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*Full Length Research Paper*

# **Rapid and efficient plant regeneration from shoot apical meristems of finger millet [*Eleusine coracana* (L.) Gaertn.] via direct organogenesis**

**Asunta Mukami<sup>1</sup>, Alex Ngetich<sup>2</sup>, Cecilia Mweu<sup>2</sup>, Mutemi Muthangya<sup>1</sup>, Richard O. Oduor<sup>3</sup>, Mathew Ngugi<sup>3</sup> and Wilton Mbinda<sup>4\*</sup>**

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**A simple and efficient plant regeneration system via direct organogenesis was established in finger millet using *in vitro* derived shoot apical meristems. Six varieties; GBK-043128, GBK-043094, GBK-043050, GBK-043137, GBK-043122 and GBK-043124 were evaluated. MS medium was used for cotyledonary germination. Maximum number of shoots (84.33%) was observed in variety GBK-043128 while GBK-043094 had the least germination efficiency (62.67%). Shoot apical meristems from three-day old seedlings were evaluated for their potency of shoot induction on varied 6-benzylaminopurine (BAP) concentrations. Highest shoot induction was observed in medium supplemented with 1.75 mg/l BAP in GBK-043050 (3.00) whereas GBK-043094 (1.28) had the least response in medium supplemented with 1.0 mg/l BAP. To induce rooting, *in vitro* regenerated plants cultured on MS medium was supplemented with different concentrations of indole-3-acetic acid. The highest response in root induction, with a larger number of roots (10.28), was observed in MS medium supplemented with 4.0 µM IAA. Statistical analysis indicated that plant regeneration response varied greatly among the varieties. *In vitro* germinated plants were successfully transferred to the greenhouse after hardening, with 300 shoots developing into fertile plants, which were indistinguishable with wild type plants. This plant regeneration system has potential for production of transgenic finger millet crops.**

**Key words:** Direct organogenesis, finger millet, root induction, shoot apical meristems.

## **INTRODUCTION**

Finger millet [*Eleusine coracana* (L.) Gaertn.] is an important cereal crop worldwide which is cultivated on

more than 4 million hectares with an annual production of at least 4.5 million tons of grain. Due to its ease of

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cultivation, low fertilizer requirements and high adaptability, finger millet is considered a food security and major staple food crop for millions of subsistence and rural communities in semi-arid regions of Asia and Africa (Chivenge et al., 2015). Besides its direct use as table and feed stock, finger millet is also a candidate for the production of renewable plant products such as ethanol (Tekaligne et al., 2015). Compared to other cereals, finger millet has outstanding nutritional qualities as the grain is rich in calcium, phosphorus, iron, cysteine, tyrosine, tryptophan and methionine (Latha et al., 2005). Finger millet is primarily consumed as porridge in Africa but in South Asia as bread, soup, roti (flat bread) and to make beer. Other new food merchandise made of finger millet that have become common among younger generation include vermicelli, pasta, noodles, sweet products, snacks and different bakery products.

Despite its major importance, finger millet has not received significant attention from plant biotechnologists. The plant's growth and productivity is greatly constrained by agronomic practices, pests and diseases, low soil fertility, labour intensity, high weed infestation, low yielding varieties, lodging, poor attitude to the crop, salinity and drought emanating from climate change (Mgonja et al., 2007). In light of the utilization of finger millet-based items, interest for the crop is expanding over the world (Pathi et al., 2013) and enhancing and developing finger millet varieties with desirable qualities and tolerance to various environmental pressures through genetic engineering is therefore a critical need. However, for genetic engineering to occur, a reliable and effective plant regeneration system have to be initially established. Plant tissue culture technology is thus a significant biotechnological instrument for the transfer of genetic traits to overcome crop yield losses due to various biotic and abiotic stresses (Kumar et al., 2015b). Finger millet has long been considered recalcitrant for plant tissue culture (Gupta et al., 2017). To the best of the researchers' knowledge, the current finger millet regeneration systems available were based on somatic embryogenesis and are limited by low regeneration frequencies and long regeneration periods (Dey et al., 2012).

Direct and indirect shoot organogenesis from different explants are reportable as effective explants for several plant species. Direct organogenesis regeneration from explants, omitting the callus induction segment, is admirable notably in modern plant tissue culture technology wherever reducing costs of regeneration systems, minimising somaclonal variation and increasing rapidity are fundamental components of consideration (Burner and Grisham, 1995). The success of plant regeneration especially through direct regeneration is dependent on choice of explant and maturation and conversion of the explant into plants. Direct regeneration therefore, represents a promising tool for plant regeneration because is a rapid and an effective

approach. The present work reports a rapid and efficient direct plant regeneration system for African finger millet varieties using *in vitro*-derived shoot apical meristems as explants, without an intermediate callus phase. For multiple shoot induction and regeneration, the concentration of cytokinin was optimized. This procedure is rapid, reliable and reproducible and can immensely be used for genetic transformation of finger millet in the future.

## MATERIALS AND METHODS

### Plant material and explants preparation

Six Kenyan preferred finger millet varieties were used: GBK-043128, GBK-043094, GBK-043050, GBK-043137, GBK-043124 and GBK-043122. The seeds of these varieties were procured from Kenya Agricultural Research and Livestock Organization, Gene Bank, Nairobi, Kenya. The seeds were surface sterilized by washing them with sterile distilled water followed by incubating them with 70 % (v/v) ethanol for two min then transferred to 20% sodium hypochlorite containing a drop of Polysorbate 20 for 23 min. Surface sterilized seeds were rinsed thrice with double distilled water and germinated aseptically on Murashige and Skoog (MS) basal medium (Murashige and Skoog, 1962) supplemented with 30% sucrose. The medium pH was adjusted to 5.8 before adding 3 g/l gelrite, followed by autoclaving at 121°C for 15 min under 15 kPa. The cultures were incubated at 25±2°C in the dark for germination for three days. The germination percentage of the six varieties was calculated after 3 days of culture.

### Shoot induction and elongation.

Aseptically grown 3-day-old shoot tips, comprising of the apex and a part of mesocotyl, were excised and utilized as explants. The shoot tips (4-6mm) were cultured on shoot induction medium (SIM) comprising of MS basal medium supplemented with 30% sucrose and different concentrations of 6-benzylaminopurine (BAP) (1, 1.5, 1.75 and 2 mg/l). The culture bottles were incubated in the growth room with 16/8-hours light/dark at 26±2°C for 12 days. Twelve days after culture, the formed shoots were transferred to fresh SIM and incubated in the growth room with 16/8-hours light/dark at 26±2°C for a further 12 days to elongate the induced shoots. The percentages of the number of shoots that formed in each shoot clump and the mean number of shoots induced in each explant were calculated following 24 days of culture.

### Root induction

The elongated shoots (5-6 cm long) were transferred to rooting medium comprising of MS basal medium supplemented with 30% sucrose and various concentrations of indole-3- acetic acid (IAA; 1, 2, 3, 4 and 5 µM). The plantlets were then incubated in the growthroom under light 16/8-hours light/dark at 26±2°C to induce rooting. The total number of roots initiated per shoot was calculated after 28 days of culture.

