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Original Paper

Collaborative Exploration for *Panicum maximum* Genetic Resources in Kenya, March, 2012

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Summary

Guineagrass (*Panicum maximum* Jacq.) is a one of most important tropical forage grass species. This grass exhibits apomixis (asexual) propagation through seeds, this poses difficulties for developing improved cultivars by breeding. Therefore, numerous accessions have been collected and characterized, and many cultivars have been released directly using apomictic accessions. Although guineagrass has been collected and genetically analyzed, the key factor in guineagrass improvement is finding sexual lines. Phylogenetic analysis of guineagrass reveals that the Kenya is a center of diversity for guineagrass, therefore, it is thought sexual lines may exist there. A molecular marker selection method has been developed to identify sexual plantlets without the need for cytological analysis or progeny tests.

Although numerous apomictic accessions have been collected, only a few sexual lines have been identified, and these do not have superior agronomical characteristics. This narrow genetic base has prevented recurrent selection of sexual lines themselves and also breeding and crossing with superior apomictic lines. Thus, agronomical superior sexual lines are required. This visit to Kenya was conducted to initiate a new search for sexual varieties of guineagrass in its native habitat. Based on observations made there appears to be the possibility of finding abundant sexual lines.

During this preliminary expedition, a total of 14 native *P. maximum* sites were found and recorded. At each site, several morphological varieties were recorded. The Kenyan Genebank has already collected 1300 accessions of guineagrass and we hope to identify new sexual lines among these accessions. We recognized also that KARI-Katumani has 56 accessions from dividing tillers of native guineagrass, these materials should also be checked for new sexual lines.

KEY WORDS：Guineagrass, forage, apomixes, sexual line

Introduction

Guineagrass is one of the major forage grasses in tropical and semitropical regions. Guineagrass belongs to the family *Poaceae*, subfamily *Panicoideae*, and tribe *Paniceae*, and forms an agamic complex with other two species, *Panicum infestum* Anders and *Panicum trichocladum* K. Schum. However, only a few sexual accessions have been identified as *P. maximum* (Muir and Jank 2004). Guineagrass exhibits persistence, good yield and high quality in moderately drought prone tropical and sub-tropical regions. It is used as hay and silage for dairy and meat cattle. Guineagrass is most diverse in Africa and probably where it originated. It exhibits wider climatic adaptation than other cultivated pastures of the other tropical and semitropical regions (Muir and Jank 2004).

Guineagrass grows well in tropical and semitropical climates such as regions with an altitude range of 0-2400 m a.s.l. (Clayton and Renvoize 1982), from 16.3 ° N ′ (Russell and Webb 1976) to more than 25° S′ (Muir et al. 2001), and with annual rainfalls from 700 mm (Muir et al. 2001) to 1100 mm (Skerman and Riveros 1990). In addition, the optimal growth temperature of guineagrass ranges 19.1-22.9° C (Russell and Webb 1976). Because guineagrass is native to tropical Africa and Madagascar (Clayton and Renvoize 1982), larger diversity of the native guineagrass has been found in East Africa, which is considered the center of origin (Combes and Pernès 1970). Also, molecular phylogenetic analysis reveals that the center of diversity of guineagrass is Kenya (Ebina et al. 2007).

Guineagrass is characterized as an apomictic species. Apomixis in guineagrass has been classified as gametophytic aposporous type (Warmke 1954). Most guineagrass accessions are autotetraploid (2n = 4x = 32), and reproduce through facultative aposporous apomixis. Only a few completely sexual guineagrass accessions were found in East Africa, and these sexual plants are identified as diploid (2n = 2x = 16) or tetraploid (Nakajima et al. 1979). In natural habitats, 7 % of guineagrass genotypes reproduce sexually (Pernès 1975). It is considered that diploid sexual forms intercross with *P. maximum*, *P. infestum* and *P. trichocladum* by spontaneous haploidization and recurrent tetraploidization (Savidan and Pernès 1982). Therefore, it is thought that tetraploid sexual lines of *P. maximum* could be found in natural populations. Natural populations can be classified into three types: (i) monomorphic; (ii) polymorphic discontinuous; and (iii) polymorphic continuous (Pernès 1975). Polymorphic discontinuous; and polymorphic continuous populations must be a result of the crossing between sexual lines themselves or sexual and apomictic lines.

