Inheritance of resistance to (NGR1) pathogen isolates of *Pyricularia grisea* in GULU-E finger millet last resistant variety of Uganda

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Abstract

Finger millet is basis for food security which directly supports the livelihoods of rural majority living in marginal areas in East Africa. Gene action and heritability of blast resistance in GULU-E finger millet was determined from crosses between GULU-E as female parent mated to four susceptible genotypes, using the North Carolina 1 crossing design, to determine nature of resistance. Inoculation of finger millet with a fungus, *Pyricularia grisea,* which causes blast, was done using one potentially most virulent local pathogen isolate (NGR1) identified from Ngora district, in Odwarat parish, which is one of the pathogen hotspots of eastern Uganda . It was identified following isolate screening trial for virulence in Makerere University, during 2012b. F_1, F_2 and backcrosses were evaluated under controlled conditions. Disease reaction indicated that resistance was 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 a probability level of 0.05. Broad-sense heritability estimated by the variance components method was high (88.8%) on entry mean basis. Selection for resistant progeny derived from crosses between GULU-E and DR21 finger millet 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 disease epidemic, need to be determined, since they are important variables for durable resistance.

Key words: Broad sense heritability, durable resistance, 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. These consist of traditional and new cultivars grown by farmers; however, new high yielding cultivars have been characterised by lack of durability of resistance to blast disease caused by fungus, *pyricularia grisea*, one of the key biotic constraint to finger millet production in East Africa. Lack of sustainability of resistance is caused by break-down of a single gene, which confers resistance (Takan *et al.*, 2011). The frequency of break-down of varieties is predicted to increase following the recent variability in weather and this will affect livelihoods of people who depend on finger millet .The breakdown can occur immediately after release or sometimes even before breeding lines reach on- farm. Durability of resistance is a vital component of food security, therefore it is imperative to breed for varieties that are stable.

There are efforts in Malawi 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 and deployment of resistance genes. GULU-E is local variety released in 1960's and grown in north eastern agro-ecology of Uganda and has maintained it's 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 a breeding plan for efficient transfer of resistance into breeding lines, thus leading to development of durable finger millet varieties for release (Getachew Gashaw *et al.*, 2013). Therefore, the purpose of this study was to determine gene action in GULU-E as a basis of a breeding strategy for incorporating blast resistance on to elite breeder's lines.

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), using North Carolina 1 crossing design producing half-sib progenies that were selfed to F_2. Inoculation was done using one potentially most virulent local pathogen isolate (NGR1) identified from Ngora district, one of the pathogen hotspots of eastern agro-ecology and major finger millet growing area (Adipala.E and Wandera. 2001 and Takan *et al.,* 2004). 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 high humidity at 96% and temperature of 22^{oc} necessary for infection. The trial was laid in randomised complete block design (RCBD), replicated five times. Data were taken on five plants per pot. Means subjected to analysis of variance, regression analysis, Chis-square test of

Table 1. Description of parents used to derive crosses for resistance to *Pyricularia grisea* in selected drought tolerant finger millet genotypes in Serere research institute

Genotype	Origin	Description under artificial infection
GULU-E	Gulu in northern Uganda, landrace variety	Resistant to blast
KATFM1	Kenya Commercial Variety , drought tolerant from Katurumani Institute	Susceptible to blast
DR21	Breeders line from Serere research institute	Breeders line from Serere research institute, moderately susceptible
IE2790	ICRISAT line from India	Susceptible to blast
KABALE	Kabale in south west Uganda, land race	Moderately susceptible to blast

Source: Aru (2014)

goodness of fit and component of generation means.