### Hardening and acclimatization

Rooted plants were washed with double distilled water to remove medium on the plantlets. The plants were thereafter transferred into peat moss in plastic cups (11×15 cm) for hardening for 5 days

**Table 1.** Germination efficiencies of six finger millet varieties.

Variety	Number of seeds	Germination efficiency
GBK-043137	100	84.33± 0.048 <sup>a</sup>
GBK-043128	100	82.33±0.034 <sup>a</sup>
GBK-043124	100	72.70±0.10 <sup>ab</sup>
GBK-043122	100	62.67±0.013 <sup>b</sup>
GBK-043094	100	62.67±0.013 <sup>b</sup>
GBK-043050	100	80.00± 0.012 <sup>a</sup>

Means (± SE) followed by different alphabets in each column are significantly different ( $P \leq 0.05$ ) using Fishers LSD.

following which the plants were then transferred to soil in pots and incubated in greenhouse for acclimatization. The plants were watered regularly and data on survival rate of plants recorded after 4 weeks of culture. Plant survival rate was calculated as:

$$\text{Survival rate} = \frac{\text{surviving plants}}{\text{total plantlets}} \times 100$$

#### Experimental design and data analysis

A completely randomized block design with three replications of 15 explants per replication was employed. MS basal medium excluding plant growth regulators was used as a negative control. Observations for any morphological changes formed on the cultures were made periodically and recorded at regular intervals. Data on the number of rooted shoots were chronicled after 28 days of culture on rooting medium. After 30 days of transfer of the plantlets to plastic cups, the survival percentage was recorded. The variability in data was expressed as mean ± standard error (SE). The data collected were analysed using one-way analysis of variance (ANOVA) with Minitab statistical computer software.17. Means were separated using the Fisher's protected LSD test at a confidence level of 95% ( $p \leq 0.05$ ).

## RESULTS

### Effects of MS medium on germination

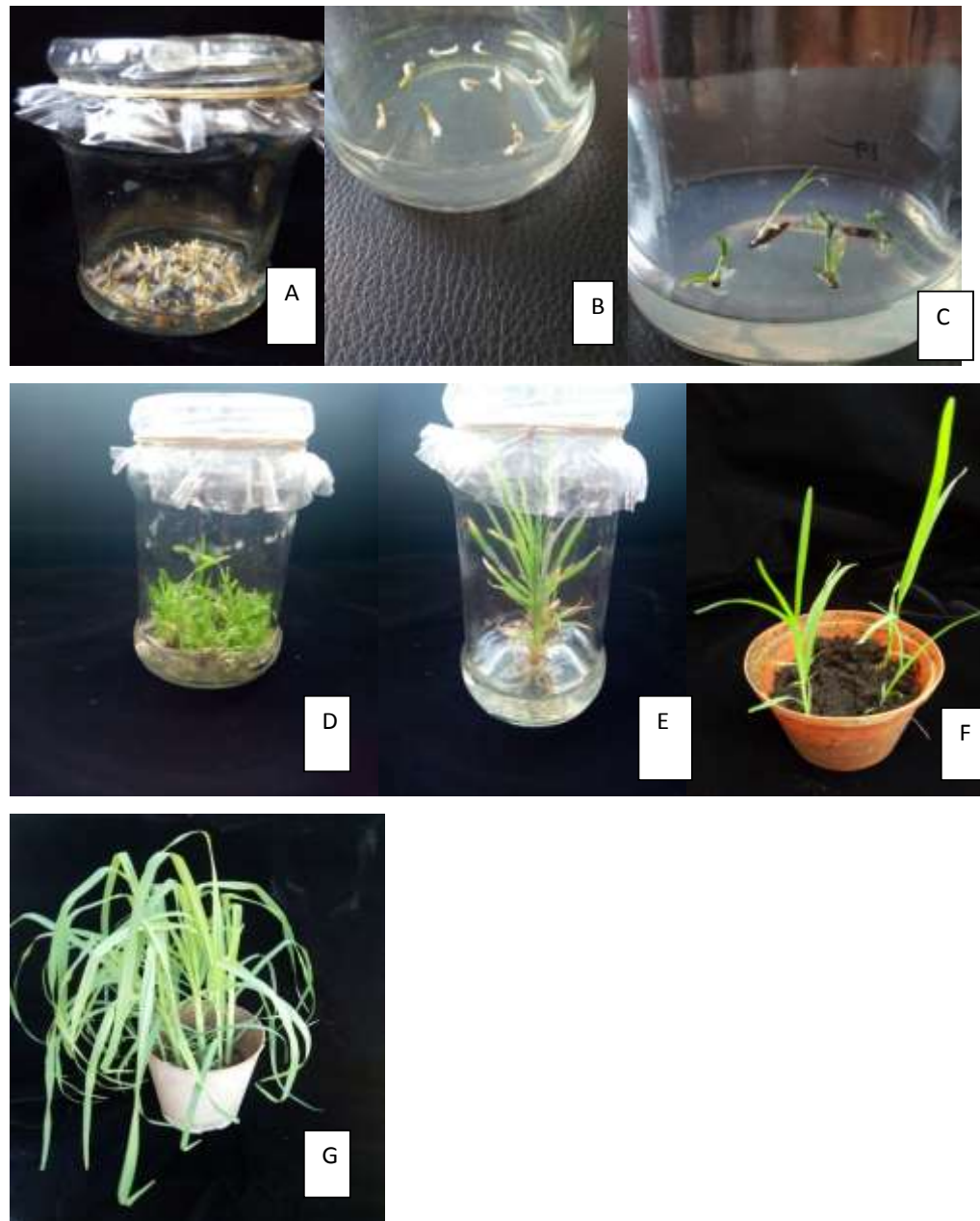
The seeds of the six selected finger millet varieties were germinated on MS basal medium containing with 30% sucrose and different germination efficiencies recorded after three days of incubation in dark (Figure 1A). GBK-043137 had the highest germination efficiency of 84.33% followed by GBK-043128, GBK-043050 and GBK-043124 with seed germination efficiencies of 82.33, 80 and 72.70% respectively. Varieties GBK-043122 and GBK-043094 had the least germination efficiencies of 62.67% (Table 1). Significantly higher difference in germination was observed for varieties GBK-043137, GBK-043128, GBK-043050, GBK-043124, GBK-043122 and GBK-043094 (Table 1). However, for varieties GBK-043137, GBK-043128, GBK-043050 there was significantly lower difference in germination rate (Table 1).

### Effect of induction of shoot apical meristems

When 3-day-old meristemic shoot tips, consisting of the apex and part of mesocotyl were cultured on MS basal medium containing various concentrations of BAP, shoot induction was observed within 1 day of incubation. After one day of incubation, the explants induced shoot which were white in color (Figure 1B) and formed a single leaf-like structure which thereafter became green (Figure 1C) and formed multiple shoots after 24 days (Figure 1D). The six finger millet varieties tested exhibited remarkably different regeneration responses depending on the concentration of BAP. The best shoot induction was observed in medium containing 1.75 mg/l BAP (Table 2). Shoot apical meristems explants of all finger millet varieties tested responded well to different concentrations of BAP tested in the shoot induction medium (Figure 1B). However, shoot induction response and number of shoots per explant also varied based on the variety and BAP concentration in the shoot induction medium. Statistical analysis of variance indicated that significant differences were observed among the varieties in plant regeneration response. Induction medium supplemented with 1.75 mg/l BAP exhibited significantly better response of shoot induction than the other BAP concentration tested, ranging from 3.00 to 1.28 shoots per explant. The variety GBK-043050, showed significantly superior response of shoot induction; GBK-043128, GBK-043124, GBK-043137 and GBK-043122 produced a moderate response; GBK-043094 produced a significantly lowest response of 1.62 shoots per explant in shoot induction medium supplemented with 1.75 mg/l BAP (Table 2). The lowest performing media was 1.0 mg/l on GBK-043094 with an average number of shoots of 1.28±0.13 (Table 2). The height of the plant varied between 5 and 6 cm.