In the 1940s, many *Panicum* germplasms was collected and the cultivar “Gatton” was released as a result (Edye and Miles 1976). In the 1970s the Plant Introduction Center in Georgia, USA, collected and characterized guineagrass germplasm mainly from South Africa (Hanna et al. 1973). French and Japanese scientists discovered sexual materials from East Africa (Combes and Pernès 1970 ; Nakajima et al. 1979; Hojito and Horibata 1982; Tsurumi et al. 1997). Due to limited number of sexual tetraploid lines, tetraploid sexual lines were generated through the colchicine chromosome doubling method in order to utilize them efficiently for breeding programs (Nakagawa and Hanna 1992). However, artificial sexual tetraploid lines do not exhibit good persistency. Also, natural sexual lines are not good enough to directly use for cultivars, and this has prevented breeding improvement of guineagrass.

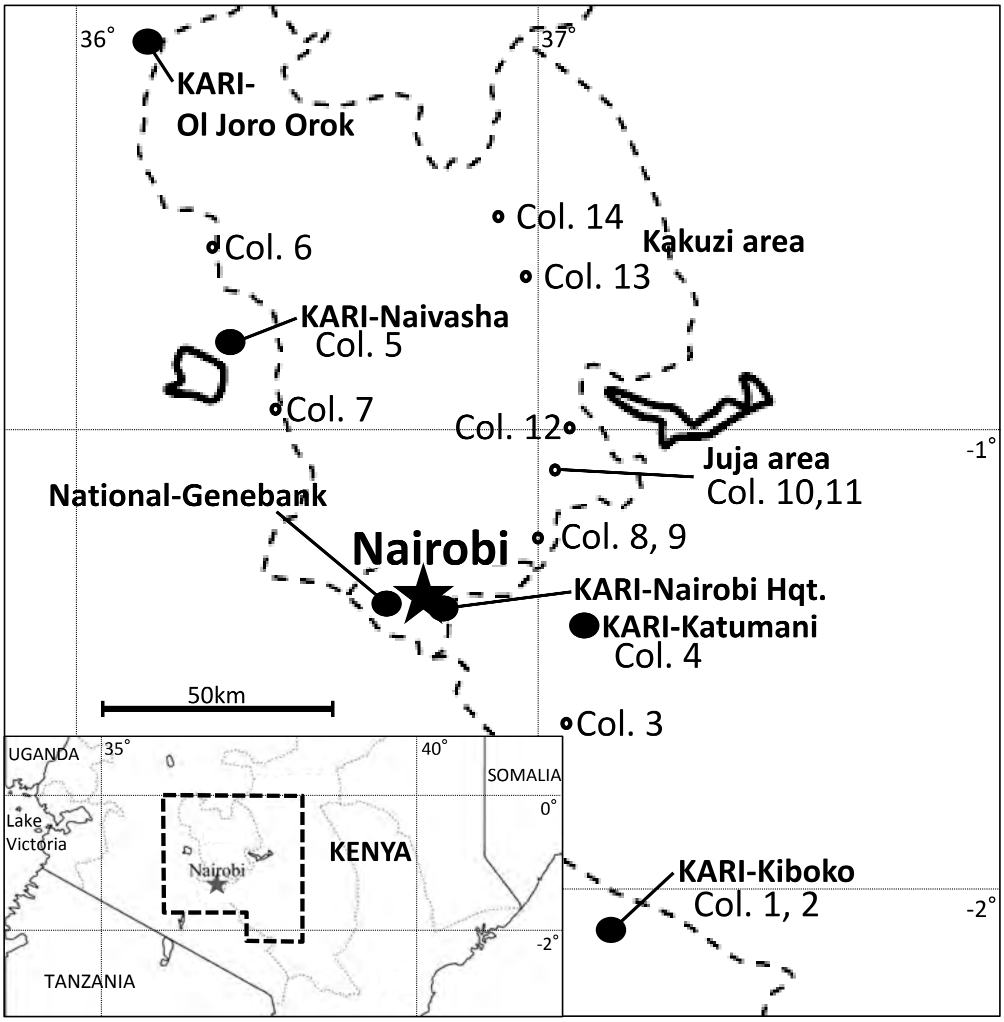

Fig. 1. Maps of Collection Sites and Visited Institutions in Kenya.
A left below map is a broad area map indicating area of the exploration with dashed line. A upper map indicates detailed map of the exploration. (○) indicates collection sites and (●) indicates visited institutions. The Sites number are also indicated in Table 2 (as K number) and photos with detailed descriptions.

Table 1. Itinerary of the survey in Kenya, 2012

day	M/DD*		Exploring activities	Stay town
1	3/4	Sun	Depart Japan	
2	3/5	Mon	Arrive in Kenya; visit KARI	Nairobi
3	3/6	Tue	Visit KARI-Headquarter, Ministry of Livestock, National-Genebank	Nairobi
4	3/7	Wed	Visit KARI-Kiboko	Machakos
5	3/8	Thu	Visit KARI-Katumani	Nairobi
6	3/9	Fri	Visit KARI-Naivasha and KARI-Ol Joro Orok	Nairobi
7	3/10	Sat	Visit Juja (Compure girls)	Nairobi
8	3/11	Sun	Rest	Nairobi
9	3/12	Mon	Visit KARI-Headquarter, ILRI, Japanese embassy	Nairobi
10	3/13	Tue	Visit KARI-Headquarter, Depart Kenya	
11	3/14	Wed	Arrive in Japan	

*M/DD ; for month/date

