African Journal of Plant Science

Volume 8 Number 3, March 2014

ISSN 1996-0824

Academic Journals

ABOUT AJPS

The **African Journal of Plant Science (AJPS)** (ISSN 1996-0824) is published Monthly (one volume per year) by Academic Journals.

African Journal of Plant Science (AJPS) provides rapid publication (monthly) of articles in all areas of Plant Science and Botany. The Journal welcomes the submission of manuscripts that meet the general criteria of significance and scientific excellence. Papers will be published shortly after acceptance. All articles published in AJPS are peer-reviewed.

Submission of Manuscript

Please read the **Instructions for Authors** before submitting your manuscript. The manuscript files should be given the last name of the first author

Click here to Submit manuscripts online

If you have any difficulty using the online submission system, kindly submit via this email ajps@academicjournals.org.

With questions or concerns, please contact the Editorial Office at ajps@academicjournals.org.

Instructions for Author

Electronic submission of manuscripts is strongly encouraged, provided that the text, tables, and figures are included in a single Microsoft Word file (preferably in Arial font).

The **cover letter** should include the corresponding author's full address and telephone/fax numbers and should be in an e-mail message sent to the Editor, with the file, whose name should begin with the first author's surname, as an attachment.

Article Types
Three types of manuscripts may be submitted:

Regular articles: These should describe new and carefully confirmed findings, and experimental procedures should be given in sufficient detail for others to verify the work. The length of a full paper should be the minimum required to describe and interpret the work clearly.
Short Communications: A Short Communication is suitable for recording the results of complete small investigations or giving details of new models or hypotheses, innovative methods, techniques or apparatus. The style of main sections need not conform to that of full-length papers. Short communications are 2 to 4 printed pages (about 6 to 12 manuscript pages) in length.

Reviews: Submissions of reviews and perspectives covering topics of current interest are welcome and encouraged. Reviews should be concise and no longer than 4-6 printed pages (about 12 to 18 manuscript pages). Reviews are also peer-reviewed.

Review Process

All manuscripts are reviewed by an editor and members of the Editorial Board or qualified outside reviewers. Authors cannot nominate reviewers. Only reviewers randomly selected from our database with specialization in the subject area will be contacted to evaluate the manuscripts. The process will be blind review.
Decisions will be made as rapidly as possible, and the journal strives to return reviewers' comments to authors as fast as possible. The editorial board will re-review manuscripts that are accepted pending revision. It is the goal of the AJFS to publish manuscripts within weeks after submission.

Regular articles

All portions of the manuscript must be typed double-spaced and all pages numbered starting from the title page.

The Title should be a brief phrase describing the contents of the paper. The Title Page should include the authors' full names and affiliations, the name of the corresponding author along with phone, fax and E-mail information. Present addresses of authors should appear as a footnote.

The Abstract should be informative and completely self-explanatory, briefly present the topic, state the scope of the experiments, indicate significant data, and point out major findings and conclusions. The Abstract should be 100 to 200 words in length.. Complete sentences, active verbs, and the third person should be used, and the abstract should be written in the past tense. Standard nomenclature should be used and abbreviations should be avoided. No literature should be cited.
Following the abstract, about 3 to 10 key words that will provide indexing references should be listed.

A list of non-standard **Abbreviations** should be added. In general, non-standard abbreviations should be used only when the full term is very long and used often. Each abbreviation should be spelled out and introduced in parentheses the first time it is used in the text. Only recommended SI units should be used. Authors should use the solidus presentation (mg/ml). Standard abbreviations (such as ATP and DNA) need not be defined.

The Introduction should provide a clear statement of the problem, the relevant literature on the subject, and the proposed approach or solution. It should be understandable to colleagues from a broad range of scientific disciplines.

Materials and methods should be complete enough to allow experiments to be reproduced. However, only truly new procedures should be described in detail; previously published procedures should be cited, and important modifications of published procedures should be mentioned briefly. Capitalize trade names and include the manufacturer's name and address. Subheadings should be used. Methods in general use need not be described in detail.

Results should be presented with clarity and precision. The results should be written in the past tense when describing findings in the authors' experiments. Previously published findings should be written in the present tense. Results should be explained, but largely without referring to the literature. Discussion, speculation and detailed interpretation of data should not be included in the Results but should be put into the Discussion section.