Inoculation and disease assessment

Inoculum preparation was according to nature protocols for preparation of long-term stocks of virulent *magnaporthe grisea* (David, 2008). Inoculation was done at 55 days after planting (DAP), corresponding with field resistance, when race-specific genetic factors are more strongly expressed compared seedling stage. At maturity stages (from flower initiation to physiological maturity), proper identification of traits with good adaptations which constitute components of yield in finger millet is possible such as number of fingers, length of fingers and size of seed. In breeding for durable resistance, it is important to accumulate genes for race specific resistance, resulting in decrease of fitness or aggressiveness of the pathogen, which reduces development of disease epidemics (Mukankusi *et al.,* 1999). Data were taken on area of the leaf covered by lesions (Takan *et al*., 2002) and on number of 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 (Adipala and Wandera, 2001).

Statistical analysis

Inheritance of blast resistance was computed using regressions analysis, Chis-square test of goodness of fit and estimation of components of generation means based on theoretical expectations (Singh and Chaudhary, 2007)

Results

Variability for flag leaf infection

There was significant variance between genotypes (P<0.001, CV = 25.6 %). The interval from inoculation to infection of the flag leaf provided means of differentiating genotypes (Table 2).

Heritability of blast resistance and type of gene action

Results from mid-parent regression on F_2 progenies was significant, with moderate coefficient of regression (b = 0.28), which is narrow sense heritability (Table 3). Genotypes; IE2790, F_2 GULU-E X KATFM1, BCF_1DR21 X GULU-E and

KATFM1 had the lowest number of days to infection of the flag below the grand mean of 21 days for the population. Meanwhile F_2GULU-E X KABALE, F_1GULU-E X DR21 had an average of 31 days from inoculation before infection of the flag leaf (Table 4). Analysis of change of mid parent from F_1 showed presence of heterosis on either direction for days to infection of flag leaf (Table 5).

Nature of genetic resistance

Analysis of genes leading to these expression based on segregation ratios of resistant to susceptible genotypes (R: S) and testing their frequencies, we found that

Table 2. Analysis of variance for genetic differences among F_1S, F_2S, backcrosses and parents of crosses between GULU-E to susceptible finger millet genotypes based on period from inoculation with *Pyricularia grisea* 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. Regression of mid-parent values on F2 progenies for infection of the flag leaf by chlorotic lesions for estimating narrow –sense heritability the degree to which the genes for flag leaf resistance to are passed to offspring

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 , R^2= Coefficient of determination =1-error MS/total MS is fraction of variation accounted for ,b = regression coefficient ,Ho = b = 0, **Significance of the regression (P<0.01)

Table 4. Days after inoculation with *Pyricularia grisea* to infection of flag leaf from F_1S, F_2S, backcrosses & parents crossed to GULU-E the resistant host in Makerere University Kabanyolo

Genotype (Crosses)	Mean
BC1F$_1$DR21 X GULU-E	18.4
BC1F$_1$GULU-E X DR21	25.4
F$_2$IE2790 X GULU-E	11.2
F$_1$GUL-E X IE2790	21.6
F$_1$GULU-E X KABALE	31.8
F$_1$GUL-E X DR21	31.0
F$_1$ GULU-E X IE2790	21.2
F$_1$ GULU-E X KAFM1	22.8
F$_2$ GULU –E X IE2790	20.0
F$_2$GULU-E X KABALE	27.8
F$_2$GULU-E X KATFM1	13.0
GULU-E	21.2
IE2790	10.8
KABALE	25.2
KATFM1	18.8
Grand mean	21.65

F_2GULU-E X IE2790 and F_2GULU-E X KATFM1 fitted into 13R:3S segregation ratio indicating the presence of dominant epistasis (Table 6).Genotypes F_2 GULUXKABALE &F_2 GULU-EXDR21 fitted in to (15R:1S), an evidence of duplicate gene interaction (Table 7).

Discussion

Mode of inheritance

Mid-parent regression on F_2 progenies was significant implying that the deviations from regression were significant. The regression equation does not properly explain the relationship between the mean of mid-parent and that of F_2 population. There could be other unaccounted factors such physiological, structural and environmental influencing the phenotypic expression (disease response) not genetically backed. These need to be understood and incorporated in to breeding or integrated in to disease management.

