyan variety - drought tolerant	Susceptible to blast
3	DR21	Breeding line from Serere Research institute	Susceptible to blast
4	IE2790	ICRISAT line from India	Susceptible to blast
5	KABALE	KABALE in south west Uganda, land race	Moderately susceptible to blast

Source: Aru (2014)

hotspots of eastern agro-ecology of Uganda. It was identified following isolate screening trial for virulence in Makerere University during 2012B (Aru *et al*., 2014).

The F_1, F_2, parents and some successful backcrosses making all together 16 entries were evaluated under controlled conditions. The trial was laid in Randomized complete block design (R.C.B.D), replicated five times and data was taken on five plants per pot and the means subjected to analysis of variance and other statistical analysis.

Inoculation and disease assessment

The procedure for inoculum preparation was according to (Nature protocols, 2008) for preparation of long-term stocks of virulent *magnaporthe grisea.* Inoculation was done at 55days after planting (DAP), since this corresponds with field resistance when race-specific genetic factors are more strongly expressed compared to seedling stage. At maturity stages proper identification of traits with good adaptations would be possible. In breeding for durable resistance, it is important to accumulate genes for race specific resistance resulting in decrease in fitness or aggressiveness of the pathogen, which reduces development of disease epidemics (Johnson, R.1976, Tewsbury and Nabban, 2001). Data was collected on area of the leaf covered by lesions (Takan *et al*., 2002).

When less 30 % of the leaf is covered by lesion was rated as resistant. Data were also taken and on number days from inoculation to infection of flag leaf (appearance of monogenic chlorotic lesions). These variables indicate the rate of pathogen growth in the plant. The difference between inoculation and infection reflects the differences in the growth rate of the pathogen in the host and is component of partial resistance (Parleviet, 1978).

Statistical analysis

Study of gene action involved in the inheritance of blast resistance was determined using regressions analysis, Chis-square test of goodness of fit and estimation of components of generation means based on theoretical expectations (Hayman, 1958).

Results

There was significant variance between genotypes at $P<0.001$ with high C.V of 25.6 %. The interval from inoculation to infection of the flag leaf provided means of differentiating genotypes (Table 2).

Heritability of blast resistance

The results of mid-parent regression on F_2 progenies indicated close relationship, with moderate narrow sense heritability of 0.28 and very high coefficient of determination of 0.72 (Table 3). Genotypes; IE2790, F_2 GULU-E X KATFM1, BCF_1DR21 X GULU-E and KATFM1 had the lowest mean number of days to infection of flag leaf below the grand mean of 21 days for the population. Mean while F_2 GULU-E X KABALE, F_1 GULU-E X DR21 took an average of 31 days from inoculation before infection of the flag leaf (Table 4).

Nature of gene action

Analysis of change of mid parent from F1 showed presence of heterosis to either direction (Table 5). On further analysis of genes leading to these expression based on segregation rations of resistance to susceptible reaction (R: S) and testing their frequencies, we found that F_2 GULUE X

Table 2. ANOVA for F_1S, F_2S, backcrosses & parents based on period from inoculation to infection of the flag leaf

Source of variation	DF	SS	MS	VR	Fpr
Rep	4	250.45	62.61	2.03	
Genotype	15	2990.6	199.37	6.48***	<0.001
Residual	60	1847.15	39.79		
Total	70	5088.2			
L.S.D (5%)=7.01,		C.V=25.6%		S.E =5.54	

*** Significant = p<0.001

Table 3. Analysis of variance for mid-parent regression on F2 progenies for blast reaction for determining narrow –sense heritability (h^2n)

Source of variation	DF	SS	MS	F
Regression	1	2.93	2.93	58.6**
About regr (error)	2	0.1	0.05	
Total	3	3.03		

C.V% = 8.1, R^2 = 0.72, b = 0.28 = h^2n = narrow sense heritability, R^2 = Coefficient of determination = 1-(errorMS)/ (total MS) is fraction of variation accounted for, b = regression coefficient, Ho=b=0, **Significance of the regression (p<0.01)

IE2790 and F_2 GULUEX KATFM1 fitted into 13R:3S segregation ratio indicating the presence of dominant epistasis (table 6).On the other hand F_2 GULU X KABALE &F_2 GULU-E X DR21 fitted in to (15R:1S), this showed evidence of duplicate gene interaction (Table 7).

