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Full Length Research Paper

Occurrence, distribution and severity of finger millet blast caused by *Magnaporthe oryzae* in Kenya

Margaret Odeph[1], Wycliffe Wanjala Luasi[2], Agnes Kavoo[3], Cecilia Mweu[1], Matthew Ngugi[3], Fredah Maina[4], Naomi Nzilani[3] and Wilton Mwema Mbinda[5]*

[1]Institute for Biotechnology Research, Jomo Kenyatta University of Agriculture and Technology, Nairobi, Kenya.
[2]Department of Biochemistry, Microbiology and Biotechnology, Kenyatta University, Nairobi, Kenya.
[3]Department of Horticulture, Jomo Kenyatta University of Agriculture and Technology, Nairobi, Kenya.
[4]Kenya Agricultural and Livestock Research Organization, Nairobi, Kenya.
[5]Department of Biochemistry and Biotechnology, Pwani University, Kilifi, Kenya.

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Finger millet is a food crop that provides nutritional security and is climatically resilient for farming and agricultural diversification. However, its quality and yield remain low due to biotic and abiotic factors, the greatest of which is blast disease caused by *Magnaporthe oryzae*. We surveyed the occurrence, distribution and severity of finger millet blast in five counties, namely, Busia, Bungoma, Kisii, Machakos and Makueni, in Kenya. Blast disease occurrence was determined by observing disease symptoms of different plant parts on each farm, and symptoms were recorded as either present or absent. Severity was evaluated based on the disease symptoms on plant fingers, leaves and necks and generally at the whole farm scale. Distribution was assessed based on the number of farms sampled for blast per county, and global positioning systems coordinates were recorded. Blast occurrence was 100%, with a uniform distribution pattern on all the farms surveyed across all the counties. Busia County had the highest disease severity at 82.3%, while Makueni had the lowest severity at 61%. Pearson's correlation test revealed no statistically significant correlation between blast severity and plant parts infected ($p < 0.05$), with Busia (74.2%) having the highest number of plants showing symptoms of blast on fingers, followed by Bungoma (57.1%), Makueni (57%), Machakos (56%) and Kisii, 53.3%. This study reveals that finger millet blast is rampant in all the counties surveyed and is widely distributed in Kenya. This information is helpful in understanding the geographical distribution, occurrence and severity of *M. oryzae*.

Key words: Finger millet blast, occurrence, severity, distribution, *Magnaporthe oryzae*.

INTRODUCTION

Crop transformation and diversification are an ideal mechanism for transforming agriculture from a stable system to a more useful system. At present, the area under which some cereal crops such as finger millet [*Eleusinecoracana* (L.) Gaertn.] are cultivated is rapidly declining; yet this crop plays a significant role in meeting both the dietary and economic needs of most subsistence farmers living in arid and semiarid areas in sub-Saharan

*Corresponding author.E-mail: wilton.mbinda@gmail.com.

Africa and Asia (Babu, 2011). In addition, this traditional food crop acts as an important plant genetic resource for agriculture that provides a resource for food-security deprived farmers living in such areas (Lata, 2015). Finger millet is an excellent source of minerals and nutrients such as methionine, iron and calcium (Patro et al., 2018) that are especially useful to expectant women, nursing mothers and children (Manyasa et al., 2019). Despite its importance as a staple crop, finger millet production is still low compared with that of other cereals. Globally, the production of finger millet accounts for a minimal 30.73 million tonnes of millet; 37% of which is produced by India. Among other small millets, finger millet production only accounts for 11%. In India, finger millet production accounts for an estimated 81% of millet production with an annual production turnover of approximately 2.78 tons (Babu, 2011; Sakamma et al., 2018). Among the main finger millet growing regions in India, Karnataka State is the leading producer of finger millet, but this production only accounts for 58% of the global production. Despite this scenario, only a few Indians are aware of its health benefits and nutritional value (Chandra et al., 2016). In East Africa, cultivation of this crop accounts for more than 50% of the area of small millet, which only reflects a 25% increase over 30 years within the region. This expansion, though minimal, is due to increased domestic demand, improved regional trade and increased market prices in comparison to those of other cereals (Manyasa et al., 2019). Studies in East Africa have shown that finger millet is grown on over 800,000 ha; 470,000 ha in Uganda, 350,000 ha in Tanzania, and 77,890 ha in Kenya (Mwema et al., 2013). In Kenya, this crop is mainly grown in low input subsistence systems on 0.5 to 1 ha farms, with western regions being the highest producers of this crop. Having been replaced by maize in the 20th century finger millet is perceived more like the “poor–man-crop” or ‘bird seed thus it is not a priority crop for most of the farmers (Handschuch and Wollni, 2016).

Finger millet crop production and productivity are impeded by both abiotic and biotic factors such as drought, high temperatures, nutrient stress, declines in land fertility, high labor requirements for weeding and low-yielding disease-susceptible varieties (Saha et al., 2016). Insects also damage this grain only slightly due to the minute size of the seed, but birds and striga weed pose significant threats (Mgonja et al., 2007). However, worldwide, blast disease caused by *M. oryzae* is the major biotic constraint that affects finger millet production and productivity, leading to low grain quality and poor yields. The disease was first reported in Asia more than 3,000 years ago, and it has now spread to several countries across the globe. This disease is highly adaptable to different environmental conditions, such as irrigated lowlands, rain-fed uplands or even deep water rice paddies (Srivastava et al., 2014). Substantial yield losses have frequently occurred due to this blast fungus since it infects different parts of the plants. Extensive damage to the aerial parts of plants, especially the panicle, leading to withered or blasted finger, neck and head portions of the crop have been observed. This greatly lowers the positive agronomic traits exhibited by the crop eventually causing losses of between 28-36% whereas in endemic areas this could rise up-to 80-90% (Ramakrishnan et al., 2016)

This situation is worsened by the fact that *M. oryzae* is able to produce mycelia on infected living leaves or survive in dead plant debris in the soil for a long time. In addition, preexisting environmental conditions, such as high mean temperatures with relatively high humidity and damp leaves exacerbate this situation. Additionally, the presence of infected related weed hosts such as *Eleusine indica*, *E. africana*, *Digitaria* spp., *Setaria* spp. and *Doctylocterium* spp. may act as disease reservoirs for the next crop thus intensifying disease spread (Gashaw et al., 2014). On top of this, high amount of nitrogen content either in the plant or soil as a result of nitrogenous fertilizer application increases blast incidence thus predisposes the crop to blast infection. Disease incidence and severity are more intense during heavy rainy seasons and highly humid months than during the hot dry seasons (Onyango, 2016). Blast disease management involves practices such as plant quarantine, pathogen inoculum elimination and implementation of good agricultural practices that include intercropping, rotating crops, implementing good farm hygiene, understanding and fighting virulence mechanisms of pathogens, using biological control methods and using biotechnological techniques to improve plant performance. Chemical control methods are also employed but may be too expensive for poor farmers (Shittu, 2018).

Since the identification of blast disease in 1933 in Kenya, information on finger millet blast pathogen diversity and characteristics in East Africa has remained insufficient (Takan et al., 2012). Currently in Kenya, most of the studies have not provided a quantitative measurement of finger millet blast occurrence, distribution and severity, although blast disease has been established as the greatest constraint on finger millet production. These disease assessment techniques would be critical in determining the geographical distribution and disease status throughout the country to prioritize research. This study was therefore carried out with the aim of determining the occurrence, distribution and severity of finger millet blast caused by *M. oryzae* in five counties in Kenya. This knowledge will be a good resource for the determination of effective and sustainable disease management strategies, blast epidemiology and varietal selection procedures against blast for different geographic regions in Kenya.

MATERIALS AND METHODS

Study area and survey methodology

A survey of finger millet blast disease was conducted in the five

counties of Busia, Bungoma, Kisii, Machakos and Makueni in Kenya in May 2018 and February and June 2019. These counties represent some of the main finger millet growing areas. The location of each farm was recorded using a GPS device.

Sampling and survey methodology

Purposeful sampling was adopted, and both symptomatic and asymptomatic plants were collected. Finger millet crops were physically examined for the presence of diamond-shaped lesions on the leaves and neck, while a rusty brown appearance was observed on the finger. The finger millet samples collected included leaf, finger and neck, which were then stored in brown sugar bags to avoid contamination. Three plants were collected per farm: 1 asymptomatic plant and 2 severely infected plants at random. The number of farms sampled per county depended on the availability of finger millet.

Disease intensity was evaluated on the basis of occurrence, distribution and severity. Disease occurrence was based on the presence or absence of disease on the farms surveyed. Disease severity was based on the symptoms of blast on the plant parts (finger, leaf and neck) as well as disease symptoms spread on the whole farm. Disease severity data were recorded and scored as less severe (0-25%) = 1, moderately severe (30-60%) = 2 and very severe (70% and above) = 3. The identity of the *M. oryzae*was determined using PCR. GPS data were used to determine farm locations and disease density per county.

Data analysis

Survey data were analyzed using the Pearson product-moment correlation coefficient with SPSS version 25 software (IBM Corp, NY, USA) to assess the extent of the statistical relationship between the linearly related variables using the following equation:

$$r = \frac{N\sum xy - (\sum x)(\sum y)}{\sqrt{[N\sum x^2 - ([N\sum x)^2][(N\sum y^2 -)(N\sum y)^2]}}$$

where r = Pearson r correlation coefficient; N= number of observations; ∑xy= sum of the products of the paired scores; ∑x =sum of the x scores; ∑y= sum of the y scores; $\sum x^2$= sum of the x squared scores; $\sum y^2$= sum of the y squared scores. xand y represent the county-level severity on the whole farm and severity per plant part, respectively.

Maps were designed to show the extent of disease spread within the different counties surveyed to portray disease distribution within the counties.

RESULTS

Farming practices

Finger millet farming practices across the five counties were somewhat similar. The crop was grown for subsistence where low-input cropping systems were utilized. The farms were mostly managed by female farmers, with the highest number of farmers in Kisii(77%), Busia (65%), Bungoma (61.5%) and Machakos, and Makuenihad the least number of farmers at 50% (Table 1).

Soil fertility was enhanced on most of the farms surveyed by farm yard manure with Busia and Machakos having the highest fertility at 90%, Bungoma and Makueni at 85% and Kisii with the lowest fertility at 80%. A few farmers, however, used compost manure and inorganic fertilizers as well (Table 1). According to farmer interviews, organic fertilizers were applied prior to crop planting to avoid scorching. Kisii County had the highest percentage (92%) of farmers using seeds carried forward from the previous harvest, followed by that of Bungoma (Table 1). Machakos County had zero percentage carryover of seeds because the farms were undergoing a pilot research program from International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), so the seeds were supplied by the organization. The fields surveyed varied in size, but the majority of farms with finger millet crops were less than an acre (0.404 ha), as was observed during sampling.

Intercropping finger millet with other crops was a key cultural practice among the farmers in all the counties visited. The predominant crops used in intercropping were cereals such as maize, beans and cowpeas; fruits such as mangoes, oranges, guavas and pawpaw; and tubers such as cassava and sweet potatoes. Makueni County was the farm with the most intercropping at90% of the farms being intercropped with cereals, and Busia and Bungoma had the next highest about of farms with intercropping at 85%. Machakos had the least number of farms with intercropping at 60%. Farms intercropped with fruits were mainly in Makueni with 85% implementing intercropping and Machakos with 80% implementing intercropping and no intercropping in Kisii, Bungoma and Busia (Table 1). Farmers across the counties agreed that the reason for growing finger millet was mainly for food with 90% of the farmers in Kisii and Bungoma counties in agreement and 85% of the farmers in Machakos, Busia and Makueni in agreement. In addition, some farms in Busia, Bungoma and Kisii grew this crop for cultural reasons at 30, 10 and 10% of the farms, respectively (Table 1). With regard to soil fertility practices, farm yard manure application was the key method used by the farmers. Busia and Machakos had 90% of the farms using this method, followed by those in Makueni and Bungoma at 85% and Kisii at 80%. Inorganic fertilizer application was not a common practice across the counties with 10% of farms using inorganic fertilizer in Makueni and Kisii and 5% inMachakos, Bungoma and Kisii (Table 1).

Finger millet waste was fed to livestock, burnt, left on the farm to dry or placed in cowsheds. In Busia, 80% of the farmers fed waste to the animals; in Bungoma, Kisii and Machakos 75% of the farmers implemented this practice; and in Makueni, 70% of the farmers in Makueni implemented this practice. The amount of water burned in decreasing order per county was as follows: Kisii 18%, Bungoma and Makueni 10%, Busia and Machakos 5%. Most farmers across the counties also laid the waste in the livestock shed as follows: Busia 10%, Bungoma 10%,

Table 1. Finger millet farming patterns in Busia, Bungoma, Kisii, Machakos and Makueni counties in Kenya.

Parameter (in %)	Busia	Bungoma	Kisii	Machakos	Makueni
Female farmers	65	61.5	77	50	50
Male farmers	35	38.5	33	50	50
Farming as main source of income	80	77	85	100	70
Formal employment as source of income	20	33	15	0	30
Seeds obtained from owner	80	85	92	0	80
Seeds obtained from neighbors	0	0	0	0	2
Seeds bought from market	0	0	0	0	10
Seeds bought from agricultural organizations	33	15	8	100	8
Source of food	85	90	90	85	85
Farms with very severe blast symptom	84.6	71.4	58.3	80	30
Farms with moderately severe blast symptom	15.4	14.3	41.7	20	70
Farms with less severe blast symptom	0	14.3	0	0	0
stable crop for climate variability	0	0	0	15	10
storage friendly	0	0	0	0	3
cultural reasons	30	10	10	0	0
commercial use	0	0	0	0	2
intercropped with other cereals	85	85	80	60	90
intercropped with Fruits	0	0	0	80	85
intercropped with tubers	85	85	85	60	65
mixed crop farming only	10	0	0	0	10
mixed farming	100	100	100	100	90
fertilizer application	5	5	10	5	10
compost manure application	10	10	10	5	5
Farm yard manure application	90	85	80	90	85
wastes fed to livestock	80	75	75	70	75
wastes left on farm	5	5	5	10	10
wastes laid in livestock shed	10	10	2	10	5
wastes burnt	5	10	18	5	10

Machakos 10%, and Makueni 10%, while Kisii Countyhad the least number of farmers using this method. Most farmers across the counties left the waste on the farms to dry as a last resort. The percentage values of the farms using this method were as follows: Busia 5%, Bungoma 5%, Kisii 5% and Makueni 10%, and Machakos 10% (Table 1).

Blast disease occurrence and distribution

Finger millet blast disease occurred on all the farms visited in Busia, Machakos, Makueni, Bungoma and Kisii, representing a 100% disease occurrence on all the farms visited (Table 2). Finger millet blast disease was sampled from two main agroe-cological zones (western and eastern) represented by Bungoma, Busia, Kisii, Machakos and Makueni counties as shown in the spatial analytical map (Figure 1). According to the results of the surveyed areas, finger millet blast disease was distributed across all the counties surveyed. However, the density of disease distribution varied across the counties. In Busia and Bungoma counties, the farms were closely distributed; consequently, the disease distribution followed the same pattern. In Machakos and Makueni, the farms were far apart, and as a result, the disease also tended to follow a spatial distribution pattern. Although in Kisii County, the farms were fairly far apart, blast disease was present at a high density.