Discrimination of sexual and apomictic guineagrass has been developed by cytological methods (Nakagawa and Hanna 1992), and by a molecular base progeny test (Ebina and Nakagawa 2001). However, these methods take time and requires maintaining of the sexual lines in testing fields. All of the sexual lines found were crossed with apomictic lines seeds collected and stored. These seeds are segregating and to re-find sexual lines also has the same difficulties. Linkage analysis of guineagrass apomixis has been done and apomictic markers have been found (Ebina et al. 2005). Further, several of the apomictic markers were converted to STS markers, and adapted to other apomictic accessions and segregation populations. These markers enabled site diagnostics using a simple DNA marker for apomixis using plantlets gDNA. The LAMP method has also been developed (Mori et el. 2004), and Hem resistant KOD DNA polymerase (Takagi et al. 1997) will support identification of apomictic plants.

We attempted to discovered new natural tetraploid sexual accessions during this preliminary expedition to Kenya. The observations during the expedition were consistent with the hypothesis that many sexual tetraploid lines occur in Kenya. The establishment of on-site diagnostics enable us to finding new variable sexual tetraploid guineagrass accessions.

Survey Methods

The itinerary of the survey is shown (Table 1). The survey routes and collection sites are shown (Fig. 1). The survey was conducted according to the planned regions as follows: Kiboko, Machakos, Naivasha, Ol Joro Orok and Juja (Compure - girl's school).

Landscape of survey sites, information of investigated and collected sample traits, such as plant height, basal stem diameter, length of panicle were recorded. Samples were collected and sites and plants were photographed.

Results and Discussions

On 5th March, Japanese group arrived in Nairobi. Kenyan and Japanese researchers had a survey planning meeting on 5th and 6th March, 2012.

National Genebank
(6th March, 2012)

Previously collected accessions from earlier collaboration between Kenya and Japan were shared and kept in both Genebanks. Prior to this survey, we checked the previous collection storage and list at the National Genebank. Almost all of the stocks, especially latest 1993 collection were safely conserved in the Genebank and the collection list including all of the crops stocked in the National Genebank could be checked on the database of the National Genebank.

