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Skip-row planting of maize and sorghum in semi-arid Ethiopia

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Soil water deficits during grain fill constrain crop production in semi-arid areas of Ethiopia. Skip-row planting is a means of saving soil water for grain fill while tie-ridging can improve soil water availability throughout the season by reducing runoff. The hypotheses were that where rainfall ceases before or during early grain fill 1) maize (Zea mays L.) and sorghum (Sorghum bicolor L. Moench) yield can be increased by skip-row planting, 2) skip-row planting and tie-ridging interact positively, and 3) productivity can be further increased by planting an early maturity crop in the skip-row area. Skipping a row after planting one or two rows resulted in similar yields compared with planting all rows. Maize yield was 43% greater with tie-ridge compared with flat tillage. There was no tillage by skip-row interaction. Productivity was increased by 20% when a relatively short season bean (Phaseolus vulgaris L.) was planted in the skipped rows of both maize and sorghum as cereal yield was not affected but bean added to productivity. Tie-ridging presents an opportunity for increasing maize yield. Skip-row planting for similar conditions is unlikely to increase productivity unless bean or another crop is planted in the skip-row area.

Key words: Dry bean, intercrop, Sahel, soil water deficits, late season stress, tie-ridging.

INTRODUCTION

Rainfall often ceases before or during early grain fill of maize and sorghum in many semi-arid areas of Ethiopia. Severe stress due to soil water deficits during grain fill is common resulting in low grain weight, grain yield, and harvest index. Such stress has been estimated to account for more than 300,000 Mg year⁻¹ grain yield loss for grain sorghum in Ethiopia (Wortmann et al., 2009).

Skip-row planting is a means of delaying root access to available soil water until later growth stages as the root system extends (Milroy et al., 2004). Within-row plant density is commonly increased with skip-row planting to compensate for fewer planted rows and the crop is more likely to experience stress during the vegetative stage while having greater soil water availability during grain fill (Nielsen et al., 2007). Skip-row planting may therefore result in increased kernel weight, improved harvest index, and increased grain yield where soil water deficit stress is common during grain fill. Skip-row planting is expected to

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result in reduced yield where terminal stress due to soil water deficit is not severe or not common but may increase yield and safeguard against crop failure when such stress is severe (Whish et al., 2005). Potential for increased yield with skip-row planting increases as frequency of severe soil water deficits during grain fill increases, accompanied by potential for deep rooting depth and good soil available water holding capacity. Increased evaporative loss of soil water with skip-row planting is a concern, although saving more deep soil water for later in the season can compensate for these losses (Myers et al., 1986; Spackman et al., 2000). Maintenance of good crop residue ground cover to reduce soil water evaporation enhances the potential of skip-row planting (Klein et al., 2007).

In Australia, skip-row planting was advantageous when mean sorghum grain yield was less than 2.5 Mg ha\(^{-1}\) because of soil water deficits (Spackman et al., 2000). Clark and Knight (1996) observed increased sorghum grain yield with skip-row planting when mean grain yield was less than 2 Mg ha\(^{-1}\), but decreased grain yield when mean yield was more than 3 Mg ha\(^{-1}\). In Ethiopia, planting two rows alternated with a skipped row resulted in increased sorghum grain yield in northern Ethiopia where mean yields were less than 2.5 Mg ha\(^{-1}\) but not in the Central Rift Valley where mean yields were more than 2.5 Mg ha\(^{-1}\) (Mesfin et al., 2010).

On the US Great Plains, skip-row planting resulted in a corn and sorghum grain yield advantage compared with conventional planting if mean grain yield was less than 4 Mg ha\(^{-1}\) but a disadvantage when yield was more than 5 Mg ha\(^{-1}\) (Vigil et al., 2008). Planting maize skipping alternate pairs of rows had more yield compared with planting all rows if soil water deficits limited yield to 4.7 Mg ha\(^{-1}\) and skipping alternate rows was advantageous to 6.9 Mg ha\(^{-1}\) (Lyon et al., 2009). In Nebraska, skipping alternate rows was advantageous for grain sorghum when soil water deficits limited grain yield to less than 4.5 Mg ha\(^{-1}\) (Abunyewa et al. 2010). Water use of sorghum was more and less efficient with skipping alternate rows compared with planting all rows in Nebraska when mean in-season precipitation was less than 2 and more than 2.5 mm day\(^{-1}\), respectively (Abunyewa et al., 2011).

Runoff loss of water with intense rainfall events can be reduced by tie-ridging with inter-row furrows of 20 to 30-cm depth blocked with earthen ties spaced according to the slope of the land, water infiltration rate, and expected intensity of rainfall (Lal, 1977; Gusha, 2002; Gebrekidan, 2003; Pendke et al., 2004). Sorghum grain yield in northern Ethiopia was 62% more with tie-ridging compared with flat planting (Brhane et al., 2006). Mesfin et al. (2009) reported 33 and 18% more sorghum grain yield in northern and Central Rift Valley locations of Ethiopia, respectively, with tie-ridging compared with flat planting. Highland pulse grain yield was increased with tie-ridging by 31 to 96% in northern Ethiopia (Brhane and Wortmann, 2008). In eastern Kenya, maize and cowpea (Vigna unguiculata [(L.]) yield was inconsistently increased with tie-ridging (Miriti et al., 2007). The tie-ridging effect was enhanced with skip-row planting of sorghum in northern Ethiopia but this interaction did not occur in the Central Rift Valley (Mesfin et al., 2009). The tie-ridging by skip-row planting interaction has not been previously investigated for maize.

Smallholder farmers of Ethiopia are reluctant to leave land unplanted as required with skip-row planting. Planting an early maturing crop with a relatively shallow root system, such as bean, in the skip-row area of maize or sorghum may preserve benefits of skip-row planting while giving some bean yield. Workayehu and Wortmann (2011) found that maize planted skipping alternate rows with bean in the skipped row gave a mean land equivalent ratio of 1.6. In another study, a maize intercrop was 55% more productive compared with the sole crops (Bhatnagar and Chaplot, 1991).

The objectives of this research were to determine the impact of skip-row planting and tie-ridge tillage on maize yield, and of skip-row planting of maize and sorghum with an intercrop of bean on yields in an important semi-arid agricultural production area of the Central Rift Valley in Ethiopia. The hypotheses addressed were that where water deficit stress is severe during grain fill 1) maize and sorghum yield can be increased by skip-row planting to save soil water for the reproductive stage, 2) there is a positive interaction of skip-row planting with tie-ridging, and 3) productivity can be further increased by planting an early maturity crop in the skip-row area.

**MATERIALS AND METHODS**

Trials were conducted in 2006 to 2010 for study I and in 2010 to 2012 for study II at Melkassa Agricultural Research Center of the Ethiopia Agricultural Research Institute located at 8°24’N, 39°12’E, and 1500 m elevation. The study I trial of 2009 was not completed because of severe drought stress. The soil was a calcareous clay loam of volcanic parent material classified as a Typic Haplustand with low wet aggregate stability, a propensity for crusting, more than 1-m rooting depth, and slopes ranging from 0.02 to 0.04 m m\(^{-1}\). Soil pH was 7.4 and soil organic matter was 12 g kg\(^{-1}\) for the 0 to 20-cm depth. Cumulative rainfall for the growing season of June to October was more than the long term average and ranged from 635 mm in 2011 to 818 mm in 2012 (Figure 1) with a mean of 65% falling in July and August. The trial site was moved each year. The previous crops were onion (Allium cepa L.), maize, and dry bean in 2009, 2010, and 2011, respectively.

The complete factorial combination of treatments for study I consisted of three or four planting treatments and two tillage treatments. The maize planting treatments, based on 0.75 m inter-row spacing, were: planting all rows; skipping alternate rows; skipping alternate pairs of rows; and planting two rows alternated with a skipped row. The treatment of planting two rows alternated with a skipped row was included in 2008 and 2010 only. The tillage treatments were flat and tie-ridge tillage. The complete factorial combination of treatments for study II was in a split plot design with corn or sorghum as the main plots and three planting practices as the sub-plots including planting all rows, planting two rows alternated with a skipped row, and planting two rows alternated with bean planted is the skipped row. Treatments included a bean sole
crop. Study II was planted with tie-ridging. Individual plot size was 10 × 6 m for each study and each had three replications.

The entire experimental area was tilled with a tractor drawn plow and then ridged with a tractor drawn 4-furrow ridger before planting and application of the treatments. Ridges were of 0.30 m height with 0.75 m spacing that were tied at the ends of the plots to create the tie-ridges. Planting followed the on-set of the rains and occurred during the first two weeks of July. Harvest was in November. Maize and sorghum were planted in the furrow. Ridges were reshaped by ridging the soil around the lower stems of the plants and retied at weeding time.