The distribution of diseased plants followed a uniform pattern on the farms visited across all the counties surveyed. However, in some farms that were intercropped with large fruit trees, more blast symptoms were observed than those on other farms. In addition, farms that were on the windward side had more blast symptoms than those that were on the leeward side. Regarding the plant parts infected, the fingers were more infected than the leaves and neck. The number of farms

Table 2. Finger millet blast disease occurrence in sampled Kenyan counties in 2018 and 2019.

Agro-ecological zone	County	Number of farms surveyed	Blast occurrence	Percentage blast disease occurrence
Western	Busia	>200	+	100
	Bungoma	13	+	100
	Kisii	13	+	100
Eastern	Machakos	5	+	100
	Makueni	10	+	100

(+) sign indicates presence of blast disease.

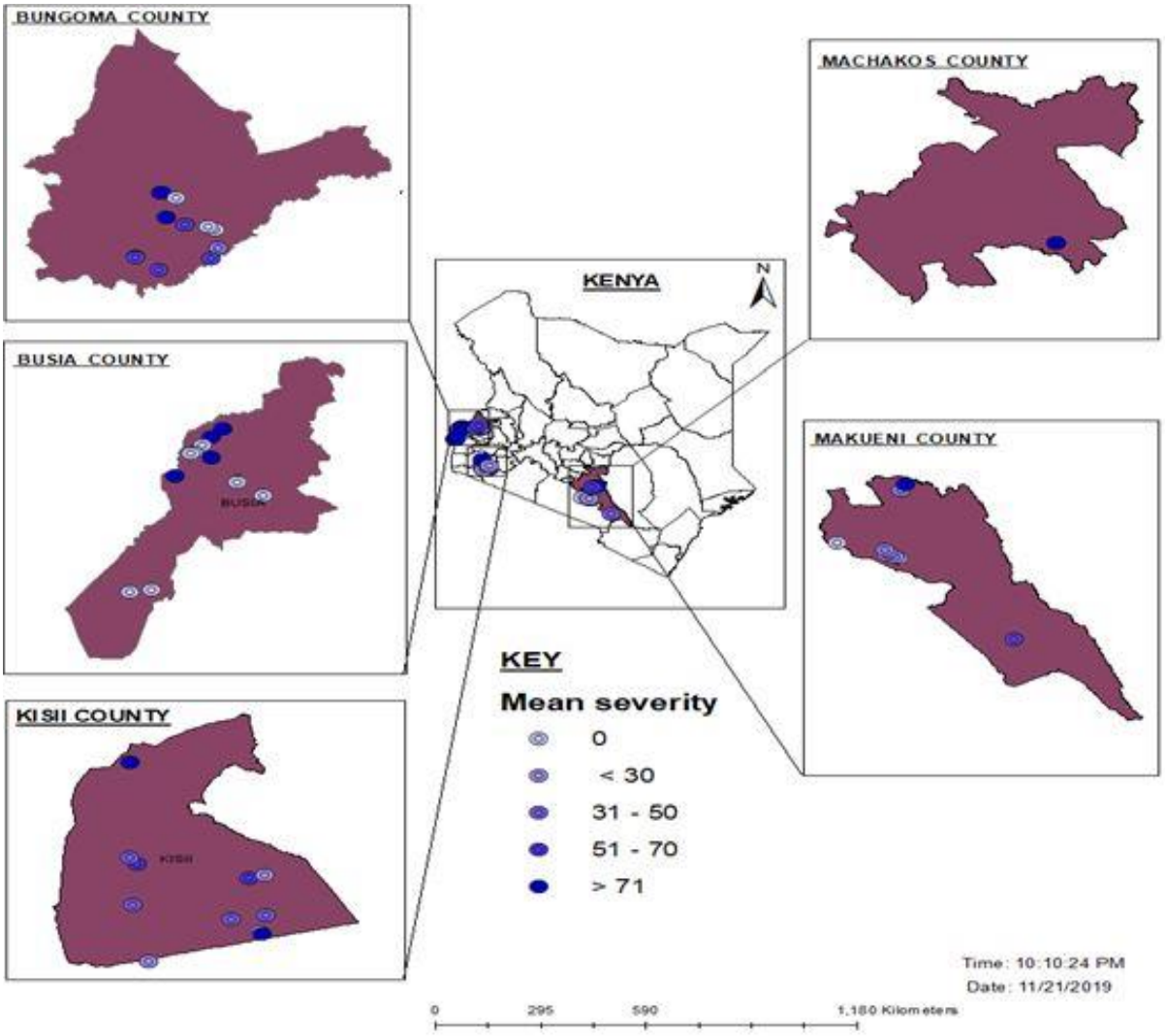

Figure 1. Finger millet blast disease sampling sites in Kenya. The surveyed areas represent the major finger millet growing areas. The blue spots with different shades represent sampled areas with varying blast disease severity.

surveyed varied among the counties, with Busia having the highest number of farms sampled (>200), followed by Bungoma (13) and Kisii (13), Makueni (10) and Machakos (5) (Table 2).

Blast severity

Busia county recorded the highest finger millet blast severity (82.3%), based on the symptoms of the whole

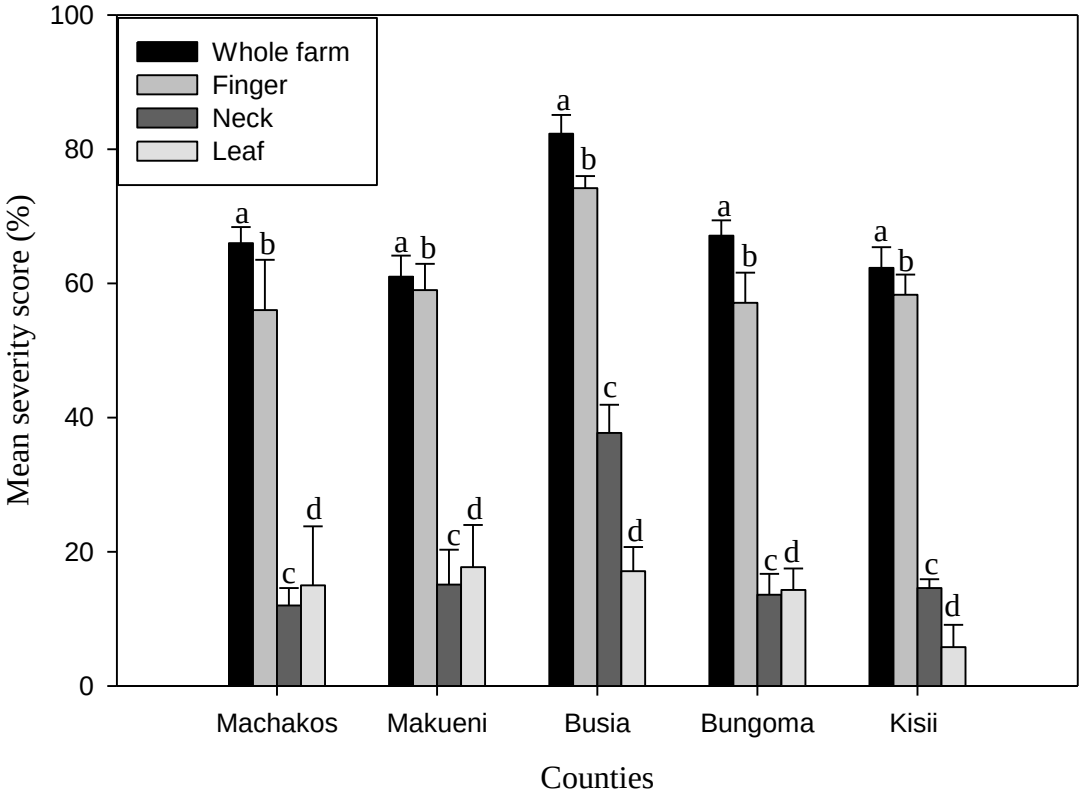

Figure 2. Percentage finger millet blast severity across the five counties for the whole farm and individual plant parts affected. Means ± standard error followed by different alphabets in each column are significantly different (P≤0.05) using Fishers LSD.

Table 3. Pearson correlation table for finger millet blast severity.

County	Whole farm mean severity	Finger severity	Neck severity	Leaf severity
Machakos	66^{a} ±2.4	56^{ab} ±7.5	12^{b}±2.6	15±8.8
Makueni	61^{a}±3.14	59^{ab} ±3.9	15.1^{b}±5.2	17.7±6.3
Busia	82.3^{a} ±2.8	74.2^{ab} ±1.8	37.7^{b}±4.2	17.1±3.6
Bungoma	67.1^{a} ± 2.3	57.1^{ab}±4.5	13.6^{b}±3.1	14.3±3.2
Kisii	62.3^{a} ±3.1	58.3^{ab} ±3	14.6^{b}±1.3	5.8±3.3

[a]Means are significantly correlated at p<0.05; [b] means are significantly correlated at p<0.01.

farm, followed by Bungoma and Machakos (67 and 66%, respectively), while Makueni had the lowest severity (61%) (Supplementary Material 1 and Figure 2). In terms of infected plant parts, Busia County also had the highest severity of infected fingers at 74.2%, neck at 37.7% and leaves at 17.1% (Supplementary material 1 and Figure 2). Pearson's correlation test revealed a statistically significant correlation of whole farm finger millet blast disease symptoms between the counties surveyed (Table3). A significant association (p< 0.01) existed between blast severity on a whole farm and severity on plant parts infected in the different counties surveyed. The whole farm blast severity symptoms were significantly higher in Busia than in Makueni. The blast disease symptoms were most severe in Busia but decreased across the counties with 67% in Bungoma, 66% in Machakos, 62.5% in Kisii and 61% in Makueni (Supplementary Material 1).

Pearson's correlation test also showed that blast severity was significantly associated with plant parts infected (p< 0.05) (Supplementary material 1). The blast disease symptoms on fingers decreased in severity

across the counties, with Busia (74.2%) having the highest number of plants showing symptoms of blast on fingers, followed by Bungoma (57.1%) and Makueni (57%), Machakos (56%) and Kisii 53.3%. Within Busia County, in comparison to other counties, Teso South sub-county had more farms with plants showing blast symptoms on fingers at 80%, Bunyala at 77.5% and Nambale at 66.7% (Table 2). In Bungoma, Kanduyi sub-county had the highest number of plants with blast symptoms on fingers (72%), Kabuchai (70%) and Webuye west (60%). In Kisii County, Nyaribarichache subcounty had the highest number of plants with blast symptoms on fingers at 66.7%, Bomachogechache at 62% and Bobasi at 60%. In Machakos County, Mwalasub-county had 66% of its plants with blast symptoms on the finger. In Makueni County, the Kibwezi East and Mbooni East sub-counties had the highest percentages, with 70% of plants showing symptoms of blast on fingers, Kibwezi West and Nzaui with 60%, Makueni with 56.7% and Kilome with 50% (Supplementary material 1).

Generally, there was a positive correlation among blast symptom severity on the whole farm and blast symptoms on the fingers of the crop on all the farms surveyed. On farms with higher blast disease symptoms on the whole farm, the fingers of the crop also showed a higher level of infection (0°08’50.2’’N 34001’56.4’’E Bunyala sub-county 90% severity on the whole farm and 80% severity on the fingers) compared with those on farms with lower percentages (0°54'09.4”S 34°53'08.2”EBobasi 40% severity on the whole farm and 40% severity on the fingers) (Supplementary material 1). The Pearson correlation tests further revealed a positive correlation ($p<0.05$) between finger blast severity and neck blast severity (Table 3). Plants with higher severity on the finger than on the other parts also had higher severity on the neck (Supplementary material 1). However, there was no correlation between severity on finger and severity on leaf.

There was a significant difference ($p<0.01$) in neck blast severity across the different counties surveyed. Similarly, there was a significant statistical correlation of neck blast severity with the different counties surveyed (Table3). Neck blast symptom severity decreased across the counties. Busia had the highest severity at 37.7%, Makueni at 15%, Kisiiat14.6%, and Bungoma at13.6% and Machakos at 12% (Supplementary material 1). However, there was variation in the severity within the different sub-counties. Teso south had the highest severity at 40%, Bunyala at 37.7% and Nambale at 31.7% in Busia county. In Bungoma, Kabuchai sub-county had the highest at 18.3%, Kanduyi at 16% and Webuye West at 11%. In Kisii, the Nyaribarichache subcounty had the highest neck severity, at 15%, Bomachogechache at 11% and Bobasi at 9%. Mwala sub-county in Machakos had a severity of 12%. In Makueni, Mbooni east had the highest neck blast symptom severity at 50%, with Nzauiat 21%, Kibwezi east at 15%, Kibwezi west and Kilome at 10% each and Makueni at 4.7% (Table 2). No significant correlation was found between neck blast symptom severity and leaf blast symptom severity.

DISCUSSION

The occurrence of finger millet on all the farms surveyed across different counties suggests that blast disease is highly prevalent in the main finger millet growing areas in Kenya. This scenario is consistent with the results of earlier studies by Oduori (2008) and Gashaw et al. (2014), who revealed that the disease was highly prevalent in Busia, Teso and Kisii districts, causing 10-80% yield losses in both Kenya and Uganda. Similarly, Owere (2013) showed that finger millet blast in Uganda is endemic to all finger millet growing areas with the disease severity being higher in some areas than in others depending on weather conditions. Zhang et al. (2016) have equally shown that blast disease is highly prevalent in areas where host plants are cultivated worldwide. Over 85 countries in both developing and developed nations have reported the incidence of blast especially on finger millet and rice: the key hosts for *M. oryzae*. Although the disease is widespread in all finger millet growing areas, farmers still have limited knowledge about it, thus posing a great challenge to management of the disease.