KARI-Kiboko and surrounding area
(7th March, 2012)

KARI-Kiboko experimental station (site 1) is around 100 km south-east of Nairobi, neighboring to the Chyulu Hills National Park along the Mombasa Rd, (lat. 02° 15' 09'' S; long. 37 ° 43' 40'' E, alt. 1025 m). In this station, a low maintenance green zone was covered with

P. maximum (Photo 1). According to the morphological appearance, at least two types of *P. maximum* were found. One has an obvious larger inflorescence having thin greenish spikelets (Photo 2), and the other has a small inflorescence with denser purplish spikelets (Photo 3).

Near the KARI-Kiboko experimental station, there is a well-established experimental field (site 2) (lat. 01° 44' 15'' S, long. 43° 44' 00'' E, alt. 984 m). Under the shade of acacia (*Acacia* spp.), several plants of *P. maximum* had escaped from the field, but morphological appearance were different from the original plants in the field (Photo 4). Native *P. maximum* tends to be under acacia trees.

On the Mombasa Rd side (site 3), (lat. 01° 59' 51'' S, long. 37° 21' 27'' E, alt. 1245 m), around young acacia trees, native *P. maximum* could be found (Photo 5). The inflorescence (Photo 6) exhibit a different type among the native *P. maximum* based on the previous survey (Photo1-4).

The survey sites around KARI-Kiboko were close each other, however, the native *P. maximum* plants exhibit different morphological appearances, also different types of native *P. maximum* plants existed in the same place (Photo 1-3).

KARI-Katumani and surrounding area
(8th March, 2012)

KARI-Katunami experimental station is near Nairobi along the Mombasa Rd, (lat. 01° 35' 18'' S, long. 37° 14' 20'' E, alt. 1610 m). At this station, 56 native ecotypes were collected by Kenyan researchers and evaluated. The best clones were propagated vegetatively (Photo 7), distributed to several other KARI experimental stations, and performance trials were carried out at these stations. The experiments objective was to determine accessions of *P. maximum* for severely arid areas of Kenya (Photo 8). The each of the 56 native ecotypes also are conserved in the station, thus these plant could be directly used for the future survey for sexual material plant of guineagrass.

KARI-Naivasha and surrounding area
(9th March, 2012)

KARI-Naivasha experimental station (site 5) is around 100 km north-west of Nairobi, neighboring Lake Naivasha National Park along the Nakuru-Nairobi Rd in the African Great Rift Valley, (lat. 00° 41' 27'' S, long 36° 24' 10'' E, alt. 1902 m). Here an experimental field at the station, one accession from Kenyan coastal area was conserved (Photo 9). High altitude may be a cause of loss of plants in the experimental field. Also, from Naivasha to Nakuru (site 6), high altitude area, in this survey no native *P. maximum* was found (Photo 10). Photograph 10 indicates no *P. maximum* in the area, (lat. 00° 28' 09'' S, long. 36° 21' 28'' E, alt. 2139 m).

Returning from Nakuru to Nairobi, we checked the altitudinal limit of native guineagrass. At the road side of a cliff of the African Great Rift Valley (site 7), (lat. 00° 59' 34'' S, long. 36° 35' 59'' E, alt. 1905 m), several native *P. maximum* were found (Photo 11, 12). Surrounding vegetation was represented by natural semi-arid vegetation (Photo 12). At this site three different morphological types of native *P. maximum* were observed (Photo 13). The one has large inflorescence type with thin and purple spikelets, and the other two have comparatively compact inflorescence type with denser and purple spikelet. The other two compact inflorescence types could be distinguished by long dense trichome on greenish culm or short trichome on purplish culm (Photo 14). Sites 5 and 6 (alt. 2139-1902 m), had no *P. maximum*, but site 7 at an altitude of 1905 m quite variable *P. maximum* was found. Thus, the altitude of 1900 m is considerable altitude limit for native *P. maximum* in the west highlands from Nairobi.