Plant stands were thinned to desired density by manual uprooting at about two weeks after emergence. The targeted maize and sorghum plant densities were 6.7 plants m⁻² with greater within-row densities with skip-row planting. Bean was also planted in the furrow at 25 and 6.3 plants m⁻² for sole crop and intercrop, respectively. The maize, sorghum, and bean cultivars were Melkassa II, Meko, and Awash Melka, respectively. Melkassa II, Meko, and Awash Melka require 125, 115, 90 to 95 days to maturity, respectively, at 1500 m elevation. Fertilizer was band-applied at the rate of 20 kg ha⁻¹ each of N and P at planting time as di-ammonium phosphate and 23 kg ha⁻¹ of N was side-dress applied as urea for all plots. The sites were manually weeded twice. No pesticides were used for pest and weed control.

Five randomly selected plants per row were measured to determine maize and sorghum plant height at maturity. The maize and sorghum plants from 7 m of row from the center three rows of each plot (15.75 m²) were cut at the soil surface and air-dried after counting the plants and ears. Grain was shelled from the ears and the cobs were combined with the other stover to obtain stover dry weight. Air-dried grain water content was not affected by planting treatments and was found to average 123 g kg⁻¹ for maize and bean and 118 g kg⁻¹ for sorghum. Grain yields were expressed at 150 g kg⁻¹ water content.

The air-dried weight of 100 kernels was determined. Kernel ear⁻¹ and panicle⁻¹ were calculated. The harvest index was calculated as the ratio of grain yield to the above-ground biomass yield with grain and stover water content adjusted to a water content of 80 g kg⁻¹.

For P2S1 with bean treatments, the one bean row in the area for yield determination was harvested by uprooting the plants and threshing after air-drying. Bean grain yield was also expressed at 150 g kg⁻¹ water content.

The analyses of variance for study I were conducted combining 2006 with 2007 data and 2008 with 2010 data using Statistix 9 (Analytical Software, Tallahassee, FL). The ANOVA for study II was combined for 2010-2012. Grain yield was related to yield components using regression and correlation analyses. Means were compared with the ANOVA protected LSD means separation test. Treatment effects and relationships were considered significant at P less than 0.05.

RESULTS

Study I

There were no treatment effect on days to anthesis (X = 81 in 2006-7 and X = 69 in 2008 and 2010) and days to physiological maturity (X = 135 in 2006-7 and X = 120 in 2008 and 2010). Mean plant height was 11 cm taller with tie-ridging compared with flat tillage but not affected by row configuration (Table 1). In 2006 and 2007, grain yield and grain yield components were not affected by interactions. Grain yield and kernel weight were increased by 29 and 19% with tie ridge compared with flat tillage, respectively. Tillage effects were not significant for the other yield components in these years. Grain yield was 24% less with skipping alternate pairs of rows compared with planting all rows. Grain yield and its components were not different with skipping alternate rows and planting two rows alternated with a skipped

Figure 1. Growing season (May to October) monthly rainfall for trials conducted to evaluate skip-row planting and tie-ridging effects on maize in central Ethiopia.
Grain yield was affected by the year x tillage interaction because yield was 43 and 17% less in 2008 and 2010, respectively, with flat tillage compared with tie-ridging (Table 1). Grain yield was not affected by other interactions but was consistently more with tie-ridging compared with flat tillage with a mean increase of 43%. Grain yield was consistently less with skipping alternate pairs of rows compared with other row configurations but planting all rows, skipping alternate rows, and planting two rows alternated with a skipped row had similar mean yields.

The increase in ear plant⁻¹ was greater with planting all rows and planting two rows alternated with a skipped row compared with skipping alternate rows and skipping alternate pairs of rows in 2008 than in 2010 (Table 1).

The main effect of tie-ridging compared with flat tillage was a 49% increase in grain yield, a 13% increase in kernel weight, and a 7% increase in ears plant⁻¹. Mean grain yield and ear plant⁻¹ were 28 and 7% less with skipping alternate pairs of rows compared with planting all rows. Grain yield and its components were not different with skipping alternate rows and planting two rows alternated with a skipped row compared with planting all rows.

Stover yield was more in 2006 and 2008 and less in 2007 with tie-ridging compared with flat tillage with no significant tillage effect in 2010 (Table 1). The reduction in stover yield with skipping alternate pairs of rows compared with planting all rows was greater in 2007 than in 2006, with a mean reduction over all four years of 20% with skipping alternate pairs of rows compared with

<table>
<thead>
<tr>
<th>Factor level</th>
<th>Plant height (cm)</th>
<th>Ear plant⁻¹</th>
<th>Kernel ear⁻¹</th>
<th>100 kernel weight (g)</th>
<th>Grain yield (Mg ha⁻¹)</th>
<th>Stover yield (Mg ha⁻¹)</th>
<th>HI</th>
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<tr>
<td><strong>2006 and 2007; three row configurations‡</strong></td>
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<td>2006</td>
<td>242</td>
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<td>2007</td>
<td>241</td>
<td>0.90</td>
<td>347</td>
<td>23.6</td>
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<td>0.88</td>
<td>359</td>
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<td>246</td>
<td>0.92</td>
<td>350</td>
<td>25.6</td>
<td>4.93</td>
<td>4.34</td>
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<tr>
<td><strong>Row configuration</strong></td>
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<tr>
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<td>0.92</td>
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<td>25.2</td>
<td>4.60a</td>
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<td>315</td>
<td>27.3</td>
<td>3.96</td>
<td>7.16</td>
<td>0.34</td>
</tr>
<tr>
<td><strong>Row configuration</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PF††</td>
<td>252</td>
<td>0.89a</td>
<td>332</td>
<td>26.0</td>
<td>3.63a</td>
<td>6.91a</td>
<td>0.33</td>
</tr>
<tr>
<td>P1:S1</td>
<td>252</td>
<td>0.90a</td>
<td>302</td>
<td>26.8</td>
<td>3.48a</td>
<td>6.36ab</td>
<td>0.33</td>
</tr>
<tr>
<td>P2:S2</td>
<td>244</td>
<td>0.83b</td>
<td>284</td>
<td>23.6</td>
<td>2.62b</td>
<td>5.58b</td>
<td>0.31</td>
</tr>
<tr>
<td>P2:S1</td>
<td>240</td>
<td>0.86ab</td>
<td>321</td>
<td>26.2</td>
<td>3.51a</td>
<td>7.13a</td>
<td>0.31</td>
</tr>
</tbody>
</table>

Ns, *, **, ***: Not significant and significant at P = 0.05, 0.01, and 0.001, respectively. Letters denote differences within columns and sets of years using the ANOVA-protected LSD 0.05 means comparison test.
planting all rows. Mean harvest index for the four years was 13% greater with tie-ridge compared with flat tillage but was not affected by interactions or row configuration.

Variation in kernel ear\(^{-1}\) accounted for more variation in grain yield compared with other yield components. The significant Pearson correlation coefficients of yield components with grain yield were 0.18, 0.68, and 0.49 for ear m\(^{-2}\), kernel ear\(^{-1}\), and 100-kernel weight, respectively. In total, variation in yield components accounted for 99% of the variation in grain yield: Grain yield = -9.64 + 0.206 * 100-kernel weight + 0.014 kernel ear\(^{-1}\) + 0.801 ear m\(^{-2}\).

**Study II**

Maize and sorghum plant height, ear or panicle m\(^{-2}\), kernel wt., and stover yield were not affected by treatments (Table 2). Grain yield and harvest index of sorghum were also not affected by treatments. Maize grain yield was 30% more for planting all rows compared with planting two rows alternated with a skipped row in 2011 and kernel ear\(^{-1}\) was 40% more in 2012 with planting all rows compared with planting two rows with bean planted in the skipped row but these variables were not affected in other years (Table 3). Maize harvest index was low and high, respectively, in 2010 and 2011 with planting all rows compared with planting two rows alternated with a skipped row. Sorghum panicle m\(^{-2}\) was 12% more with planting two rows alternated with a skipped row compared with the other planting arrangements in 2010 but otherwise not affected by treatments.

Maize kernel ear\(^{-1}\) and kernel m\(^{-2}\) were highly correlated (\(r = 0.97\) and both were related to maize grain yield (\(r = 0.86\)). Kernel weight was not related to maize yield but ear m\(^{-2}\) was related to yield (\(r = 0.50\)). Kernel m\(^{-2}\) accounted for 73% of the variation in maize yield. Sorghum kernel panicle\(^{-1}\) and kernel m\(^{-2}\) were highly correlated (\(r = 0.96\), both were related to sorghum grain yield (\(r = 0.83\) and 0.85, respectively), and together accounted for 96% of the variation in yielding. Kernel weight and panicle m\(^{-2}\) were not related to sorghum yield.