We observed that finger millet blast distribution varied from farm to farm and county to county. Based on these results, finger millet blast distribution was clustered into two main categories, densely and sparsely distributed. In the western agroe-cological zone (Busia, Bungoma and Kisii counties), the disease distribution was denser than that in the eastern zone (Machakos and Makueni counties), which exhibited a relatively sparse distribution. This observation could be attributed to the different farm management practices and agroclimatic conditions prevalent on the farms and/or counties. Onyango (2016) revealed that appropriate ecological requirements including adequate rainfall that is well distributed all through the year play a significant role in profiling blast disease distribution. Machakos and Makueni counties are semiarid regions and thus receive lower annual rainfall amounts (830 and 834 mm, respectively) and have lower humidity levels than those in Busia (1691 mm), Bungoma (1628 mm) and Kisii (1922 mm). High rainfall conditions with higher humidity levels are favorable conditions for blast fungi survival. Another possibility for the increased blast disease distribution within the western agro-ecological zone could be related to the origin of the finger millet pathogen. *M. oryzae* populations that colonize finger millet and rice in sub-Saharan Africa have shown unique patterns of genetic diversity, mating-type distribution, fertility status and host compatibility. This pattern emulates the different histories and patterns of

cultivation of these crops within East Africa (Takan et al., 2012). Shittu (2018) also confirmed that the frequency of occurrence of *M. oryzae* isolates possessing the transposable DNA elements such as *grh* element varied significantly due to difference in lineage. The study further revealed that the presence of these elements among the rice blasts isolates corresponded to gene flow between rice and other *M*. *oryzae* hosts. This has provided good knowledge in the understanding of the population dynamic patterns of *M. oryzae* from different hosts. This is supported by earlier studies on the genetics of finger millet blast fungus which revealed a repetitive DNA element grasshopper (*grh)* only in populations of *M. oryzae* from finger millet in Japan, Nepal and India as well as some in West African countries (Viji et al., 2000; Tanaka et al., 2007). However, PCR analysis showed that some indigenous blast populations did not have this element and were unique from the Asian blast populations. The *M. oryzae* isolates that had the *grh* element were recent introductions, and most of the pathogen population in East Africa lacked the *grh* repeat element (Takan et al., 2012; Shittu, 2018). There is thus a high likelihood that the blast pathogen could have been an introduction through the western route possibly through cross-border transfer of finger millet seeds given that finger millet originated from Uganda and that Busia is a border point. This scenario could explain why Busia tends to have a higher disease distribution compared to that of the remaining regions. The presence of certain haplotypes in various locations within Kenya and Uganda further confirms that pathogen movement is highly likely linked to environmental human impacts (Lenne et al., 2007). Furthermore, the disease spread within the western region of Kenya could have occurred through the purchasing of seeds from the market and borrowing from neighbors.

Our results on blast disease severity also revealed that there were variations from farm to farm and county to county due to the different farm management practices and geographical and environmental conditions prevalent in each county. As with the blast disease distribution, severity also followed a similar trend, and the western agro-ecological zones had more farms with severe infections compared with those in the eastern zone. The surveyed farms in Busia County are between altitudes of approximately 1000-1300 m above sea level, with a mean temperature of 22°C. This county is a humid lower midland zone with well-drained soils that are suitable for agriculture. *M. oryzae* infestation tends to be more severe in this area because of high humidity conditions. Furthermore, because of the proximity of Busia to Uganda, there is the possibility of cross-border infection (Onziga, 2015). Bungoma (1200-1400 m above sea level) and Kisii (1500-1800m above sea level) have similar agroclimatic conditions to those of Busia County. Bungoma and Busia counties are within close proximity, so the chances of farmers sharing diseased seeds are higher. Kisii County may be further away, but the climatic conditions of Kisii, Bungoma and Busia are similar. Besides Kisii county experiences cloudy skies with frequent rains and drizzles: conditions that allow dew accumulation on leaves for a longer period. Such conditions increase blast incidence thus predisposes the plant to blast infection. All three counties experience high humidity, which is a favorable condition for blast fungus to thrive. Moreover, the tropical climate favors the accumulation of blast spores in the air throughout the year which favors continuous disease development (Onyango, 2016).

Machakos (1000-1600 m above sea level) and Makueni (1200-1600 m above sea level) counties are both semiarid regions experiencing low rainfall with low humidity. The extent of disease severity within the farms in these two counties was not substantially different from that in Kisii and Bungoma. In addition to climatic conditions, farm management practices also contributed to disease spread. Most finger millet farms in these two counties had large fruit trees surrounding the farms or near the finger millet farms. Such trees reduce the much needed light intensity and instead increase humidity within the farms, creating favorable microclimatic conditions for the blast pathogen. Whereas the essence of intercropping is to maximize supplementary resource utilization including canopy and root architecture, nutrient fixation and resource sharing on the other hand these can be at the detriment of the plant (Daryanto et al., 2020) as observed by the presence of the large fruit trees present in the finger millet farms. According to Gashaw et al. (2014), presence of microclimatic conditions such as increased humidity due to large tree canopies in the farms predisposes the finger millet plants to blast infection. In addition, most of the farms were on the windward side so that when the wind blows, it facilitates the spread of the pathogen spores thus exposing more plants to blast infection.

Good farm management practice is a gateway to a good harvest and reduced disease infection rates (Meena et al., 2017). Some farmers, especially in Kisii, Makueni and Bungoma, employed appropriate management practices, such as proper spacing, crops rotation, enhancing soil fertility a few months before planting with organic fertilizer and timely weeding. On such farms, there was notably lower disease severity at the whole farm level. There is thus a need to train farmers on finger millet farm proper management techniques to minimize the severity of disease.

Conclusion

Finger millet blast caused by *M. oryzae* occurred throughout the counties in this study; thus, this pathogen is widely distributed in Kenya at high severity levels. However, both the distribution pattern and severity differed across the counties surveyed. The majority of

farmers were not aware that blast infection is a disease. Thus, there is an urgent need to increase farmers' awareness about blast disease. In addition, the development of an integrated disease management strategy that includes resistant varieties and good agronomic practices is needed to safeguard the livelihood of subsistence farmers in Kenya. We are currently performing genetic diversity of the fungi and the finger millet grown in the study areas to relate our results.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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SUPPLEMENTARY MATERIAL

Finger millet blast symptom severity and plant part infected in sampled farms in Kenyan sub counties in 2018-2019.

County	latitude	longitude	Sub county	Severity score Scale 1-3	% blast severity in the whole farm	Main part of plant infected	% blast severity per plant part			Level of severity
							finger	Neck	leaf	
	0°27'26.8''N	34°07'07.6''E	Teso south	3	90	f, n, l	80	60	30	very severe
	0°33'41.0''N	34°11'12.3''E	Teso south	3	90	f, n, l	80	60	20	very severe
	0°34'59.9''N	34°12'32.3''E	Teso South	2	80	f & n	70	50	10	moderately severe
	0°34'60.0''N	34°12'26.1''E	Teso south	3	90	f, n, l	80	50	25	very sever
	0°32'26.3''N	34°10'11.0''E	Teso south	2	90	f & l	80	10	40	very severe
	0°31'17.9''N	34°09'13.4''E	Teso south	2	80	f & n	80	35	10	very severe
Busia	0°31'03.9''N	34°08'57.1''E	Teso south	2	80	f& n	70	15	40	very severe
	0°26'40.7''N	34°14'23.1''E	Nambale	2	60	f, n	70	30	10	moderately severe
	0°26'26.7''N	34°14'07.7''E	Nambale	2	70	f, n	60	35	5	very severe
	0°24'20.6''N	34°17'11.6''E	Nambale	2	70	f, n	70	30	5	very severe
	0°30'19.2''N	34°11'11.9''E	Teso south	3	90	f, n, l	70	40	20	very severe
	0°08'50.2''N	34°01'56.4''E	Bunyala	2	90	f & n	80	40	5	very severe
	0°09'10.1''N	34°04'30.4''E	Bunyala	2	90	f& n	75	35	5	very severe
Mean					**82.31±2.8**		**74.2±1.8**	**37.7±4.2**	**17.1±3.6**	
	0°31'49.6"N	34°34'44.9"E	Kanduyi	2	70	f & n	70	35	5	very severe
	0°31'40.1"N	34°34'44.8"E	Kanduyi	2	70	f& n	70	25	5	very severe
	0°37'02.3"N	34°40'29.3"E	Kanduyi	1	80	f	60	10	5	very severe
	0°37'07.0"N	34°40'29.3"E	Kanduyi	2	70	f & l	70	5	30	very severe
	0°29'39.5"N	34°37'29.3"E	Kanduyi	2	70	f & l	60	5	30	very severe
Bungoma	0°33'16.6"N	34°44'20.8"E	Webuye west	1	60	f	50	5	5	moderately severe
	0°31'29.6"N	34°43'29.6"E	Webuye west	1	70	f	40	5	5	very severe
	0°31'29.6"N	34°43'28.6"E	Webuye west	3	70	f,n&l	70	30	10	very severe
	0°36'10.4"N	34°43'58.4"E	Webuye west	1	50	f	40	5	5	less severe
	0°36'40.7"N	34°43'09.5"E	Webuye west	1	50	l	20	5	30	less severe
	0°42'10.4"N	34°37'41.9"E	Kabuchai	2	70	f&l	70	5	30	very severe
	0°42'14.8"N	34°37'45.8"E	Kabuchai	2	**80**	f& n	**70**	**20**	**5**	very severe
	0°41'28.7"N	34°39'25.9"E	Kabuchai	1	**50**	l	**40**	**5**	**30**	moderately severe
	0°38'09.8"N	34°38'17.9"E	Kabuchai	3	**80**	f & n	**70**	**30**	**5**	very severe
Mean					**67.1±2.3**		**57.1±4.5**	**13.6±3.1**	**14.3±3.2**	

Supplementary material contd.

Kisii	0°54'09.4"S	34°53'08.2"E	Bobasi	1	**40**	f	**40**	**5**	**2**	moderately severe
	0°54'27.0"S	34°53'18.6"E	Bobasi	3	**80**	f,n&l	**70**	**40**	**20**	very severe
	0°52'26.0"S	34°53'33.7"E	Bobasi	1	**60**	**f**	**50**	**10**	**5**	moderately severe
	0°52'51.2"S	34°50'51.0"E	Bobasi	2	**60**	**f & n**	**50**	**20**	**2**	moderately severe
	0°46'37.6"S	34°43'27.5"E	Bomachogechache	2	**70**	**f & n**	**70**	**20**	**5**	very severe
	0°46'00.5"S	34°42'50.8"E	Bomachogechache	1	**60**	**f**	**60**	**5**	**5**	moderately severe
	0°51'14.8"S	34°43'09.1"E	Bomachogechache	1	**60**	**f**	**50**	**5**	**5**	moderately severe
	0°57'29.2"S	34°44'22.2"E	Bomachogechache	2	**70**	**f & n**	**60**	**20**	**5**	very severe
	0°57'28.8"S	34°44'20.8"E	Bomachogechache	1	**50**	**f**	**50**	**5**	**5**	very severe
	0°35'19.7"S	34°42'50.4"E	Nyaribarichache	1	**80**	**f**	**60**	**5**	**5**	very severe
	0°48'19.4"S	34°52'12.0"E	Nyaribarichache	2	**70**	**f &n**	**70**	**30**	**5**	very severe
	0°47'57.8"S	34°53'27.2"E	Nyaribarichache	1	**50**	**f**	**70**	**10**	**5**	very severe
Mean					**62.3±3.1**		**58.3±3**	**14.6±1.3**	**5.8±3.3**	
Machakos	$1^0$30'40.7''S	$37^0$36'18.2''E	Mwala	1	**60**	**f**	**50**	**10**	**5**	moderately severe
	$1^0$30'40.7''S	$37^0$36'18.2''E	Mwala	1	**60**	f	**60**	**10**	**5**	moderately severe
	$1^0$30'40.7''S	$37^0$36'18.2''E	Mwala	1	**70**	l	**30**	**5**	**50**	very severe
	$1^0$30'40.7''S	$37^0$36'18.2''E	Mwala	1	**70**	f	**70**	**15**	**5**	very severe
	$1^0$30'40.7''S	$37^0$36'18.2''E	Mwala	1	**70**	f	**70**	**20**	**10**	very severe
Mean					**66±2.4**		**56±7.5**	**12±2.6**	**15±8.8**	
Makueni	$1^0$55'09.5"S	$37^0$13'10.5''E	Kilome	1	**50**	f	**50**	**10**	**5**	moderately severe
	$2^0$00'48.0"S	$37^0$28'51.3"E	Nzaui	3	**70**	f,n&l	**60**	**40**	**20**	very severe
	$2^0$00'48.0"S	$37^0$28'51.3"E	Nzaui	1	**50**	f	**50**	**2**	**30**	moderately severe
	$2^0$00'19.4"S	$37^0$28'05.8"E	Kibwezi west	1	**60**	f	**50**	**10**	**10**	moderately severe
	$1^0$59'09.6"S	$37^0$25'51.3"E	Makueni	1	**50**	f	**60**	**4**	**11**	moderately severe
	$1^0$58'35.0"S	$37^0$26'05.7"E	Makueni	2	**60**	f& l	**50**	**5**	**5**	moderately severe
	$1^0$57'50.9"S	$37^0$25'28.7"E	Makueni	2	**60**	f &l	**60**	**5**	**10**	moderately severe
	$2^0$29'56.9"S	$37^0$58'38.8"E	Kibwezi East	3	**70**	f, n&l	**65**	**15**	**11**	very severe
	$1^0$36'26.5"S	$37^0$29'27.2"E	Mbooni East	2	**60**	f &l	**55**	**10**	**5**	moderately severe
	$1^0$33'53.1"S	$37^0$30'35.0"E	Mbooni East	3	**80**	f, n&l	**90**	**50**	**70**	very severe
Mean					**61±3.1**		**59±3.9**	**15.1±5.2**	**17.7±6.3**	

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Wild blackberry (*Rubus* spp.) differ in duration to reproductive phase under conventional production in Kenya

Kenneth Omondi Ouma*, Robert Morwani Gesimba and Joseph Ngwela Wolukau

Department of Crops, Horticulture and Soils, Faculty of Agriculture, Egerton University, P.O. Box 536-20115, Egerton, Kenya.

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The purpose of this study was to determine the duration from planting to reproductive phase, of wild blackberry (*Rubus* spp.) collected from the wild. It was carried out at the Horticulture Research and Teaching Farm, Egerton University, Njoro, Kenya. The experimental layout was a randomized complete block design (RCBD) replicated three times; treatments were four wild blackberry species and one cultivated cultivar. The days to first flowering were significantly shorter in cultivated species *Rubus fruiticosus* at 126 days after planting while wild species *Rubus pinnatus, Rubus steundneri, Rubus volkensis* and *Rubus apetalus* took much longer time (341, 333, 332 and 227 days, respectively). Compared to other wild species *R. apetalus* took a significantly shorter time to flowering than all the other wild species. Analysis also showed that cultivated species, *R. fruiticusos* had significantly the longest harvesting duration compared to all wild species planted in the present study. It is concluded that wild blackberry species under conventional production practices have varying days to reproductive phase. However, more studies are necessary with a view to reducing the days to reproductive phase for the wild species to compare favourably with the cultivated types.

Key words: Wild blackberry, reproductive phase, conventional production.

INTRODUCTION

Blackberries were earlier considered as wild fruits and belong to the family of Rosaceae. Currently, gardening of this species is of great significant in a number of countries (Clark and Finn, 2014). With regards to nutritional benefits, blackberry has beenclassified second after Blue berry (*Vaccinium sp.)* among the berry fruit species in the world. They are rich in numerous nutrients such as vitamins, minerals, anti-oxidants and dietary fibers that are vital for health (Ding et al., 2006; Tulipani et al., 2008). They belong to the genus *Rubus* that comprises a high diversity of species like: *Rubus ursinus, Rubus fruiticosus* and *Rubus argutus,* besides they are among the soft and aggregate fruitsthat are healthy and nutritious to the human diet (Tulio et al., 2008; Hirsch, 2013). Blackberries are extensively cultivated on over 20,000 ha worldwide, mainly in Europe and North

*Corresponding author. E-mail: ken8omondi@gmail.com.

America (Strik et al., 2007).