Table 5. Mean disease severity of parents (p1and p2), F_1, F_2 and mid parent values and measurement of heterosis as change from performance of mid-parent values from F_1 based on period after inoculation with *Pyricularia grisea* to infection of flag leaf

Cross	p1	p2	F1	BCF1	F2	Change of MP from F1 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 (Singh and Chaudhary, 2007). $F_1 = MP = F_2$ if genes are primarily additive. $F_2 = (F_1+MP)/2$, $BC_1(P^1)=(F_1+P^1)/2$ $BC_1(P^2) = (F_1+P^2)/2$ if both dominance & additive are present. MP = Mid parent, BC = Backross,p1 = parent 1 p2 = parent 2. Mid-parent heterosis (%) = $(F_1-MP)/MP$ x 100

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Table 6. **Analysis of gene interactions using Chi-square (X²) test to determine departure of observed frequencies from theoretical expectations (13R:3S) classical dominant epistasis model**

Crosses	Phenotype	Observed	Expected	Chr-Sqr 0.05
F_2 GULU x KATFM1	R	24	20.3	0.67
	S	1	4.69	2.9
	Total	25	24.99	3.57*
F_2 GULU x KABALE	R	24	20.3	0.024
	S	1	4.69	0.011
	Total	25	24.99	0.035ns
F_2 GULU x IE2790	R	13	20.3	2.63
	S	11	24.99	8.49
	Total	24	24.99	11.09*
F_2 GULU x DR21	R	17	20.3	0.54
	S	8	1.57	2.34
	Total	25	24.9	2.88ns

Based on scale of 1-5 disease severity ratings for leaf blast modified from Takan *et al.,* 2011. Scores 1-3 for severity were considered resistant(R) that is 30% of leaf area covered by lesions and scores(4-5) severity as susceptible(S) with more than 30% leaf area covered by lesions. Significant goodness of fit *P<0.05. 30% leaf area covered by lesions (Takan *et al.,* 2011). Significant goodness of fit *P<0.05,***P<0.001

Table 7. **Analysis of gene interactions using Chi-square (X²) test to determine departure of observed frequencies from theoretical expectations (15R:1S) classical duplicate gene interaction**

Cross	Phenotype	Observed	Expected	Chr - Sqr
F_2GULU X KATFM1	R	24	23.4	0.02
	S	1	1.57	0.21
	Total	25	24.97	0.23ns
F_2GULU X KABALE	R	21	23.4	0.25
	S	4	1.57	3.76
	Total	25	24.97	4.01*
F_2GULU X IE2790	R	13	23.4	4.62
	S	11	1.57	56.64
	Total	24	24.97	61.26**
F_2GULU X DR21	R	17	23.4	1.75
	S	8	1.57	26.33
	Total	25	24.97	28.08***

Disease severity rating scale of 1-5; Scores 1-3 severity were considered resistant (R) that is 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.,* 2011). Significant goodness of fit *P<0.05,***P<0.001

Combined analysis of variance of generations showed significance, implying presence of genetic variability for selection at early generations. The genetic components of this variance were both non-additive and additive. The additive variance being controlled by many genes is influenced by environment, which is supported by moderate narrow sense heritability(Holland *et al.*, 2003). Low narrow sense heritability could have also been due to the small sample size; therefore, it is necessary to verify these findings using a, large population. But nevertheless, presence of heterosis on either direction indicates presence of two types of gene action.

Nature of genetic resistance

Although blast resistance is controlled mainly by major genes (Oduori, 2008), from this study it's expression in some crosses could have depended on the system of polygenic modifiers, depending on the genetic background and environment. GULU-E X KABALE and GULU-E X DR21 have good specific combining ability that could be exploited in development of improved varieties based on expression of low infection. The expression of chlorotic-lesion resistance is enhanced in a background containing genes for lesion-number resistance and flag leaf resistance. There is evidence from several variance estimation studies that epistasis is common in self pollinated crops such as finger millet. Dominance variance might also contain epistatic variances if epistasis is present. This is in agreement with the findings from Pswarayi A and Vivek. B., (2008) that genes with major or minor effect will function differently in different genetic background.