Bean pod plant\(^{-1}\), kernel pod\(^{-1}\), stover yield, and harvest index were not affected by treatments (Table 3). Intercrop bean yield was 31% of sole crop bean yield. Bean yield was less suppressed by intercropping with sorghum compared with maize in 2010 but yields were similar for the two intercrops in 2011 and 2012. Bean kernel weight was 11% less with intercropping compared with sole cropping in 2011. The maize: bean land equivalent ratio

---

**Table 2. Analysis of variance results for the effect of planting pattern on maize, sorghum, and bean in the Central Rift Valley of Ethiopia.**

<table>
<thead>
<tr>
<th>Factor level</th>
<th>Plant height (cm)</th>
<th>Ear (m(^{-2}))</th>
<th>Kernel (ear(^{-1}))</th>
<th>Kernel weight (mg)</th>
<th>Grain yield (Mg ha(^{-1}))</th>
<th>Stover yield (Mg ha(^{-1}))</th>
<th>HI</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Maize</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2010</td>
<td>5.53</td>
<td>219</td>
<td>247</td>
<td>3.09</td>
<td>7.84</td>
<td>0.26</td>
<td></td>
</tr>
<tr>
<td>2011</td>
<td>202</td>
<td>273</td>
<td>218</td>
<td>3.87</td>
<td>7.05</td>
<td>0.33</td>
<td></td>
</tr>
<tr>
<td>2012</td>
<td>181</td>
<td>236</td>
<td>213</td>
<td>3.37</td>
<td>6.81</td>
<td>0.29</td>
<td></td>
</tr>
<tr>
<td>Planting (P)</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td></td>
</tr>
<tr>
<td>Year x P</td>
<td>ns</td>
<td>ns</td>
<td>*</td>
<td>ns</td>
<td>ns</td>
<td>***</td>
<td></td>
</tr>
<tr>
<td><strong>Sorghum</strong></td>
<td>Plant height (cm)</td>
<td>Panicle(^{-1})</td>
<td>Kernel panicle(^{-1})</td>
<td>Kernel weight (mg)</td>
<td>Grain yield (Mg ha(^{-1}))</td>
<td>Stover yield (Mg ha(^{-1}))</td>
<td>HI</td>
</tr>
<tr>
<td>2010</td>
<td>6.10</td>
<td>960</td>
<td>38.3</td>
<td>2.25</td>
<td>5.71</td>
<td>0.28</td>
<td></td>
</tr>
<tr>
<td>2011</td>
<td>5.76</td>
<td>1229</td>
<td>36.1</td>
<td>2.61</td>
<td>8.20</td>
<td>0.23</td>
<td></td>
</tr>
<tr>
<td>2012</td>
<td>6.39</td>
<td>1684</td>
<td>29.0</td>
<td>3.07</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Planting</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td></td>
</tr>
<tr>
<td>Year x P</td>
<td>ns</td>
<td>ns</td>
<td>*</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td></td>
</tr>
<tr>
<td><strong>Bean</strong></td>
<td>Pod plant(^{-1})</td>
<td>Kernel pod(^{-1})</td>
<td>Kernel weight (mg)</td>
<td>Grain yield (Mg ha(^{-1}))</td>
<td>Stover yield (Mg ha(^{-1}))</td>
<td>HI</td>
<td></td>
</tr>
<tr>
<td>2010</td>
<td>23.3</td>
<td>4.93</td>
<td>188</td>
<td>1.65</td>
<td>2.33</td>
<td>0.39</td>
<td></td>
</tr>
<tr>
<td>2011</td>
<td>14.9</td>
<td>6.59</td>
<td>175</td>
<td>0.93</td>
<td>0.56</td>
<td>0.62</td>
<td></td>
</tr>
<tr>
<td>2012</td>
<td>158</td>
<td>1.68</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Planting</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>***</td>
<td>***</td>
<td>ns</td>
<td></td>
</tr>
<tr>
<td>Year x P</td>
<td>ns</td>
<td>ns</td>
<td>*</td>
<td>**</td>
<td>ns</td>
<td>ns</td>
<td></td>
</tr>
</tbody>
</table>

ns, * , ** , *** : Not significant and significant at P = 0.05, 0.01, and 0.001, respectively.
was 1.42, 1.43, and 1.01 in 2010, 2011, and 2012, respectively. The sorghum:bean land equivalent ratio was 1.19, 1.53, and 1.19 in 2010, 2011, and 2012, respectively. Overall mean land equivalent ratio for planting two rows with bean planted in the skipped row was 1.30.

**DISCUSSION**

In all trials, there was little rainfall after flowering. Tie-ridge compared with flat tillage resulted in more maize grain yield in all years with planting all rows which is generally consistent with results for sorghum and pulses in northern Ethiopia (Brhane et al., 2006; Brhane and Wortmann 2008; Mesfin et al., 2010). Stover yield was increased with tie-riding in two of four years. Tie-riding is potentially widely beneficial to maize production on moderately sloping land with soil of medium texture and weak aggregate stability.

Results from the above cited studies indicate the benefits of tie-riding for vertic soils that have slow infiltration when wet. All of these studies were done on moderately sloping land. The risk of ridges breaking during heavy rainfall when used on steeply sloping land is of concern and the practice should be used in such cases only if other practices are in place, such as terraces or vegetative barriers, that reduce runoff or otherwise stabilize the slope against erosion. Tie-riding requires tillage but tillage is already the usual practice to break soil crusts and for weed control.

Skip-row planting did not result in increased maize yield. Grain yield was generally well above 2.0 to 2.5 Mg ha\(^{-1}\), the upper grain yield level at which skip-row planting was found to be advantageous compared with planting all rows for grain sorghum when practiced without good crop residue cover (Spackman et al. 2000; Clark and Knight 1996). Mean grain yields were less but near the 4.7 Mg ha\(^{-1}\) yield level identified by Lyon et al. (2009) for skip-row planting to be advantageous to maize yield under no-till conditions with good ground cover on the US Great Plains.

However, even with flat tillage where mean maize grain yield was 3.1 Mg ha\(^{-1}\) in the current study, skip-row planting did not have an advantage. There also was no advantage to planting two rows alternated with a skipped row planting of sorghum compared with planting all rows. Greater kernel weight and harvest index of maize and sorghum would be expected if stress were reduced during grain-fill; these were not increased by skip-row planting indicating that this practice was not effective in reducing late season stress. Therefore, skip-row planting is not likely to have an advantage for maize and sorghum production in Ethiopia unless considerably more severe late season soil water deficit stress conditions occur than encountered in this study. More severe stress does commonly occur with sorghum production in northern Ethiopia where yield was greater with planting two rows alternated with a skip row compared with planting all rows (Mesfin et al., 2010).

There may be greater advantage with skip-row planting if some form of reduced tillage or no tillage is practiced coupled with maintaining ground cover by crop residues sufficient to reduce evaporation and to reduce the potential for erosion. However, crop residues are highly valued and account for 30 to 40% of the value of the sorghum crop in Ethiopia (Wortmann et al., 2009). Therefore, fields in semi-arid areas are nearly bare of

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**Table 3.** Planting configuration by year interaction effects on maize, sorghum, and bean in the Central Rift Valley of Ethiopia. PF and P2:S1 are planting configurations with all rows planted and two planted alternated with one skipped row, respectively.

<table>
<thead>
<tr>
<th></th>
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<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Maize yield (Mg ha(^{-1}))</td>
<td>Maize kernel (ear(^{-1}))</td>
<td>Sorghum panicle (m(^3))</td>
<td>Bean grain yield (Mg ha(^{-1}))</td>
<td>Bean kernel weight (mg)</td>
<td></td>
</tr>
<tr>
<td>PF</td>
<td>2.77(^a)</td>
<td>4.38(^a)</td>
<td>3.84(^ab)</td>
<td>193(^c)</td>
<td>299(^a)</td>
<td>286(^ab)</td>
</tr>
<tr>
<td>P2:S1</td>
<td>3.46(^bc)</td>
<td>3.37(^bc)</td>
<td>2.90(^bc)</td>
<td>251(^ab)</td>
<td>226(^bc)</td>
<td>220(^bc)</td>
</tr>
<tr>
<td>P2:S1 with bean</td>
<td>3.03(^bc)</td>
<td>3.85(^ab)</td>
<td>3.32(^bc)</td>
<td>213(^bc)</td>
<td>296(^a)</td>
<td>203(^c)</td>
</tr>
<tr>
<td>PF</td>
<td>0.23(^c)</td>
<td>0.42(^d)</td>
<td>0.31(^b)</td>
<td>5.76(^bc)</td>
<td>5.74(^bc)</td>
<td>6.38(^ab)</td>
</tr>
<tr>
<td>P2:S1</td>
<td>0.31(^b)</td>
<td>0.31(^b)</td>
<td>0.31(^b)</td>
<td>6.55(^a)</td>
<td>5.53(^c)</td>
<td>6.38(^ab)</td>
</tr>
<tr>
<td>P2:S1 with bean</td>
<td>0.30(^b)</td>
<td>0.32(^b)</td>
<td>0.29(^bc)</td>
<td>6.00(^bc)</td>
<td>6.00(^bc)</td>
<td>6.42(^bc)</td>
</tr>
<tr>
<td>Bean, sole crop</td>
<td>3.11(^a)</td>
<td>1.49(^b)</td>
<td>2.83(^a)</td>
<td>180(^ab)</td>
<td>189(^a)</td>
<td>157(^a)</td>
</tr>
<tr>
<td>P2:S1, maize</td>
<td>0.63(^c)</td>
<td>0.74(^cd)</td>
<td>0.71(^cd)</td>
<td>191(^a)</td>
<td>167(^bc)</td>
<td>163(^bc)</td>
</tr>
<tr>
<td>P2:S1, sorghum</td>
<td>1.23(^bc)</td>
<td>0.57(^d)</td>
<td>0.72(^cd)</td>
<td>193(^a)</td>
<td>170(^bc)</td>
<td>156(^d)</td>
</tr>
</tbody>
</table>

\(Ns, *; **; ***; ****: Not significant and significant at \(P = 0.05, 0.01, \) and 0.001, respectively. Letters denote differences within columns using the ANOVA-protected LSD 0.05 means comparison test.\)
crop residue when land is prepared for planting. Any remaining crop residue is commonly consumed by termites once the rains begin. Despite much promotion, practice of conservation agriculture by smallholders is rare in semi-arid areas of Ethiopia.