Blackberry is a mild climate fruit crop that can acclimatizefast to diverse ecological conditions, such as woodlands, scrubs, sides of hills, a long hedge rows and can be invasive within a relatively short time across large areas. Moreover, blackberry tolerates drought and warmer conditions compared to raspberries, flowers and produces purple, black or red fruits (Crocker et al., 1998).

The health benefits are attributed to presence of high nutritional content of dietary fiber, vitamin C, K, folic acid, and manganese which is an essential mineral (Sariburun et al., 2010). They are also notable for their phytochemical content that is nutritious and antioxidant strength which is higher in ranking among fruits,that are known to destroy free radicals that harm cells and can lead to cancer, such compounds are ellagic acid, tannins, ellagitannins, quercetin, gallic acid, anthocyanins, and cyanidins (Hager et al., 2008; Overall et al., 2017). The phenolic compounds found in blackberries have also been linked to a reduced risk of cancer and cardiovascular diseases which are degenerative (Reyes-Carmona et al., 2005).

Production of blackberry has improved significantly in the United States of America (USA) (Siriwoharn et al., 2004). In Kenya, there are 84 species of wild blackberries (Chittaranjan, 2011). While the number of cultivated species and acreage in Kenya is unknown, however, in U.S.A, 15 species are in cultivation with production of 65,171 tonnes from 7,159 ha of land. North America and European Union in particular have become a common outlet market for blackberry fruits; this expansion have been enjoyed due to a combination of factors like improved cultivars, expanded marketing efforts and fruit availability (Strik et al., 2007).Generally increased berry consumption is due to its potential health benefit and in food market that are functional (Ding et al., 2006; Tulipaniet al., 2008; Kaumeet al.,2012). However, in Kenya there is little or no information on the performance in terms of reproductive phase, flowering and fruiting time after planting of wild blackberry species in comparison to the cultivated types that have been imported. The duration that wild blackberry takes to reach reproductive phase is unknown as they grow wild without keen interest.Theobjective of this study was to evaluate the duration it takes from planting to reproductive phase of four wild blackberry species and one cultivated cultivar when grown under conventional cultivation.

MATERIALS AND METHODS

Study site

The research was carried out at the Horticulture Research and Demonstration Field, of Egerton University Njoro Kenya. Geographically it lies at a latitude of 0°23`S longitude 35°35`E and an altitude of ≈2238m above sea level, receiving an annual rainfall that ranges from 1200 to 1400 mm, while the average maximum and minimum temperatures range from 19 to 22°C and 5 to 8°C, respectively. According to Jaetzold and Schmidt (2006) the soils are characterized as well-drained dark reddish clays classified as *Mollicandosols*.

Planting material, experimental design and treatment

Wild blackberry plant materials were collected from the wild in Nakuru County (36.0800 E,0.3031 S) and Baringo County (35.7412 E, 0.4897 N) in July 2015 and later taken to a botanist to identify the species after which they were propagated through splits cutting. The seedling of the different wild blackberry species *R. steundneri, R. apetalus, R. volkensis, R. pinnatus, R. fruiticosus* were then planted in the field in December of 2015 at a spacing of 1.0×1.0m (a stand of 10,000 plants per hectare).

The experiment was conducted in randomized blocks containing 5 treatments (species) with three blocks and 16 plants per experimental unit. The trees were cultivated according to the recommendation of Campagnolo and Pio (2012).

Data collection

Days to first and 50% flowering

The number of days from transplanting to first day of flower appearance was determined, while days to 50% flowering were taken when the lateral in each tagged plant had at least 5 flowers.

Flowering duration

The duration of blooming was determined by counting the number from the day of first bloom to the last day of blooming.

Fruit set percentage

Percent fruit set was determined by the number of fruits expressed as a percent of the total number of flowers per cane.
Percent fruit set =(Number of mature fruits per cane *100)/Total number of flowers per cane.

Fruit maturity

The days to fruit maturity was determined by how long it takes the colourto change from red to black or purple or any other colour on the tagged fruits.

Harvesting duration

Harvesting duration was determined as the period from first harvest to the last day of harvesting, which was carried out everyday from the first day of fruit maturity from the grown blackberry.

Data analysis

The Procunivariate procedure of SAS (Version 9.1; SAS Institute, Cary, NC) was used to check for normality of the data before statistical RCBD analysis. Data were then subjected to analysis of variance (ANOVA) using the GLM procedure of SAS at $P \leq 0.05$. Data were analyzed using the model:

$$Yij = \mu + \alpha i + \beta j + \varepsilon ij$$

Where, Yij is the Blackberry species response, μ is the overall

Table 1. Days to first flowering, days to 50% flowering, fruit set %, days to fruit maturity and harvesting duration of different wild blackberry species.

Treatment	Days to first flowering	Days to 50% flowering	Fruit set %	Days to fruit maturity	Harvesting duration
R. apetalus	227.17^{b}	267.33^{b}	84.47^{a}	34.37^{ab}	175.83^{b}
R. volkensis	332.67^{a}	367.00^{a}	39.52^{c}	35.12^{a}	87.17^{c}
R. steundneri	333.67^{a}	364.33^{a}	0.00^{d}	0.00^{c}	0.00^{d}
R. pinnatus	341.00^{a}	374.33^{a}	0.00^{d}	0.00^{c}	0.00^{d}
R. fruiticosus	126.83^{c}	154.17^{c}	55.83^{b}	33.18^{b}	269.83^{a}

Means followed by the same letter(s) within a column are not significantly different at P≤ 0.05 according to HSD Turkey's test.

mean, αi is the effect due to the ith treatment, βj is the effect due to the jth block, εijis the random error term. Means for significant treatments at the F test were separated using Tukey's honestly significant difference (THSD) test at P ≤ 0.05.

RESULTS

Days to first flowering (Table 1) were significantly shorter in *R. fruiticosus* while there were no significant differences in the number of days to first flowering, among wild species *R. volkensis, R. pinnatus* and *R. steundneri,* which took longer days to first flowering. Wild species *R. apetalus* had the shortest days to flowering compared to the other wild species.A similar result was also observed for the days to 50% flowering, whereby *R. fruiticosus* had a significantly shorter daysto all the wild species grown while *R. apetalus* had also a significantly shorter days to 50% flowering in comparison to other wild species grown. Percent fruit set also varied significantly with the wild species *R. volkensis* having a lower percent fruit set followed by cultivated species *R. fruiticosus,* and wild species *R. apetalus* had the highest percent fruit set.Two wild species *R. steundneri* and *R. pinnatus* did not set any fruit during the study. Days to fruit maturity did not show significant variation between *R. apetalus* and *R. volkensis*, however cultivated species *R. fruiticosus* differed significantly from all the wild species. Harvesting duration varied significantly among all the species with cultivated species *R. fruiticosus* exhibiting the longest harvesting duration, while the shortest was observed in *R. volkensis*.

DISCUSSION

The number of days to first and 50% flowering of the blackberryspecies grown were significantly different. Duration to first and 50% flowering in fruit crop is important because it determines when fruit will be ready for harvesting. The change from vegetative to flowering stage in plants is controlled by environmental factors such as temperature and light (Guo et al., 1998; Mouradov et al., 2002). Many other environmental factors such as mineral nutrition and abiotic stress affect flowering time (Lae-Hyeon et al., 2017). The variation in the number of days to flowering is as a result of different chilling requirements (Dale et al., 2003), and it has been described as basic climatic factor for flowering and fruit set (Elloumi et al., 2013) that has a strong effect on phenological stages (Javanshah, 2010). Temperate fruit cultivars with set chilling requirements, show great variability in the flowering period from year to year under warm winter conditions (Petri et al., 2008). Cultivated species *R. fruiticosus* might have responded better to the climatic conditions in the present study thus the fewer days to first and 50% flowering compared to all the wild species.

Flowering time in plants can also be due to genetic factors (Mouradov et al., 2002; Simpson et al.,2003; Putterill et al., 2004) as there are specific genes responsible for early or late flowering in plants. The significant difference in flowering time could be due to the genetic make-up of the different blackberry species planted during this study. Cultivated species could have had genetic factors related to early flowering while the other species had genes which took a longer time to respond to early flowering.

Flower bud initiation in blackberry may be influenced by accumulation of carbohydrates (Crandall et al.,1974), even though there has been no systemic study to establish causal relationship or to examine the interacting effects of irradiance and cane carbohydrates reserves on blackberry flower bud differentiation (Takeda et al.,2003). For the current study, this could be the reason for the difference in time of flowering, therefore cultivated species *R. fruiticosus* accumulating enough carbohydrate for early flower bud initiation compared to the wild species that were grown during the same study.

According to Takeda et al.(2002), flower bud initiation is generally thought to respond to short day in biennial blackberry cultivars and the rate of flower bud initiation varies with the type of cultivar and prevailing winter temperatures. This study shows that cultivated blackberries that were planted together with the wild species have shorter chilling requirement hence the shorter duration to first and 50% flowering. Flowering

time can also be manipulated by exogenous application of gibberellic acid (GA) as this has been shown to positively regulate the expression of flowering signal integrator genes such as *SOC1* and *LFY* (Blazquez and Weigel, 2000; Moon et al., 2003). Though in this study, there was no exogenous application of GAs, therefore this could not be the reason for the different flowering time.

Fruit set variation was observed in the different blackberry species; wild blackberry *R. apetalus*had the highest percent fruit set while cultivated *R. fruiticosus*and wild species *R. volkensis*had lower percent fruit set. Fruit set variation as proposed by MacDaniels (1922) suggested that all buds of brambles have the potential to develop into fruit buds at the same time. Carew et al.(2000) observed that full flowering potential is often inhibited by cultural conditions,more advanced buds that have completed differentiation may suppress some buds (Takeda et al., 2003). Internal shading on early senescence and abscission may also arrest some buds (Wright and Waister, 1984).Fruit set is determined by the adaptability of a species to a given location; it is affected by moisture content of the soil and nutrient availability. Reproductive bud development and differentiation rate in blackberry are reliant on climate and the time of floral bud differentiation and blooming (Moore and Caldwell, 1985). Wild species *R. apetalus* adapted well to the environmental conditions it was grown followed by cultivated species *R. fruiticosus* and wild species *R. volkensis.* Plants that look normal and healthy may at times produce a lot of flower but fail to set fruits completely, but more often it may be partial, with misshapen berries, whose appearance range from nearly normal to some with only a single drupelet (Mohammad, 1996). Such conditions may be due to virus or fungal infection, damage caused by insects, genetic abnormality or all those combined causes (Mohammad, 1996). In the present study, lack of fruit set in *R. steundneri* and *R. pinnatus* could be due to a combination of all those factors that leads no fruit set.

The number of days from flower opening to fruit maturity did not show any significant difference apart from the two species which did not set fruit. Variation of days from first bloom to fruit maturity depends on cultivar and climatic conditions (Salgado and Clark 2015). A comparable result was also reported by Hussain et al. (2016) where the duration between flower bud and ripe berry stages of blackberry cultivars ‘Tupy’ and ‘Xavante’ was quite similar, 29.6 and 31 days, respectively. Striket al.(2012) also found out that the number of days from first bloom to first fruit was unaffected by cultivar or treatment and average was 36 and 43 days in 2004 and 2005, respectively. This is an indication that the days from initial bloom to fruit maturity is not influenced by species for this study and in other studies citationtherein.

Harvesting duration for the different blackberry species was significant with *R. fruiticosus* having the longest harvesting duration, while the shortest in harvesting duration was *R. volkensis*. The longer harvesting duration is due to the ability of a plant species to utilize its carbohydrates or photosynthates efficiently therefore the longer duration of flowering. In some species, the harvesting duration was shorter and is due to lack of fruit set, diseases and environmental condition. Phenological characteristics especially flowering period,days to fruit maturity harvesting period vary due to cultivars and ecological conditions (Rosati et al., 1993). In another study by Campagnolo and Pio (2012) on 11 blackberry cultivars, the longest harvesting duration of cultivar “Amoravermelha” was 161 and 245 days, in 2009/2011 and 2010/2011 respectively, which was shorter than the results of the current study of the different blackberry species while the shortest harvest duration was for cultivar “Chactow” at 42 and 38 days in 2009/2011 and 2010/2011, respectively. Duration of harvest varies from one species to the other due to the adaptability of that species to a given environmental conditions.

Conclusion

This study shows that different blackberry species cultivated in Kenya differ in the days to reproductive phase, wild species *Rubus apetalus* had the shortest days flowering followed by *Rubus volkensis*in comparison to the cultivated species *Rubus fruiticosus.* Harvesting duration also was long in *R. apetalus* and *R. volkensis*but not longer than the cultivated species. This is an indication that the two wild species can be adopted for cultivation through further improvement under convectional production.

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CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Effects of chemical fertilizer types and rates on tuber yield and quality of potato (*Solanum tuberosum L.*) at Assosa, Western Ethiopia

Desta Bekele[1]*, Girma Abera[2] and Amsalu Gobena[2]

[1]Ethiopian Institute of Agricultural Research, Assosa Agricultural Research Center, Assosa, Ethiopia.
[2]School of Plant and Horticulture Science, College of Agriculture, Hawassa University, Hawassa, Ethiopia.

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Potato tuber yield and quality is constrained by a number of biotic and abiotic factors, among which low soil fertility is the prime one. Thus, the objective of the study was to evaluate the effects of chemical fertilizer types and rates on tuber yield and quality of potato at Assosa, Western Ethiopia. The treatments consisted of control, three rates of NP combinations (55 kg N and 45 kg P_2O_5, 110 kg N and 90 kg P_2O_5, 165 kg N and 135 kg P_2O_5 kg ha^{-1}), one NPK combination (110 kg N, 90 kg P_2O_5 and 69 kg K_2O ha^{-1}), formula 2 (100 % NPSB + 91.9 N kg ha^{-1} and 200% NPSB + 128.8 N kg ha^{-1}) and formula 4 (100 % NPSZnB + 93.1 N kg ha^{-1} and 200% NPSZnB + 131.2 N kg ha^{-1}). It was observed that higher marketable tuber yield (30.03 t ha^{-1}) and total tuber yield (34.58 t ha^{-1}) were obtained with the application of NPK (110 kg N + 90 kg P_2O_5 + 69 kg K_2O ha^{-1}) implying that K is an important limiting nutrient besides N and P in the study area. Therefore, NPK fertilizer application is recommended for high yield and quality tuber production in Assosa area, western Ethiopia.

Key words: Chemical fertilizer, potato, tuber, yield.

INTRODUCTION

Potato is the fourth most important crop after rice, wheat, and maize, and has historically contributed to food and nutrition security in the world (FAOSTAT, 2015; FAO, 2015). The crop is also rich in several micronutrients and vitamins, especially vitamin C; a single medium sized potato of 150 g provides nearly half of the daily adult requirement (100 mg) (FAO, 2008).

Potato is a versatile crop that can be cultivated in diverse environments and is currently grown in 100 different countries. Africa takes 10% of potato production in hectares from the world (FAOSTAT, 2015), while Kenya takes the highest potato production in Eastern Africa. Potato production in Kenya increased from 1,084,412 tons in 2004 to 2,192,885 tons in 2013 (FAOSTAT, 2015). Ethiopia has about 70% of the available agricultural land suitable for potato production (Gebremedhin et al., 2008a). Since the highlands are also home to 88% of Ethiopia's population (Gebremedhin et al., 2008b) potato could play a key role in ensuring national food security. Potato is one of the tuber crops grown in Benishangul Gumuz region of Ethiopia, whereby the number of farmers growing potato is increasing from time to time in the region. The number of farmers growing potato in Benishangul Gumuz region was approximately

*Corresponding author. E-mail: hundachala17@gmail.com.