The Discussion should interpret the findings in view of the results obtained in this and in past studies on this topic. State the conclusions in a few sentences at the end of the paper. The Results and Discussion sections can include subheadings, and when appropriate, both sections can be combined.

The Acknowledgments of people, grants, funds, etc should be brief.

Tables should be kept to a minimum and be designed to be as simple as possible. Tables are to be typed double-spaced throughout, including headings and footnotes. Each table should be on a separate page, numbered consecutively in Arabic numerals and supplied with a heading and a legend. Tables should be self-explanatory without reference to the text. The details of the methods used in the experiments should preferably be described in the legend instead of in the text. The same data should not be presented in both table and graph form or repeated in the text.

Figure legends should be typed in numerical order on a separate sheet. Graphics should be prepared using applications capable of generating high resolution GIF, TIFF, JPEG or Powerpoint before pasting in the Microsoft Word manuscript file. Tables should be prepared in Microsoft Word. Use Arabic numerals to designate figures and upper case letters for their parts (Figure 1). Begin each legend with a title and include sufficient description so that the figure is understandable without reading the text of the manuscript. Information given in legends should not be repeated in the text.

References: In the text, a reference identified by means of an author‘s name should be followed by the date of the reference in parentheses. When there are more than two authors, only the first author‘s name should be mentioned, followed by ’et al‘. In the event that an author cited has had two or more works published during the same year, the reference, both in the text and in the reference list, should be identified by a lower case letter like ’a‘ and ’b‘ after the date to distinguish the works.

Examples:

Abayomi (2000), Agindotan et al. (2003), (Kelebeni, 1983), (Usman and Smith, 1992), (Chege, 1998; 1987a,b; Tijani, 1993,1995), (Kumasi et al., 2001)
References should be listed at the end of the paper in alphabetical order. Articles in preparation or articles submitted for publication, unpublished observations, personal communications, etc. should not be included in the reference list but should only be mentioned in the article text (e.g., A. Kingori, University of Nairobi, Kenya, personal communication). Journal names are abbreviated according to Chemical Abstracts. Authors are fully responsible for the accuracy of the references.

Examples:

Chikere CB, Omoni VT and Chikere BO (2008). Distribution of potential nosocomial pathogens in a hospital environment. Afr. J. Biotechnol. 7: 3535-3539.

Moran GJ, Amii RN, Abrahamian FM, Talan DA (2005). Methicillinresistant Staphylococcus aureus in community-acquired skin infections. Emerg. Infect. Dis. 11: 928-930.

Pitout JDD, Church DL, Gregson DB, Chow BL, McCracken M, Mulvey M, Laupland KB (2007). Molecular epidemiology of CTXM-producing Escherichia coli in the Calgary Health Region: emergence of CTX-M-15-producing isolates. Antimicrob. Agents Chemother. 51: 1281-1286.

Pelczar JR, Harley JP, Klein DA (1993). Microbiology: Concepts and Applications. McGraw-Hill Inc., New York, pp. 591-603.

Short Communications

Short Communications are limited to a maximum of two figures and one table. They should present a complete study that is more limited in scope than is found in full-length papers. The items of manuscript preparation listed above apply to Short Communications with the following differences: (1) Abstracts are limited to 100 words; (2) instead of a separate Materials and Methods section, experimental procedures may be incorporated into Figure Legends and Table footnotes; (3) Results and Discussion should be combined into a single section.
Proofs and Reprints: Electronic proofs will be sent (e-mail attachment) to the corresponding author as a PDF file. Page proofs are considered to be the final version of the manuscript. With the exception of typographical or minor clerical errors, no changes will be made in the manuscript at the proof stage.

Fees and Charges: Authors are required to pay a $550 handling fee. Publication of an article in the African Journal of Plant Science is not contingent upon the author's ability to pay the charges. Neither is acceptance to pay the handling fee a guarantee that the paper will be accepted for publication. Authors may still request (in advance) that the editorial office waive some of the handling fee under special circumstances

African Journal of Plant Science

Table of Content: Volume 8 Number 3, March 2014

ARTICLES

academicJournals

Vol. 8(3), pp. 140-146, March 2014
DOI: 10.5897/AJPS2013.1137
Article Number: DD4872C46906
ISSN 1996-0824

http://www.academicjournals.org/AJPS

African Journal of Plant Science

Full Length Research Paper

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

Tewodros Mesfin[1], Jibril Mohammed[1], Abuhay Taklete[1], Feyera Merga[1] and Charles Wortmann[1,2]*

[1]Melkassa Agriculture Research Center-EARI, P. O. Box 436, Adama Ethiopia
[2]University of Nebraska-Lincoln, 369 Keim Hall, Lincoln EN 68583-0915, Ethiopia.