Similarly, shoot clumps developed on all varieties when sub-cultured in shoot induction medium containing BAP. The shoot clumps produced were significantly more shoots and the shoots were also longer. The best response was observed in MS basal medium containing 1.75 mg/l BAP with 12 total shoots (Table 3). However, shoot clumps sub-cultured to the same induction medium containing 1.0 mg/l BAP and no response of shoot





**Figure 1.** (A) Three day-old finger millet seedlings germinated on plant growth-regulators free MS medium; (B) Initiation of shoots from shoot apical meristems(4-6 cm) inoculated on MS medium containing BAP; (C) Shoots formed in 12 days; (D) Multiple shoots formed in 24 days; (E) Root development in MS medium containing IAA; (F) Acclimated plantlets in plastic cups containing sterile peat moss; (G) Two weeks after hardening off on peat moss

multiplication was observed on MS basal medium lacking plant growth regulators. The shoot multiplication response also varied among the 6 varieties evaluated (Table 3).

#### Root induction

Rooting occurred after two weeks when the elongated

shoots (5-6 cm in height) were cultured on MS basal medium. Rooting responses varied based on the variety and concentration of IAA. Majority of the varieties produced optimal root induction response on MS supplemented with 3  $\mu\text{M}$  IAA. The variety GBK 043124 showed significantly higher response of root induction (8.89 roots); GBK043137, GBK043128, GBK043122 and GBK043094 produced moderate response; GBK 043050



**Table 2.** Shoot induction on BAP after 12 days of six finger millet varieties.

Variety	BAP (mg/l)			
	1.0	1.5	1.75	2.0
GBK-043137	1.70±0.23 <sup>b</sup>	1.98±0.11 <sup>ab</sup>	2.02±0.11 <sup>b</sup>	1.88±0.28 <sup>b</sup>
GBK-043128	1.55±0.08 <sup>b</sup>	2.22±0.11 <sup>ab</sup>	2.40±0.31 <sup>ab</sup>	1.60±0.21 <sup>b</sup>
GBK-043124	1.67±0.32 <sup>b</sup>	1.96±0.15 <sup>ab</sup>	2.01±0.15 <sup>b</sup>	2.02±0.16 <sup>ab</sup>
GBK-043122	1.84±0.29 <sup>ab</sup>	1.75±0.17 <sup>b</sup>	2.07±0.41 <sup>ab</sup>	1.72±0.07 <sup>b</sup>
GBK-043094	1.28±0.13 <sup>b</sup>	1.89±0.29 <sup>ab</sup>	1.62±0.29 <sup>b</sup>	1.74±0.13 <sup>b</sup>
GBK-043050	2.38±0.26 <sup>a</sup>	2.71±0.65 <sup>a</sup>	3.00±0.39 <sup>a</sup>	2.61±0.39 <sup>a</sup>
Control	0.00±0.00 <sup>c</sup>	0.00±0.00 <sup>c</sup>	0.00±0.00 <sup>c</sup>	0.00±0.00 <sup>c</sup>

Means (± SE) followed by different alphabets in each column are significantly different ( $P \leq 0.05$ ) using Fishers LSD

**Table 3.** Shoot multiplication on BAP after 24 days of six finger millet varieties.

Variety	BAP (mg/l)			
	1	1.5	1.75	2.0
GBK-043137	3.60±0.15 <sup>a</sup>	4.13±0.19 <sup>b</sup>	4.43±1.44 <sup>cd</sup>	3.77±0.23 <sup>c</sup>
GBK-043128	3.30±0.32 <sup>a</sup>	3.63±0.09 <sup>bc</sup>	10.33±0.88 <sup>ab</sup>	6.00±0.58 <sup>bc</sup>
GBK-043124	2.00±0.00 <sup>b</sup>	3.00±0.00 <sup>c</sup>	8.00±1.53 <sup>bc</sup>	7.33±0.33 <sup>ab</sup>
GBK-043122	3.83±0.32 <sup>a</sup>	3.43±0.55 <sup>bc</sup>	4.13±0.63 <sup>cd</sup>	4.00±0.57 <sup>bc</sup>
GBK-043094	3.00±0.58 <sup>a</sup>	3.47±0.29 <sup>bc</sup>	4.00±1.08 <sup>d</sup>	3.70±0.59 <sup>c</sup>
GBK-043050	3.67±0.29 <sup>a</sup>	5.00±0.00 <sup>a</sup>	12.00±2.31 <sup>a</sup>	10.00±2.89 <sup>a</sup>
Control	0.00±0.00 <sup>c</sup>	0.00±0.00 <sup>d</sup>	0.00±0.00 <sup>e</sup>	0.00±0.00 <sup>d</sup>

Means (± SE) followed by different alphabets in each column are significantly different ( $P \leq 0.05$ ) using Fishers LSD.

produced the significantly low response of 4.25 roots. However, root induction in varieties GBK-043124 and GBK-043050 was highest achieved in 4 and 2  $\mu\text{M}$  with 10.28 and 5.70 roots, respectively. Root induction was least achieved in MS medium supplemented with 1 and 5  $\mu\text{M}$  across the six varieties (Table 4).

### Hardening and acclimatization

The rooted plants were transferred to peat moss and maintained in growthroom for 5 days. Following the 5 days culture in growthroom, the plants were transferred to soil in plastic pots with 100% survival rate and incubated in the greenhouse where the plants were watered regularly. All plant regenerated via direct organogenesis grew well and exhibited phenotypic homogeneity and same growth characteristics when compared to field-grown finger millet plants derived from seeds (Figure 1G).

### DISCUSSION

Genetic engineering techniques are increasingly becoming important to achieve rapid improvements in

finger millet cultivars. To successfully achieve this objective and efficiently produce bioengineered crops, improvements to the existing laborious and time-consuming protocols for *in vitro* regeneration need to be established. In this study, procedures for rapid and effective shoot-regeneration of six Kenyan farmer preferred finger millet varieties that have potential for application in genetic engineering experiments was optimized. In order to establish rapid and efficient plant regeneration procedures that could be used in genetic engineering experiments, hormone regimes which have previously been reported in successful finger millet transformation were tested. The choice of shoot organogenesis over somatic embryogenesis is because it is fast and also circumvents prolonged callus stages, therefore minimising chances of somaclonal variation (Karp, 1991). More so, shoot apical meristems are also easily handled compared to other explants and can be induced to produce multiple shoots (Arockiasamy and Ignacimuthu, 2007).

The shoot apical meristems derived from mature seeds was used as an initial explant for efficient and reproducible direct regeneration protocol for finger millet. The successful use of shoot apical meristems explants in this study implies that they are a better choice for plant regeneration in cereals when compared to other explants.

**Table 4.** Root induction on IAA after 28 days of six finger millet varieties.

Variety	IAA ( $\mu\text{M}$ )				
	1	2	3	4	5
GBK-043137	4.33 $\pm$ 0.17 <sup>ab</sup>	7.13 $\pm$ 0.35 <sup>a</sup>	8.03 $\pm$ 0.42 <sup>ab</sup>	4.50 $\pm$ 0.25 <sup>b</sup>	3.39 $\pm$ 0.59 <sup>ab</sup>
GBK-043128	3.70 $\pm$ 0.29 <sup>bc</sup>	6.50 $\pm$ 0.12 <sup>ab</sup>	7.86 $\pm$ 0.29 <sup>ab</sup>	4.65 $\pm$ 0.80 <sup>b</sup>	2.63 $\pm$ 0.18 <sup>bcd</sup>
GBK-043124	4.54 $\pm$ 0.27 <sup>a</sup>	6.74 $\pm$ 0.54 <sup>ab</sup>	8.89 $\pm$ 0.11 <sup>a</sup>	10.28 $\pm$ 0.20 <sup>a</sup>	4.00 $\pm$ 0.00 <sup>a</sup>
GBK-043122	3.88 $\pm$ 0.07 <sup>abc</sup>	4.41 $\pm$ 0.51 <sup>c</sup>	7.60 $\pm$ 0.48 <sup>b</sup>	4.81 $\pm$ 0.51 <sup>b</sup>	3.23 $\pm$ 0.44 <sup>abc</sup>
GBK-043094	2.51 $\pm$ 0.39 <sup>d</sup>	4.44 $\pm$ 0.50 <sup>c</sup>	6.25 $\pm$ 0.25 <sup>c</sup>	5.25 $\pm$ 0.30 <sup>b</sup>	2.47 $\pm$ 0.15 <sup>cd</sup>
GBK-043050	3.48 $\pm$ 0.42 <sup>c</sup>	5.70 $\pm$ 0.49 <sup>b</sup>	4.25 $\pm$ 0.53 <sup>d</sup>	3.15 $\pm$ 0.52 <sup>c</sup>	2.01 $\pm$ 0.13 <sup>d</sup>
Control	0.00 $\pm$ 0.00 <sup>e</sup>	0.00 $\pm$ 0.00 <sup>e</sup>	0.00 $\pm$ 0.00 <sup>d</sup>	0.00 $\pm$ 0.00 <sup>d</sup>	0.00 $\pm$ 0.00 <sup>e</sup>

Means ( $\pm$ SE) followed by different alphabets in each column are significantly different ( $P \leq 0.05$ ) using Fishers LSD.