3,277 in 2015/2016 and increased to 8,359 farmers in 2016/2017 (CSA, 2016/2017).

Potato is one of the important tuber crops that has great contribution to food security and cash income for farm households in Ethiopia. Potatoes bulk higher tuber yield in shorter time. However, it requires higher nutrients since it has shallow root systems and shorter growing season (Nigussie, 2001). In addition to N and P, potato demands higher amounts of potassium fertilizer, since it is involved as an important regulator and quality nutrient that is supposed to regulate several enzymes for plant biochemical reactions in the plant cell. Potassium improves the quality of tuber size and dry matter content of potatoes. Thus, balanced application of mineral fertilizer maximizes potato yield and reduces N and P losses to the environment.

In view of this, the national soil data base, the Ethiopia Soil Information Systems (EthioSIS) soil fertility mapping project in Ethiopia identified deficiencies of K, S, Zn, B and Cu in addition to N and P in major Ethiopian soils and hence recommended customized and balanced fertilizer (EthioSIS, 2013) applications for sustainable crop production including potato. Balanced fertilization not only guarantees optimal crop production, better food quality and benefits for the growers, but is also the best solution for minimizing the risk of nutrient losses to the environment. Nutrients such as N, P, K, S, Zn and B can often be included relatively cheaply in new fertilizer formulas; when targeted to deficient soils. These nutrients can dramatically improve fertilizer-use efficiency and crop profitability (Esayas, 2015).

However, there is little information on the impact of different types of fertilizers on the growth and productivity of potato and their interaction on the availability of micro nutrients in Ethiopia. According to Bekabil et al. (2011), there is lack of knowledge and information with regard to balanced macro- and micro-nutrient types and rates in fertilizer blends as a major input to enhance crop productivity. According to EthioSIS (2013), Assosa area soils are deficient with N, P, S, Zn and B; and fertilizer application practices in the region have been mainly based on blanket recommendations, since limited studies were carried out. There is little information on the response of the crop to different fertilizer types and chemical fertilizers under the conditions in the Benishangul Gumuz region. Thus, this study was conducted with the objectives to evaluate the effect of chemical fertilizer types and rates on potato tuber yield, yield components and tuber quality and to determine economically optimum and agronomically efficient types and rate (s) for potato production at Assosa area, western Ethiopia.

MATERIALS AND METHODS

Description of the study site

The experiment was conducted at Asossa Agricultural Research Center (AsARC) in 2017 main cropping season under rain fed conditions at Benishangul Gumuz Regional State of Ethiopia. The AsARC is located from 10º 01' 25'' to 10º 02' 50'' N latitude and from 34º 33' 50'' to 34º 34' 35'' E longitude. The experimental site is located at an altitude of about 1553 m above sea level. Benishangul Gumuz Regional State is geographically located between 9° 30' to 11° 39'' N latitude and 34° 20' to 36° 30'' E longitude covering a total land area of 50,000 km^2. The experimental site is located between 10° 02' 05'' N latitude and 34° 34' 09'' E longitudes at 4 km east of Asossa town and at 660 km west of Addis Ababa.

Asossa has a unimodal rainfall pattern, which starts at the end of April and extends to mid-November, with maximum rainfall received in June, July, August, September, and October. The total annual average rainfall of Asossa is 1275 mm. The minimum and maximum temperatures are 14.33 and 28.43ºC, respectively. The dominant soil type of Asossa area is Nitosols and Fluvisols with the soil pH ranging from 5.1 to 6.0 (EARO, 2004). The major crops cultivated in the study area are mango, sorghum, maize, finger millet, soybean and potato (EARO, 2004).

Experimental procedures

The experimental field was well prepared during May and June 2017 using tractor and then human labour. Ridges were prepared after the plot was harrowed and levelled following the recommended ridge spacing (75 cm between ridges) for potato planting. Medium-sized (40-60 g) and sufficiently sprouted potato tubers (with 2-3 cm long sprouts) were planted on one-third position of the top of the ridges at the specified spacing (30 cm). Planting was done on 24 June 2017. All agronomic activities like weeding, hoeing and earthing up were done based on the plant requirements.

Experimental materials

The improved potato variety namely Belete (CIP-393371.58) released in 2009 by Holeta Agricultural Research Centre (HARC) was used as a planting material (MoARD, 2009). Belete is the prominent variety adapted and selected for Assosa area by the Assosa Agricultural Research Center (AsARC), which has a wide range of environmental adaptation in Ethiopia. It requires from 110 up to 120 days for physiological maturity and is resistant to late blight (Wassu, 2014).

Nitrogen Phosphorus Sulphur and Boron (NPSB) (Formula 2) and Nitrogen Phosphorus Sulphur Zinc and Boron (NPSZnB; Formula 4) blended fertilizer rates, NPK and national blanket recommended N and P rates selected for Assosa area were used following the EthioSIS soil fertility map (EthioSIS, 2013). Nitrogen shortage in blended fertilizers was adjusted by Urea. Chemical fertilizer, potassium from KCl source and phosphorus from TSP (Triple Super Phosphate) source were basal applied at planting and nitrogen was applied twice. Half of nitrogen was applied at planting time, while the rest half was applied at 40 days after planting as side dressing.

Treatments and experimental design

The treatments consisted of control, two levels of formula 2: NPSB (100% and 200%) rates, two levels of formula 4:NPSZnB (100% and 200%) rates and three levels of recommended NP (50%, 100% and 150% NP) rates and recommended NPK. Half of recommended NP (55 kg N and 45kg P_2O_5 ha^{-1}), full of recommended NP 100% (110 kg N + 90 kg P_2O_5 ha^{-1}) and 150% recommended NP (165 kg N + 135 kg P_2O_5 ha^{-1}) and full recommended NP and K (110 kg N + 90 kg P_2O_5 + 69 kg K_2O ha^{-1})

Table 1. Detailed nutrient contents of the chemical fertilizers used as treatments.

TN	Fertilizer type	Rates (kg ha^{-1})	Compound fertilizers' nutrient contents (%)
1	Control	0	0
2	Half R NP	55 N + 45 P_2O_5	55 N kg + 45 kg P_2O_5
3	Full R NP	110 N + 90 P_2O_5	110 N kg + 90 kg P_2O_5
4	150% Recommended NP	165 N + 135 P_2O_5	165 N kg + 135 kg P_2O_5
5	Recommended NPK	110 N + 90 P_2O_5+69 K_2O	110 kg N + 90 kg P_2O_5 + 69 kg K_2O
6	F2:100% NPSB	100% + 91.9 N	18.1 N + 36.1 P_2O_5 + 6.7 S + 0.71 B
7	F2:200% NPSB	200% + 128.8 N	36.2 N + 72.2 P_2O_5 +13.4 S + 1.42B
8	F4:100% NPSZnB	100% + 93.1N	16.9 N + 33.8 P_2O_5 + 7.3 S + 2.23 Zn + 0.67B
9	F4 :200% NPSZnB	200% + 131.2 N	33.8 N + 67.6 P_2O_5 + 14.6 S + 4.46 Zn + 1.34 B

TN= Treatment Number; F2= Formula 2; F4= Formula 4.

were used as treatments. Additional N was added to most of the chemical fertilizers to meet the N shortages in the chemical (Table 1). The experiment was carried out in a Randomized Complete Block Design (RCBD) with three replications. The nine treatments were assigned to each plot randomly. The total numbers of plots were 27 and each plot had a gross area of 11.25 m^2 with 3 m length and 3.75 m width. A spacing of 0.75 x 0.30 m was used in each plot having 50 plants where spacing between plots and adjacent blocks were 1 and 2 m, respectively.

Soil sampling and analysis

Twelve surface soil samples (0-30 cm) were randomly collected following diagonal sampling technique from the entire experimental field before planting. The collected samples were bulked into one composite sample, then air dried ground and passed through a 2-mm sieve for analysis of selected physico-chemical properties, while organic carbon and total N was determined from soil samples sieved by 0.5-mm sieve. Bulk density was determined using soils sampled by core sampler.

Thus, soil samples were subjected for physico-chemical analysis (soil texture, organic carbon, soil pH, total N, available P, exchangeable K, S, Zn, B and CEC). The soil particle size distribution was determined using the hydrometer technique (Ryan and Rashid, 2001) while the soil textural class was identified from textural triangle (Motsara and Roy, 2008). The cation exchange capacity (CEC) was determined using 1N-neutral ammonium acetate method (Jackson, 1967). Soil pH was determined in a 1:2.5 soil to water ratio using a glass electrode attached to a digital pH meter (Page, 1982).

Organic carbon content of the soil was determined following the wet oxidation method of Walkley and Black (1934). Total nitrogen was determined according to the Kjeldahl method (Dewis and Freitas, 1984). Exchangeable potassium was extracted using 1N neutral ammonium acetate methods at pH 7 (Hesse, 1971) and determined with a flame photometer. Available phosphorus was determined by the Olsen method (Olsen et al., 1954). Sulfur was determined by aqua regia digest–turbidimetric method, while Zn was determined by ash extract–flame atomic absorption.

Data collection and measurements

Data were collected from the three middle rows, leaving aside plants in the border rows in order to avoid edge effects. Data were recorded on different phonological and growth parameters, including yield and yield components, and tuber quality parameters.

Days to 50% flowering

The number of days to 50% flowering was recorded (Shiri-e-Janagrad et al., 2009).

Leaf area index (LAI)

This was obtained by dividing the value of the leaf area by the area of the land occupied by the plant using the following formula by Diwaker and Oswalt (1992):

$$Leaf area\ index = \frac{LA}{A} XN$$

Where, LA = mean leaf area of the plant (cm^2); A = the area (cm^2) occupied by one plant in the cropping area; and N = number of leaves on the plant. Leaf area was calculated as a product of the width and leaf length based on pre-measured width and length of leaves from five plants randomly selected and tagged at flowering stage multiplied by a constant (0.674).

Leaf area =W*L*0.674,

Where, 0.674 is the correction factor according to [Sakllova, 1979 quoted by Djilani and Senoussi (2013)].

Above ground dry biomass (t ha^{-1})

This refers to the dry weight of leaves, stems and branches. It was determined from 5 randomly taken plants from the central rows just before senescence (at physiological maturity). Dry weight of the samples was taken after they were air-dried followed by oven-drying at 70°C (CIP, 1984).

Underground dry biomass (g)

This refers to the dry weight of roots, stolons, and tubers. It was determined from 5 randomly taken plants from the central rows at harvesting time. Sample dry weights were taken after air-drying the samples followed by oven-drying at 70ºC (CIP, 1984).

Marketable tuber yield (ton ha^{-1})

The weight of tubers, which were free from diseases, insect pests,

and greater than or equal to 25 g in weight, was recorded as marketable tuber yield.

Unmarketable tuber yield (ton ha^{-1}): the weight of diseased and/or rotting and small-sized (less than 25 g in weight) tubers was recorded.

Total tuber yield (ton ha^{-1})

The total weight of both marketable and unmarketable tubers.

Tuber quality parameters

Tuber dry matter content (%)

Five fresh tubers were randomly selected from each plot and weighed. The tubers were sliced and dried in an oven at 70ºC for 24 hours. The dry matter percent was calculated according to the following formula (Williams and Woodbury, 1968).

$$Tuber\ Dry\ matter\ Content = \frac{\text{Weight of sample after drying (g)}}{\text{Int ial weight of sample (g)}} \text{ X } 100$$

Tuber size distribution in weight (g)

This refers to the proportional weight of tubers in size categories. All tubers from five randomly taken plants were categorized into small (less than 39 g); medium (39-75 g), and large (greater than 75 g) according to Lung'aho et al. (2007).

Partial budget analysis

Partial budget analysis was performed following CIMMYT (1988) procedures. For economic evaluation, dominance analysis, cost and marginal rate of return (MRR) were calculated according to the procedures of CIMMYT (1988). The process of calculating the marginal rates of return of alternative treatments, proceeding in steps from the least costly treatment to the most costly, and deciding if they are acceptable to farmers, is called marginal analysis (CIMMYT, 1988). The field price (6 Birr kg^{-1}) for potato tuber yield at the time of harvest and Urea (8.24 Birr kg^{-1}), TSP (12.75 Birr kg^{-1}), potassium chloride (9.08 Birr kg^{-1}), NPSB (11.02 Birr kg^{-1}) and NPSZnB (11.70 Birr kg^{-1}) were used for partial budget analysis.

Statistical analysis

Data were subjected to analysis of variance (ANOVA) according to Proc Mixed SAS version 9.0 (SAS, 2004). The treatments mean value differences were separated by using Least Significant Difference at 5% level of significance.

RESULTS AND DISCUSSION

Selected physicochemical properties of the experimental soil

The physicochemical properties are presented in Table 2. The results revealed that the soil was clay loam in texture with strongly acidic in reaction (pH 5.5). According to Fageria et al. (2011) optimum growth of potato was found within the soil pH range of 5.2 to 6.5. Furthermore, the clay loamy soil texture was suitable for the production of potato and other major crops due to its good ability to retain nutrients and soil moisture (water). The experimental soil had medium CEC, 22.93 Cmol(+) kg^{-1} soil (Hazelton and Murphy, 2007), but low in total nitrogen, 0.15% (EthioSIS, 2014). The available phosphorus (4.52 mg kg^{-1}) was low as per Olsen et al. (1954). Exchangeable potassium (9.98 mg kg^{-1}), available sulfur (3.01 mg kg-1), and zinc (0.34 ppm) were very low at the experimental site in accordance with EthioSIS (2014). According to Jones (2003), soil boron content was low (0.61 ppm). The organic carbon (1.75%) was medium at the experimental site (Tekalign, 1991). These results signify that the soils require additional supply of plant nutrients to enrich the soil and make them available to the plants.

Potato phenology and growth parameters

The results revealed that the applied chemical fertilizer types and rates significantly ($p< 0.01$) influenced days to 50% flowering, plant height, leaf area index and underground dry biomass of potato; but not number of main stems and above ground biomass (Table 3). Application of 110 kg N + 90 kg P_2O_5 + 69 kg K_2O ha^{-1} significantly increased plant height by 16-21 cm, compared to other treatments. It was observed that plants treated with control had the shortest shoot length compared to the rest of the treatments. However, shoot elongation was slightly higher with plants treated with unblended fertilizer (average mean difference of 2.3 cm) than blended fertilizers. Additionally, significant increase in plant height was observed when K_2O was supplied together with N and P_2O_5 as compared to control and other treatments without the involvements of K_2O. This shows that potash contributes to increased cell division, and elongation results in higher canopy development. This is in line with Marschner (1995) who reported that potassium enhanced cellular growth and development.