Received 24 November, 2013; Accepted 20 February, 2014

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

*Corresponding author. E-mail: cwortmann2@unl.edu.

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

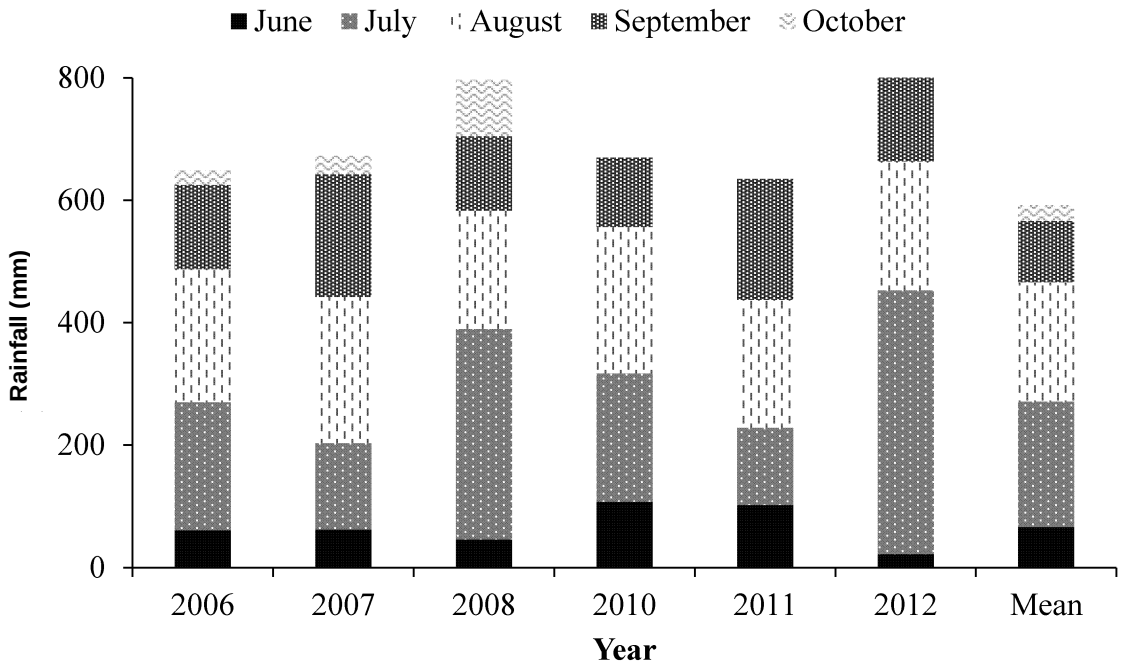

Figure 1. Growing season (May to October) monthly rainfall for trials conducted to evaluate skip-row planting and tie-ridging effects on maize in central Ethiopia.

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 m^2) 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 ($\overline{X}$ = 81 in 2006-7 and $\overline{X}$ = 69 in 2008 and 2010) and days to physiological maturity ($\overline{X}$ = 135 in 2006-7 and $\overline{X}$ = 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

Table 1. Maize performance as affected by tie-ridging and skip-row planting in the Central Rift Valley of Ethiopia. PF, P1:S1, P2:S1B and P2:S1, and P2:S2 are planting configurations with all rows planted, single planted and skipped rows alternated, two planted alternated with one skipped row with and without bean planted in the skipped row, and planted with skipped pairs of rows alternated, respectively.