This study therefore confirms previous reports by Ramakrishnan et al. (2014) and Ceasar and Ignacimuthu (2010). Mature seeds derived explants are better source material for tissue culture research than others because of the availability of seeds, ease of storage of seeds, and homogeneity quality of the explants (Yang et al., 2013). To induce shoots, different concentrations (1.0, 1.5, 1.75 and 2.0 mg/l) of BAP were tested separately. Cytokinins play important role in shoot growth. BAP is commonly used for *in vitro* regeneration of cereals and other monocot plants (Ramakrishnan et al., 2013). BAP concentration of 1.75 mg/l was found to be more effective in direct shoot induction of six finger millet varieties. The genotype dependent variations in shoot induction among finger millet varieties observed in this study were also noted in previous studies for monocot cereal plants (Pazuki and Sohani, 2013).

Shoot clumps obtained from the shoot induction medium were subcultured to shoot elongation medium with various concentrations of BAP. The medium supplemented with 1.75 mg/l BAP produced more number of shoots of 10.33 shoots per explant. To the best of the researchers' knowledge, this is the highest number ever reported in finger millet. Report by Pande et al. (2015) stated that optimal multiple shoot induction response was recorded on MS basal media supplemented with 3.0 mg/l of BAP, while Satish et al. (2015) reported 8.3 shoots per explants of finger millet variety 'CO(Ra)-14' in MS basal medium containing 17.6  $\mu\text{M}$  6-BAP, 0.9  $\mu\text{M}$  2,4-dichlorophenoxyacetic acid (2,4-D) in combination with 750 mg/l proline, 500 mg/l casein enzymatic hydrolysate and 2 mg/l glycine. When compared to other monocots, Pilahome et al. (2014) reported 30.33 shoots per explant of Sakon Nakhon in MS medium containing 50  $\mu\text{M}$  6-BAP in rice while Muoma et al. (2008) reported 5.7 shoots from inbred lines of Kenyan maize KAT and TLO8 from shoot apices on MS basal medium containing 26.64  $\mu\text{M}$  BAP, 296  $\mu\text{M}$  adenine and 9  $\mu\text{M}$  2,4-D. Earlier research work on maize genotypes from stated 4.3 shoots (CM300) and 1 to 3 shoots (LM5) in MS medium supplemented with 4.4  $\mu\text{M}$

BA and 2.8  $\mu\text{M}$  IAA from 14-day-old immature embryos (Rakshit et al., 2010; Manivannan et al., 2010). Similarly, research work by Pathi et al. (2013) described 9 shoots from mature embryo in maize genotype HQPM-1 on MS basal medium containing 8.8  $\mu\text{M}$  BA, 4.6  $\mu\text{M}$  Kinetin and 2.6  $\mu\text{M}$  1-naphthaleneacetic acid (NAA).

The direct regeneration system reported in here is rapid, effective and proficient and offers mass multiplication of finger millet within 7 weeks. Results from various work on other plants including *Zea mays* (Ramakrishnan et al., 2014), *Curcuma attenuata* (Kou et al., 2013), *Hippophae rhamnoides* (Sriskandarajah and Lundquist 2009), *Metabriggsia ovalifolia* (Ma et al., 2010), *Primulina tabacum* (Yang et al., 2012), and *Pulsatilla koreana* (Lin et al., 2011) using the two-stage tissue culture system has also proven it to be efficient in other plants.

In order to achieve the *in vitro* rooting of regenerated shoots, indole-3-acetic acid was used at different concentrations (1, 2, 3, 4 and 5  $\mu\text{M}$ ). This study found 3  $\mu\text{M}$  to be the best concentration for four finger millet varieties; GBK-043137, GBK-043128, GBK-043122, GBK-043094. Varieties GBK-043124 and GBK-043050 however, showed best root induction at 4 and 2  $\mu\text{M}$ , respectively. Interestingly poor rooting was observed in 1 and 5  $\mu\text{M}$  across all the varieties. These results indicate that *in vitro* rooting in finger millet can be induced with IAA at concentrations between 2 and 4  $\mu\text{M}$ . Peat moss was used for acclimatization and hardening of the rooted plants because of its high water retention capacity recorded in previous study (Ngetich et al., 2018). Consequently, all the plants were successfully acclimatized and hardened with 100% survival rate. This high survival rates could also be attributed to the well-developed root system and greenhouse conditions. Plants were regenerated within 52 days.

## Conclusion

This study outlines a rapid efficient, simple and

reproducible protocol for shoot regeneration of several finger millet varieties using shoot apical meristems as explants. The system developed in vitro plant regeneration protocol is potentially useful for plant genetic transformation and gene function studies of finger millet.

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## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

## ABBREVIATIONS

**BAP**, 6-Benzylaminopurine; **IAA**, Indole-3-acetic acid; **MS**, Murashige and Skoog basal medium; **SAM**, shoot apical meristems.

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*Full Length Research Paper*

# **Assessment of morphological characteristics among upland rice (*Oryza sativa* and *Oryza glaberrima*) germplasm**

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Rice is an important staple food crop that feeds over half of the global population and has become the cereal that provides a major source of calories for the urban and rural poor in Africa. This work aimed to evaluate the morphological of rice (*Oryza sativa* and *Oryza glaberrima*) germplasm. In the present study, 14 quantitative traits were used across 48 accessions or genotypes obtained from Central Agricultural Research Institute (CARI), Liberia and Plant Genetic Resources Research Institute (PGRRI), Ghana. In this research, Completely Randomized Design was conducted to study the genetic variability among the 48 genotypes or accessions obtained from CARI, Liberia and PGRRI, Ghana. Field data taken included 14 quantitative traits scored using the IRRI descriptor list. Analysis of variance revealed highly significant difference ( $P \leq 0.01$ ) among the accessions for all quantitative traits studied. Four significant principal components analysis were identified and accounted for 55.3%. PC1 had Eigen-value of 18.5%, whereas PC2 accounted for Eigen-value of 14.5%. PC3 contributed 12.2% whereas PC4 had 10.1%. Correlation analysis indicated the length of ligule was highly significant and positive leaf width blade. Similar observation was made with grain length and length of ligule. Some accessions in the biplot showed longer vector distances, while shorter vector distances were observed referencing PC1; Gh1578 recorded the longest vector distances while Gh1526 and LAC 23-1, recorded the moderate distances from the vector origin at the similarity coefficients at 90%. The highly distant genetic diversity was found between ACSS37 and ACSS1 from Ghana and Liberia. Cluster X was the largest of all the clusters while Clusters VII and VIII were the second largest clusters with seven accessions each. The outcome of this study should be useful for the management of the germplasm conservation and future rice genetic improvement. However, all the accessions may be cultivated over time at different locations on the field to ascertain their stability and purity.