Increasing application of combined N and P at rates ranging from 0 to 55kg N + 45 kg P_2O_5 ha^{-1}, 110 kg N + 90 kg P_2O_5 ha^{-1} and165 kg N + 135 kg P_2O_5 ha^{-1} increased plant height by 9, 15, and 20%, respectively over the control. This finding is in line with the results of Singh and Ragav (2000) who reported that a significant interaction between N and K, leading to the better utilization of N in presence of K, contributed to an increased plant height. The results are also in agreement with Firew et al. (2016) who reported that increasing nitrogen and phosphorus rates increased the height of potato plants.

Increasing N and P application ranging from 0 to 165 kg N + 135 kg P_2O_5 kg ha^{-1} prolonged the days required

Table 2. Growth parameters of potato as influenced by chemical fertilizer types and rates at Assosa, western Ethiopia.

Fertilizers applied kg ha^{-1}	NMS	PH (cm)	DF	LAI	AGDB ton ha^{-1}	UGDB (ton ha^{-1})
0 (Control)	2.33	53.17^{c}	50.00^{d}	1.93^{d}	0.74	0.782^{d}
55N + 45P_2O_5	3.27	57.77^{bc}	51.00^{cd}	2.29^{cd}	1.12	3.06^{c}
110 N + 90 P_2O_5	3.47	60.93^{bc}	52.33^{bc}	3.25^{ab}	1.64	4.31^{bc}
165 N + 135 P_2O_5	3.83	63.77^{b}	54.33^{a}	3.12^{abc}	1.92	5.06^{ab}
110 N + 90 P_2O_5 + 69 K_2O	3.17	73.93^{a}	52.33^{bc}	3.12^{abc}	2.05	6.31^{a}
100% NPSB + 91.9 N	3.6	63.2^{b}	52.00^{bc}	3.59^{ab}	1.79	4.41^{bc}
200% NPSB + 128.8 N	3.33	65.03^{ab}	53.67^{ab}	3.65^{a}	1.80	5.05^{ab}
100% NPSZnB + 93.1 N	3.67	58.5^{bc}	51.67^{dc}	2.71^{bcd}	1.79	3.35^{bc}
200% NPSZnB + 131.2 N	3.7	60.33^{bc}	52.67^{abc}	3.63^{a}	2.50	2.69^{c}
LSD 5%	Ns	5.18	1.68	0.91	Ns	1.78
CV %	19.39	8.39	1.86	17.39	36.13	21.07

Means followed by the same letter within a column are not significantly different at 5% level of significance; LSD = least significant difference; and CV = Coefficient of Variation; NMS = Number of Main Stem; PH = Plant Height; LAI = Leaf Area Index; AGDB = Above Ground Dry Biomass (ton ha^{-1}); UGDB = Under Ground Dry Biomass (ton ha^{-1}); DF = 50 % Day to Flowering.

to attain 50% flowering of potato by about 4 days as compared to the control. This is perhaps because of the fact that the applied N enhanced the photosynthetic activity and prolonged the vegetative growth of potato, while optimum P facilitates cell division and promotes root development of potato. This result is in line with the findings of Zelalem et al. (2009) and Israel et al. (2012) that noted excessive vegetative growth and delayed flowering due to high nitrogen levels.

Plants treated with 200% NPSB +128.8 kg ha^{-1} N produced 89% higher leaf area index as compared to controls. However, leaf area index was slightly higher with plants treated with chemical fertilizers. This might be due to the combination of macro with micro nutrients like Zn and B fertilizer blends to NPS. These results are in line with the findings of Salam (2004) who reported that B increased plant growth, leaf area index, and root length of crops. Above ground dry biomass was not significantly ($p>0.05$) affected by chemical fertilizer types and rates applied (Table 3).The present result is in contrary to the finding of Israel et al. (2012) who stated that increasing phosphorus and nitrogen fertilizer rates increased above ground dry biomass yield significantly

The highest above ground dry biomass (2.5 ton ha-1) was recorded with the application of 200% NPSZnB + 131.2 N, while the least was recorded with the control (0.74 ton ha^{-1}). The underground dry biomass yields (6.31 t ha-1) was obtained with application of NPK (110 kg N + 90 kg P2O5 + 69 kg K2O ha^{-1}) whereas the lowest underground dry biomass yields (0.782 tha^{-1}) was obtained from the control (Table 3). Plants treated with potassium together with recommended NP fertilizers showed significant response as compared to other treatments. This is due to the fact that potassium application activates number of enzymes involved in photosynthesis, carbohydrate metabolism and assists in the translocation of carbohydrates from leaves to tubers. Potassium increases the yield by increasing the number and yield of large sized tubers. According to Asmaa and Hafez (2010) higher application of potassium resulted in higher underground biomass production in potato as compared to lower K application.

Increasing application of chemical fertilizers NPSB and NPSZnB from 100 to 200% NPSB with N adjustment also increased underground dry biomass yield linearly as compared to the control. This present finding is in agreement with that of Zelalem et al. (2009), Israel et al. (2012) and Mulubrhan, (2004) who reported that interaction of nitrogen and phosphorous significantly influenced tuber dry weight of potatoes.

Potato tuber yield response to chemical fertilizers

Analysis of variance showed that marketable and total tuber yields were significantly ($P < 0.05$) influenced by chemical fertilizers types and rates, while unmarketable tuber yield was not affected significantly ($P > 0.05$) (Table 4). Maximum marketable tuber yield (30.03 t ha^{-1}) was obtained from application of NPK (110kg N + 90 kg P_2O_5 + 69 kg K_2O ha^{-1}) whereas minimum marketable tuber yield (10.23 t ha^{-1}) was recorded from the control. Increasing N and P fertilizers rates from 0 to 150% NP (165 kg N+ 135 kg P_2O_5 ha^{-1}) increased marketable tuber yield by 114% as compared to control. Thus, the application of K with NP resulted in higher marketable tuber yields when compared to NP and chemical fertilizers rates. This is due to potassium increases tuber yield, size of tuber, resistance against drought and diseases.

Increasing chemical fertilizers rates of NPSB and

Table 3. Influence of the application of chemical fertilizer types and rates on yield and yield components of potato at Assosa, western Ethiopia.

Fertilizer applied kg ha^{-1}	MTW (g)	TTNPH	MTY (ton ha^{-1})	UMTY (ton ha^{-1})	TTY(ton ha^{-1})
0 (Control)	52.96^{b}	7.13	10.23^{c}	4.94	15.17^{c}
55 N + 45	58.92^{b}	8.73	17^{bc}	3.53	20.54^{bc}
110 N + 90 P_2O_5	56.04^{b}	10.33	19.35^{b}	4.97	24.32^{b}
165 N + 135 P_2O_5	66.54^{b}	10.07	21.88^{ab}	5.29	27.18^{ab}
110 N + 90 P_2O_5 + 69 K_2O	104.4^{a}	10.07	30.03^{a}	4.55	34.58^{a}
100% NPSB + 91.9 N	55.67^{b}	11.33	20.81^{b}	6.35	27.16^{ab}
200% NPSB + 128.8 N	61.52^{b}	12.67	22.25^{ab}	5.28	27.54^{ab}
100% NPSZnB + 93.1 N	66.12^{b}	8.8	17.12^{bc}	3.86	20.99^{bc}
200% NPSZnB + 131.2 N	68.68^{b}	9.73	18.74^{b}	4.67	23.41^{b}
LSD 5%	24.15	NS	8.27	NS	7.92
CV%	21.26	20.28	24.23	22.56	18.65

Means followed by the same letter within a column are not significantly different at 5% level of significance; Ns = Not Significant; LSD = least significant difference; and CV = Coefficient of Variation; MTW = Mean Tuber Weight (g); TTNPH = Total Tuber Number per Hill MTY = Marketable Tuber Yield (ton ha^{-1}); UMTY = Unmarketable Tuber Yield (ton ha^{-1}); TTY = Total Tuber Yield (ton ha^{-1}).

NPSZnB from 100 to 200% NPSB with adjusted N increased marketable tuber yield as compared to control. Generally, increasing the rates of chemical fertilizers with adjusted N increased marketable tuber yield. The current result agrees with work of many researchers who stated that increasing N and P rates increased marketable tuber yield (Israel et al., 2012; Burtukan, 2016).

The combined application of NPK (110 kg N + 90 kg P_2O_5 + 69 kg K_2O ha^{-1}) fertilizers improved total tuber yield by 128% as compared to the control. Increasing nitrogen and phosphorus fertilizer rates from 0 to 150% NP (165 kg N + 135 kg P_2O_5 ha^{-1}) increased total tuber yield of potato by 79% as compared to the control. This finding is similar to that of Mulubrhan (2004) who stated that increasing nitrogen and phosphorus application increased total tuber yield.

Many previous studies reported growth, production and tuber quality increase (Al-Moshileh and Errebi, 2004) and yield and quality of potato tubers improvement (Asmaa and Hafez, 2010; Naz et al., 2011; Wassie and Shiferaw, 2011). In addition to the above stated benefits, potassium enhances nitrogen use efficiency by favoring protein formation. Wassie and Shiferaw (2011) also reported potato yield increased by 114-341% on Nitosols due to K fertilizer application.

Potato tuber quality in response to chemical fertilizer

Specific gravity, small and medium size tubers were non-significantly (P>0.05) affected by chemical fertilizer rates and types (Table 5). These results are in agreement with several findings which reported non-significant response in specific gravity of tubers due to N and P fertilizer application (Zelalem et al., 2009; Simret et al., 2010). The present finding of small and medium tuber size is contrary to the findings of Firew et al. (2016) who reported that small and medium sized tubers were affected by nitrogen and phosphorus fertilizer rates.

On the other hand, large sized tubers were significantly (P<0.05) affected by chemical fertilizer types and rates (Table 5). This result is in agreement with the findings of Habtam (2012) who reported that potassium increased the yield of large sized tubers. Increasing application of fertilizers from 0 to 150% NP (165 kg N + 135 kg P_2O_5 ha^{-1}) increased yield of large-sized tubers by 163% as compared to the control. This result is in line with the findings of Firew et al. (2016) who stated that increasing N and P increased large tuber-sized yield of potatoes. Increase in the yield of large-sized tubers due to potassium fertilization may be ascribed to the stimulating effect of potassium on photosynthesis, phloem loading and translocation, as well as synthesis of large molecular weight substances within storage organs that may be contributing to the rapid bulking of the tubers (Singh, 1999). Additionally, it could also be due to the role K plays in carbohydrate formation, transformation and movement of starch from leaves to tubers (Vander, 1981). The increase of chemical fertilizer (NPSB and NPSZnB) application from 100 to 200% with adjusted N, increased yield of large-sized tubers as compared to the control.

Tuber dry matter content was significantly (P < 0.05) affected by application of fertilizer rates and types (Table 4). The highest tuber dry matter content (26.8%) was obtained with the application of NPK (110 kg N+90 kg P_2O_5+ 69 kg K_2O ha^{-1}). The highest dry matter was obtained due to potassium application with NP fertilizers, since K is known to play a significant role on tuber dry matter content. Similarly, Tawfik (2001) reported that

Table 4. Quality parameters of potato as influenced by chemical fertilizers types and rates at Assosa, western Ethiopia season.

Fertilizers (kg ha^{-1})	Potato tuber size distribution in t ha^{-1}			SG	TDMC %
	SSTY (<39 g)	MSTY (39-75 g)	LSTY (>75 g)		
Control	1.77	6.74	6.66^{c}	1.053	24.92^{ab}
55 N +45 P_2O_5	2.66	6.32	11.55^{bc}	1.071	24.41^{ab}
110 N+90 P_2O_5	3.56	6.38	14.38^{bc}	1.074	21.23^{c}
165 N+ 135 P_2O_5	3.68	5.99	17.5^{ab}	1.085	23.15^{bc}
110 N+90 P_2O_5+ 69 K_2O	2.41	5.37	26.8^{a}	1.103	26.8^{a}
100% NPSB+ 91.9 N	4.64	6.66	15.86^{bc}	1.102	22.29^{bc}
200% NPSB + 128.8N	4.56	6.46	16.52^{b}	1.059	22.64^{bc}
100% NPSZnB + 93.1N	2.05	6.2	12.74^{bc}	1.111	23.33^{ab}
200% NPSZnB + 131.2 N	4.03	7.07	12.31^{bc}	1.072	24.34^{ab}
LSD 5%	Ns	Ns	9.45	Ns	2.97
CV %	47.27	30.71	36.59	4.99	7.25

Means followed by the same letter within a column are not significantly different at 5% level of significance; Ns = Not Significant; LSD = least significant difference; and CV = Coefficient of Variation; SSTY = Small size tuber yield; MSTY = Medium size tuber yield; LSTY = Large size tuber yield; SG = Specific Gravity; TDMC = Tuber dry matter Content in percentage.

potato plants fertilized with high K had significantly higher dry matter content than those fertilized with low K. On the other hand, a significant reduction in percent dry matter content can be due to an increase in the application rate of N fertilizer (Kanzikwera et al., 2001). This may be attributed to the fact that high rates of N stimulate top growth more than tuber growth, thereby delaying tuber formation and maturity. This result is coherent with the findings of Allison et al. (2001) that the balanced nutrient management with nitrogen, potassium and phosphorous fertilizers led to highest tuber specific gravity and tuber dry matter in potato.

Partial budget analysis

The results of the partial budget analysis revealed that the highest net benefit of Birr 156,262.8 ha^{-1} was recorded with NPK (110 kg N, 90 kg P_2O_5 and 69 kg K_2O ha^{-1}) fertilizer application; whereas, the lowest net benefit of Birr 55,260 was obtained from the control treatment (Table 6). Based on partial budget analysis, it is advisable to apply full recommended NPK to get optimum yield of potato for Assosa area. Maximum yield and minimum cost evidently lead to high income. For every 1.00 Birr invested for the application of potassium combined with NP, farmers can obtain 1.00 Birr recovery, and an additional 42.5 Birr ha^{-1} by applying recommended NPK (110 kg N, 90 kg P_2O_5, 69 kg K_2O ha^{-1}) (Table 6).

When the new technology surpassed the conventional practice, it is said to be undominated. In contrary, when the new technology yields lower benefit, then the technology is said to be dominated (CIMMYT, 1988). The dominant analysis revealed that the net benefit of some treatments was un-dominated. Thus, unfertilized plot, half of recommended NP (55 kg N + 45 kg P_2O_5 ha^{-1}), two rates of blended fertilizers 100% NPSB + 91.9 kg N ha^{-1}, 200% NPSB +128.8 kg N ha^{-1} and full recommended NP (110 kg N, 90 kg P_2O_5, 69 kg K_2O ha^{-1}) treatments were undominated (Table 6). These results indicated that the net benefit increased with increasing the total cost that varies (Figure 1). So, farmers select un-dominated treatments compared to dominated treatments.

The analysis indicated that all un-dominated treatments were above minimum acceptable marginal rate of return for farmers to accept the fertilizer recommendation. It is important to note that the acceptable minimum marginal rate of return for farmers to accept the fertilizer recommendation is 50-100% (CIMMYT, 1988). Thus, the application of NPK (110 kg N, 90 kg P_2O_5 and 69 kg K_2O ha^{-1}) resulted in the highest net benefit and higher marginal rate of return (4246 %) and higher tuber yield. So, application of 110 kg N, 90 kg P_2O_5 and 69 kg K_2O ha^{-1} is advisable for farmers to use for profitable potato production at Assosa area.