The planting two rows with bean planted in the skipped row is promising for maize and sorghum production where bean or another legume of similar growth habit is adapted. Bean is an important food and market crop in Ethiopia. Other pulses may replace bean at elevations where bean is less well adapted.

Conclusion

Tie-riding is likely to result in improved maize yields for semi-arid areas of Ethiopia on moderately sloping land with soil of medium texture and weak aggregate stability, including much of production in the Central Rift Valley of Ethiopia. There is no indication that skip-row planting will result in increased maize or sorghum grain yield in the Central Rift Valley of Ethiopia but other information indicates that skip-row planting is appropriate where late season soil water deficits are more severe such as in northern Ethiopia and in many places throughout the Sahel. Planting bean or another pulse crop of similar growth habit in the skip-row area increases productivity relative to planting all rows of maize or sorghum alone.

Conflict of Interest

The author(s) have not declared any conflict of interests.

ACKNOWLEDGEMENT

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REFERENCES

Cloning and expression of anthocyanidin synthase (ANS) gene from peel of mango (*Mangifera indica* Linn)

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Fruit coloring is a major source of anthocyanin and carotenoid in mango. The anthocyanidin synthase (ANS) is a key enzyme in the anthocyanin biosynthesis. The ANS gene was cloned from mango peel by homologous cloning method. This manuscript reported characterization of ANS from mango peel that comprised 1,262 bp full-length cDNA with open reading frame (ORF) of 1,056 bp and encoding a protein of 351 amino acids. The theoretical molecular weight of the deduced amino sequence of ANS was 39.8 kDa. It was found that the gene encoding for the protein had close relationship with mountain grape (*Vitis vinifera*), cocoa (*Theobroma cacao*), mulberry (*Morus alba*), litchi (*Litchi chinensis*), and other fruit trees through phylogenetic analysis. Expression of ANS was maximum in the green rather than the yellow and red fruit peel. Expression was down-regulated in mature fruit, there was no response to fruit coloration which was affected by anthocyanin.

**Key words:** Cloning, expression, anthocyanidin synthase (ANS), mango.

INTRODUCTION

The anthocyanins are one of the most widespread studied members of the tricyclic flavonoid family of secondary metabolites in plants (Grotewold, 2006). The anthocyanins contribute to some of the most important pigments in flowers and fruits for flower pollination or fruit and seed dispersal, and also the precursors of tannin oligomers present in tea and red wine, which have long-established biomedicinal properties, including inhibition of cell proliferation and antimutagenic, antimicrobial, anti-inflammatory, antioxidant and antihypertensive properties (Pool-Zobel et al., 1999; Harborne and Williams, 2000; Akhisa et al., 2003; Parejo et al., 2004). The structural genes that encode enzymes involved in the anthocyanin pathway and many regulatory genes for transcriptional regulation of the structural genes have been cloned and characterized from a wide variety of plants (Mol et al., 1998; Holton and Cornish, 1995; Lesnick and Chandler, 1998).

Anthocyanidin synthase (ANS), an enzyme of the biosynthetic pathway to anthocyanin, catalyzes the reaction(s) from the colorless leucoanthocyanidins to the colored anthocyanidins. The ANS proteins belong to the 2-oxoglutarate iron-dependent oxygenases and were cloned first from *Perilla frutescens* (Saito et al., 1999). In a series of studies on recombinant ANS from *Arabidopsis thaliana* evidence was presented for the initial oxidation of the substrate at C-3 (Welford et al., 2001).

Furthermore, the ANS formed predominantly quercetin...
and cis and trans-dihydroquercetin (DHQ) with cyanidin being a minor product only (Welford et al., 2001; Turnbull et al., 2003), and the product pattern from (2R, 3S, 4S) cis-leucocyanidin vs. that from (2R, 3S, 4R)-trans-leucocyanidin implied that cis-DHQ, trans-DHQ and cyanidin resulted mostly from the unnatural (2R, 3S, 4R)-trans-leucocyanidin (Turnbull et al., 2003). Moreover, co-crystallization of the ANS with Fe²⁺, 2-oxoglutarate and racemic trans-DHQ or enantiomerically pure (2R, 3R)-DHQ as a substrate analogue in the absence or presence of molecular oxygen supported the stereoselective C-3 hydroxylation of the substrate and surprisingly revealed two molecules of the substrate analogue in the active site with (2R, 3R)-trans-DHQ closest to the iron atom, whereas either enantiomer was bound at the other location (Wilmouth et al., 2003). Clearly, additional data are required to define the substrate specificity of ANS. Mango (Mangifera indica Linn) is one of the delicious seasonal fruits grown in the tropics, and cultivated in many regions of India and now distributed wide across the world in many continents. Mango fruit is one of the most popular, nutritionally rich fruits with unique flavor, taste, and health promoting qualities making it a common ingredient in new functional foods often labeled “The king of the fruits.” Internally, it has juicy flesh features, orange-yellow in color with numerous soft fibrils radiating from the husk. Its flavor is pleasant and rich, and tastes sweet with mild tartness. Outer skin of mango is smooth and green in unripe, but turns into golden yellow, bright yellow or orange-red when ripe depending upon the cultivar.

In the present study, we reported the isolation of cDNA clone coding for an ANS gene from mango. This is the first report on ANS gene from mango peel, these studies add to the understanding of the biosynthesis of anthocyanin in tropical fruit, with significant practical implications for enhancing the specific biomedical anthocyanin contents in mango by genetic engineering.

**MATERIALS AND METHODS**

**Plant materials**

Mango (M. indica Linn) cv “Guifei” cultivar was grown at Tropical Crops Genetic Resources Institute, Chinese Academy of Tropical Agricultural Sciences in Danzhou, China. Before the commercially mature stage, green, yellow and red peels were taken from at least 20-30 fruit at a month interval throughout the growing season. Fresh fruit samples were taken to laboratory and immediately frozen in liquid nitrogen and stored at -80°C.

**cDNA of ANS cloning and expression**

Total RNA was isolated from mango peel sample according to the method of Wan and Wilkins (1994). Reverse transcription was achieved with oligo-d(T)₁₆ primers by using 3 μg of total RNA from peel of mango litchi and the primeScript II 1st strand cDNA synthesis kit (TaKaRa). To isolate partial cDNA clone, two oligonucleotides designed by on the basis of conserved amino acid sequences of several available ANSs were used: forward primer (5’- AAGTACATCCATCCAGCTCATYTTG ‘3’); reverse primer (5’- GAATCCAAAGGATCCGGGAAYGAGA ‘3’). The PCR reaction was carried out according to the following conditions: 94°C, 3 min (1 cycle); 94°C, 30 s; 55°C, 30 s; 72°C, 1 min (30 cycles); 72°C, 5 min (1 cycle). Product of 280 bp, whose identity was confirmed by sequencing was amplified. The 3’RACE and 5’RACE of ANS gene was obtained according to 3’-Full RACE Core Set ver.2.0 and 5’-Full RACE Kit (TaKaRa in Dalian, China). For the analysis of ANS expression in three phases of mango peel, RT-PCR was performed for 30 cycles to determine the linearity of the PCR. The thermal cycling parameters used for the RT-PCR for all genes were as follows: 94°C for 30 s, 56°C for 30 s, 72°C for 100 s; followed by 72°C for 10 min. The cDNA was amplified from 30 ng of total RNA, using specific primers sets for, ANS-F 5’-ATTATGGCAGTGTATTCAATCGGGT3’ ANS-R 5’-GGGAGGAGGCTGGAAGTGCAGTA3’. As a positive control, actin7 fragment was amplified under the same RT-PCR conditions, using the primer pair: Actin7-F 5’-AATGGGAACCTGGAATGTACAGGC3’, TGCCAGATCTTTCTCCATCTCCATCCA3’.

**Sequence analysis**

Nucleotide and amino acid sequence comparisons were performed using the LASERGENE DNA software package (DNASTar, Madison, WI). Sequence similarity searching was performed using the BLASTN and BLASTX, nucleic and protein databases at NCBI (http://www.ncbi.nlm.nih.gov). The phylogenetic analysis of ANS from mango and other species was carried out by alignment using the bioEdit software (http://www.mbio.ncsu.edu/BioEdit/bioedit.html). The phylogenetic and molecular evolutionary analysis used MEGA (molecular evolutionary genetics analysis) version 5.0 (Kumar et al., 2004).