**Key words:** Correlation, morphological, quantitative, qualitative, accessions.

## **INTRODUCTION**

Rice (*Oryza sativa* L.,  $2n = 24$ ), a member of Poaceae (Gramineae) is the world's most important staple food crop that feeds over half of the global population (Ishimaru et al., 2017). It is cultivated in tropical and

subtropical regions. Rice is grown in more than 114 countries, over an area of 161.4 m ha in a wide range of ecosystems under varying temperature and water regimes with the production of 466.7 mt (on milled basis)

(FAO, 2011). According to Ansah et al. (2017), approximately 20 million farmers are engaged in rice production in sub-Saharan Africa (SSA) and about 100 million people depend on it directly for their livelihoods on the continent.

Rice is rapidly becoming a staple food in the African diet; and its production in SSA continues to be outpaced by consumption as a result of low and stagnated production. Imported rice accounts for 50% of sub-Saharan Africa's rice requirement (Notarnicola et al., 2017). Rice is no longer a luxury food but has become the cereal that constitutes a major source of calories for the urban and rural poor. Rice production in SSA has been bedeviled with conditions such as environmental degradation due to pesticide usage, excessive water usage, and nutrient contamination, methane emission and ammonia volatilization and these conditions require urgent attention (Luther et al., 2017).

A wide range of technologies are available and can be used as tools for reducing these adverse consequences of rice production; but they are, however, not extended to majority of rice growers or farmers (Notarnicola et al., 2017). Self-sufficiency in rice production is, however, declining as demand increases. Little attention has been paid to the improvement of Liberian and Ghanaian rice germplasm evaluation and the genetics of some quality traits. Thus, there is very little information available on the genetic diversity of Liberian and Ghanaian rice germplasm for crop improvement and conservation purposes. There is an urgent need to increase and improve the production of rice in Africa in order to meet up with the high demand. The need for increasing rice cultivation depends not only on cultural/traditional practices, but also, on their inbuilt genetic potential to withstand stresses. A successful breeding programme will depend on the genetic variability of a crop for achieving the goals of improving the crop and producing high yielding varieties (Onyia et al., 2017). The first step in achieving this is to evaluate and characterize available rice germplasm or genotypes at the morphological stage.

## METHODOLOGY

### Planting materials and experimental design

Forty-eight (48) upland rice accessions were evaluated from Ghana and Liberia. Thirty-five Ghanaian genotypes were obtained from Plant Genetic Resources Research Institute (PGRI) at Bunso Ghana, while 13 Liberian accessions were obtained from the Central Agricultural Research Institute (CARI), Suakoko in Bong County, Liberia. Experiment was carried out at the Insectary Laboratory, Faculty of Agriculture, Kwame Nkrumah University of Science and Technology (KNUST) and pots were arranged in

complete randomized design with three replicates. The compound fertilizer of NPK (15-15-15) was applied by ring method seven days after planting at the rate of 1.5 g in each pot as a first dose and second dose of NPK was applied one month after the first at the same rate per pot. The third dose was the urea fertilizer. The urea was the third fertilizer applied in the maximum tillering stage one month after the second dose of NPK. Pesticide Lambda Master 2.5 EC (25 g lambda-cyhalothrin/liter) at a dosage of 100 mL/15 L of water (600 mls/ha) were used for pest outbreak. Manual weeding and birds watching were done at tillering and reproductive stages using bird net till harvesting time.

### Data collection

Evaluation of the rice accessions was carried out for different morphological parameters representing the vegetative growth stage of rice. Trait selection and measurement techniques were based on IRRI standard evaluation system of rice (Hien et al., 2007). Culm diameter at basal internode (Culm diameter (mm) was measured using vernier calipers and the mean was computed), leaf length of blade (length of the topmost leaf blade below the flag leaf on the main culm in centimeters), leaf width (width of the widest portion of the blade on the leaf below the flag leaf in centimeters), flag leaf length width (leaf length was measured from the base to the tip of the flag leaf, rounded off to the nearest millimetre, while the width was measured at the widest part of the flag leaf and recorded to the nearest mm), and panicle number per plant (total number of tillers) were counted and recorded at the maturity stage before harvest), ligule length (length from the base of the collar to the tip of the ligule in millimeters), plant height (plant height (cm) was measured from soil surface to tip of the plant at reproductive stage using the measuring tape), productive tillers per plant (productive tillers/plant was obtained by counting the number of tillers per plant and averaged across replications for each accessions during the maturity stage). *awn length (mm)* (the awn is a long slender extension of the lemma in rice) was also measured. It was measured from the tip of the spike to the tip of the longest awn), panicle length of main axis (the panicle length of main axis was measured from the base of the panicle to the tip of the lemma or palea using the measuring tape), one hundred grain weight (one hundred well developed seeds were randomly selected per replication for each accession. The seeds were obtained from the harvested samples of accessions after harvest; dried to 13% moisture content and weighed on a balanced precision scale (METTER PM 400) to determine the 100 grain weight, grain length (the grain length was measured as the distance from the base of the lowermost glume to the tip (apiculus) of the fertile lemma or palea), and grain width (to obtain the grain width, it was measured as the distance across the fertile lemma and palea at the widest point using the callipers at post-harvest stage).

### Data analysis

All recorded agro-morphological traits were analyzed using two complementary procedures; Microsoft Excel was used to record and organize the data. The quantitative data were subjected to Analysis of Variance (ANOVA) using the GenStat Statistical package version (12<sup>th</sup> edition, VSN international, Hemel Hempstead) to calculate the relationship between the traits of the genotypes.

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Least Significant Difference (LSD) at 5% was used to separate the treatments means. MINITAB®17 Statistical Software was used to perform the principal component analysis (PCA).

## RESULTS

Fourteen quantitative traits were evaluated against 48 genotypes of rice received from Liberia and Ghana (Table 1). The evaluation of morphological traits usually reveals important traits which are essential to characterize the genetic resources. The mean, standard error, range, coefficient of variation, standard error of deviation, F probability of the least significant difference at 5% were computerized for 14 quantitative characters are shown in Table 2. The 14 quantitative traits were performed to determine the relative contribution on different traits to the total variation in rice. Among the 14 quantitative traits studied, productive tillers, grain width, sterile lemma length and grain length had variations of 42.0, 41.6, 28.1 and 24.2 (Table 2). On the other hand, leaf blade length, and flag leaf obtained the lowest variation among the traits (Table 2).

The principal components analysis based on the 14 quantitative traits was performed individually to determine the relative contribution of the different traits to the total variation in rice. The PCA is an ordination of multivariate technique that allows the use of biplots to visualize the relationship between the accessions and measured traits.

Four significant principal components were identified and accounted for 55.3% of the total variation. PC1 had Eigen-value of 0.44, explaining 18.5% of the total variation (Table 3). Quantitative traits such as panicle per plant (0.43), panicle length of the main axis (0.43), flag leaf (0.34), sterile lemma length (0.39), leaf length of ligule (0.27) and plant height (0.25) contributed greatly to PC1, which accounted highest for the total variation (Table 3). PC2 depicted proportion of variance as 14.5%, while PC3 contributed 12.2% to the total variation and PC4 had 10.1% to the total variation (Table 3). PC2 was associated with leaf length of blade (0.48), sterile lemma length (0.43), grain width (0.34), plant height (0.33) and flag leaf (Table 3). PC3 was associated with Culm diameter at the basal internode (0.47), awn length (0.46), leaf width of blade (0.41), and flag leaf (w) (0.35). The fourth PC had 0.16 as its Eigen-value and it explained 10.1% of the total variation. PC4 was associated with leaf width of blade (0.62), grain weight of 100 fully developed grain (0.45), leaf length of ligule (0.32), and grain length (0.29) (Table 3).