Juja and surrounding area
(10th March, 2012)

In East Nairobi, on the roadside, also several different morphological types of *P. maximum* were found. Site 8 (Photo 15) and nearby site 9 (Photo 16) were at the same latitude and longitude (alt. 1541m). The plant of native *P. maximum* of site 8 was not flowering, but the plant of native *P. maximum* of site 9 had already flowered. Although genetic particularity could be determined after genotyping by DNA tools, the observation of these two different types in close proximity was consistent with the composite population of site 1 and site 7.

Slightly further east of Nairobi, in a burnt field after corn cultivation (site 10 and 11), (lat. 01° 03' 34'' S, long. 37° 01' 31'' E, alt. 1541m), several plants of another type of *P. maximum* were found (Photo 17, 18 and 19). These plants occurred as weed in cultivated corn. Since these plants were totally dormant due to aridity, the differences among the plants could not be observed. Observations made at various sites in close proximity reveal the diversity of guineagrass in Kenya. At site 8 (Photo 15) plants exhibit young vegetative growth, at site 9 (Photo 16) plants are post-flowering and at site 10 (Photo 17-19) plants are dormant. Near a farm, on the road side, (lat. 01° 00' 02'' S, long. 37° 04' 28'' E, alt. 1519 m), adjacent to a small scale farm, another type of several native plants of *P. maximum* were found (Photo 20 and 21).

On the road side in Kakuzi area, adjacent dense tall trees (Photo 22 and 23), survey site 13 and 14, (lat. 00° 55' 46'' S, long. 37° 10' 21'' E, alt. 1494m), *P. maximum* plants were grow along with the road. Here trees provide shade most of the day. Each of the plants culm length, thickness and inflorescence length were measured (Photo 24, Table 2). Native plants of *Brachiaria* spp. were associated with *P. maximum*, especially in near east of Nairobi.

Conclusion

We surveyed for new natural tetraploid sexual accessions of guineagrass during this preliminary expedition in Kenya. In many sites, especially in site 1, 7, 8, 9 and 10, several different types of *P. maximum* were found, they have clear morphological differences. At site 1 and 7, these existed as composite population in the same site, and in the site 8, 9 and 10, obviously different types of *P. maximum* occurred in close proximity. These observations are consistent with the hypothesis that sexual tetraploid lines occur in center of diversity for guineagrass in Kenya.

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和文摘要 (Japanese summary)

本報告は，世界の熱帯圏で広く牧草として利用されているギニアグラス (*Panicum maximum* Jacq.) の数少ない野生の有性生殖個体を発見するための予備調査の結果を示している．ギニアグラスはアポミクシスであり，母株と同じ遺伝子型の無性生殖種子で繁殖するため，交雑による育種が困難で,かつ野生の有性生殖個体は現在までのところ全世界で数系統しか発見されていない．そのため，アポミクシス自生株の収集が盛んに行われてきており，これらには豊富な自然変異が含まれている．これらの豊富な変異は自然交雑による変異と考えられ，有性生殖個体の存在を裏付けるものである．しかしながら，これまで収集されてきた有性生殖個体が少ないため，はっきりしない点が多い．豊富な変異を含むアポミクシス自生株の遺伝変異解析から，ケニアに最も豊富な変異が含まれることがこれまでの研究から明らかになってきた．また，近年 DNA を用いた直接的でかつ少量の葉からすぐに判定が可能な手法が開発されてきた．そのため，ケニアの自生状況を確認するとともに，DNA の簡易判定手法の応用の可能性を検討するため，探索を行った．その結果，同一収集地点または近接収集地点で，形態的に明らかに遺伝変異を含んでいると考えられる個体を複数の地点で確認することができた．このことは，有性生殖株の存在を強く示唆するものであり，DNA 簡易判別手法の応用により即座な判定を行うことができれば，希少な野生有生殖株を収集蓄積することが可能であり，これらは，今後のギニアグラスの牧草あるいはエネルギー作物としての育種の重要な基盤となることが期待される．

