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Macropropagation technique for production of healthy banana seedlings

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Abstract Banana (*Musa* spp) is one of the most important crops providing food security, nutrition and income for many smallholder farmers in sub-Saharan Africa. However, production is hindered by scarcity of high quality seedlings, insect pests and diseases. To improve availability of quality seedlings macropropagation technology is being introduced in Kenya. This study was carried out to generate knowledge to enhance understanding and adoption of the technology. A survey was done initially to identify the key pests and pathogens of banana in Central and eastern regions of Kenya. Macropropagation nurseries were established in six sites in farmers' fields representing different agroecological zones. Corms of banana varieties Kampala, Sweet banana, Cavendish and Uganda Green obtained in accordance with established quality assurance protocols were propagated. The health of the seedlings produced was monitored. *Fusarium oxysporum* f. sp. *cubense* was isolated from less than 1% corms of sweet banana and Kampala varieties. *Radopholus similis* was isolated from all the varieties but its incidence was highest (46%) in the Cavendish variety. Endophytes and non pathogenic microorganisms were isolated from more than 90% of the corms. Over 98% of the propagated corms produced healthy seedlings and only less than 1% of the corms propagated rotted in the propagation media due to non pathogenic causes. In areas with high weevil infestation it was difficult to obtain corms with the standard required for macropropagation. The information obtained shows that macropropagation technique effectively produces healthy banana seedlings. Practitioners will need to observe quality control measures to ensure production of high quality seedlings.

Key words: Banana, health, macropropagation, seedlings

Introduction

Bananas are the fourth most important crop in the developing world after rice and wheat. They are rich in carbohydrates. The world's largest consumers of banana are East and South Africa with an annual per capita consumption of 400-600 kg (Karamura *et al.*, 1998). Bananas are a source of income for resource poor farmers. Its production is hindered by pests and diseases, lack of clean planting material, decline in soil fertility and scarcity of land, among other factors.

Banana production in Kenya has declined significantly below the potential capacity. The decline can be attributed mainly to an increase in the prevalence of pests and diseases combined with lack of effective control strategies (Kahangi *et al*., 2002). Naturally regenerated suckers are highly preferred by farmers but these often harbour pests and diseases which are spread within and between farms leading to reduced productivity and a shortened lifespan of new plantations.

The most important diseases and pests of banana include Fusarium wilt, Black leaf sigatoka, Bacterial wilt, Banana Streak Virus, nematodes and the banana weevil. Fusarium wilt (panama) caused by *Fusarium oxysporum f. sp. cubense* is regarded as the most destructive banana disease. It is capable of wiping out entire plots of susceptible varieties Gros Michel and Sweet banana. However, it has not been reported on East African highland bananas (EA-AAA). Black leaf sigatoka caused by *Mycosphearella fijiensis* causes premature leaf drying and subsequent incomplete filling of fingers. Bacterial wilt caused by *Xanthomonas vasicola* pv. *musacearum* is one of the most lethal disease (Valentine *et al*., 2006) attacking many banana varieties.

Demand for pest free and high quality planting materials has been on the increase for initiating new plantations and expanding the existing orchards. As a result, Tissue Culture (TC) was introduced in 1997, but its adoption has been low due to high costs of seedlings, capital requirements and skills involved. To address the demand for affordable seedlings, macropropagation technology has been introduced as an alternative to TC. The technology requires little capital and skill for implementation. Macropropagation has the potential to produce 50-60 shoots per sucker in 4-5 months (Singh *et al*., 2011). The technology has been adopted in Cameroon where it is has contributed to improved quantity and quality of planting material.

The costs and infrastructure needed to set up macropropagation facilities are within reach of many small holders hence nurseries can be put up near farmers fields and seedlings acquired at a lower cost**.** The technology has been introduced recently in East Africa. It uses the principle of overcoming apical dominance and exposing lateral buds to allow rise of many shoots. The buds are stimulated to develop almost simultaneously into suckers which are detached and hardened before planting them in the field. This can be done at farm level making it possible for the farmers to have choice of variety. Locally available materials are used for construction of the nurseries thus the seedlings are affordable to the farmers. Some aspects of the technology however require further research to

support its deployment. This study was initiated to investigate the effectiveness of macropropagation technology in producing disease free banana seedlings.

Materials and Methods

A survey was carried out in six districts in Eastern and Central Kenya. It was done in 7 districts, Muranga, Mathioya, Kirinyaga East, Kirinyaga Central, Kirinyaga West, Embu East, Meru Central and Imenti South with a minimum of 10 farms in each district. The purpose was to determine the most important diseases and pests affecting banana and the agronomic practises carried out. Farms were selected randomly at 3-5 km apart. A photo card aided identification of the diseases by the farmers and a questionnaire was used to collect the required information. A transect walk was done diagonally in the plantation for inspection of plants to identify any that showed disease symptoms. Samples of diseased materials were taken to the laboratory for isolation and identification of pathogens. Their importance as pests was determined through pathogenicity tests.