**RESULTS**

**Isolation of the anthocyanin synthase (ANS) gene from mango**

To obtain ANS from mango, the 280 bp cDNA ANS fragments were isolated with two oligonucleotides primers by RT-PCR. The entire length of the cDNA ANS was gotten with the rapid amplification of cDNA ends (RACE) reactions. The 920 and 530 bp cDNA ANS fragments were gotten by the 3’-RACE and 5’-RACE reactions.

The isolated ANS cDNA had a 1,262 bp full-length with ORF of 1,056 bp, and encoding a protein of 351 amino acids (Figure 1). The theoretical molecular weight and isoelectric point of the deduced amino sequence of ANS were 39.8 kDa and 5.68, respectively.

**Sequence analysis of anthocyanidin synthase (ANS) from mango**

A search for amino acid sequence homology revealed high sequence identity (Arabidopsis thaliana, GenBank number NM_118417.1, Vitis vinifera, GenBank Accession number ABV82967.1, Pyrus pyrifolia, GenBank...
Accession number ADP09379.1, *Litchi chinesis* GenBank Accession number HQ402913.1) between ANS and its closest sequence homolog (Figure 1). An alignment of the deduced amino acid sequences of all these proteins indicated that ANS contain a conserved sequence with other plant in the same domain. Phylogenetic analysis was performed on ANS protein sequences. The tree confirms the conclusions that ANS of mango was most closely related to *Morus alba* (GenBank Accession number AEN55613.2), *V. vinifera* (GenBank Accession number ABV82967.1), and *Litchi chinensis* (GenBank Accession number HQ402913.1). ANS of mango from *Medicago truncatula* (GenBank Accession number ABU40983.1), and *Anthurium andraeanum* (GenBank Accession number ABK76317.1) was more distant related in this work clusters. It formed two separate branches, one branch mainly included fruit and crops, for example *Vitis, Litchi, Malus* and *Fragopyrum*, interesting, other branch mainly included flower of plants, for example, *Glycine max, M. truncatula* and *Ipomoea trifida* (Figure 2).

### Expression of ANS and measurement of anthocyanin in different stages in mango peel

Expression of ANS was the highest in the green stage, and the lowest in the yellow stage (Figure 3). A close correlation was also observed between the ANS gene expression and enzyme activity (data not shown). In this study, ANS activity was detected, and changed over the growth period. Anthocyanin was measured in the three phenotypes for peel (green, yellow and red). The green stage examined contains lowest detectable amounts of

![Figure 1. Amino acid sequence of ANS comparison between mango and other species. Sequence analysis of amino acid region proposed to determine specificity.](image-url)
Expression of ANS was measured by RT-PCR at three developmental stages. Expression of ANS was the highest in the green stage, and the lowest in the yellow stage. Values are the average ±SD of three replicants.

Total anthocyanin concentration in the three stages of mango peel. The accumulation of anthocyanin in the peel started at the yellow stage and was 1.12 mg/100 g. The anthocyanin was 8-fold higher in red peel as compared to green peel (Figure 4).

**DISCUSSION**

Some mango cultivars do not have anthocyanin in their shaded skin, however, anthocyanin was tested in “Guifei”
mango. Although several structural genes as well as some regulatory genes in the anthocyanin pathway of mango had been isolated, there are currently no reports on the ANS gene in anthocyanin pathway of mango. In this experiment, we initially investigated the sequence characterization, expression patterns of ANS from mango and analyzed the contents of total anthocyanin. The results indicated that the ANS is one of the key enzymes in anthocyanin-pigmentation pathway of mango, and it is responsible for the formation of anthocyanin in the outer mango peel. Molecular evolutionary trees deduced from amino acid sequences indicated that the enzymes in anthocyanin biosynthesis form a distinct group in the supper family of 2-oxoglutarate-dependent enzymes. ANS had close relationship with mountain grape (V. vinifera), cocoa (Theobroma cacao), mulberry (M. alba), litchi (Litchi chinensis), and other fruit trees through phylogenetic analysis. ANS, a key enzyme of the biosynthetic pathway to anthocyanin, catalyzes the reaction(s) from the colorless leucoanthocyanidin to the colored anthocyanidin. So the present research on isolation and characterization analysis of ANS from mango will provide an alternative to controlling the overall metabolic flux to the target products by appropriate genetic engineering strategies.

Conflict of Interests

The author(s) have not declared any conflict of interests.

ACKNOWLEDGEMENTS

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Full Length Research Paper

Groundnut rosette disease symptoms types distribution and management of the disease in Uganda

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Groundnut rosette disease (GRD), caused by a complex of three agents: groundnut rosette assistor luteovirus, groundnut rosette umbravirus, and the associated satellite RNA, is a major groundnut disease in Uganda. Two main symptom types, chlorotic rosette and green rosette occur. A nationwide survey covering 23 districts was done in 2012 and 2013 to ascertain the predominant GRD symptom types, GRD incidences and severity, farmers’ knowledge and their GRD coping mechanisms, the current groundnut seed system and farming practices. Data were analysed using SPSS and Chi-square tests of association. Mean GRD severity scores were geo-referenced and plotted on the Uganda map. Most respondent (52%) were females. Other than Northern Uganda, most regions grow groundnut landraces. Major seed sources were home saved and marketed. Thirty six percent of farmers grew groundnuts after cereals as recommended. All the farmers sampled knew about and had seen both rosette symptoms types, which were more visible during the second rains. A whole 42% of the farmers have no coping mechanism against GRD. The current knowledge of GRD did not have a significant effect on its management, seed source, varieties grown or gender of the farmers. The green rosette type predominates, making Uganda a green rosette belt.

Key words: Arachis hypogaea L., groundnut rosette virus, green rosette, yellow rosette.

INTRODUCTION

Groundnut (peanut, Arachis hypogaea L.) is the second most important legume, after common beans, grown by smallholder farmers in Uganda (Okello et al., 2010, 2013). The crop also represents a significant source of income that contributes to food security and alleviates poverty. Groundnut seeds contain 40 - 50% high quality edible oil, 20 - 50% easily digestible protein and 10 - 20% carbohydrate depending on the variety. Groundnut is also a nutritional source of vitamin E, niacin, falacin, calcium, phosphorus, magnesium, zinc, iron, riboflavin, thiamine and potassium (Savage and Keenan, 1994).

Groundnut rosette disease (GRD), which is endemic to sub-Saharan Africa (SSA) and its off-shore islands, is widespread and one of the most destructive disease...
of groundnuts (Waliyar et al., 2007). GRD is the most important disease of groundnuts in Uganda (Okello et al., 2010). GRD was first documented at the beginning of the twentieth century in present-day Tanzania and South Africa (Hayes, 1932) and since then has been reported in all groundnut-growing regions of SSA and in Madagascar (Naidu et al., 1998, 1999; Storey, 1935; Storey and Bottomley, 1928). The disease is caused by a complex of three agents: groundnut rosette assistor luteovirus (GRAV), groundnut rosette umbravirus (GRV) and a satellite RNA (Sat-RNA) associated with GRV (Naidu et al., 1999). Two main symptom types of the disease occur (Hayes, 1932, Smartt, 1961; Hull and Adams, 1968) chlorotic rosette (Murant et al., 1988; Naidu et al., 1999; Storey and Bottomley, 1928) and green rosette (Murant et al., 1988). Both types of symptoms are attributed to variants of the Sat-RNA (Murant and Kumar, 1990). Chlorotic rosette has been the predominant form, while green rosette has been reported in the western and southern regions of Africa (Naidu et al., 1998). To date, there is no report on the distribution of the GRD symptom types in Uganda (Wangai et al., 1999). The aphid, *Aphis craccivora* Koch, transmits both forms of GRD in a persistent and circulative manner (Hull, 1964). Either symptom type can cause up to 100% loss in pod yield if the infection occurs before flowering (Naidu et al., 1999; Okello et al., 2010).

In recent years, efforts to control GRD have focused on improving cropping practices to delay the onset and spread of both the vector and the disease and on breeding for host-plant resistance. Cropping practices have led to effective management practices for controlling GRD (Naidu et al., 1998); however, the approach is seldom feasible for the subsistence farming systems of SSA (Deom et al., 1999). Efforts in breeding for host-plant resistance and evaluation of the global collection of groundnut germplasm have contributed to the identification and development of several groundnut germplasm lines with acceptable levels of field resistance to GRD (Olorunju et al., 1991; 2001; van der Merwe and Subrahmanyam, 1997; Subrahmanyam et al., 1998). Since 1995, the Uganda National Groundnut Improvement Programme has released 13 rosette resistant commercial varieties. The goal of this work was to determine the knowledge and management practices used for GRD, document the groundnut seed systems, and analyze the prevalence and distribution of GRD symptom types in widespread districts of Uganda.

