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# The inheritance of resistance to finger millet blast disease

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## Abstract

Finger millet is a very important cereal crop in Uganda. It is a major staple food crop and also provides cash for both the rural and urban people. Of the many diseases attacking the crop, blast (*Piricularia grisea* (Cooke) Sacc.)is the most important causing yield losses ranging from 10 – 90% across the country depending on the environmental conditions, varietal differences or cropping systems. Breeding for resistance has widely been recognised as the most effective control strategy for blast in finger millet. However, the mode of inheritance to the disease resistance has not been adequately studied. Two cultivars with distinct markers for grain/glume colour and head shapes and known blast reaction were used to study the mode of inheritance of resistance to blast in experiments conducted at Serere and Ngetta. It was found that a purple grain/glume colour conferred blast resistance more than a tan colour, and that a compact head shape also conferred blast resistance more than an open head. Higher levels of resistance were observed where the two characters were present together. The results also showed that resistance to blast is both dominant and additive.

Key words: Blast, grain colour, head shape, host resistance, phenotype, *Pyricularia grisea*, progeny

## Introduction

Finger millet *(Eleusine coracana* (L) Gaertn) is an important cereal food crop in Uganda being second to maize. It is produced on an average of 300,000 ha yielding 450 metric tons annually (FAO, 1999). The crop is grown virtually in all parts of the country but with more concentration in the Eastern, Northern and parts of Western Uganda where it is a major staple food. In these areas, finger millet is also emerging as an important cash earner to the rural people. The crop is nutritionally superior to rice and wheat, having good malting properties as a result of high levels of amyloses (Purseglove, 1988). It also has high levels of vitamins and minerals making it the most suitable food for pregnant women, lactating mothers and children.

Unfortunately, finger millet is vulnerable to diseases. A wide range of fungal, bacterial and viral diseases have been reported on finger millet (Rachie and Peters, 1977; Adipala – Ekwamu, 1980). The most important of these diseases is blast caused by the pathogen *Piricularia grisea* (Cooke) Sacc. (Teleomorph *Magnaporthe grisea*).

The disease is very destructive and economically important causing over 50% losses in yield, especially in wet seasons (Adipala and Mukiibi, 1991). Field studies have recorded losses of 10 – 90% in Uganda (Emechebe, 1975). High rainfall, temperature (25 – 30°) and humidity (over 90%), are the most important predisposing factors.

*Pyricularia grisea* attacks finger millet at all stages of plant development from seedling to grain formation. The symptoms at seedling stage appear as small, brown, circular to elongated spots on the leaves which later coalesce into large elongated or spindle shaped lesions. The centres of these lesions eventually develop greyish mycelial fungal growth consisting of conida and conidiophores of the fungus. Severe infections may lead to seedling death. The leaf blast phase of the disease tends to be less severe. Crop loss is always greatest when the disease appears on the peduncle and/or finger causing neck and head blast respectively during flowering and grain formation. Depending on the

severity of infection, this may lead to total inhibition of grain formation or production of shrivelled seed. Rath and Misra (1975) reported that neck blast causes increase in spikelet sterility.

Blast screening nurseries in Serere (eastern Uganda) and elsewhere have shown a wide range of variability in resistance to blast, ranging from from almost complete resistance to total susceptibility. Variability in resistance has been attributed to differences in plant and grain colour, head shapes and maturity periods. Generally, dark seeded cultivars are more resistant than white seeded ones. Also, compact headed cultivars are more resistant than open headed ones. The very early maturing cultivars are more susceptible than the late maturing ones. In rice, which is attacked by *Piricularia oryzae* resistance to blast can be unreliable, with previous resistant cultivars showing high levels of susceptibility in the field within a few years of release (Kiyosawa, 1972). There have been numerous explanations advanced for the instability of resistance. These can be grouped into two broads themes: inadequate exposure of breeding materials to diverse pathogen populations, and hypervariability of the pathogen.