Pearson correlation among the 14 quantitative traits were highly significant as positive correlation was observed between length of ligule and leaf width of blade ( $r = 0.93$ ). Relationship between sterile lemma length and length of ligule was highly positively correlated ( $r = 0.92$ ) (Table 4). Plant height was significantly positively correlated ( $r = 0.69$ ). Grain length and length of ligule is highly significantly positively correlated ( $r = 0.99$ ) (Table

4). However, correlation between culm diameter at basal internode and length of ligule, sterile lemma length and culm diameter at basal internode, 100 grain weight of fully developed grain and culm diameter at basal internode, plant height and grain length were positively correlated (Table 4). Correlation between awn length and culm diameter at basal internode, panicle length of the main axis and length of ligule, plant height and 100 grain weight of fully developed grain were negatively correlated (Table 4).

The principal components analysis based on PCA biplot of the rice accessions revealed diverse grouping pattern among the 14 quantitative traits (Figure 1). While some accessions showed longer vector distances, shorter vector distances were observed for others. For PC1, Gh1578 recorded the longest vector distances. Gh1526 and LAC 23-1, recorded the moderate distances from the vector origin while GH3623, GH1588 and LAC23-2-3 recorded the shortest. GH1570 and GH1550 had the longest distances for PC2; whereas GH5173 and LAC 23-12 had similar vector in PC2 and were separated from the rest of the accessions (Figure 1).

A dendrogram was constructed for the 48 rice genotypes based on their morphological characteristics. Figure 2 shows that at a similarity index of 68%, the accessions clustered into 7 main clusters. The most distant genotype were Gh1540 and LAC23-1, which were found in the first and last of position of the dendrogram. Cluster I was the largest of all the clusters and contained 20 accessions with five sub-clusters. Cluster III was the second largest cluster with 16 accessions including two sub-clusters and Cluster IV had 8 accessions with two sub-clusters respectively. Cluster VI had three accessions with two sub-clusters and next was Cluster II which had two accessions only. Clusters V and VII had one accession each, which were LAC23-9 and GH1540. Cluster VI had three accessions and comprised two sub-clusters. Accessions in the same cluster have the same morphological characteristics and sub-clusters indicate that the accessions have some distinct traits from other members of the clusters. The 48 rice accessions (from Liberia and Ghana) showed no distinctive morphological characteristics based on geographical origin, as the analysis showed no group of accessions from either of the geographical locations divergently clustered.

## DISCUSSION

In the present study, a set of rice genotypes from Liberia and Ghana were subjected to diversity analysis based on variation in morpho-phenological traits. To meet the future rice demand in Liberia and Ghana with the increasing population, one option is to increase the productivity per unit area of the land, thus the identification of more yield related agro-morphological characters is very much important. Plant height in rice is

**Table 1.** Accessions of rice's, their sources and collection countries.

<b>Accession number</b>	<b>Name</b>	<b>Source</b>
1	LAC 23-1	AFRICA RICE LIBERIA
2	LAC 23-2-2	AFRICA RICE LIBERIA
3	LAC 23-2-3	AFRICA RICE LIBERIA
4	LAC 23-3	AFRICA RICE LIBERIA
5	LAC 23-4	AFRICA RICE LIBERIA
6	LAC 23-5	AFRICA RICE LIBERIA
7	LAC 23-6	AFRICA RICE LIBERIA
8	LAC 23-7	AFRICA RICE LIBERIA
9	LAC 23-8	AFRICA RICE LIBERIA
10	LAC 23-9	AFRICA RICE LIBERIA
11	LAC 23-10	AFRICA RICE LIBERIA
12	LAC 23-11	AFRICA RICE LIBERIA
13	LAC 23-12	AFRICA RICE LIBERIA
14	GH 1510	PGRRI GHANA
15	GH 1515	PGRRI GHANA
16	GH 1518	PGRRI GHANA
17	GH 2204	PGRRI GHANA
18	GH 2193	PGRRI GHANA
19	GH 1512	PGRRI GHANA
20	GH 1521	PGRRI GHANA
21	GH 1534	PGRRI GHANA
22	GH 1538	PGRRI GHANA
23	GH 1526	PGRRI GHANA
24	GH 1528	PGRRI GHANA
25	GH 1536	PGRRI GHANA
27	GH 1594	PGRRI GHANA
28	GH 2144	PGRRI GHANA
29	GH 1593	PGRRI GHANA
31	GH1588	PGRRI GHANA
32	GH 1587	PGRRI GHANA
33	GH 1580	PGRRI GHANA
34	GH1581	PGRRI GHANA
35	GH 1578	PGRRI GHANA
36	GH 1575	PGRRI GHANA
37	GH 1572	PGRRI GHANA
38	GH 1579	PGRRI GHANA
39	GH 1584	PGRRI GHANA
40	GH 3623	PGRRI GHANA
41	GH 1576	PGRRI GHANA
42	GH 1550	PGRRI GHANA
43	GH 1598	PGRRI GHANA
44	GH 1570	PGRRI GHANA
47	GH 1573	PGRRI GHANA
48	GH 1523	PGRRI GHANA
49	GH 1549	PGRRI GHANA
50	GH 1524	PGRRI GHANA
51	GH 1540	PGRRI GHANA
52	GH 1571	PGRRI GHANA



**Table 2.** Summary statistics of 14 quantitative traits measured on 48 rice accessions from Liberia and Ghana.

Traits	Mean $\pm$ S.E	Range	CV (%)
Culm (DABI)	7.19 $\pm$ 1.03	1.79 – 13.00	14.3
Flag leaf (FLL)	34.04 $\pm$ 2.25	13.17 – 67.50	6.6
Leaf (LOB)	51.47 $\pm$ 1.44	34.67 – 71.83	2.8
Leaf (LOL)	1.83 $\pm$ 0.17	1.13 – 2.50	9.5
Leaf (WOB)	1.50 $\pm$ 0.16	1.17 – 2.00	10.5
Panicle (PNPP)	11.47 $\pm$ 1.10	4.67 – 25.00	9.6
Plant height, cm (PH)	129 $\pm$ 11.14	66.00 – 187.5	8.6
Produ. Tillers /plt	11.06 $\pm$ 4.6	3-29	42.0
Awn Length (AL)	8.04 $\pm$ 0.12	1.5 – 62.00	14.3
Grain Length(GL)	1.46 $\pm$ 0.35	0.96 – 2.08	24.2
Grain width (GW)	1.55 $\pm$ 0.65	1.16 – 3.10	41.6
Grain (WOFDG)	2.17 $\pm$ 0.40	1.10 – 2.88	18.4
Panicle (LOMA)	24.58 $\pm$ 3.27	19.47 – 30.63	13.3
Sterile (LL)	1.30 $\pm$ 1.23	1.00-9.00	28.1

WOB=Leaf width of blade; LOL=leaf length of ligule; FLL= flag leaf length; DABI=culm diameter at basal internode; AL= awn length; LOMA= panicle length of the main axis; WOFDG=100 grain weight of fully developed grain; GL= grain length; GW=grain weight; PH= plant height; Sterile (LL) = lemma length.