Breeding strategy

A combination of major resistance with quantitative resistance becomes a promising breeding strategy for reducing the rate of disease epidemic. Both forms of resistance are present in GULU-E and are responsible for preserving its durability overtime. The fact that resistance breakdown frequency due to major gene is high, this cultivar could have been naturally eliminated because one gene could not contain the necessary variability for resistance to such a variable pathogen given that finger millet is highly self pollinating crop. Therefore, from the study, it is possible to accumulate genes for race specific resistance in host cultivars through modified at backcrossing that might reduce development of disease epidemics in some areas. Major genes together can exhibit additive gene effect and when dominant genes become more frequent they tend increase on the days to infection of the flag leaf and overall rate of growth of the pathogen slowed. The frequency of dominant genes against virulent pathogen populations in our environment can be accumulated through modified backcrossing at F_3. The findings are in line with the hypothesized breeding strategy for breeding for durability in pepper cultivars (*Capsicum annum*) attacked by potato virus Y (Poty virus) (Ruth *et al.*, 2001). 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.

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References

Adipala, E. and Wandera. 2001. Variation in pathogenicity of Uganda finger millet pyricularia grisea isolates. *Africa Crop Science Conference Proceedings* 5:369-379.

Aru, J. 2014. On genetic improvement of drought tolerant finger millet for blast resistance in Uganda. Msc. Thesis. Makerere University, Kampala, Uganda. 69pp.

Getachew Gashaw, Tesfaye Alemu and Kassahum Tesfaye. 2013. Chararcterization of finger millet Blast Pathogen and its management using Bio-controls and fungicides. First Bio-innovate regional conference February, 25-27 2013. UNCC-ECA Addis Ababa, Ethiopia.

Holland, J.B.W.E. and Cervantez-Martinez, C.T. 2003 Estimating and interpreting heritability for plant breeding:An update. *Plant Breeding Rewiews* 22:9-112.

Mukankusi, C., Adipala, E., Kyamanya, S., Epieru, G., Odeke, V., Warren, H.L. and Wilson, H.R 1999. Effect of host type, time of planting,spacing of groundnut rosette and *cercospora* leaf spot diseases in eastern Uganda. *African Journal of Plant Protection.*

Oduori, C. 2008. Breeding investigations of finger millet characteristics including blast disease and *striga* resistance in western Kenya (PhD) Thesis in Plant Breeding. University of KwaZulu-Natal Republic of South Africa. 202pp.

Pswarayi, A. and Vivek, B. 2008. Combining ability amongst CIMMYT's early maturing maize (*Zea mays* L.) germplasm under stress and on stress conditions and identification of testers. *Euphytica* 162:353-362

Ruth, M., Solomon., B. and Hugh, B. 2001. A review of host major gene resistance to potato viruses. *Heredity* 86:8-16.

Singh, R.K.and Chaudhary, B.D. 2007. Biometrical methods in quantitative genetic analysis. Kalyani publishers, New Delhi, India. 774pp.

Takan, J.P., Muthumeenakshi, S., Sreenivasaprasad, S., Akello, B., Obilana, A., Bandyopadhyay, R., Coll, R., Brown, A.E. and Talbot, N.J. 2002. Characterisation of finger millet blast pathogen populations in East africa and strategies for disease management. In: Plant Pathology: Global Food Security, BSPP conference, Imperial College, London, 8-10.

Takan, J.P., Akello, B., Esele, P., Manyasa, E.O., Obilana, A.B. and Audi, P.O. 2004. Finger millet blast pathogen diversity and management in East Africa: A summary of project activities and outputs. *International Sorghum and Millets Newsletter* 45:66-69.

Takan, J.P. 2007. A study on the genetic diversity, sexuality and pathogenicity of the finger millet blast pathogen *Magnaporthe grisea* populations in two East African countries. PhD Thesis, University of Exeter, UK. 315pp.