Conclusion

The results showed that increasing N and P application rates delayed flowering of potato as compared to the control treatment. Application of chemical fertilizers, NP (110 N + 90 P_2O_5 kg ha^{-1}) with K (69 kg K_2O) increased potato height by about 39% as compared to the control. Increasing application of chemical fertilizer types and rates revealed significant ($p < 0.05$) influence on leaf area index, underground dry biomass, total tuber yield, marketable tuber yield and mean tuber weight. Applications of 50%NP, 100% NPK, 100% NPSB with

Table 5. Dominance analysis with chemical fertilizers types and rates application on potato tuber yield production at Assosa, western Ethiopia.

Fertilizers (kg ha^{-1})	AVMTY kg ha^{-1}	10% Ad.MTY kg ha^{-1}	GB	TCV	NB	B:C
Control	10230	9210	55260	0	55260	-
55 N, 45 P_2O_5	17000	15301	91806	2502.91	89303.09	35.68
100% NPSB, 91.9 N	20810	18728	112368	3063.19	109304.8	35.68
100% NPSZnB, 93.1 N	17120	15411	92466	3152.69	89313.31D	28.33
110 N, 90 P_2O_5	19350	17412	104472	4824.97	99647.03D	20.65
200% NPSB, 128.8 N	22250	20028	120168	4903.59	115264.4	23.51
100% NPSZnB, 93.1 N	18740	16863	101178	5095.21	96082.79D	18.86
110 N, 90 P_2O_5, 69 K_2O	30030	27022	162132	5869.17	156262.8	26.62
165 N, 135 P_2O_5	21880	19695	118170	7147.48	111022.52D	15.53

AVMTY kg ha^{-1} = Average Marketable Tuber Yield kg ha^{-1}, 10% Ad. MTY = Adjusted Marketable Total Yield; GB = Gross Benefit, TVC = Total Cost that Vary; NB = Net Benefit; D = Dominated.

Table 6. Marginal rate of return of un-dominated treatments as influenced by chemical fertilizers types and rates with potato tuber yield at Assosa, western Ethiopia.

Applied fertilizers kg ha^{-1}	TCV	MC	NB	MB	MRR%
0	0	-	55260	-	-
55 N, 45 P_2O_5	2502.91	2502.91	89303.09	34043.09	1360
100% NPSB, 91.9N	3063.19	560.28	109304.8	20001.71	3570
200% NPSB, 128.8 N	4903.59	1840.4	115264.4	5959.6	324
110N, 90 P_2O_5, 69 K_2O	5869.17	965.58	156262.8	40998.4	4246

TCV = Total Cost that Vary, MC = Marginal cost, MB = Marginal Benefit, NB = Net benefit, MRR = Marginal rate of return.

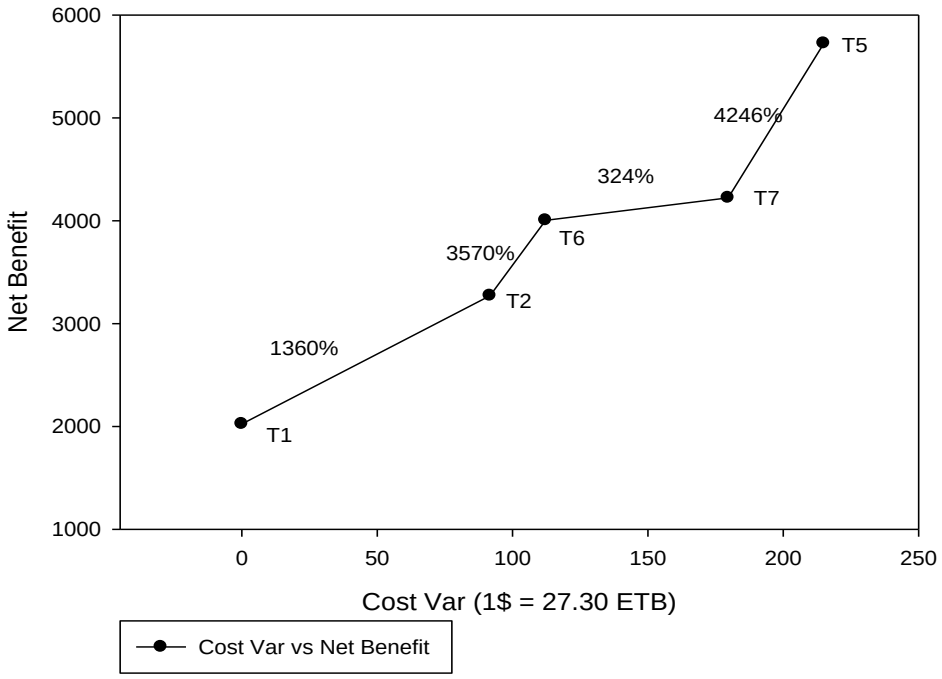

Figure 1. Net benefit curve and marginal rate of return of potato as influenced by chemical fertilizer types and rates at Assosa area Western, Ethiopia. T1 = Control; T2 = Half of recommended NP; T5 = Full recommended of NPK; T6 = 100% NPSB + 91.9 N kg ha^{-1}; T7 = 200% NPSB +128.8 kg N ha^{-1}, the exchange rate of 1 $ is 27.30 Ethiopian Birr.

adjusted N and 200%NPSB with adjusted N fertilizers resulted in higher net benefits with the acceptable marginal rate of return (above 100%). The partial budget analysis revealed that application of 110 kg N, 90 kg P_2O_5 and 69 kg K_2O ha^{-1} resulted in the highest net benefit and marginal rate of returns. In nutshell, on the basis of marketable tuber yield, net benefit and MRR, we recommend 110 kg N, 90 kg P_2O_5 and 69 kg K_2O ha^{-1} fertilizers for profitable and sustainable potato production at Assosa area, western Ethiopia.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Genetic divergence analyses of lowland rice genotypes in North Western Ethiopia

Alamir Ayenew[1*], Tiegist Dejene[2] and Fisseha Worede[3]

[1]Ethiopian Institute of Agricultural Research, Mehoni Research Center, Ethiopia.
[2]Bahir Dar University, College of Agriculture and Environmental Sciences, Ethiopia.
[3]Ethiopian Institute of Agricultural Research, Fogera Rice Research and Training Center, Ethiopia.

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Information on the nature and magnitude of genetic variability is one of the prerequisites for a successful breeding program in selecting genotypes with desirable characters. To estimate the level of genetic divergence of lowland rice genotypes, a field experiment was conducted using thirty-six genotypes during 2017 main cropping season. The experiment was laid out 6x6 simple lattice design. Analyses of variances showed significant differences among traits tested, except harvest index. Principal components PC1, PC2 and PC3 accounted for 64.16% of the total variation. The highest inter-cluster distance was exhibited between cluster I and IV (D^2 = 769.77), cluster I and VI (D^2=485.33), cluster I and III (D^2=232.35), cluster III and IV (D^2=312), cluster III and VI (D^2=197), cluster IV and VI (D^2=746.51), cluster VI and V (D^2=259.51) and cluster VI and VII (D^2=140.15). The crosses between genotypes selected from cluster I with clusters IV, VI and III; cluster IV with clusters III, VI and V are expected to produce better genetic recombination and segregation in their progenies. The cluster means for cluster VI and I were characterized by early heading and early maturity. While cluster I, II, III, and IV were characterized by high mean performance for grain yield for most of its components.

Key words: Cluster analysis, divergence, genetic distance, principal component, rice.

INTRODUCTION

Information on the nature and magnitude of genetic variability is one of the prerequisites for a successful breeding program in selecting genotypes with desirable characters (Dudly and Moll, 1969). It is of great importance for breeders to know the genetic divergence, which classifies a sample of subjects based on a set of different variables into different groups such that similar subjects are put in the same group (Fellahi et al., 2013). It also sorts genotypes into clusters, so the degree of association may be strong between members of the same cluster and weak between members of different clusters. Cluster analysis is performed using measure of similarity levels and Euclidean distance (Everitt, 1993).

Different researchers have grouped rice genotypes using cluster analysis. For example, Sinha and Mishra (2013) reported that using cluster analysis, thirty-four rice genotypes were grouped into five different clusters. In additional approaches, Padmaja et al. (2010) determined

*Corresponding author. E-mail: alamiray12@gmail.com.

Table 1. List of the thirty- six lowland rice genotypes tested at Fogera, Ethiopia.

G.N.	Genotype name	G.N.	Genotype name
1	Aromatic-1	19	SCRID091-20-2-2-4-4
2	Edirne	20	SCRID091-24-3-2-2-3
3	Halilbey	21	SCRID091-38-3-1-3-1
4	Osmancik-97	22	SCRID090-60-1-1-2-4
5	Trakya	23	SCRID090-72-3-3-5
6	Tunka	24	SCRID090-164-2-1-2-1
7	Suitouchuukanbohonnou 11	25	SCRID090-177-2-4-3-4
8	Condai	26	SCRID090-18-1-2-2-1
9	Pepita	27	SCRID091-20-3-1-3-4
10	Saegyejinmi	28	SCRID122-5-2-1-1-3
11	Lunyuki	29	SCRID122-13-1-1-4-3
12	Hangamchal	30	SCRID122-72-1-1-2
13	Hawaghaelo-2	31	SCRID198-73-5-1-3
14	Namcheobyeo	32	Ediget
15	Samgangbyeo	33	X-jigna
16	SCRID091-10-1-3-2-5	34	Abay
17	SCRID091-15-2-2-1-1	35	Erib
18	SCRID091-18-1-5-4-4	36	Shaga

G. N=genotypic number.

the genetic diversity of 150 genotypes of rice by using Mahalanobis D^2 statistics, 13 clusters were determined. As utilized in this research study, principal component analysis reflects the importance of the highest contributor to the total variation at each axis of differentiation (Sharma, 1998). The major importance of principal component analysis is reducing the number of dimensions of a dataset with a number of correlated traits without missing any of the information (Jolliffe, 2002). Among the various sources of useful evidence, information of genetic divergence is important for breeders to improve yield, select drought tolerance rice genotypes and disease resistance. Therefore, the objective of this study was to determine the extent of genetic difference of low land rice genotypes and to identify important traits for selective genotypes.

MATERIALS AND METHODS

Planting materials

A total of thirty-six rice *(Oryza sativa* L.) genotypes that introduced from Asian and African rice considered for this study. The genotypes were found from Fogera National Rice Research and Training Center, Ethiopia (Table 1).

Experimental design and procedure

The experiment was laid out in a 6x6 simple lattice design. The dimension of an individual plot area was 1.2 m width x 2.5 m length (3 m^2) with six rows for each entry. The spacing between blocks, plots and rows were 1, 0.5 and 0.2 m, respectively. The experimental field was well tilled and planting rows were prepared using a hand-pulled row-maker. Planting was done with the seed rate of 64 kg/ha (19.2 g/plot) and 3.2 g/row. Both Di-ammonium phosphate (DAP) and Urea fertilizers were applied at the rate of 100 kg/ha. The total dose of Urea was equally divided into three, and applied at planting, mid tillering and panicle initiation stages.

Data collection

Data were collected on plot and plant basis according to standard evaluation systems for rice (IRRI, 1998). Plant height (cm), panicle length (cm), number of panicles per plant, number of spikelet per panicle and number of grains per panicle were measured from ten randomly selected plants in the middle four rows of each plot. Data for days to 50% heading, Grain-filling period and days to 85% physiological maturity were collected from plot basis. However, Thousand-seed weight (g), biomass yield (kg/ha) and grain yield were collected on the four central rows.

Statistical analysis

Analysis of variance (ANOVA)

The collected data for all quantitative traits were controlled to analysis of variance for simple lattice design. Analysis of variance also performed using Proc lattice and Proc GLM procedures of SAS version 9.2 (SAS Institute, 2008) after testing the significant difference. Model for simple lattice design is:

$$Y_{ijr} = \mu + A_r + G_{ij} + B_{ir} + B_{jr} + e_{ijr},$$

Where Y_{ijr}= the value observed for the plot in the r^{th} replication containing the genotype G_{ij}, μ= grand mean, G_{ij}= genotype effect in the i^{th} row and j^{th} column, Ar= replication effect, B_{ir}=i^{th} block effect, B_{jr}=j^{th} block effect, e_{ijr}, = the plot residual effect.

Table 2. The mean squares for different sources of variation for 12 traits of low land thirty-six rice genotypes evaluated under rainfed condition.

Traits	Mean squares					
	Genotypes (36)	Blocks (6)	Error	CV (%)	R^2	LSD
DH	124.54**	62.8*	21.25	4.57	0.90	3.56
GFP	67.96**	25.65*	26.71	4.81	0.85	3.39
DM	83.37**	11.75^{ns}	24.46	3.67	0.85	3.25
PH	294.15**	17.95^{ns}	10.33	4.17	0.98	2.11
NPP	2.86**	0.41^{ns}	0.45	12.48	0.91	0.44
PL	4.49**	0.9^{ns}	1.39	6.98	0.86	0.77
NSPP	1.73**	1.16*	0.40	7.64	0.88	0.41
NGP	280.58**	263.76**	83.1	13.23	0.87	5.99
TSW	3.6**	0.54^{ns}	0.78	5.4	0.9	0.58
BY	0.22**	0.01^{ns}	0.013	5.0	0.96	0.07
GY	85.94**	4.64^{ns}	4.75	5.4	0.96	1.43
HI	84.15^{ns}	25.85^{ns}	46.08	20.17	0.77	

*, ** = significant at 0.05, 0.01 probability levels. ns= Non-significant, GY= grain yield, DH= number of days to heading, DM= number of days to mature, GFP= grain filling period, PL= panicle length, PH= plant height, NPP= number of panicles per plant, NSPP= number of spikelets per panicle, NGP= number of grains per panicle, BY= biomass yield, HI= harvest index, TSW= thousand-seed weight.

Principal component analysis (PCA)

The principal component analysis was computed by using the princomp procedure of 9.2 version of statistical analysis system (SAS, 2008). Statistical analysis was computed by taking all factors at a time. The investigation of suitable multivariate technique for analyzing data for all the characters is considered. The following formula was computed scores on the first component which extracted by a principal component analysis.

PC1=b11(X1) +b12+···b1p=(Xp)

Where, PC1 = the subject's score on principal component 1 (the first component extracted), b1p = the regression coefficient (or weight) for observed variable p, as used in creating principal component 1 and Xp = the subject's score on observed variable p.

Cluster analysis

Clustering the genotypes into different groups was carried out by average linkage method. The appropriate number of clusters was determined from the values of Pseudo F and Pseudo T^2 statistics using the procedures of SAS computer software version 9.2 to group sets of genotypes into similar clusters (SAS, 2008).

Genetic divergence analysis

The genetic difference between groups was determined using the Mahalanobis (D^2) statistics (Mahalanobis, 1936). The D^2 analysis was performed based on the mean values of all traits by using SAS software program. In matrix notation, the distance between any two groups was estimated from the following relationship.