Inheritance of resistance to blast in finger millet has not been adequately studied. Yet efficient utilisation of breeding materials requires knowledge of modes of inheritance. This study was therefore instituted to provide information on the nature of resistance to the blast disease. It has been argued that like for other cereals, incorporation of genetic resistance is the best choice for disease management in finger millet and other cereals (Agrios, 1997).

## Materials and methods

*Development of breeding population*

Two cultivars DR 3 SS and E 11 with distinct characters of grain/glume colour, head shapes and known blast reaction were selected for the study (Table 1). The two cultivars were chosen in such a way that factors controlling other traits were basically similar, so that the differences observed if any would primarily be a result of grain colour, head shape and blast reaction.

In order to make all possible crosses between the two cultivars, diallel crosses with their reciprocals were made. Hot water emasculation method was used in which the finger millet heads were dipped in hot water at 50°C for 2 minutes, just before flowering. $F_1$ plants were derived from crosses between parents. $F_2$ plants were derived by selfing $F_1$ plants. $BC_1$ plants were obtained by backcrossing $F_1$ plants to each of the parents. All the crosses were made in the greenhouse, at Serere.

*Pathogen isolation and inoculum preparation*

The pathogen was isolated from infested finger millet tissue by inducing sporulation on moist blotter paper in petri-dishes. Prior to plating, the infected seeds and glumes were surface sterilised by immersion in 10% sodium hypochlorite solution for about 11 seconds and wrinsed in sterile distilled water (SDW) thrice. The blotters were then put into the deep freezer for 12 hours at minus 4.5°C. The purpose of deep – freezing was to suppress the possible germination of the seeds during incubation. After deep freezing, the samples were then incubated under alteranting 12/12 hours under alternating near ultra-violet light (NUV) and darkness for seven days. The resultant spores were streaked on Agar plates and incubated at 28° C for 7 days. Single germinating conidia were picked and colonies grown on Potato dextrose agar (PDA) plates, and then stored as stock isolates.

*Table 1. Description of finger millet varieties used in the blast resistance studies.*

| Variety | Blast reaction | Grain/Glume colour | Head shape |
|---|---|---|---|
| DR 3 SS | Resistant | Purple | Compact |
| E 11 | Susceptible | Tan | Open |

Stock isolates were re-cultured at Serere on PDA media with streptomycin added at 10 mg per 250 ml of medium to control bacteria. The plates were placed under continuous fluorescent light for 7 days at 28°C to induce sporulation. Conidia were scrapped from the incubated plates into 50ml of sterile distilled water. Spore suspensions were then filtered through nylon cloth. Spores from the resulting suspension were counted with a haemocytometer and appropriately diluted with sterile distilled water to make an inoculum suspension of 1 x $10^6$ spores per millilitre. Two to three drops of Tween 20 were added to the inoculum as a wetting agent.

*Disease and phenotypic evaluation*

Disease and phenotypic evaluation were done at two locations, Serere and Ngetta in eastern and northern Uganda. The two locations are rather similar in weather conditions but with Serere being wetter (1230 mm per annum) than Ngetta (1100 mm). All the populations ($P_o$, $F_1$, $F_2$ and $BC_1$) were planted in the field to assess their reaction to blast disease and evaluate the phenotypes. The experiments were planted in a completely randomised design with two replications. Plot sizes consisted of two five-meter row plots for $P_o$, $F_1$, and $BC_1$ progenies and ten five-meter row plots for $F_2$ progenies. The objective was to obtain at least 60 plants for observation in $P_o$, $F_1$ and $BC_1$ and 300 plants in each $F_2$ per replication. Three hundred plants per replication were selected for the $F_2$ population because of the greater variation expected in this segregating generation.

Grain/glume colour and head shapes of the parental lines were observed to ensure that the lines were true breeding. These observations were also made on the $F_1$ plants to determine if they were actual crosses or were parental self-pollinations. Studies on gene dominance and inheritance patterns were carried out on the $F_2$ and $BC_1$ progenies. The identification of colour and head shape as dominant or recessive was determined by phenotypic classification of parental lines (Po), $F_1$, $F_2$ and $BC_1$ populations. Every individual panicle in $F_2$ and $BC_1$ populations were studied for the various phenotypic classifications and disease reaction. Only one panicle was examined among those that had tillered.