Table 2. Recorded guinea grass in Kenya

No.	Coll. No.	Coll. Date (March)	Species name	Status*1)	Local name	Sample*2)	Locality Province, (Village)	Latitude	Longitude	Altitude (m)	Condition*3)	Collection	Remarks
K1	2012Kenya 01	7	*Panicum maximum*	1	–	In	Kiboko	S02–15–09.2	E37–43–40.7	1025	3–1–2–1–4	Photos	Plant length including flower (panicle) : 1.2 m Basal stem diameter : 3 mm Flower (panicle) length : 26 cm
K2	2012Kenya 02	7	*Panicum maximum*	1	–	In	Kiboko	S01–44–15.0	E43–44–00.0	984	3–1–1–2–4	Photos	Plant length including flower (panicle) : 1.0 m Basal stem diameter : 3 mm Flower (panicle) length : 20 cm
K3	2012Kenya 03	7	*Panicum maximum*	1	–	In	Kiboko	S01–59–51.6	E37–21–27.0	1245	3–1–1–2–4	Photos	Plant length including flower (panicle) : 1.5 m Basal stem diameter : 4 mm Flower (panicle) length : 50 cm
K4	2012Kenya 04	8	*Panicum maximum*	1	–	In	Katumani	S01–35–18.5	E37–14–20.2	1610	5–1–2–2–4	Photos	Plant length including flower (panicle) : 2.2 m Basal stem diameter : 9 mm Flower (panicle) length : 60 cm
K5	2012Kenya 05	9	*Panicum maximum*	1	–	In	Naivasha	S00–41–27.9	E36–24–10.3	1902	3–1–1–1–4	Photos	Plant length including flower (panicle) : 0.3 m Basal stem diameter : ND Flower (panicle) length : ND
K6	2012Kenya 06	9	*Panicum maximum*	1	–	In	Naivasha	S00–28–07.9	E36–21–28.6	2139	5–1–2–1–4	Photos	Plant length including flower (panicle) : ND Basal stem diameter : ND Flower (panicle) length : ND
K7	2012Kenya 07	9	*Panicum maximum*	1	–	In	Naivasha	S00–59–34.4	E36–35–59.0	1905	6–2–3–2–3	Photos	Plant length including flower (panicle) : 1.3 m Basal stem diameter : 4 mm Flower (panicle) length : 21 cm
K8	2012Kenya 08	10	*Panicum maximum*	1	–	In	Juja	S01–03–09.7	E37–00–45.1	1541	3–1–1–2–3	Photos	Plant length including flower (panicle) : 1.5 m Basal stem diameter : 7 mm Flower (panicle) length : 20 cm
K9	2012Kenya 09	10	*Panicum maximum*	1	–	In	Juja	S01–03–09.7	E37–00–45.1	1541	3–1–N–2–3	Photos	Plant length including flower (panicle) : 1.6 m Basal stem diameter : 9 mm Flower (panicle) length : 41 cm
K10	2012Kenya 10	10	*Panicum maximum*	1	–	In	Juja	S01–03–14.8	E37–01–31.0	1541	3–1–N–2–N	Photos	Plant length including flower (panicle) : 2.3 m Basal stem diameter : 5 mm Flower (panicle) length : 35 cm
K11	2012Kenya 11	10	*Panicum maximum*	1	–	In	Juja	S01–03–14.8	E37–01–31.0	1541	3–1–N–2–N	Photos	Plant length including flower (panicle) : 2.2 m Basal stem diameter : 5 mm Flower (panicle) length : 34 cm
K12	2012Kenya 12	10	*Panicum maximum*	1	–	In	Juja	S01–00–02.7		1519	3–1–1–2–4	Photos	Plant length including flower (panicle) : 2.3 m Basal stem diameter : 9 mm Flower (panicle) length : 37 cm
K13	2012Kenya 13	10	*Panicum maximum*	1	–	In	Kakuzi	S00–55–46.4	E37–10–21.9	1494	5–2–2–2–3	Photos	Plant length including flower (panicle) : 1.6 m Basal stem diameter : 3 mm Flower (panicle) length : 34 cm
K14	2012Kenya 14	10	*Panicum maximum*	1	–	In	Kakuzi	S00–55–46.4	E37–10–21.9	1494	5–2–2–2–3	Photos	Plant length including flower (panicle) : 2.3 m Basal stem diameter : 5 mm Flower (panicle) length : 43 cm