Factor level	Plant height (cm)	Ear plant^{-1}	Kernel ear^{-1}	100 kernel weight (g)	Grain yield (Mg ha^{-1})	Stover yield (Mg ha^{-1})	HI
2006 and 2007; three row configurations‡							
Year							
2006	242		363	23.6	3.96	4.89	0.42
2007	241	0.90	347	23.6	4.78	3.72	0.53
Tillage							
Flat tillage	236	0.88	359	21.6	3.81	4.27	0.44
Tie-ridge	246	0.92	350	25.6	4.93	4.34	0.50
	**	ns	ns	*	*	Ns	**
Row configuration							
PF	246	0.92	339	25.2	4.60a	4.78a‡	0.47
P1:S1	238	0.91	374	23.9	4.39ab	4.29ab	0.49
P2:S2	240	0.88	352	21.7	3.50b	3.84b	0.46
	Ns	ns	ns	ns	*	**	Ns
2008 and 2010; four row configurations§							
Year							
2008	286	0.87	419	25.9	3.87	6.22	0.37
2010	207	0.87	200	25.4	2.74	6.77	0.27
Tillage							
Flat tillage	240	0.84	304	24.2	2.65	5.83	0.30
Tie-ridge	252	0.90	315	27.3	3.96	7.16	0.34
	*	**	ns	**	***	***	*
Row configuration							
PF††	252	0.89a	332	26.0	3.63a	6.91a	0.33
P1:S1	252	0.90a	302	26.8	3.48a	6.36ab	0.33
P2:S2	244	0.83b	284	23.6	2.62b	5.58b	0.31
P2:S1	240	0.86ab	321	26.2	3.51a	7.13a	0.31
	ns	*	ns	ns	***	**	ns

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.

row compared with planting all rows.

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

Factor level	Plant height (cm)	Ear (m^{-2})	Kernel (ear^{-1})	Kernel weight (mg)	Grain yield (Mg ha^{-1})	Stover yield (Mg ha^{-1})	HI
Maize							
2010		5.53	219	247	3.09	7.84	0.26
2011	202	6.38	273	218	3.87	7.05	0.33
2012	181	6.36	236	213	3.37	6.81	0.29
Planting (P)	ns	ns	ns	ns	ns	ns	ns
Year x P	ns	ns	*	ns	*	ns	***
Sorghum	**Plant height (cm)**	**Panicle $plant^{-1}$**	**Kernel $panicle^{-1}$**	**Kernel weight (mg)**	**Grain yield (Mg ha^{-1})**	**Stover yield (Mg ha^{-1})**	**HI**
2010		6.10	960	38.3	2.25	5.71	0.28
2011	161	5.76	1229	36.1	2.61	8.20	0.23
2012	150	6.39	1684	29.0	3.07		
Planting	ns	ns	ns	ns	ns	ns	ns
Year x P	ns	ns	*	ns	ns	ns	ns
Bean		**Pod $plant^{-1}$**	**Kernel pod^{-1}**	**Kernel weight (mg)**	**Grain yield (Mg ha^{-1})**	**Stover yield (Mg ha^{-1})**	**HI**
2010		23.3	4.93	188	1.65	2.33	0.39
2011		14.9	6.59	175	0.93	0.56	0.62
2012				158	1.68		
Planting		ns	ns	ns	***	***	ns
Year x P		ns	ns	*	**	ns	ns

ns,*, *, **, ***: Not significant and significant at P = 0.05, 0.01, and 0.001, respectively.

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 yield. 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 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.

Factor level	2010	2011	2012	2010	2011	2012
	Maize yield (Mg ha^{-1})			Maize kernel (ear^{-1})		
PF	2.77^{c}	4.38^{a}	3.84^{ab}	193^{c}	299^{a}	286^{ab}
P2:S1	3.46^{bc}	3.37^{bc}	2.90^{bc}	251^{abc}	226^{abc}	220^{bc}
P2:S1 with bean	3.03^{bc}	3.85^{ab}	3.32^{bc}	213^{bc}	296^{a}	203^{c}
	Maize HI			Sorghum panicle (m^{-2})		
PF	0.23^{c}	0.42^{a}	0.31^{b}	5.76^{bc}	5.74^{bc}	6.38^{ab}
P2:S1	0.31^{b}	0.31^{b}	0.31^{b}	6.55^{a}	5.53^{c}	6.38^{ab}
P2:S1 with bean	0.30^{b}	0.32^{b}	0.29^{bc}	6.00^{bc}	6.00^{bc}	6.42^{ab}
	Bean grain yield (Mg ha^{-1})			Bean kernel weight (mg)		
Bean, sole crop	3.11^{a}	1.49^{b}	2.83^{a}	180^{ab}	189^{a}	157^{c}
P2:S1, maize	0.63^{d}	0.74^{cd}	0.71^{cd}	191^{a}	167^{bc}	163^{bc}
P2:S1, sorghum	1.23^{bc}	0.57^{d}	0.72^{cd}	193^{a}	170^{bc}	156^{c}

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.

was 1.42, 1.43, and 1.01 in 2010, 2011, and 2012, respectively. The sorghum:bean land equivalent ratio was 1.19, 153, 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-ridging in two of four years. Tie-ridging 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-ridging 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- ridging 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

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-ridging 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

This is a contribution of the Ethiopia Institute of Agricultural Research and the University of Nebraska-Lincoln Agricultural Research Division. The research was partly funded by the Hatch Act and by the U.S. Agency for International Development through INTSORMIL under the terms of Grant No. LAG-G-00-96-900009-00.