**Table 3.** Principal components analysis based on the 14 quantitative traits.

Variable	PC1	PC2	PC3	PC4
Leaf Length of Blade	0.25	-0.48	-0.04	-0.08
Leaf Width of Blade	0.07	0.03	0.41	-0.62
Leaf Length of Ligule	0.27	0.01	-0.18	0.32
Flag Leaf Length	0.34	0.28	0.04	0.11
Flag Leaf Width	0.11	0.33	0.35	0.01
Culm Diameter at Basal Internode	-0.15	0.11	-0.47	-0.28
Panicle Number per Plant	-0.43	-0.29	0.01	0.18
Sterile Lemma Length	-0.39	-0.43	0.09	-0.04
Awn Length	0.21	0.11	-0.46	-0.26
Panicle Length of the Main Axis	0.41	-0.14	-0.28	-0.05
100-Grain Weight of Fully Developed Grain	0.27	-0.22	0.05	-0.45
Grain Length	-0.29	0.29	-0.26	-0.29
Grain Width	0.19	0.34	0.23	0.09
Plant Height	0.25	-0.33	0.18	0.27
Eigenvalue	0.44	0.31	0.24	0.16
Proportion	0.03	0.02	0.02	0.01
Cumulative	0.95	0.97	0.99	1.00

complex character and the end product of several genetically controlled factors called internodes (Cheema et al., 2018). Tall plant type is very typical of landrace genotypes which exceed in their capacity to support panicle growth by large stem reserve mobilization. Ali et al. (2008) observed relatively greater range in plant height than the other characters. The smallest plant height was recorded for accession GH1571 cm and

accession GH1550 cm recorded the highest value. This was true because the semi dwarf plant type was extensively utilized in the rice (*O. sativa*) cultivars throughout the world. However, depending on the part of the world with improvement in farmers' lives, there is a growing desire to combine desirable characteristics of tall varieties' with yielding ability and a new type of architecture: intermediate plant height as stated by Zafar

**Table 4.** Pearson correlation coefficients among the quantitative traits studied.

Trait	LOB	WOB	LOL	L	W	DABI	PAN	LL	AWN	LOMA	WOFGD	GL	GW
WOB	-0.316												
LOL	-0.237	0.93**											
FLL	0.44**	-0.21	-0.12										
FLW	-0.10	0.17	0.18	0.22									
DABI	0.02	0.37*	0.38*	-0.05	-0.13								
PAN	0.22	0.04	0.04	-0.04	-0.16	0.14							
SLL	0.46**	0.97**	0.92**	-0.31*	0.11	0.33*	-0.02						
AL	-0.05	0.05	0.08	0.03	-0.14	0.05	-0.32*	0.07					
LOMA	0.78**	-0.14	-0.02	0.54**	0.09	0.21	0.19	-0.30*	0.08				
WOFDG	-0.33*	0.98**	0.94**	-0.29*	0.114	0.37*	-0.03	0.97**	0.06	-0.16			
GL	-0.43*	0.98**	0.93**	-0.26	0.13	0.37**	-0.01	0.99**	0.09	-0.25	0.98**		
GW	-0.45**	0.97**	0.93**	-0.27*	0.13	0.34**	-0.04	0.99**	0.08	-0.28*	0.98**	0.99**	
PH	0.71	-0.28**	-0.23*	0.39	-0.05**	-0.09	0.14	-0.40	-0.22*	0.69**	-0.31*	0.38**	-0.38**

$P \leq 0.05^*$ ;  $P \leq 0.01^{**}$ ; WOB=Leaf width of blade; LOL=Leaf length of ligule; FLL= Flag leaf length; FLW=Flag leaf width; DABI=Culm diameter at basal internode; PAN=panicle number per plant; SLL=Sterile lemma length; AL= Awn length; LOMA= Panicle length of the main axis; WOFGD=100 grain weight of fully developed grain; GL= Grain length; GW=grain weight; PH= plant height.

et al. (2017). Tiller is one of the main attributing plant traits as indicated by Abbasi et al. (2015). Based on Table 2 statistical data analyzed, there was high significant difference of  $P < 0.011$ . The coefficient of variation and standard deviation recorded next 42.0% and 3.53 respectively. The accessions had a great variability with a high range (3-29) for number of productive tillers (Table 2). Better tillering capacity is a desirable feature to upgrade the yield potential of upland varieties. Ray et al. (2016) generally indicated that when rainfall is plentiful and the soil has good water-retention capacity, the high-tillering and short-statured varieties definitely respond better to nitrogen and yield higher than do the taller types. The accessions that produced more productive tillers will contribute to increased yield in a breeding program and could be selected as base genotypes for further improvement. In breeding applications, according to Chen et al. (2014), grain size is usually evaluated by the grain weight, which is positively correlated with several characters including grain length, grain width and grain thickness. It is a major determinant of grain weight; one of the three components (number of panicles per plant, number of grain per panicle and grain weight of grain yield). The grain length ranged from 0.96 to 2.08 cm and width 1.16 to 3.10 cm (Table 2), thus inferring rice accessions were largely long-grain. Although the preference for rice grain characteristics varies with consumer groups, long and slender grains are generally preferred and are good valuable attributes that could be exploited to improve the grain characteristics of local rice accessions (Cuevas et al., 2016).

Similar variability were reported by Javed et al. (2015) who studied 12 genotypes of coarse rice to check their yield performance in Kallar tract and reported highly significant variation for different traits. This variation in the

grain yield might be due to the environment and genetic constitution of accessions (Xie et al., 2015) or the correlation of grain yield per plant with various yield contributing characteristics such as; fertility of soil, flag leaf area, number of grains per panicle and grain weight which showed positive correlations. Similarly, Jones et al. (2015) reported positive correlation among number of panicles per plant, panicle length, number of grains per panicle, 100-grain weight and grain yield per plant-type. The grain shape character also showed the highest variation in studies conducted in Pakistan by Siddiqui et al. (2007). Core collection is important in germplasm characterization. Accessions selected for this study were 48 in total from Liberia and Ghana. Among the accessions studied, 18 out of the 48 accessions were distant from the rest, and were selected to constitute a core collection for further improvement. The concept of a core collection was introduced by Krueger et al. (2015) with the intent of using the core collection to minimize the cost of germplasm conservation while ensuring maximum genetic diversity.

According to Wijayawardhana et al. (2015), cluster analysis has the singular efficacy and ability to identify crop accessions with highest level of similarity. The dendrogram obtained from the present study also proved the above statement in terms of similarity existing among accession further. Baloch et al. (2016) also proved that agro-morphological traits can be used effectively to characterize the rice cultivars. Wangpan et al. (2018) have reported a similar variability of rice varieties. Results of the present study have shown similarities to the findings of Wangpan et al. (2018) in terms of dendrogram analysis, clustering groups and PCs analysis with some exceptions. These exceptions were identified in terms of variations in cluster formation and grouping behaviors of