Blast reaction was evaluated on field inoculated plants. The panicles of all the populations were inoculated at head emergence by spraying the with spore suspensions until runoff. The inoculated panicles were bagged in pollinating bags for 4 days to maintain high humidity. Disease evaluation on the inoculated plants was conducted using a 1 – 5 scale where 1 = no visible symptoms, 2 = 1 – 10% of susceptible plant parts affected, 3 = 11 – 25% of susceptible plant parts affected, 4 = 26 – 50% of susceptible plants parts affected and 5 = over 50% of susceptible plant parts affected. A minimum of 20 plants per phenotype per generation was used to obtain a blast rating. In the final analysis, blast reaction was rated as follows: resistant (Ratings 1.0 – 2.5), moderately resistant (Ratings 2.6 – 3.5) and susceptible (Ratings 3.6 – 5.0).

*Statistical analysis*

Since no apparent differences were observed in either phenotypes or blast values in the two replications in each site, their observations were pooled for statistical analysis. To determine their dominance and inheritance and to establish linkage with blast reaction, phenotypic ratios were selected for goodness of fit by a chi-square test for the observed to the expected number of phenotypes within each of the $F_2$ and $BC_1$ populations. An analysis of variance was used to determine location and phenotypic effects. Fisher's LSD at 5% probability level was used to determine differences among the means.

## Results

Phenotypes from crosses with minimal influence from other traits were selected for studying grain/glume colour and head shapes. Blast ratings differed for each of the phenotypic classifications (Table

2).

All $F_1$, $F_2$ and $BC_1$ progeny with purple glume colour and compact heads had low blast ratings (1 – 2) indicating resistance. The $F_2$ and $BC_1$ progenies that segregated for tan glume colour and open heads were susceptible (ratings 4 – 5). The $F_2$ and $BC_1$ progeny that segregated for tan glume colour and compact head or purple glume colour and open head had moderate disease ratings (3 – 3.5).

All the $F_1$ were purple coloured and compact headed and showed high levels of resistance. This indicated that resistance is dominant and is governed by purple glume colour and compact head. Genotypes with purple colour and an open head were more resistant than those with tan glume colour and compact head . This suggested that although both purple colour and compact head conferred blast resistance, purple colour conferred greater resistance than compact head. However, there appeared to be an additive effect, because when both purple colour and compact head were present, higher levels of resistance were observed.

The inheritance of purple colour and compact head were studied using the same parents and was tested for goodness of fit by chi-square analysis (Table 3). All the $F_1$ progenies had purple grain/glume colour and compact head shape. The $F_2$ progenies segregated in the expected 27:9:21:7 ratio for purple compact: purple open: tan compact: tan open: respectively. The $F_1$ x $P_2$ progenies segregated in the expected 3:1:3:1 ratio for similar phenotypic classification. $F_1$ x $P_1$ progeny all were purple, and compact. These ratios showed that purple grain/glume colour and compact head shape were inherited in a dominant manner.

## Discussion and conclusions

In Uganda, blast is still the most important biotic constraint to finger millet production (Adipala, 1992) and considerable work has been done on breeding for resistance to the disease (Esele and Odelle, 1995). It is widely recognised that resistance is the cheapest and most effective strategy for the control of blast in finger millet (Rachie and Peters 1977; Esele, 1989; Viswanath and Seetharam, 1989; Pande *et al* .,1995 ).

