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RESISTANCE OF NAPIER GRASS CLONES TO NAPIER GRASS STUNT DISEASE

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ABSTRACT

Napier grass (*Pennisetum purpureum* Schumach) is the major livestock fodder under intensive and semi-intensive systems in East Africa. However, the productivity of the grass is constrained by Napier grass Stunt Disease (NSD). The purpose of this study was to identify Napier grass clones with resistance to NSD. Seven introduced Napier grass, from the International Livestock Research Institute (ILRI) gene bank in Ethiopia, and 3 locally available clones in Uganda, were screened for resistance to NSD. Napier grass Stunt Disease severely infected plants tended to have more tillers ($r = 0.84$, $P<0.001$), but reduced biomass ($r = -0.70$, $P<0.001$) and height ($r = -0.88$, $P<0.001$). The reaction of the clones ranged from highly susceptible (clones 16795 and 16792), moderately susceptible (P99, KW4, 16806, 16803, 16785, local and 16814) to resistant (16837). Clone P99 was moderately susceptible to NSD but its harvestable biomass was least affected by disease infection, thus could be, in the interim, utilised in integrated NSD management as the search for more resistant clones is expedited.

Key Words: Disease severity, *Pennisetum purpureum*

RÉSUMÉ

Le Napier (*Pennisetum purpureum* Schumach) est un fourrage important pour l'élevage dans les systèmes intensifs et semi-intensifs en Afrique de l'est. Par ailleurs, la productivité de cet aliment fait face au problème de productivité de cette herbe qui est affecté par la maladie du rabougrissement de Napier (NSD). L'objet de cette étude était d'identifier les clones du Napier introduit et localement disponible avec résistance au NSD. Sept Napiers introduits en provenance du la banque de gène de l'Institut International de Recherche sur l'Elevage (ILRI) en Ethiopie, et trois clones locaux disponibles en Ouganda étaient évalués pour leur résistance à cette maladie dans un essai au champ au 'National Crops Resources Research Institute (NaCRRI)' à Namulonge en Ouganda. La réaction de ces clones variait de hautement susceptible (16795 et 16792), modérément susceptible (P99, KW4, 16806, 16803, 16785 et 16814) à résistant (16837 et local). La maladie de rabougrissement des plants sévèrement infectée tendaient d'avoir significativement plus de talles ($r = 0.84$, $P<0.001$) mais avec de la biomasse ($r = -0.70$, $P<0.001$) et hauteur ($r = -0.88$, $P<0.001$) réduites. Les clones P99 et 16837 modérément susceptibles au NSD mais dont leur biomasse n'était pas affecté par by l'infection de la maladie, pourrait être, dans l'intérim, utilisés dans la gestion intégrée du NSD pendant que la recherche des clones plus résistants continue.

Mots Clés: Maladie sévèrity, *Pennisetum purpureum*

INTRODUCTION

Napier grass (*Pennisetum purpureum* Schumach), also known as elephant grass, is the principal fodder crop in intensive and semi intensive smallholder livestock production systems in East Africa (Staal *et al.*, 1998). Presently, Napier grass stunt disease (NSD) caused by a phytoplasma of group 16SrXI, '*Candidatus* Phytoplasma *oryzae'*; the rice yellow dwarf phytoplasma (Nielsen *et al.*, 2007) is limiting Napier grass production in eastern Africa and many smallholder farmers have reported losses of up to 100% of their crop, forcing them to either reduce the number of animals or purchase fodder from elsewhere (Arocha and Jones, 2010).

Development of NSD management strategies is still in its infancy, and includes mainly cultural measures like weeding, rouging infected plants, applying organic and inorganic fertilisers and planting disease free Napier grass cuttings (Orodho, 2006). Use of resistant clones may constitute the only defense against NSD, particularly because Napier grass is extensively grown on non-commercial basis and most times grows in the wild. The desired clones would be those with resistance to NSD and/or the insect vectors that transmit the phytoplasma.

The starting point in searching for resistance is knowing the existing levels of resistance among a well characterised and inventoried germplasm; information which is lacking in the case of Napier grass in some East African countries such as Uganda. This situation necessitates assembling Napier grass clones from different sources to increase the possibility of identifying materials with resistance to NSD and other desirable attributes. The collection of *Pennisetum purpureum* and its *Pennisetum glaucum* hybrids from several African countries and USA made by the International Livestock Research Institute (ILRI) in Ethiopia (Wouw *et al.*, 1999) offers the most dependable source of Napier grass materials.

The objective of this study was to identify Napier grass clones with resistance to NSD.