*1) 1; Wild, 4; Landrace
*2) In; Individual, P; Population (seeds)
*3) Topography–Site–Stoniness–Soil texture–Drainage
Topography 1; swamp, 2; flood plain, 3; plain level, 4; undulation, 5; hilly, 6; mountainous, 7; other (specify)
Site 1; level, 2; slope, 3; summit, 4; depression
Stoniness 1; none, 2; low, 3; medium, 4; rocky
Soil texture 1; sand, 2; loam, 3; clay, 4; silt, 5; highly organic
Drainage 1; poor, 2; moderate, 3; good, 4; excessive

Photo 1. *P. maximum* at KARI-Kiboko (Photo1, K1)

Photo 5. *P. maximum* in site K3, Mombasa Rd. at Kiboko (Photo5, K3)

Photo 2. *P. maximum*; greenish floweret type at KARI-Kiboko (Photo2, K1)

Photo 6. Inflorescence in site K3, Mombasa Rd. at Kiboko (Photo6, K3)

Photo 3. *P. maximum*; purplish floweret type at KARI-Kiboko(Photo3, K1)

Photo 7. *P. maximum* test field at KARI-Katumani (Photo7, K4)

Photo 4 . *P. maximum* under the Acacia tree at Kiboko exemplar field (Photo4, K2)

Photo 8. *P. maximum* test field at KARI-Katumani, attacked by deer (Photo8, K4)

Photo 9. *P. maximum* field at KARI-Naivasha (Photo9, K5)

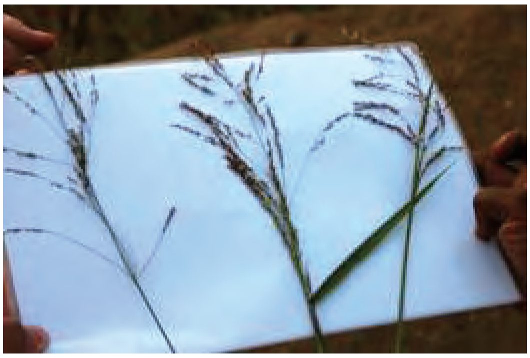

Photo 13. Inflorescence of three different types of *P. maximum* at Nakuru to Nairobi (Photo 13, K7)

Photo 10. Naivasha to Nakuru (Photo10, K6)

Photo 14. Pubescence and colors of node of three different types of *P. maximum* (Photo14, K7)

Photo 11. *P. maximum* at Nakuru to Nairobi (Photo11, K7)

Photo 15. *P. maximum* on site K8, at Kirathe Farm Juja (Photo15, K8)

Photo 12. Natural vegetation Background of *P. maximum* at Nakuru to Nairobi (Photo12, K7)

Photo 16. *P. maximum* on site K9, at Kirathe Farm Juja (Photo16, K9)

Photo 17. *P. maximum* on site K10, at remains of corn field in Juja farmer (Photo17, K10)

Photo 21. *P. maximum* at Road side small farm at Juja (Photo21, K12)

Photo 18. *P. maximum* on site K10 (Photo18, K10)

Photo 22. *P. maximum* on site K13, at a narrow margin between forest and road Kakuzi area (Photo22, K13)

Photo 19. *P. maximum* on site K11, at the other remains of corn field Juja area (Photo19, K11)

Photo 23. *P. maximum* on site K14, at same as and near site K13 (Photo23, K14)

Photo 20. *P. maximum* at Road side small farm at Juja (Photo20, K12)

Photo 24. Measuring the traits at site K14 (Photo 24, K14)