D^2ij= (Xi -Xj) S-1 (Xi – Xj)

Where, D^2ij = the square distance between any two accessions i and j; Xi and Xj = the vectors for the values for accession i^{th} and j^{th} genotypes; and S-1= the inverse of pooled variance covariance matrix within groups.

Testing the significance of the squared distance values obtained for a pair of clusters was taken as the calculated value of x^2 (chi-square) and tested against the tabulated X^2 values at n-2 degree of freedom at 1 and 5% probability level, where n= number of characters used for clustering genotypes.

RESULTS AND DISCUSSION

Analysis of variance

The analysis of variance showed that genotypes were highly significant ($p < 0.01$) for number of grains per panicle, days to heading, grain-filling period, days to maturity, thousand-seed weight, plant height, panicle length, number of spikelets per panicle, number of grains per panicle, biomass yield and grain yield indicating the existence of variability among the tested genotypes. However, harvest index showed non-significant variation on the tested genotypes (Table 2).

Principal component analysis

Principal component analysis reflects the importance of the largest contributor to the total variation at each axis for differentiation (Sharma et al., 1998). The result of the principal component analysis of the thirty-six rice genotypes by using 11 quantitative characters is displayed in (Table 3). The result showed that a total of 11 PCs were extracted. However, the first three principal components, with eigenvalues greater than one, contributed

Table 3. Vector loadings and percentage of explained variation by the first three PCs.

Variable	PC1	PC2	PC3
DH	0.15	**0.59**	-0.02
GFP	0.03	-0.35	**0.51**
MD	0.21	0.41	**0.42**
PH	0.33	-0.24	**-0.42**
NPP	**-0.32**	0.22	-0.06
PL	**0.37**	-0.11	-0.35
NSPP	**0.42**	0.02	0.30
NGP	**0.47**	-0.09	0.24
TSW	-0.05	**-0.32**	0.27
BY	0.26	**0.35**	-0.08
GY	**0.36**	-0.13	-0.16
Eigenvalues	3.27	2.30	1.49
Proportion (%)	29.72	20.91	13.53
Cumulative (%)	29.72	50.63	64.16

PC= Principal Component, GY= grain yield, DH= number of days to heading, MD= number of days to mature, GFP= grain-filling period, PL= panicle length, PH= plant height, NPP= number of panicles per plant, NSPP= number of spikelets per panicle, NGP= number of grains per panicle, BY= biomass yield, TSW= thousand-seed weight.

64.16% of the total variability. PC1 with eigenvalue of 3.27 contribute 29.72% of the total variability, PC2 with eigenvalue of 2.3 contribute 20.91% and PC3 with eigenvalues of 1.49 contributed to 13.53% of the total variability (Figure 1). Similar finding was obtained by Worede et al. (2014) explained 73.5% of the total variability in rice using the first three PCs.

Variables important in the PC1 were number of grains per panicle (0.47), number of spiklets per panicle (0.42), number of panicles per plant (-0.32), panicle length (0.37) and grain yield per hectare (0.36) thus both vegetative and reproductive characters contributed to the component. The second principal component was associated with days to heading (0.59), thousand-seed weight (-0.35) and biomass yield (0.35), this component is weighted by phenological and yield contributing traits. PC3 is related to grain-filling period (0.51), days to maturity (0.42) and plant height (-0.42); both phenological and vegetative characters contributes positively and negatively to the component. Similar results have been observed by Sanni et al. (2012) and Sinha and Mishra (2013).

Cluster analysis

The clustering pattern of the thirty-six rice genotypes into seven clusters as shown in Table 4 revealed that the genotypes of heterogeneous origin were frequently present in same cluster. Although the genotypes originated in same place or geographic region were also found to be grouped together in the same cluster, the instances of grouping of genotypes of different origin or geographical regions in same cluster were observed in case of all the clusters. This indicated lack of any definite relationship or correlation between genetic diversity and geographic origin of the rice genotypes evaluated in the present study. Therefore, the selection of parental material for hybridization programme simply based on geographic diversity may not be a rewarding exercise (Fellahi et al., 2013). The choice of suitable diverse parents based on genetic divergence analysis would be more fruitful than the choice made on the basis of geographical distances. This finding is in conformity with the previous reports encouraging lack of parallelism between genetic and geographic diversity in rice. Cheema et al. (2004) supported that the number of clusters formed, number of genotypes in the clusters and superposition of the genotypes within the clusters indicated the possibility of genetic improvement for yield and yield components. Among the seven clusters, Cluster I was largest group consisting of ten genotypes, followed by Cluster IV having eight genotypes. Cluster VI with six genotypes, Cluster III with five genotypes, while Cluster II and VII had two genotypes each.

Average intra and inter- cluster distances (D^2)

The estimates of average intra and inter- cluster distances for seven clusters, revealed that the genotypes present in a cluster had little genetic divergence from each other with respect to aggregate effect of 11 characters under study, while much more genetic diversity was observed between the genotypes belonging to different clusters (Table 5). Since, high or optimum

Table 4. Cluster membership of thirty-six rice genotypes using morphological traits.

Cluster number	Number of genotypes	Name of genotypes
I	10	Aromatic-1, 6Tunka, Erib, SCRID091-10-1-3-2-5, Hawaghaelo-2, SCRID090-60-1-1-2-4, Abay, SCRID090-177-2-4-3-4, Osmancik-97, SCRID090-72-3-3-5
II	2	Lunyuki, SCRID090-18-1-2-2-1,
III	5	SCRID091-20-2-2-4-4, Suitou chuukanbohonnou 11, SCRID091-24-3-2-2-3, SCRID091-20-3-1-3-4, SCRID122-72-1-1-2
IV	8	Hangamchal, Condai, SCRID090-164-2-1-2-1, SCRID198-73-5-1-3, SCRID122-5-2-1-1-3, SCRID091-18-1-5-4-4, X-jigna, Shaga
V	3	Saegyejinmi, Namcheobyeo, Ediget
VI	6	Samgangbyeo, SCRID091-15-2-2-1-1, SCRID091-38-3-1-3-1, 5Trakya, Halilbey, Pepita
VII	2	Edirne, SCRID122-13-1-1-4-3

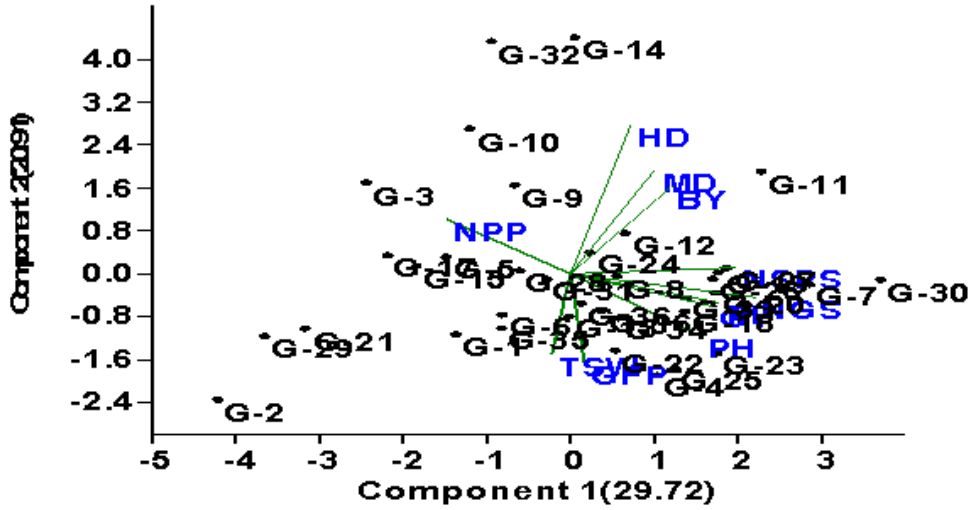

Figure 1. Principal components of lowland rice genotypes based on 11 agronomic and phenotypic traits.

genetic divergence is desired between the parents of hybridization plan for obtaining higher frequency of desirable recombinants, the chances of obtaining good segregants by crossing the little diverse genotypes belonging to same cluster are very low. In order to increase the possibility of isolating good segregants in the segregating generations, it would be reasonable to attempt crosses between the diverse genotypes belonging to clusters separated by large inter cluster distances. In the present investigation, high inter-cluster distances were shown by Cluster I from Cluster IV (769.77), VI (485.33) and III (232.35). Similarly, Cluster III recorded high order inter-cluster distances from Cluster IV (312), VI (197) and Cluster IV were obtained high class inter-cluster distance from cluster VI (746.51), V (259.51) and VII (140.15). Thus, crossing between the genotypes belonging to above stated cluster pairs is recommended for developing high yielding rice genotypes. The lowest inter cluster distance was observed between Cluster II and III (5.2), followed by Cluster II and V (4.6), Cluster II, VII (3.03). This indicated that the hybridization between the genotypes of above three cluster pairs having very low inter- cluster distances could not be rewarding due to little genetic diversity among their genotypes. These findings are similar to those of Baradhan and Thangavel (2011), Kumar and Verma (2015) (Table 5).

Cluster means

The cluster means for each of the eleven characters for seven clusters revealed that Cluster I with ten genotypes showed desirable highest cluster means for days to

Table 5. Estimate of average intra and inter-cluster distances for seven clusters in thirty-six rice genotypes.

	I	II	III	IV	V	VI	VII
I	**23.92**	40.93	232.35	769.77	192.95	485.33	140.2
II		**1.71**	5.2	16.3	4.6	10.43	3.03
III			**9.71**	312	78.33	197	42.3
IV				**32.17**	259.51	746	140.15
V					**8.06**	163.62	35.13
VI						**20.29**	58.9
VII							**4.36**

Table 6. Cluster means for yield components of thirty-six rice genotypes based on D^2 analysis.

	DH	GFP	MD	PH	NPP	PL	NSPP	NGP	TSW	BY	GY
I	93.95	40.05	134.75	75.44	5.03	16.89	8.45	70.98	17	2.17	42.18
II	106	29.5	135.5	88.43	4.16	17.45	8.19	70.17	16.56	3.16	47.03
III	104.8	32.5	137.1	82.95	4.15	17.65	9.06	82.84	16.27	2.21	44.15
IV	101.63	32.19	117.25	90.79	5.9	17.91	8.01	69.94	12.27	2.21	42.89
V	115	29.83	144.66	53.18	7.16	16.34	8.48	63.44	14.4	2.71	36.1
VI	103.25	32.75	144.66	69.4	5.51	15.35	7.72	62.92	17.12	2.09	25.73
VII	85	27.5	112.75	63.73	5.14	15.97	7.55	52.47	15.92	1.66	36.74

GY= grain yield, DH= number of days to heading, MD= number of days to mature, GFP= grain-filling period, PL= panicle length, PH= plant height, NPP= number of panicles per plant, NSPP= number of spikelets per panicle, NGP= number of grains per panicle, BY= biomass yield, TSW= thousand-seed weight.

maturity and days to heading following second highest cluster means for plant height and number of grains per panicle (Table 6). Cluster II and V had undesirable high means for late days to heading and late days to maturity along with low to moderate means for most of the characters. Cluster IV having eight genotypes showed the highest cluster means for days to heading, days to grain filling period and day to maturity, possessing high performance for most of the characters following, the second highest cluster means for plant height, number of grains per panicle and grain yield. Cluster VI with six genotypes recorded desirable highest cluster means for days to maturity, plant height, number of grains per panicle and grain yield, following second highest mean for panicle length and thousand- seed weight, possessing desirable means for remaining characters. Among the clusters showing high inter cluster distances from Cluster I, III and IV, cluster I was most important and common for all three clusters. Cluster III with five genotypes exhibited undesirable highest cluster means for days to 50% heading, grain filling period and days to maturity, while highest desirable cluster means for plant height, panicle length, number of grains per panicle, the lowest cluster means for number panicles per plant, number of spikelete per panicles and biomass yield. Cluster II, with twelve genotypes, showed undesirable lowest cluster means for grain yield per plant and second lowest cluster mean for biological yield per plant and spikelets per panicle. Cluster V with three (Saegyejinmi, Namcheobyeo, Ediget) genotypes showed undesirable highest cluster mean for days to heading and days to maturity and second highest desirable cluster mean for plant height, number of grains per panicle and grain yield, besides having desirable moderate cluster mean for number of panicle per plant, panicle length, number of spikelets per panicle and thousand- seed weight but biomass yield showed desirable lowest cluster means. In general, genotypes of Cluster VI and I were characterized by early heading and early maturity. Genotypes of Cluster I, II, III, and IV were characterized by high mean performance for grain yield for most of its components.

Conclusion

Clustering was made to categorize quantitative traits into components for the sake of understanding the share constituents contribute to major variation in the study. The cluster based on D^2 analysis on pooled mean of genotypes classified the 36 genotypes into seven clusters; which makes them to be highly divergent. The principal component analysis revealed that PC1, PC2 and PC3 accounted for 64.16% of the total variation. This result further confirmed the presence of ample genetic diversity for use in improvement program. The highest inter-cluster distance was exhibited between Cluster I and IV (D^2 = 769.77), Cluster I and VI (D^2=485.33), Cluster I and III (D^2=232.35), Cluster III and IV (D^2=312),

Cluster III and VI (D^2=197), Cluster IV and VI (D^2=746.51), Cluster VI and V (D^2=259.51) and Cluster VI and VII (D^2=140.15), indicates wider genetic divergence among the clusters. The crosses between genotypes selected from Cluster I with Cluster IV, Cluster I with Cluster VI, Cluster I with Cluster III, Cluster III with Cluster IV, Cluster IV with Cluster VI, Cluster VI with Cluster V and Cluster VI with Cluster VII are expected to produce better genetic recombination and segregation in their progenies. Therefore, these rice genotypes need to be crossed and selected to develop high yielding variety. Thus, crossing between the genotypes belonging to above stated cluster pairs is recommended for developing high yielding rice genotypes. The cluster means for each of the eleven characters for seven clusters revealed that Cluster I with ten genotypes showed undesirable highest cluster means for days to maturity and days to heading following second desirable highest cluster means for plant height and number of grains per panicle. Cluster II and V had undesirable high means for late days to heading and late days to maturity along with low to moderate means for most of the characters. Cluster IV having eight genotypes showed the highest cluster means for days to heading, days to grain filling period and day to maturity, possessing high performance for most of the characters following, the second highest cluster means for plant height, number of grains per panicle and grain yield. Cluster VI with six genotypes recorded desirable highest cluster means for days to maturity, plant height, number of grains per panicle and grain yield, following second highest mean for panicle length and thousand- seed weight, possessing desirable means for remaining characters. Cluster III with five genotypes exhibited undesirable highest cluster means for days to heading, grain filling period and days to maturity, while highest desirable cluster means for plant height, panicle length, number of grains per panicle. Cluster V with three (Saegyejinmi, Namcheobyeo, Ediget) genotypes showed undesirable highest cluster mean for days to heading and days to maturity and second highest desirable cluster mean for plant height, number of grains per panicle and grain yield. In general, genotypes of cluster VI and I were characterized by early heading and early maturity. Genotypes of Cluster I, II, III, and IV were characterized by high mean performance for grain yield for most of its components.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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