Macropropagation nurseries were established in six sites in farmers' fields on basis of the different agroecological zones; low altitude Mitunguu (1071 Meters above sea level) and Embu East (1265Masl), mid-altitude (Kerugoya 1340 Masl and Ntharene 1360 Masl) and high altitude (Mathioya 1915Masl and Meru central 1680Masl) (Table 1).

The nurseries were constructed using wooden poles; treated with preservative and polythene sheets. The roof was made using black polythene and the sides made using clear polythene to provide 50% shade. Corms were obtained through an established Macropropagation protocol. At first, certification of farms was done for corm procurement. Farms were inspected to certify that they were free from visually detectable pests and diseases that have potential to be propagated from parent to progeny. Banana plants that were flowering, maiden suckers and those that had been harvested were selected for acquiring corms for Macropropagation.

The corms were prepared for planting through paring to remove all the roots, removal of sheaths and scarification of the buds. They were washed and disinfected in 10% Jik ® (sodium hypochlorite) for ten minutes, left to dry and planted in propagators filled with sterile sawdust. The propagator was kept moist by watering after every two days for the first week initially, then twice a week thereafter. Samples were taken from the corm and checked for any latent pathogenic microorganisms. Plantlets arising from the corms were monitored for development of any disease symptoms. Corms rotting in the media were recorded, removed from the propagators and the cause determined. Plantlets were transplanted upon attaining a height of 15 cm and developing 4 leaves four weeks after rooting in the sawdust. The soil was steam-sterilized and mixed with manure in the ratio 3:1 (soil; manure). The plantlets were transplanted in polythene bags. They were monitored for any symptoms of disease development for a period of 12 weeks after transplanting. Plantlets showing disease symptoms were taken to the laboratory for identification of the causal organism.

Microorganisms isolated from the sampled were identified and pathogenicity determined. The number of healthy seedlings that were produced in each study site versus those that wilted or developed disease symptoms

Table 1. Banana production practices in the study sites.

Location	Altitude	Production practices
Mathioya	1915 M	Rainfed but use water from the river for irrigation during the dry seasons. Suckers are used as the major propagating material. Cooking bananas are most preferred. The temperatures are low (18°C -25°C) and bananas take longer (18 months) to grow, mature and produce fruit. Bananas are intercropped with crops such as maize and sweet potatoes
Meru central	1680 M	Bananas irrigated using river water. Suckers are used as the major propagating material. Cavendish variety is most preferred. Banana plantations are weeded and manure or fertilizer is added to replenish fertility.
Kerugoya	1340 M	Farmers use irrigation water from bore holes for growing bananas. Suckers are used as the major propagating material. Farmers prefer Kampala and Sweet banana varieties
Ntharene	1360 M	Bananas irrigated using river water flowing through gravity from the mountain. Farmers use irrigation water from rivers for growing bananas. Suckers are used as the major propagating material.
Embu East	1265 M	Farmers use irrigation water from bore holes for growing bananas. Suckers are used as the major propagating material.
Mitunguu	1071	Farmers use irrigation water from a river tapped by a community scheme for growing bananas. Suckers are used as the major propagating material. The temperatures are high (25°C - 30°C) and the bananas take less time (14 months) to grow, mature and produce fruit.

in the nursery and after transplanting was recorded. The data was analysed using ANOVA and means separated by what Least significant differences and Tukeys' test to determine where significant differences existed at $P \leq 0.05$, comparing between the varieties and locations.

Results

Disease occurrence in surveyed farms. The average disease incidences in all the areas surveyed (Fig. 1) showed that Fusarium wilt and Sigatoka had the highest incidences. Weevils were recoded at 17% and nematodes at 20%. Nutrient stress was recorded at 6% and Cigar end rot at 4%. Banana Xanthomonas wilt, Banana streak virus and Bunchy top virus was not observed in any of the farms surveyed. Fusarium wilt incidence was high in Kirinyaga west and Embu East districts (90%), and Kirinyaga East (80%) of the farms surveyed. Here, the most preffered varieties are Gros Mitchel and sweet banana which are susceptible to Fusarium. The disease was recorded at 70% in Meru central and 60% in Imenti south.The disease incidence was lower in Murang'a and Mathioya at 40% and Kirinyaga Central at 30%. Sigatoka was observed at 50% in all the farms surveyed. Weevils were at 52% in Imenti south, 37% in Embu East, 25% in Meru Central and less than 20% in Kirinyaga West, East and Central of the farms surveyed. There were no weevils recorded in Murang'a and Mathioya districts. These areas have high altitude thus not suitable for survival of the weevil. Nutrient stress was recorded in farms where manure and fertilizer was not adequately applied and especially in farms where Tissue Culture seedlings were used as the planting material. Cigar end rot was observed in farms where agronomic practices such as weeding and deleafing was not done.

Health of corms. Samples from the corms that were rooted in the sawdust upon isolation in the laboratory showed presence of endophytes and non pathogenic micro-organisms. Some of the endophytes isolated were *Colletotrichum gloeosporioides*, *Penicilium*, *Trichoderma*, *Alternaria* and *Aspergillus*. Saprophytes were isolated from some of the corms that were rotting in the media. Less than 1% of the corms in all the study sites rotted (Table 2). However, pathogenic *Fusarium oxysporum* f. sp. *cubense* was isolated from samples of two corms of Sweet banana in Mitunguu and Embu east and in one corm of Kampala variety. These corms gave rise to wilted plantlets which were promptly removed from the nursery.