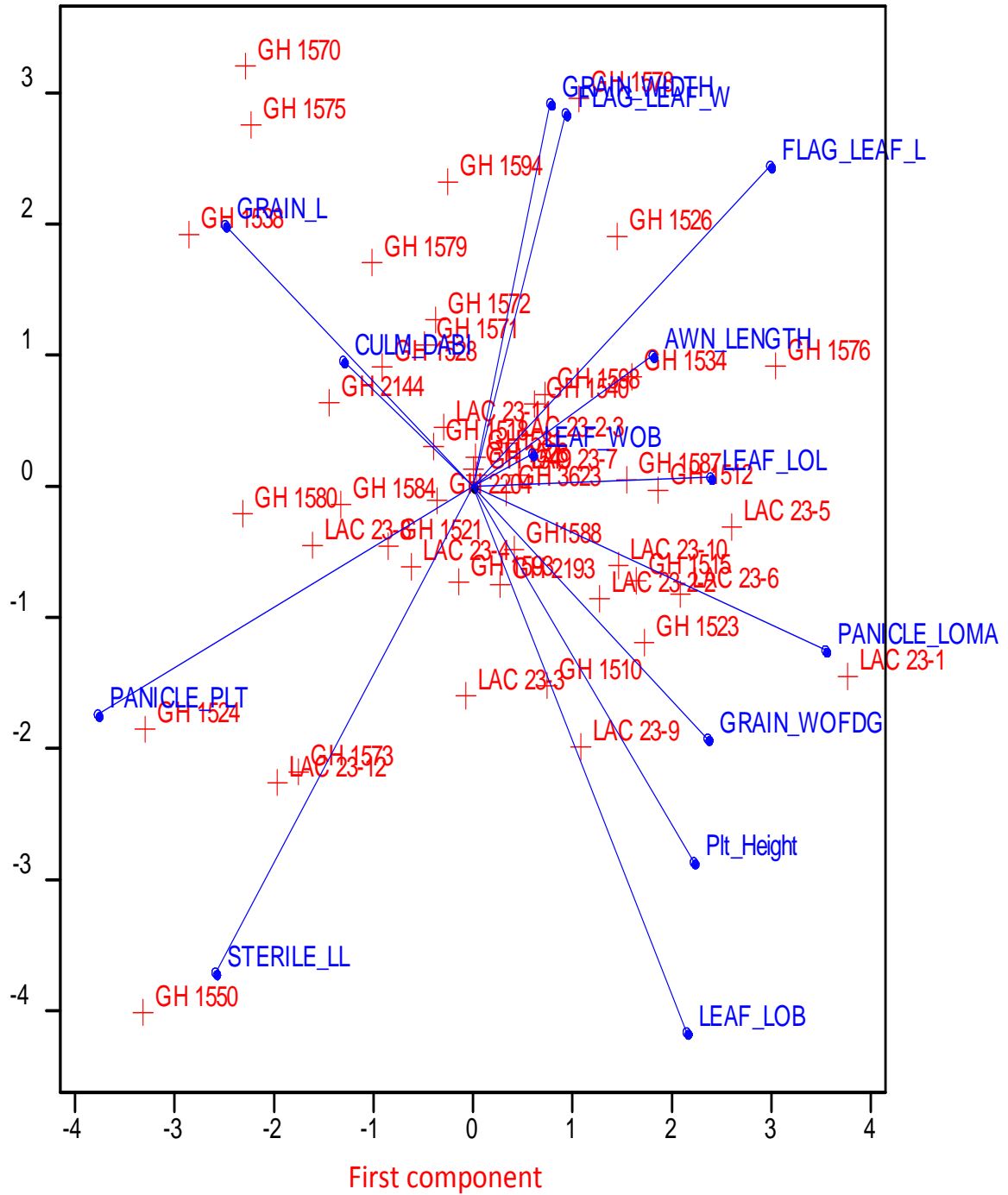
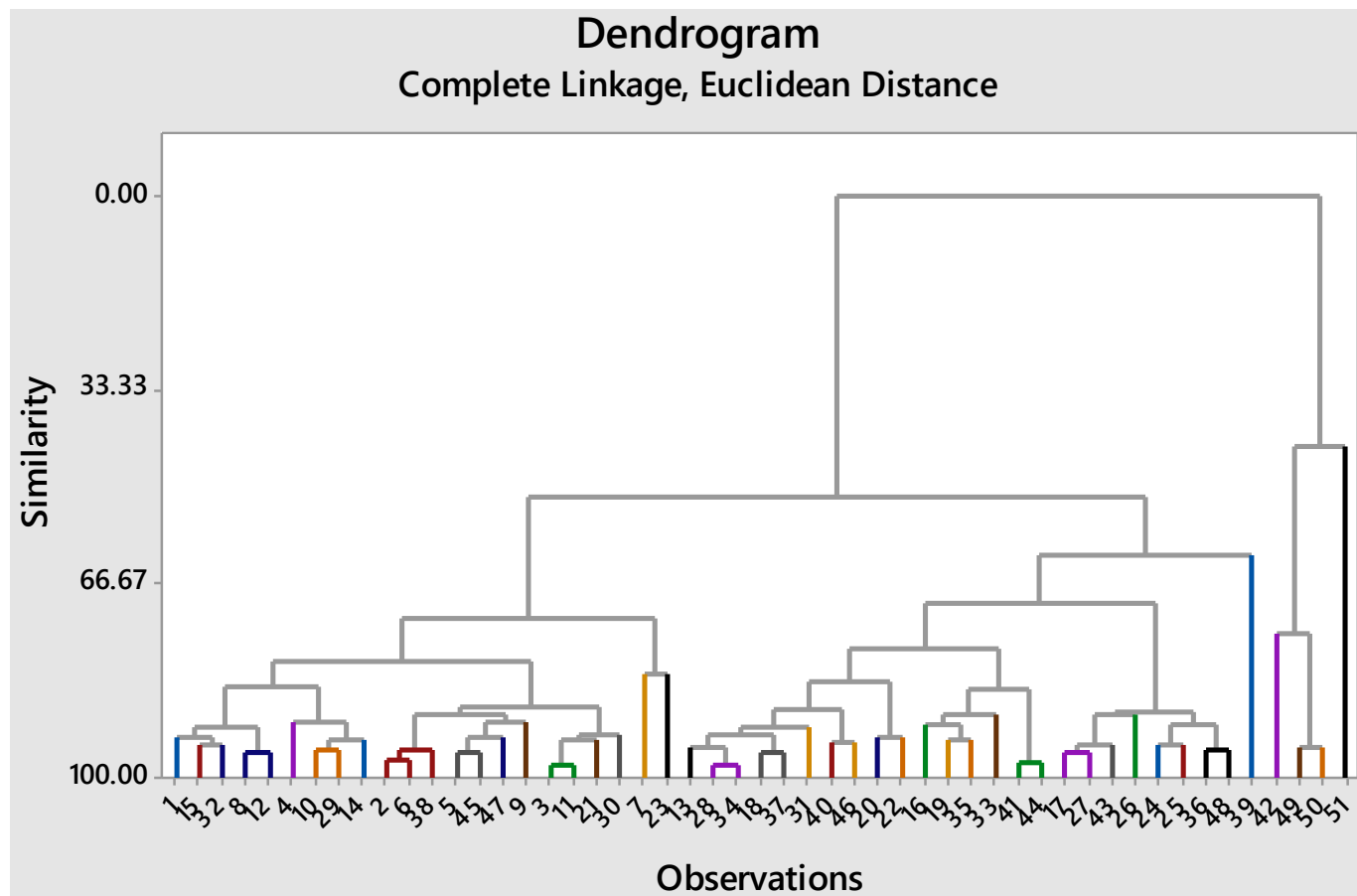


Figure 1. Grouping genotypes based on PCA biplot among the 14 quantitative traits.

their tested rice varieties. Further, these exceptions are possible due to the variations in external conditions such as soil types, soil fertility levels (Murikov et al., 2017) and soil moisture regimes (Okii et al., 2014) associated with two cropping systems. Furthermore, the genetic make-up of seed, environment and field management practices has been reported to influence the morphology of a crop

(Xiong et al., 2018). Therefore, the identification of agromorphological characters such as number of tillers, plant height, grain length, grain width and flag leaf that are important to change the rice crop architecture which have greater implication in this attempt. Hence, the data from the current study with other agromorphological data may be widely applicable in future rice crop improvement



**Figure 2.** Dendrogram generated based on the 14 traits of the forty-eight (48) rice genotypes using UPGMA.

programs.

## Conclusions

Results of the present experiment have clearly indicated the importance of agro-morphological traits to identify naturally existing distinguishable clusters. With the use of this information, plant breeders can effectively select morphologically more distinct individuals for their breeding programs. Morphological traits are used as a preliminary evaluation tool due to their easiness and can be employed as a common approach for assessing genetic variability among phenotypically distinguishable rice accessions. This study highly focused on the reproductive characters of the rice plant irrespective of the post-harvest characters. Most of the previous studies have reported the morphological variability in favor of both reproductive and post-harvest characters. Hence, the present study interprets a considerable amount of morphological diversity along with the reproductive traits of the rice plant. Therefore, rice reproductive traits can be used effectively in order to capture a considerable morphological variability associated with rice germplasm.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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