MATERIALS AND METHODS

The field experiment was conducted at the National Crops Resources Research Institute (NaCRRI) located at Namulonge in central Uganda (00 32'' N and 32 37'' E, 1150 m.a.s.l), which is a representative Napier grass growing area in Uganda. Namulonge experiences a tropical wet and mild dry climate with slightly humid conditions (averaging 65%) receiving a bimodal rainfall – averaged at 1200 mm year^{-1}. The dominant vegetation is wooded savannah, with tall trees and grasses dominated by *Pennisetum purpureum* and *Panicum maximum* (NARO, 2005).

Seven Napier grass clones acquired from the International Livestock Research Institute (ILRI – Ethiopia) field gene bank, together with 2 Napier grass clones (P99 and KW4) extensively grown locally in Uganda and a wild type - local (Table 1), were multiplied for 1 year at Buginyanya Zonal Agricultural Research Institute in Eastern Uganda, a location known to be free from NSD. Before planting the field experiment, all planting materials were tested for Napier Stunt phytoplasma in a nested PCR, using primers p1 and p6 (Deng and Hiruki, 1991) and R16F2$_n$ and R16R2$_n$ (Gundersen and Lee, 1996) to ensure that only healthy Napier grass clones were planted.

Each Napier grass clone was planted in a single line plot of 10 plants spaced 1 m between plants within a row and 2 m between rows of the different clones. A row of NSD infected Napier grass was planted along each clone row as a disease source. The experiment was laid out in a randomised complete block design in three replicates.

TABLE 1. Sources of Napier grass used in screening for resistance to Napier grass Stunt Disease in Uganda

Accession	Species	Source
16785	*Pennisetum purpureum*	Tanzania
16792	*Pennisetum purpureum*	Mozambique
16795	*Pennisetum purpureum*	Zimbabwe
16803	*Pennisetum purpureum*	Zimbabwe
16806	*Pennisetum purpureum*	USA
16814	*Pennisetum purpureum*	USA
16837	*Pennisetum purpureum x Pennisetum glaucum*	Unknown
Kw4	Unknown	Uganda
P99	Unknown	Uganda
Local	Unknown	Uganda

Napier grass Stunt Disease severity was scored 90 days after planting (DAP) using a scale of 1 – 4; where 1 = no disease symptoms; 2 = disease symptom seen with slight stunting; 3 = disease symptom seen with moderate stunting; and 4= severe disease/ stunting (Alicai *et al*., 2004). Thereafter, all the plants were cut back to a height of about 5 cm above ground and left to sprout again, mimicking a common farmers harvesting practice for Napier. Disease severity assessment and cutting back the grass was repeated at 60 days interval, the harvesting interval recommended to Napier grass farmers (Orodho *et al*., 2006), up to 510 DAP.

At 150 DAP, five fully grown tillers were selected randomly from five randomly selected Napier plants of each clone per plot, cut at the lowest node, and the stalk chopped into about 2 cm pieces using a table-knife. The stem pieces were thoroughly mixed before a 100 g sub-sample was taken. Additionally, the leaves including leaf blade and leaf sheath of each tiller were collected, cut into about 2 cm pieces, and thoroughly mixed before a 100 g a sub-sample was also taken. Each sub-sample was put in a paper bag and oven dried at 60^{o}C for five days to determine dry weight. Dry matter of leaf and stem for each clone was used to computer leaf to stem ratio.

The effect of the NSD on plant height (measured from the base to the tip of the tallest tiller), number of live tillers and harvestable biomass was determined at 510 DAP for both the symptomatic and asymptomatic plants. The impact of the disease was calculated based on the formula by Zouzou *et al*. (2008) as:

$$\text{Impact (\%)} = 100 \times (Ni - I)/Ni$$

Where:

Ni = mean severity scores of the plants without NSD symptoms; and I = mean severity scores of all plants showing NSD symptoms.

Disease severity data were used to calculate the area under disease progress curve (AUDPC) (Campbell and Madden, 1990). To test for the effect of the genotypes, relative area under disease progress curve (RAUDPC) was calculated for each clone by dividing the AUDPC with the total area under the graph (Madden *et al.,* 2007). Differences in RAUDPC, leaf: stem ratio, NSD severity and harvestable biomass yield amongst the Napier grass clones were determined using Analysis of Variance (ANOVA) of GenStat (12th Edition) Statistical Software. Where significant differences were observed, clone means were separated using Fishers Protected Least Significant Difference (LSD) at 5% level of significance.

Correlation analysis was applied to disease severity, plant height reduction (%), harvestable biomass reduction (%) and number of tillers to explore the respective interrelationships. Thereafter, Principal Component Analysis (PCA) was run based on Spearman's correlation coefficient, followed by the variance maximisation rotation (varimax) procedure to obtain a clear pattern of factor loadings for each clone. Correlation bi-plots/coefficients of the varietal factor loadings were drawn to reveal groupings among the clones using GenStat (12th Edition) software.