### MATERIALS AND METHODS

All major groundnut production areas of the country, Eastern (Bugiri, Bukedea, Busia, Iganga, Jinja, Kaberamaido, Mbale, Soroti, Tororo and Namutumba), Northern (Arua, Lira, Nwoya, Nebbi, Oyam and Pader), Central (Lyantonde and Mukono) and Western Uganda (Hoima, Isingiro, Masindi, Mbarara and Rubirizi) were sampled during the survey. Farmers’ groundnut fields were visited when the crop was between 50% anthesis and physiological maturity. Entire fields were scored for GRD severity based on a scale of 1-9 (NaSARRI scale); where, (1-3) = resistant; (4-6) = moderately resistant; (7-9) = susceptible. GRD symptom types (chlorotic and green) were visually scored and documented from field observation in addition to the farmers’ responses. Disease identification was based on the experience of the field research team and ICRI SAT disease field guides to identify GRD. Geographical position system (GPS) coordinates were recorded for each sampled field site to produce a geo-referenced map of GRD prevalence and occurrence in the Uganda. Additional, data recorded included: the previous crop in the field, the groundnut varieties grown, seed sources, stage of crop growth (age of the crop), prevalence and severity of GRD, farmer knowledge of GRD and the symptom types and GRD management schemes used by farmers.

### Data analysis

Respondents’ data were analyzed using SPSS (version 11). To study association between the presence of GRD in the field with farmer’s knowledge, socio-economic characteristics and other farm attributes, Chi-square test for association was performed. The association between farmers’ knowledge on GRD and farmers’ socio-economic background was also tested using Chi-square test for association. The mean incidence scores were then geo-referenced in Arc-GIS (Version 9) software and plotted on a map of Uganda.

### RESULTS

A total of 23 districts representing the major groundnut Agro-ecologies of Uganda were surveyed (Table 1). One hundred and eighteen farmers’ fields were sampled countrywide and the owners were interviewed. Central Uganda had the least coverage (7%) whereas Northern (40%) and East Uganda (42%) had the highest coverage.

<table>
<thead>
<tr>
<th>Region</th>
<th>Districts</th>
<th>Freq</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>North</td>
<td>Arua, Lira, Nwoya, Nebbi, Oyam, Pader</td>
<td>40</td>
<td>34</td>
</tr>
<tr>
<td>Central</td>
<td>Lyantonde, Mukono</td>
<td>8</td>
<td>7</td>
</tr>
<tr>
<td>East</td>
<td>Bugiri, Bukedea, Busia, Iganga, Jinja, Kaberamaido, Mbale, Soroti, Tororo, Namutumba</td>
<td>42</td>
<td>36</td>
</tr>
<tr>
<td>West</td>
<td>Hoima, Isingiro, Masindi, Mbarara, Rubirizi</td>
<td>28</td>
<td>24</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>118</td>
<td>100</td>
</tr>
</tbody>
</table>
Gender

Fifty two percent (52%) of the farmers surveyed were females (Table 2). Eastern and Western Uganda had the highest percentage of female respondents (71 and 61%, respectively), whereas the Northern region had the highest number of male respondents (75%). Groundnuts, like most legumes in Uganda, are predominantly grown by women farmers.

Varieties grown

With the exception of Northern Uganda, most of the groundnut varieties grown in the other regions were landraces with the Western region having the highest percentage (82%) of landraces (Figure 1). The highest percentage of farmers growing improved varieties was in Northern Uganda. This is as a result of NGOs who operate in post war Northern Uganda frequently supplying seed aid instead of other types of relief aid as the communities re-settled their villages from the two decade long insurgency. The NGOs buy improved varieties and distribute to their supported farmers.

Most farmers purchased seeds from the market (36%), others used saved seeds (23%), and 20% of the farmers procured directly from NARO (Table 3). A mere 9% of seeds were procured through the National Agricultural Advisory Services (NAADS), the official government link between research and farmers. The NAADS programme has their sponsored farmers grouped and crop priorities determined. They then provide funds and link farmers to seed sources. NAADS should be in the forefront of seed supply, connecting government supported research and farmers. Development partners (NGOs) buy seeds directly from the research institutes because of the high seed quality. Overall, twenty percent (20%) of the farmers mentioned NARO as their seed source. These farmers are supported by the NGO and they procure the seeds directly from research stations with the help of their sponsors. This trend is good for the sustainability of the seed system post NGO era. Such farmers would still recognize the research station as a source of high quality seeds.

Table 2. Gender.

<table>
<thead>
<tr>
<th>Gender of farmers</th>
<th>General</th>
<th>North</th>
<th>Central</th>
<th>East</th>
<th>West</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>52</td>
<td>25</td>
<td>50</td>
<td>61</td>
<td>71</td>
</tr>
<tr>
<td>Male</td>
<td>48</td>
<td>75</td>
<td>50</td>
<td>39</td>
<td>29</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

Figure 1. Varieties grown.
Table 3. Seed source.

<table>
<thead>
<tr>
<th>Seed source</th>
<th>General</th>
<th>North</th>
<th>Central</th>
<th>East</th>
<th>West</th>
</tr>
</thead>
<tbody>
<tr>
<td>Donors</td>
<td>1</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Farmer groups</td>
<td>3</td>
<td>3</td>
<td>0</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>Markets</td>
<td>36</td>
<td>20</td>
<td>38</td>
<td>57</td>
<td>29</td>
</tr>
<tr>
<td>Neighbors</td>
<td>6</td>
<td>5</td>
<td>13</td>
<td>5</td>
<td>7</td>
</tr>
<tr>
<td>NGO</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>Research (NARO)</td>
<td>20</td>
<td>33</td>
<td>25</td>
<td>10</td>
<td>18</td>
</tr>
<tr>
<td>Saved seed</td>
<td>23</td>
<td>25</td>
<td>25</td>
<td>7</td>
<td>43</td>
</tr>
<tr>
<td>NAADS</td>
<td>9</td>
<td>13</td>
<td>0</td>
<td>12</td>
<td>4</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

NARO (National agricultural Research Organization); NAADS (National Agricultural advisory Services) is a formal link between research and farmers.

Table 4. Growth stage.

<table>
<thead>
<tr>
<th>Growth stage</th>
<th>General</th>
<th>North</th>
<th>Central</th>
<th>East</th>
<th>West</th>
</tr>
</thead>
<tbody>
<tr>
<td>Past 50% anthesis</td>
<td>62.7</td>
<td>82.5</td>
<td>62.5</td>
<td>74</td>
<td>18</td>
</tr>
<tr>
<td>Physiological maturity</td>
<td>37.3</td>
<td>17.5</td>
<td>37.5</td>
<td>26</td>
<td>82</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

Table 5. Previous crop.

<table>
<thead>
<tr>
<th>Percentage</th>
<th>General</th>
<th>North</th>
<th>Central</th>
<th>East</th>
<th>West</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bananas</td>
<td>1</td>
<td>0</td>
<td>13</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Legumes</td>
<td>18</td>
<td>10</td>
<td>38</td>
<td>19</td>
<td>21</td>
</tr>
<tr>
<td>Vegetables</td>
<td>3</td>
<td>2.5</td>
<td>0</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>Tubers</td>
<td>30</td>
<td>30</td>
<td>13</td>
<td>50</td>
<td>4</td>
</tr>
<tr>
<td>Cotton</td>
<td>3</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Cereal</td>
<td>36</td>
<td>40</td>
<td>38</td>
<td>24</td>
<td>50</td>
</tr>
<tr>
<td>Simsim</td>
<td>4</td>
<td>10</td>
<td>0</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Sunflower</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Virgin land</td>
<td>5</td>
<td>2.5</td>
<td>0</td>
<td>2</td>
<td>14</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

Growth stage

Overall, 37.3% of the groundnuts were at physiological maturity and 62.7% had past 50% anthesis (Table 4). This was the time to rate the GRD severity since after 50% anthesis most of the plants assimilate partitioning will be towards the fruit development, hence any stress diseases would manifest themselves. The highest percentage of plants at physiological maturity was 82%, in the West. This reflects the seasonal variability throughout Uganda and the preference to grow early maturing local Spanish and Valencia groundnut botanicals in the West.

Previous crop

A high percentage of the farmers (36%) grew groundnuts after cereals (Table 5). This is a recommended practice. The 18% of the farmers who grew groundnuts after legumes need to be further discouraged of using this practice through education. If done repeatedly over more
than one season the practice leads to build up of pests and diseases that directly have impact on groundnut production.

**Knowledge of GRD**

Most respondents (98%) know and are aware of GRD (Figure 2). With exception of 5% of the respondents in Eastern Uganda, all the respondents routinely see GRD in the field (Figure 3). This observation however is not translated into the GRD management practice as reflected by the association result (Figure 9).

**Common GRD symptoms**

The green symptom type predominates (95%) in all the regions surveyed. It ranges from 100% in the Central region to 86% in the West, 97.5% in the Northern and 98% in the Eastern region. Chlorotic symptom type ranges from high (14%) in the West to not being detected in Central Uganda (Table 6). The two rosette symptom types are shown in Figure 4.

**Seasonal GRD symptoms**

The majority (53%) of the respondents mentioned that the GRD symptoms were more often observed in the second rains (Figure 5). Second rains in most parts of Uganda are short and are followed by mid- and end-of-season drought. GRD is more severe under such stressed environments.