Discussion

Combined analysis of generations showed significance (P < 0.05) this together with the presence of positive heterosis confirms presence of genetic variability. The trait was transmitted from parents to the offspring, hence potential for selection. The genetic components of this variance were additive and non additive, which was supported by moderate narrow sense heritability and presence of negative heterosis for days to infection of the flag leaf in some crosses.

Low narrow sense heritability could have been due to the small sample size; therefore it is necessary to verify these findings using a large population. But never the less presence of heterosis on either direction serves to indicate presence of two types of gene action. Although blast resistance is controlled mainly by major genes (Oduori, 2008), however, from this study its expression in some crosses could

Table 4. Mean number of days to flag leaf infection based on scale modified from Parleviet (1977)

Genotype (Crosses)	Mean	Reaction
BC1F1DR21 X GULU-E	18.4	S
BC1FIGULU-E X DR21	25.4	R
IE2790 X GULU-E	11.2	HS
F1GULU-E X IE2790	21.6	R
F1GULU-E X KABALE	31.8	HR
F1GUL-E X DR21	31.0	HR
F1 GULU-E X IE2790	21.2	R
F1 GULU-E X KAFM1	22.8	R
F2 GULU –E X IE2790	20.0	MS
F2GULU-E X KABALE	27.8	HR
F2GULU-E X KATFM1	13.0	HS
GULU-E (Resistance check)	21.2	R
IE2790	10.8	HS
KABALE	25.2	R
KATFM1	18.8	S
Grand mean	21.65	

Based on 1-5 scale; where 1 = 29-35 days from inoculation (110 days from emergency (DAE), 2 = 22-28days, 3 = 15-21days, 4 = 8-14 days, 5 = 1-7days. Scores1-3 were considered resistant. R = Resistance, HR = Highly resistant, HS = Highly resistant, S = Susceptible, HS = Highly susceptible

be depending on system of polygenic modifiers where there was positive heterosis for days to infection of the flag leaf based on change of mid parent values from F1.

There is evidence from several variance estimation studies that epistasis is common in self pollinated crops such as finger millet (Eberhart *et al*., 1966). Dominance variance might also contain epistatic variances if epistasis is present. This is in agreement with the findings from (Athwal and Waston, 1954), that genes with major or minor effect will function differently in different genetic background.

From this study, there is evidence that modifier genes could be producing minor effects on the degree of resistance or susceptibility and were also segregating in the cross together with major gene. This then was responsible for change of F_2 mean from the mid parent values on either directions. There could also be physiological/bio-chemical mechanisms responsible for increasing days to infection of the flag leaf which needs to be understood and incorporated in to breeding for resistance.

A combination of major resistance with quantitative resistance becomes a promising breeding strategy, since both forms of resistance are present in GULU-E and responsible in preserving its durability overtime. The findings are in line with the hypothesized breeding strategy for breeding for durability in pepper cultivars (*Capsicum annum*) attacked by

Table 5. Mean disease severity of parents (P1 and P2), F1, F2 and mid parent values for days to infection of flag leaf

Cross	p1	p2	F1	BCF1	F2	Change of MP from F1 as of MP %	Heterosis
IE2790 X GULU-E	10.8	21.2	11.2		20	-30	Negative
KATFM1 X GULU-E	18.8	21.2	22.8		13	14	Positive
KABALE X GULU-E	25.2	21.2	31.8		27.8	37	positive
DR21 X GULU-E	13.4	21.2	18.9	18.4	15	9	positive
Mean						10.9	

Estimation of components of generation means based on theoretical expectations (Hayman ,1958).
F_1 = MP = F2 if genes are primarily additive
F_2= (FI+MP)/2, BC_1 (P1)=(F1+P1)/2 BC_1(P2)=(F1+P2)/2 if both dominance and additive are present
MP =Mid parent, BCF = Backcross F1, p1 = parent1, p2 = parent2
Mid-parent heterosis as change of performance of mid-parent values from F1 mean
(F1-MP)/MP X100