RESULTS AND DISCUSSION

Clone 16792 and 16795 had the highest RAUDPC; while clones local and 16837 had the lowest. The disease buildup in the rest of the clones was intermediate (Fig. 1). Napier grass Stunt disease infection resulted in significant ($P<0.05$) reduction in plant height (39.1%) and harvestable biomass (49.6%) of all Napier grass clones (Table 2). The reduction in harvestable biomass and plant height significantly varied among Napier grass clones. Clone 16795, 16792 and 16806 showed the highest reduction; while clones 16837, 16785 and 16814 showed least reduction in harvestable biomass. Similarly, the highest reduction in plant height was recorded on the clones, local, 16792 and KW4, and least on clones 16837, 16785 and 16814 (Table 2). The effect of the Stunt disease infection on Napier grass is further explained by the negative significant correlations between disease severity and plant height reduction ($r= -0.88$, $P\leq0.01$) and harvestable biomass reduction ($r= -0.70$, $P< 0.001$) (Table 3). On the other hand, NSD infection caused a significant ($P < 0.001$) increase in the number of tillers (on average by 86%), which also varied by clones. Clone 16795, 16792 and 16785 produced the highest number of

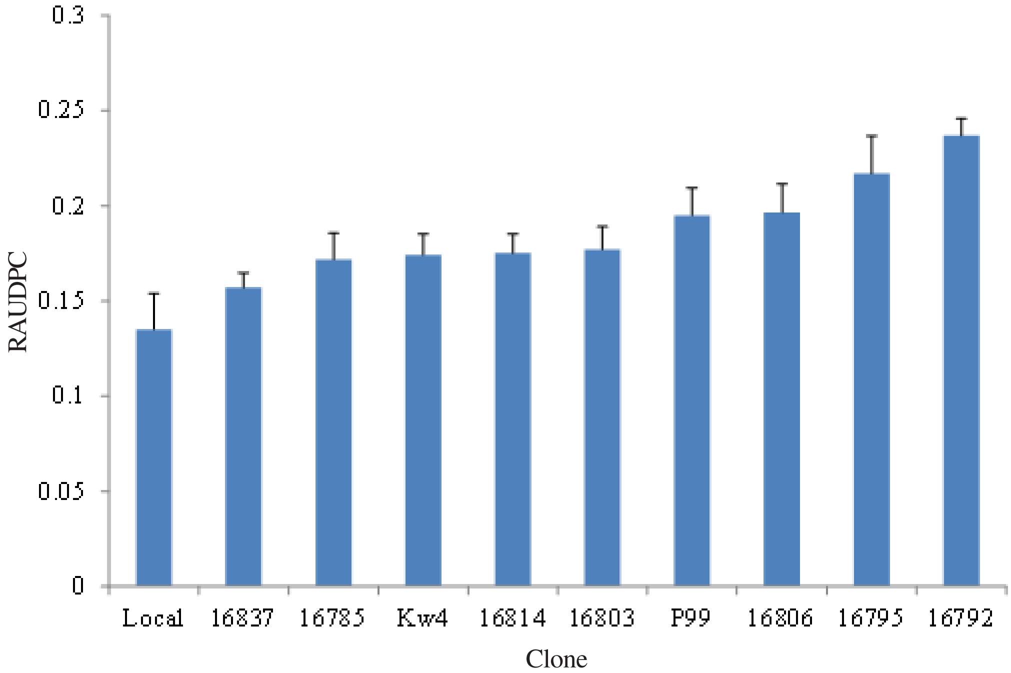

Figure 1. Napier grass Stunt Disease severity expressed as relative area under disease progress curve (RAUDPC) from trials using 10 Napier grass clones conducted at Namulonge, central Uganda during 2011 - 2012.

TABLE 2. Effect of the Stunt Disease on yield parameters of symptomatic Napier grass clones as compared to asymptomatic plants grown at Namulonge, central Uganda during 2011 - 2012

Napier clones	Weight reduction (%)	Height reduction (%)	Tiller number increase (%)
16785	37.1	42.6	110.6
16792	63.8	51.3	121.3
16795	70.4	49.7	126.6
16803	58.0	35.2	61.8
16806	60.8	27.6	94.0
16814	38.3	33.2	63.6
16837	19.6	17.6	18.9
Kw4	53.6	50.2	108.1
Local	46.6	53.4	92.6
P99	47.8	30.3	79.4
Mean	49.6	39.1	87.7
LSD (0.05)	17.35	17.04	61.32
CV (%)	20.4	25.4	42.0

tillers while clone 16837, 16803 and 16814 had the lowest number of tillers. Similarly, the number of tillers increased with disease severity ($r = 0.84$, $P< 0.001$). Leaf to stem ratio was highest in clone 16785 and lowest in local clone. Relatedly, harvastable biomass was highest in clone P99 and lowest in clone 16785. The harvestable biomass of other clones was intermediate (Table 4).