**Management of GRD**

A large percentage of farmers (42%) do nothing when GRD appears. A large percentage of respondents in the East (17%) and West (14%) do spray their groundnut crops, which are mainly landraces (Table 7). In the East, we observed landraces lines Amasoga, Mpeke mbiri, Kabonge, Mzungu, which are very susceptible to GRD (Picture 2). This could in part explain the spraying requirements.

In the West, Redbeauty a local Valencia, which is susceptible to both GRD and late leafspots, is majorly grown. The spraying regimes are targeting those two diseases. Twenty three percent (23%) of the farmers grow GRD resistant varieties. NARO has released GRD resistant varieties, which are available on station at NaSARRI in Serere and with seed companies. With education and demonstration settings, the percentage of farmers controlling GRD through the use of resistant varieties should rise significantly (Figure 6).

**Predominant GRD symptom type observed in the field by the team**

The green rosette symptom type was predominantly observed in groundnut fields (93%) in all regions sampled. The highest incidence of chlorotic rosette (18%) was observed in Western Uganda (Figure 7).

**GRD severity**

In general, GRD severity was low (1-3), falling in the resistant category. Northern Uganda had the majority of fields with the least severity scores (65%), whereas the highest severity scores were observed in Eastern Uganda. This result correlates well with the varieties grown in the various regions of Uganda (Figure 8). Farmers in Northern Uganda with the assistance of the NGOs are growing improved varieties, which are
Table 6. Common GRD symptoms.

<table>
<thead>
<tr>
<th>Rosette symptom types</th>
<th>General</th>
<th>North</th>
<th>Central</th>
<th>East</th>
<th>West</th>
</tr>
</thead>
<tbody>
<tr>
<td>Green</td>
<td>95</td>
<td>97.5</td>
<td>100</td>
<td>98</td>
<td>86</td>
</tr>
<tr>
<td>Yellow</td>
<td>5</td>
<td>2.5</td>
<td>0</td>
<td>2</td>
<td>14</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

Figure 4. Green rosette symptom (left) and yellow/chlorotic rosette symptom (right).

Figure 5. Season GRD is common.

resistant to GRD.

Association between the presence of rosette in the field, knowledge of rosette and other factors

Other than management of GRD, there were insignificant associations between the key factors tested and the presence/absence of GRD in the field (Table 8). This means that if one manages GRD, less or no disease will appear in the garden. Management can be through planting resistant varieties and following good agronomic practices (crop rotation, timely planting, right plant density).

The current knowledge of GRD was not significantly associated with GRD management, seed source, variety
Table 7. Management of GRD.

<table>
<thead>
<tr>
<th>Crop management</th>
<th>General</th>
<th>North</th>
<th>Central</th>
<th>East</th>
<th>West</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crop rotation</td>
<td>6</td>
<td>10</td>
<td>0</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>Early planting</td>
<td>4</td>
<td>10</td>
<td>0</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Intercropping</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Nothing done</td>
<td>42</td>
<td>17.5</td>
<td>50</td>
<td>55</td>
<td>57</td>
</tr>
<tr>
<td>Resistant variety</td>
<td>23</td>
<td>47.5</td>
<td>37.5</td>
<td>5</td>
<td>11</td>
</tr>
<tr>
<td>Shifting cultivation</td>
<td>2</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Spray</td>
<td>12</td>
<td>7.5</td>
<td>0</td>
<td>17</td>
<td>14</td>
</tr>
<tr>
<td>Uproot and burn</td>
<td>10</td>
<td>2.5</td>
<td>12.5</td>
<td>14</td>
<td>14</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

Figure 6. A groundnut landrace field heavily affected by rosette virus disease in Bukedea, Eastern Uganda 2012B season.

Figure 7. Predominant GRD symptom type observed in the field by the team.
Table 8. Analysis of association between the presence of rosette in the field, knowledge of rosette and other factors.

<table>
<thead>
<tr>
<th>Row factor</th>
<th>Column factor</th>
<th>Pearson chi-square value, degree of freedom</th>
<th>P-value</th>
<th>Significant association</th>
</tr>
</thead>
<tbody>
<tr>
<td>Presence/absence of Rosette in the field</td>
<td>Management of Rosette</td>
<td>61.73 df =10</td>
<td>&lt;0.001</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>Source of seed</td>
<td>8.08 df = 8</td>
<td>0.426</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>Variety grown</td>
<td>2.67 df =3</td>
<td>0.445</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>Previous crop in the field</td>
<td>15.42 df=22</td>
<td>0.843</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>Gender</td>
<td>1.90 df =1</td>
<td>0.168</td>
<td>No</td>
</tr>
<tr>
<td>Knowledge of Rosette disease</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stage of crop growth</td>
<td>1.21 df =2</td>
<td>0.546</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>Variety grown</td>
<td>2.67 df =3</td>
<td>0.445</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>Season</td>
<td>0.05 df =2</td>
<td>0.975</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>Most common rosette symptom</td>
<td>0.11 df =1</td>
<td>0.741</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>Previous crop in the field</td>
<td>8.40 df =22</td>
<td>0.996</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>Management of Rosette disease</td>
<td>2.82 df =10</td>
<td>0.985</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td>1.90 df =1</td>
<td>0.168</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>Source of seeds</td>
<td>4.96 df =8</td>
<td>0.762</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>Variety grown</td>
<td>0.11 df =3</td>
<td>0.991</td>
<td>No</td>
<td></td>
</tr>
</tbody>
</table>

Farmers were able to identify GRD in the field and the predominant season, but the knowledge is not reflected in adopting recommended technologies. The underlying factors for not putting knowledge into practice need to be addressed. Probably the existing GRD management technologies are expensive (seeds, pesticides) as compared to what farmers have (landraces). The extension educators (NAADS) need to be more visible in the ground. Researchers also need to demonstrate the superiority of GRD resistant technologies in farmers’ fields. The current groundnut seed trends of on-farm saved seeds contributing a large stake of the seed supply needs strengthening. Groundnuts seed banks should be set up in the communities and there should be active community based seed multiplication groups linked to
seeds markets and research knowledge. Such a research linked seeds multiplier could then be trained and monitored routinely to produce both foundation and quality declared seeds to meet the current demand for desired GRD resistant varieties.

**GRD severity distribution**

Northern Uganda has the least GRD severity because they mainly grow GRD resistant varieties as a result of the humanitarian aids intervention post war. GRD severity is high in the East especially the Jinja and Pallisa Area (Figure 9). There are two hotspots at Nakabango in Jinja and Ikikiki in Pallisa. In Western Uganda, GRD severity is mainly high because farmers mainly grow red landraces, which are usually susceptible to GRD.

**Conclusion**

In Uganda, GRD resulting in green symptoms predominate. This is in contrast with Wangai et al. (1999), who reported that chlorotic GRD has been the predominant form throughout sub-Saharan Africa. This finding is of utmost importance because Uganda is a major groundnut grower in SSA and a regional ICRISAT GRD trial site. The dynamics of the GRD symptomatology and the vector behavior needs further research.

Jinja and the surrounding area recorded the highest severity. This further affirms that the region is a GRD hotspot, as previously reported by Okello et al. (2010).

Most of the groundnut farmers interviewed were females who practice the recommended crop rotations. According to Kaaya and Christie (2009), groundnut production in Uganda is mainly dominated by women. This finding further strengthens the notion of the groundnut being a woman’s crop.

Northern Uganda farmers mostly grew improved varieties. This is attributed to the development partners’ (NGO and humanitarian organizations) post-war initiatives, which supplied farmers with improved seeds from research and seed companies instead of food relief. This also explains the low GRD severity levels in this region since most of the improved varieties are GRD disease resistant.
Generally, home saved seeds and markets are more visible sources of seeds than seed companies and NAADS. This unfortunate seed source trend needs to be modified to enable farmers’ access to the latest groundnut technologies.

Formal seed companies and local seed banks need to be established in Western and Southern Uganda where no GRD resistant varieties were reported to be used. The national research programme at NaSARRI needs to work with NAADS and NGOs in the areas and set up demonstration and participatory variety selection trial sites to aid in popularizing the new high yielding GRD and leafspot resistant varieties.

The dual occurrence of GRD and leaf spot diseases were reported nationwide. Leaf spot severities were very high in Western and Southern Uganda and farmers reported that they harvest their groundnut crops prematurely (2-3 weeks before physiological maturity and keep them for 3-4 weeks for colour development before stripping) because when left in the garden they dry off and the pegs become detached from the main plant and remain in the soil. Nationwide, farmers confuse leaf spot diseases with harvest indicators. A large percentage of the farmers sampled (42%) do nothing about GRD. Novel GRD and leaf spot disease resistant technologies are available and need to be rigorously disseminated through education, demonstration plots and field days.