REFERENCES

Abunyewa AA, Ferguson RB, Wortmann CS, Lyon DJ, Mason SC, Irmak S, Klein RN (2011). Grain sorghum water use with skip-row configuration in the Central Great Plains of the USA. Afr. J. Agric. Res. 6:5328-5338.

Abunyewa AA, Ferguson RB, Wortmann CS, Lyon DJ, Mason SC, Klein RN (2010). Skip-row configuration and plant population effects on sorghum grain yield and yield stability in the Central Great Plains. Agron. J. 102:296-302.

Bhatnagar GS, Chaplot PC (1991). Evaluation of intercropping of winter maize with legumes. Int. J. Trop. Agric. 9:52-55.

Brhane G, Wortmann CS (2008) Tie-ridge tillage for high altitude pulse production in northern Ethiopia. Agron. J. 100:447-453.

Brhane G, Wortmann CS, Mamo M, Gebrekidan H, Belay A (2006). Micro-basin tillage for grain sorghum production in semi-arid areas of northern Ethiopia. Agron. J. 98:124-128.

Clark LE, Knight TO (1996). Grain production and economic returns from dryland sorghum in response to tillage systems and planting patterns in the semi-arid southwestern USA. J. Prod. Agric. 9:249-256.

Gebrekidan H (2003). Grain yield response of sorghum (*Sorghum bicolor*) to tied ridges and planting methods on entisols and vertisols of Alemaya area, Eastern Ethiopia Highlands. J. Agric. Rural Dev. Trop. Sub Trop. 104:113-128.

Gusha AC (2002). Effects of tillage on soil micro relief, surface depression storage and soil water storage. Soil Till. Res. 76:105-114.

Klein R, Lyon D, Baltensperger D, Pavlista A, Shapiro C, Knezveic S, Mason S, Nelson L, Bernards M, Elmore R, Schlegel A, Vigil M, Klocke N, Melvin S. (2007). What is required to have success with rainfed skip-row corn. 64-1 In: 2007 Agronomy Abstracts. ASA Madison WI

Lal R (1977). Soil management systems and erosion control. In: Greenland D, Lal R (eds) Soil conservation and management in the humid tropics. John Wiley and Sons, Chichester NY, pp. 81-86.

Lyon DJ, Pavlista AD, Hergert GW, Klein RN, Shapiro CA, Knezevic S, Mason SC, Nelson LA, Baltensperger DD, Elmore RW, Schlegel AJ, Olson BL, Aiken RM (2009). Skip-row planting patterns stabilize corn grain yields in the central Great Plains. Crop Manage http://www.plantmanagementnetwork.org/pub/cm/research/2009/skip/ . Accessed 18 Mar 2013

Mesfin T, Tesfahunegn GB, Wortmann CS, Mamo M, Nikus O (2010). Skip-row planting and tie-ridging for sorghum production in semi-arid areas of Ethiopia. Agron. J. 102:745-750.

Mesfin T, Tesfahunegn GB, Wortmann CS, Nikus O, Mamo M (2009). Tie-ridging and fertilizer use for sorghum production in semi-arid Ethiopia. Nutr. Cycl. Agroecosyst. 85:87-94.

Milroy SP, Bange MP, Hearn AB (2004). Row configuration in rainfed cotton systems: modification of the OZCOT simulation model. Agric. Syst. 82:1-16.

Miriti JM, Esilaba AO, Bationo A, Cheruiyot H, Kihumba J, Thuranira EG (2007). Tie-riding and integrated nutrient management options for sustainable crop production in semi-arid eastern Kenya. In Bationo A, Waswa B, Kihara J, Kimetu J. (eds) Advances in integrated soil fertility management in sub-Saharan Africa: challenges and opportunities. Springer, Dordrecht, the Netherlands, pp. 435-441.

Myers RJK, Foale MA, Thomas GA, French AV, Hall B (1986). How row spacing affects water use and root growth of grain sorghum. Proc First Australian Sorghum Conf, Gatton, pp. 586-591.