Table 6. Analysis of gene interactions using Chi-square (X^2) test to determine departure of observed frequencies from theoretical expectations of (13R:3S), classical dominant epistasis model

Crosses	Phenotype	Observed	Expected	Chi-Sqr 0.05
F2GULU x KATFM1	R	24	20.3	0.67
	S	1	4.69	2.9
	Total	25	24.99	3.57*
F2GULU x KABALE	R	24	20.3	0.024
	S	1	4.69	0.011
	Total	25	24.99	0.035ns
F2GULU x IE2790	R	13	20.3	2.63
	S	11	24.99	8.49
	Total	24	24.99	11.09*
F2GULU x DR21	R	17	20.3	0.54
	S	8	1.57	2.34
	Total	25	24.9	2.88ns

Based on a scale (1-5). Disease severity ratings for leaf blast modified from Takan *et al.,* 2002. Scores (1-3) severity were considered resistant(R) scores (4-5) severity as susceptible(S). Significant goodness of fit *p<0.05

potato virus Y (Poty virus) (Baebler, 2009). This is because resistance breakdown frequency due to major gene is high and susceptibility is highly heritable, this cultivar (GULU-E) could have been naturally eliminated by the variable pathogen.

Therefore from the study, it is possible to accumulate genes for race specific resistance in host cultivars through backcrossing that might reduce development of disease epidemics in some areas.

When dominant genes become more frequent they tend increase on the days to infection of the flag leaf and overall rate of growth rate of the pathogen slowed. The frequency of dominant genes against virulent pathogen populations in our environment can be accumulated through modified backcrossing.

Conclusion

Gene action of blast resistance in GULU-E was determined from crosses between GULU-E as female parent mated to four susceptible genotypes using North Carolina 1 crossing design .The genetic components of this variation indicated both non-additive and additive variance.

A combination of major resistance with quantitative resistance is responsible for preserving durability of resistance in GULU-E cultivar. Genes with minor effect modify levels of disease resistance provided by major genes in some genetic backgrounds. Therefore a breeding strategy for durable resistance to blast should combine both forms of resistance.

From this study, it is possible to accumulate genes for race specific resistance in host cultivars that might

Table 7. Analysis of gene interactions using Chi-square (X^2) test to determine departure of observed frequencies from theoretical expectations (15R:1S), classical duplicate gene interaction

Cross	Phenotype	Observed	Expected	Chi - Sqr
F2GULU X KATFM1	R	24	23.4	0.02
	S	1	1.57	0.21
	Total	25	24.97	0.23ns
F2GULU X KABALE	R	21	23.4	0.25
	S	4	1.57	3.76
	Total	25	24.97	4.01*
F2GULU X IE2790	R	13	23.4	4.62
	S	11	1.57	56.64
	Total	24	24.97	61.26**
F2GULU X DR21	R	17	23.4	1.75
	S	8	1.57	26.33
	Total	25	24.97	28.08***

Disease severity rating scale (1-5); Scores (1-3) severity were considered resistant(R) when 30% of leaf area covered by lesions and scores (4-5) severity as susceptible (S) with more than 30% leaf area covered by lesions (Takan *et al.,* 2002). Significant goodness of fit *p<0.05, ***p<0.001

reduce development of disease epidemics in our environment. This can be through recurrent selection followed stages of backcrossing at any generation to the recurrent resistant parent to increase of frequency of dominant genes. This will contribute towards development of durable varieties.

As a recommendation, there is need to identify genetic factors directly affecting the frequency of adaptation of *pyricularia grisea* fungal pathogen to major genes and comparing them with genetic factors affecting quantitative resistance, in order to gather more information of on genes directly affecting resistance durability. This will provide basis for breeding for sustainable resistance.

Acknowlegement

Financial support by Bio-innovate and Mcknight through RUFORUM is highly appreciated. I am also grateful to Makerere University for the Msc. training, the National Semi Arid Resources Research Institute (NaSARRI) for availing the finger millet germplasm, supervision, and facilitation to participate in this conference.

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