The present results show that there is a strong relationship between glume colour and compactness. In general, the purple colour was associated with a higher blast resistance than tan colour and the compact head was more resistant to blast than loose/ open head. These findings coroborate earlier work of Esele and Odelle (1995) which showed that resistance is correlated with purple colour and compact

*Table 2. Relationship between glume/head phenotype and blast resistance in DR 3 SS ($P_1$) x E 11 ($P_2$) crosses at Serere and Ngetta.*

| | | Blast rating | | |
|---|---|---|---|---|
| Generation | Phenotype | Serere | Ngetta | Mean |
| $P_1$ | Purple, Compact | 1 | 1 | 1a |
| $P_2$ | Tan, Open | 5 | 5 | 5f |
| $F_1$ | Purple, Compact | 1.5 | 1.6 | 1.55b |
| $F_2$ | Purple, compact | 2.0 | 1.7 | 1.85b |
| | Purple, Open | 3.2 | 3.0 | 3.1c |
| | Tan, compact | 3.4 | 3.4 | 3.4d |
| | Tan, open | 4.6 | 4.5 | 4.55e |
| $P_1$x $P_1$ | Purple, compact | 1.0 | 1.0 | 1.0a |
| $P_2$ x $P_2$ | Purple, compact | 1.8 | 1.7 | 1.75b |
| | Purple, open | 3.4 | 3.3 | 3.3d |
| | Tan, compact | 3.5 | 3.5 | 3.5d |
| | Tan, open | 5.0 | 4.8 | 4.9f |
| | Mean* | $3.0_g$ | $2.9_g$ | |

*Means followed by the same letter are not significantly different at P = 0.05 using Fisher's LSD.

*Table 3. Chi-square analysis for the inheritance of grain/glume colour and head shape in the DR 3SS ($P_1$) x E 11 ($P_2$) cross at Serere and Ngetta.*

| Generation | Phenotype | Serere | | | | Ngetta | | |
|---|---|---|---|---|---|---|---|---|
| | | Ratio* | O | E | $X^2$ | O | E | $X^2$ |
| $P_1$ | Purple, Compact | 1 | ... | ... | ... | ... | ..... | .... |
| $P_1$ | Tan, Open | 1 | ... | ... | ... | ... | ... | ... |
| $F_2$ | Purple, Compact | 1 | ... | ... | ... | ... | ... | ... |
| $F_2$ | Purple, Compact | 27 | 201 | 198 | ... | 221 | 229 | ... |
| | Purple, open | 9 | 56 | 59 | ... | 61 | 68 | .... |
| | Tan, compact | 21 | 188 | 183 | ... | 197 | 211 | ... |
| | Tan, open | 7 | 50 | 54 | 0.69a | 48 | 51 | 2.1a |
| $F_1 \times P_1$ | Purple, compact | 1 | ... | ... | ... | ... | ... | ... |
| $F_2 \times P_2$ | Purple, compact | 3 | 37 | 42 | ... | 41 | 47 | ... |
| | Purple, open | 1 | 34 | 56 | ... | 30 | 52 | ... |
| | Tan, compact | 3 | 28 | 14 | ... | 29 | 15 | ... |
| | Tan, open | 1 | 31 | 18 | 8.64a | 33 | 16 | 6.2a |

* = Expected segregation ratio for the phenotypes in each generation
O = Observed number of plants
E = Expected number of plants
... = All plants observed were of the same phenotypes, hence no analysis was performed.
A = $X^2$ values were all significant at P = 0.05

heads. In many pathosystems resistance is attributed to production of biochemical compounds that may either inhibit fungal entry or growth (Agrios, 1997). In the case of *P. grisea* it appears resistance is conditioned by multiple biochemical production in resistant cultivars. Indeed, Seetharam and Ravikumar (1993) reported that the total phenol and tannin contents of resistant cultivars were generally higher than those of susceptible ones. Darker colour in fnger millet is indicative of higher phenols and tannins levels. Tannins inhibit spore germination and mycelial growth (Kambal and Bate-Smith, 1976; Hahn and Rooney, 1985).

The results of this study have also shown that resistance to blast is heritable. When the inheritance was analysed by chi-square test, the results showed complete dominance of colour and head shape. Examination of the effect of different phenotypic classifications also showed that the resistance is additive. Higher levels of resistance were observed in progenies with both purple and compact heads. Viswanath and Seetharam (1989) obtained similar results. Their study of gene action through line x tester analysis revealed the role of both additive and dominant gene action. This is very useful information for disease resistance breeding programmes. It suggests good potential for conventional breeding methods (such as recurrent selection), and early generation testing and selection for resistance.

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