Nielsen DC, Vigil MF, Caldreon FJ, Schneekloth J, Poss D, Henry WB, Benjamin JG (2007). Water extraction patterns in skip-row corn, sorghum and sunflower. 305-2. In 2007 Agronomy Abstracts. ASA, Madison, WI.

Pendke MS, Lomte MH, Gitte AU (2004). Effect of soil and water conservation practices on runoff, soil loss and yield of pigeonpea. J. Maharashtra Agric. Univ. 29:319-321.

Spackman GB, McCosker KJ, Farquharson AJ, Conway MJ (2000). Innovative management of grain sorghum in Central Queensland. Proc. Aust. Agron. Conf. Australian Soc. Accessed 3 March 2014. http://www.regional.org.au/au/asa/2001/1/a/spackman.htm

Vigil MF, Henry B, Calderon FJ, Nielsen DC, Benjamin JG, Klein B (2008). A use of skip-row planting as a strategy for drought mitigation in the west central Great Plains. Proc. Great Plains Soil Fertility Conf., Denver CO. 4-5 Mar. 2008. pp. 101-106.

Whish J, Butler G, Castor M, Cawthray S, Broad I, Carberry P, Hammer G, McLean G, Routley R, Yeates S (2005). Modeling the effects of row configuration on sorghum yield reliability in north-eastern Australia. Aust. J. Ag. Res. 56:11-23.

Workayehu T, Wortmann CS (2011). Maize-bean intercrop suppression of weeds and profitability in southern Ethiopia. Agron. J. 104:1058-1063.

Wortmann CS, Mamo M, Mburu C, Letayo E, Abebe G, Kayuki KC, Chisi M, Mativavarira M, Xerinda S, Ndacyayisenga T (2009). Atlas of sorghum (*Sorghum bicolor* (L.) Moench) production in eastern and southern Africa http://intsormil.org/smscientificpubs/Sorghum%20Atlas.pdf Accessed 6 June 2013.

academicJournals

Vol. 8(3), pp. 147-XX, March 2014
DOI: 10.5897/ AJPS2013.1121
Article Number: 45F1AC046907
ISSN 1996-0824

http://www.academicjournals.org/AJPS

African Journal of Plant Science

Full Length Research Paper

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

Zhao Zhichang*, Chen Yeyuan, Gao Aiping, Huang Jianfeng and Dang Zhiguo

Tropical Crops Genetic Resources Institute, Chinese Academy of Tropical Agricultural Sciences/Key Laboratory of Crop Gene Resources and Germplasm Enhancement in Southern China, Ministry of Agriculture, Danzhou 571737, P. R. China.

Received 11 October, 2013; Accepted 17 February, 2014

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; Akihisa 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

*Corresponding author. E-mail: zhaozhichang2001@163.com or chenyy1962@126.com

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 Fe^{2+}, 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 grow 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, fragrance, taste, and heath 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 un-ripe, but turns into golden yellow, bright yellow or orange-red when ripen 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 $(T)_{18}$ 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’ AAGTACATCCATCCAGTCATYTT ’3); reverse primer (5’ GAATCCAAGGATCCYGAGAAYGA 3’). The PCR reaction was carried out using 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 gotten 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 phages 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’ ATTATGGCAGTGTTATCAATCGGGT’3, *ANS*-R 5’ GGGAGGAGCGTGGAAAGTCGTCGTA’3. As a positive control, *actin7* fragment was amplified under the same RT-PCR conditions, using the primer pair: *Actin7*-F 5’AATGGAACTGGAATGGTCAAGGC’3, *Actin7*-R5’ TGCCAGATCTTCTCCATGTCATCCCA ’3.

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 with 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 anthocyanidin 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