The seedling production varied with varieties with Cavendish having the highest number of seedlings produced (Table 3). This was followed by Kiganda and Kampala. Sweet banana showed the least production in numbers. No seedlings died during hardening.

Discussion

Diseases and pests are the major constraints affecting banana production in Eastern and Central provinces. Fusarium wilt was high in areas where susceptible varieties are preferred by the farmers. The disease can be easily spread through infected planting material because the symptoms may not be visible in the daughter suckers. This can be rectified through planting banana varieties that are resistant to the disease such as East African Highland banana and the Cavendish sub-groups (Ploetz, 2000). Weevil incidences were higher in lower agroecological zones. This was in Mitunguu and Embu

Table 2. Number of banana corms that rotted in the nursery.

Variety	Corms planted	Corms that rotted
Kiganda	60	1
Cavendish	112	1
Sweet banana	76	2
Kampala	65	1

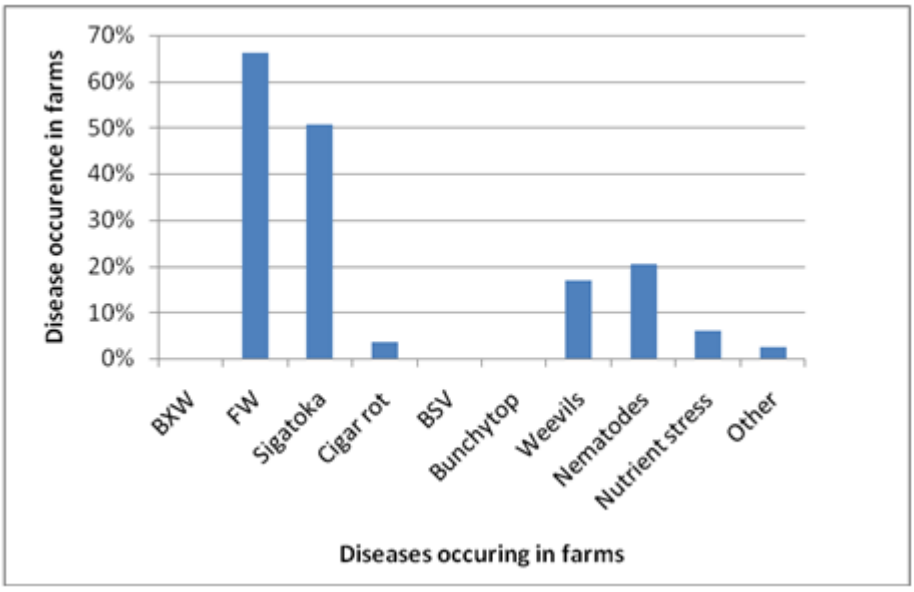

Figure 1. Average incidence of banana diseases in the farms surveyed.

Table 3. Banana seedlings produced in the study sites.

Variety	Mitunguu	Embu east	Kerugoya	Ntharene	Meru central	Mathioya
Kampala	116	90	-	98	84	80
Cavendish	122	116	78	129	102	108
S. banana	36	35	44	47	56	50
Kiganda	104	38	56	71	71	80

East. This is because the high temperature (25°C - 30°C) coupled with irrigation creates a conducive environment for weevil. Gatarayiham (2003) confirms that adult weevil population is high in low altitude areas (900 – 1100 M).

Endophytes are considered as biological control agents and potential genetic vectors in plant biotechnology (Pereiva *et al.*, 1999). Many endophytes produce antibiotics and probably inhibit the growth of latent pathogenic fungi thus giving protection to the plants. Mutual association between the endophytes and the host plants has been reported to be beneficial to plant growth (Clay & Schardl, 2002). There is a possibility that banana plantlets produced through Macropropagation benefit from the endophytes in the corm. Tenkouano (2006) found that the seedlings are less prone to post-establishment stress and loss in the field. This beneficial aspect of the microorganism lacks in Tissue culture plantlets thus they are sensitive to pathogenic attack. Latent pathogens may escape detection during certification of the farms. Therefore varieties that are resistant to FW such as Cavendish and East African Highland banana should be used for Macropropagation.

The seedlings have a high survival rate in the hardening nursery and in the field. However, less than 5% seedlings that were rootless had a lower survival rate. The variation in the number of seedlings produced across the varieties could have been due to physiological differences and temperatures. The high survival rate of the seedlings after transplanting indicates that Macropropagation technology is effective in producing healthy seedlings.

Conclusion

The effects of diseases that affect banana can be reduced by supplying healthy and affordable seedlings to farmers. Macropropagation technology can be employed to produce clean and cost effective seedlings. The seedlings need to be supplemented with a fertilizer rich in Phosphorus or well decomposed manure during transplanting to maintain the required level of fertility.

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