Principal component axes 1 and 2 accounted for 91.14% of the total variation in the data (Table 5). Plant height reduction and increase in tiller

TABLE 3. Simple correlation coefficients among parameters estimating the effect of Napier grass Stunt Disease on 10 Napier grass clones grown at Namulonge, central Uganda during 2011 - 2012

	Mean severity	Height reduction (%)	Biomass reduction (%)	Tiller number (%)
Mean NSD severity	-			
Height reduction (%)	-0.88***	-		
Biomass reduction (%)	-0.70***	0.68***	-	
Tiller number increase (%)	0.84***	-0.81***	-0.66***	-

***Significant at P<0.001

TABLE 4. Yield parameters of Napier grass clones grown at Namulonge, central Uganda during 2011 - 2012

Napier clones	Leaf : stem ratio	Harvestable biomass (kg^{-1})
16785	1.79	3648
16792	1.25	4107
16795	1.48	4304
16803	1.37	6278
16806	1.26	4626
16814	1.27	4919
16837	1.21	5856
Kw4	1.6	4600
Local	1.09	4030
P99	1.4	7100
Mean	1.38	4947
LSD (0.05)	0.36	1294.9
CV (%)	20.4	53.9

number were the most important variables contributing to Principal Component Axis (PCA) 1 (65.27%) while the second PCA accounting for 25.87% was due to plant height reduction (Table 5). A bi-plot of PCA 1 and PCA 2 revealed that the clones most affected by NSD infection were 16792 and 16795 while the least affected was clone 16837. Accordingly, the reaction of the Napier grass clones to NSD infection were grouped as resistant (clones local and 16837); moderately susceptible (P99, kw4, 16837, 16814, 16806, local, 16803 and 16785) and highly susceptible (clones 16792 and 16795) (Fig. 2). Similar observations were reported by Kison and Semmuller (2001) while evaluating resistance of root stocks of stone fruit against European stone fruit yellows.

TABLE 5. Eigen vectors of Principal Component Analysis using four parameters estimating the effect of Napier grass Stunt Disease on 10 Napier grass clones grown at Namulonge, central Uganda during 2011 - 2012

Parameters assessed	PCA1	PCA2
Height reduction (%)	0.7063	0.2383
RAUDPC	-0.1083	-0.8553
Tiller number increase (%)	0.583	-0.1618
Weight reduction (%)	0.3867	-0.4306
Eigen values	65.27	25.87

Although all Napier grass clones succumbed to NSD infection, the variability in the effect of the disease on harvestable biomass and leaf – stem ratio give hope that planting clean Napier grass cuttings of a clone with moderate reaction to NSD, but with limited – moderate effect on harvestable biomass, can ensure enough herbage for livestock feeding (Jørgensen *et al*. 2010), and when combined in an integrated manner with measures that prevent the insect vectors infesting the grass, would provide an interim strategy for managing NSD, especially in low NSD pressure areas. On that basis, clone 16837 that was least affected by NSD but had low leaf to stem ratio and harvestable biomass is less suitable. Meanwhile, clone P99 with moderate NSD severity, but high harvestable biomass (least affected by the disease) and relatively high leaf to stem ratio is a better choice for livestock farmers in the presence of NSD. Among the clones, 16837 is recommended for utilisation in breeding programmes.

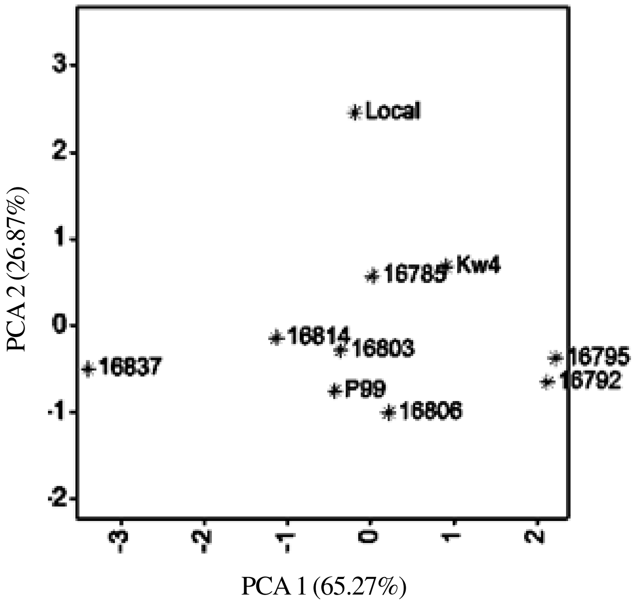

Figure 2. Bi-plot of component axes derived from Principal Component Analysis based on parameters estimating the effect of Napier grass Stunt Disease on 10 Napier grass clones grown at Namulonge, central Uganda during 2011 - 2012.

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