RNA viruses exist as “quasispecies” (Roossinck, 1977) in infected plants, and thus the population complexity of GRAV, GRV and satellite RNA in the field has the potential to be large. The potential permutations among variants of the three agents are able to form viable alternatives and their capacity to adapt to diverse and changing ecospecies is thereby enormous. With time, this continuous “evolution” of GRD agents under strong selection pressure can lead to new disease patterns. For example, in Nigeria, a clear shift occurred from green to chlorotic rosette over a period of about 20 years (Naidu et al., 1999; Yayock et al., 1976; Misari et al., 1988). The shift could be due to changes in the genome sequences of GRD agents or to different vector biotypes and cropping patterns. Routine documentation of the predominant GRD symptom types is therefore necessary. This will enhance research efforts by NaSARRI, which are geared towards development of novel strategies to support crop protection measures currently in use for management of the GRD in Uganda. This is the first report on the GRD symptom types distribution in Uganda. Since groundnuts are important and widely grown in Sudan, DR Congo, Tanzania, Rwanda and Burundi, it would be interesting to determine the GRD symptom types distribution in those countries.

Conflict of Interests

The author(s) have not declared any conflict of interests.

ACKNOWLEDGEMENTS

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King tuber mushroom: Bioconversion of fluted pumpkin, sawdust and paper

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Pleurotus tuber-regium (Rumph. Ex Fr.) Singer (King tuber mushroom) was cultivated on sawdust; mixture of sawdust and waste paper and mixture of Telfairia occidentalis Hook.F. (Fluted pumpkin) stem and waste paper in order to examine growth of the mushroom on the test substrates. The spawn of the mushroom was used to inoculate the substrates. Mycelia ramification and sclerotia production were monitored on the substrates during the research. The sclerotia produced were analyzed for protein and crude fiber content. Mycelia ramification for paper mixed with fluted pumpkin stem treatment was significantly different from sawdust and sawdust mixed with paper at P=0.05. Fluted pumpkin stem and waste paper could serve as substrates for cultivation of P. tuber-regium.

Key words: Fluted pumpkin stem, Pleurotus tuber-regium, sclerotia, waste paper.

INTRODUCTION

Pleurotus tuber-regium is found in both tropical and subtropical regions of the world (Isikhuemhen and Lebauer, 2004). It is a high valuable mushroom and researchers have reported its uses in various ways such as culinary (Chiejina and Olufokunbi, 2010), nutritional (Fasidi and Kadiri, 1993), medicinal (Oso, 1977) and nutraceutical (A fiero et al., 2013). This fungus is also useful in solving environmental problems for remediation of polluted sites (Adedokun and Ataga, 2007; Adenipekun and Fasidi, 2005)

Cultivation of P. tuber-regium requires a suitable substrate. A common substrate that has been in use by researchers is sawdust (Adedokun et al., 2003; Isikhuemhen and Lebauer 2004; Chiejina and Olufokunbi, 2010). However, wood dust may have a long term hazardous effect on health (Meier, 2013). In Nigeria, information transfer is majorly paper based although a few organizations are recently going paperless. After information extraction, papers which are found as litter all over are often gathered and burnt off. This contributes to air pollution. Likewise, remains of Telfairia occidentalis, though a useful vegetable could be a nuisance to the environment after removal of the edible tender shoot. P. tuber-regium has a wide substrate range therefore growing the mushroom on the above mentioned substrates, could both assist in solving afore mentioned

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environmental issues as well as increasing the substrate range in the mushroom industry. To this end, the focus of this paper was to examine the mycelia run and sclerotia production as well as determine the nutritional content of sclerotia of *P. tuber-regium* grown on these test substrates.

**MATERIALS AND METHODS**

**Source of samples and study sites**

The research was conducted at the Faculty of Agriculture mushroom farm in the University of Port Harcourt, Nigeria.

Sawdust was obtained from a close-by sawmill (Rumuos), fluted pumpkin stem was obtained from a close-by market (Choba), while mushroom spawns established on waste cotton were from Bezaaleel mushroom farm in Port Harcourt. Waste papers were gathered from the University of Port Harcourt environment.

**Samples preparation**

Samples were prepared according to the modified method of Stamets (2000). Sawdust (S) was composted for two weeks. 200 g of sawdust was measured into transparent bottles of dimension 7.5 x 17 x 7.5 cm. The content was sterilized at 121°C for 15 min and after cooling, it was inoculated with 2.5% spawn of the mushroom. This procedure was repeated for sawdust plus paper, (SP) (four sheets of used quarto sized paper, shredded into pieces, were added to 200 g sawdust) and paper plus pumpkin stem (PSP) where four sheets of paper was added to 100 g pumpkin stem. The samples were incubated at room temperature, 28±2°C. Three replicates were prepared. The substrate bottles were observed for mycelia initiation. Mycelia run down the bottles were by visual observation. The first treatment to complete the run was recorded. Sclerotia formed on each substrate were harvested by hand. Number of sclerotia was recorded and proximate analysis conducted for each treatment.

**Table 1.** Growth performance of *P. tuber-regium* on different treatments.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mycelia initiation (day)</th>
<th>Full mycelia run (day)</th>
<th>Sclerotia harvest (day)</th>
<th>Number of Sclerotia</th>
<th>Sclerotia fresh weight(g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sawdust</td>
<td>3.50&lt;sup&gt;a&lt;/sup&gt;</td>
<td>42.00&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>117.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.25&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Sawdust and paper</td>
<td>4.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>43.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>87.80&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.25&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Paper and pumpkin</td>
<td>4.75&lt;sup&gt;a&lt;/sup&gt;</td>
<td>29.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>104.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.50&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>LSD</td>
<td>1.9</td>
<td>11.8</td>
<td>81.0</td>
<td>0.7</td>
<td>5.3</td>
</tr>
</tbody>
</table>

Mean with the same value are not significantly different, while different values are significantly different at *P*=0.05. LSD: Least significant difference between two means.

**Table 2.** Protein, moisture and crude fiber of harvested sclerotia on different treatments.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Protein (%)</th>
<th>Crude fiber (%)</th>
<th>Moisture (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sawdust</td>
<td>10.063</td>
<td>9.639</td>
<td>57.778</td>
</tr>
<tr>
<td>Sawdust and Paper</td>
<td>8.75</td>
<td>38.02</td>
<td>41.270</td>
</tr>
<tr>
<td>Paper and Pumpkin</td>
<td>9.19</td>
<td>32.19</td>
<td>45.345</td>
</tr>
</tbody>
</table>

**Determination of nutritional contents**

Protein and crude fiber were analyzed using the standard procedure of AOAC (2002). Data was subjected to analysis of variance (ANOVA).

**RESULTS**

The mycelia of *P. tuber-regium* ramified well on sawdust, sawdust/paper mixture (SP) and fluted pumpkin stem/paper mixture (PSP). Sclerotia were also observed on the three substrates (Table 1). Mycelia run was fastest in the treatment with PSP (29 days) and significantly different at *p*=0.05 from other substrates 42 and 43 days for sawdust and SP, respectively. It was also observed that the sclerotia of the treatment with PSP were weightier than those of the two other substrates. Table 2 shows result for percentage protein, crude fiber and moisture contents of sclerotia harvested on the three substrates; the sclerotia are high in each of the parameters measured with PSP were comparable to sawdust which is widely used for cultivation.

**DISCUSSION**

The use of sawdust for mushroom cultivation is well documented by researchers (Onuoha et al., 2009; Okhuoya and Okogbo, 1990) among others. This study confirms earlier report of the use of sawdust as mushroom substrate primarily because of its abundance and availability. Few researchers have also reported the use of waste paper as substrate for mushroom cultivation both as bulk substrate and alternative for casing soil
(Baysal et al., 2003; Sassine et al., 2005). In this study, results of SP and PSP are quite interesting for mycelia run, sclerotia formation and nutritional contents. However, report on the use of fluted pumpkin stem in mushroom cultivation is limited. The use of fluted pumpkin stem for mushroom cultivation in this study is novel and observation revealed that fluted pumpkin stem could serve well as a substrate for mushroom cultivation. When compared with other substrates, mycelia ramification was significantly different and fastest for this treatment. This is probably due to the high fiber content. Furthermore, sclerotia are weightier when compared with other substrates. This may be due to the nutritional content of fluted pumpkin stem. Akanbi et al. (2007) stated the nutritional importance of fluted pumpkin as being rich in protein (29%), fat (18%) and minerals and vitamins (20%). The use of fluted pumpkin stem as a substrate for mushroom cultivation should be encouraged in view of the observation made in this study especially considering the abundance and availability of the substrate. A number of researchers (Onyango et al., 2011; Chitamba et al., 2012; Ukoima et al., 2009) have reported the advantages of using agricultural wastes as growing substrates.

Analysis of sclerotia of mushroom for protein, crude fiber and moisture content are noteworthy. It showed that the three treatments are relatively high in the parameter analyzed. This remark tallies with observation made by Oso (1977) and Mshandete and Cuff (2007). Result of fiber content is equally exiting as fiber helps to facilitate digestion in man.

Conflict of Interests

The author(s) have not declared any conflict of interests.

Conclusion

This study shows that fluted pumpkin stem and waste paper are potential substrates in mushroom cultivation. Further research is however suggested to investigate other nutritional parameters.

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African Journal of Plant Science

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