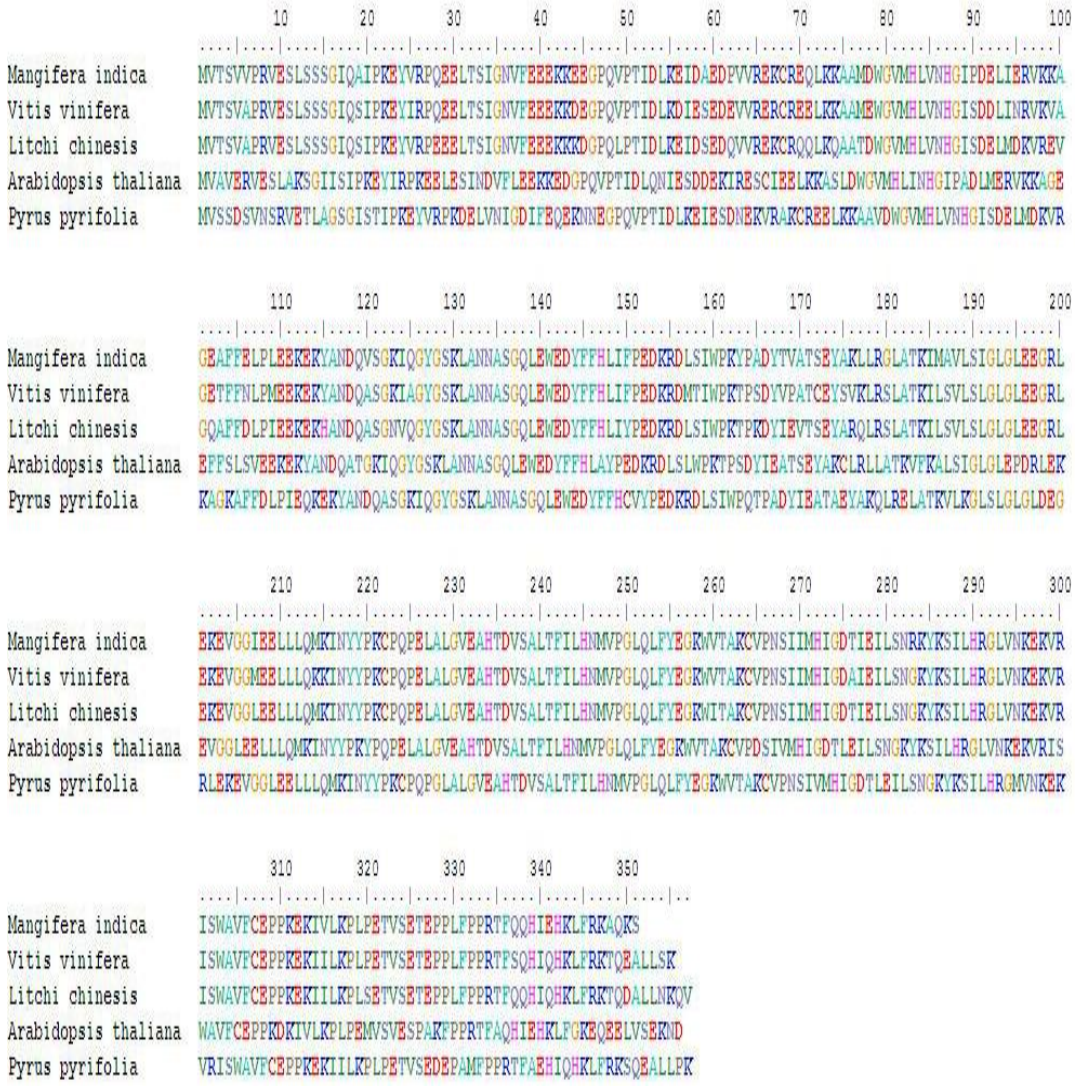

Figure 1. Amino acid sequence of ANS comparison between mango and other species. Sequence analysis of amino acid region proposed to determine specificity.

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 anthocyaninin 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

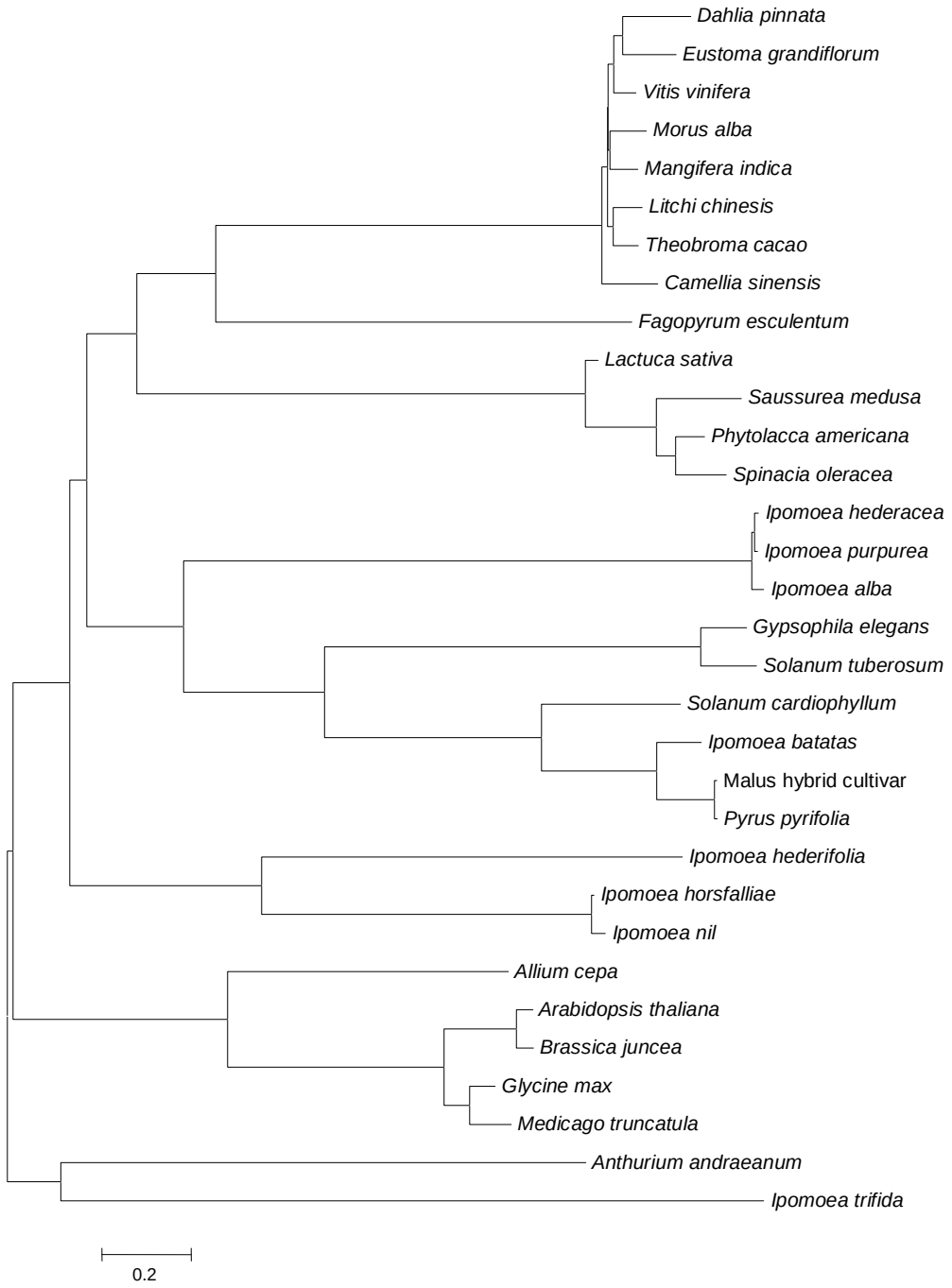

Figure 2. Phylogenetic relationship of ANS. The tree was constructed using MEGA (version 5.0). *Vitis vinifera* (ABV82967.1), *Gypsophila elegans* (AAP13054.1), *Glycine max* (AAR26525.1), *Ipomoea batatas* (ACT31916.1), *Arabidopsis thaliana* (NM_118417.1), *Anthurium andraeanum* (ABK76317.1), *Camellia sinensis* (AAV88087.1), *Allium cepa* (ABR24157.1), *Ipomoea batatas* (ABM63373.1), *Dahlia pinnata* (BAJ21536.1), *Brassica juncea* (ACH58398.1), *Ipomoea hederifolia* (BAK78919.1), *Solanum tuberosum* (AEJ90548.1), *Fagopyrum esculentum* (ADT63066.1), *Pyrus pyrifolia* (ADP09379.1), *Ipomoea nil* (BAB71811.1), *Malus* hybrid cultivar (ACP30363.1), *Solanum tuberosum (*ADP37440.1), *Eustoma grandiflorum* (BAJ08929.2), *Ipomoea trifida* (ACF59729.1), *Lactuca sativa* (BAJ10383.1), *Saussurea medusa* (AAS48200.1), *Ipomoea batatas* (ADE08370.1), *Theobroma cacao* (ADD51356.1), *Ipomoea purpurea* (AAP82030.1), *Glycine max* (ABY51685.1), *Medicago truncatula* (ABU40983.1), *Allium cepa* (ABM66367.1), *Phytolacca americana* (BAE54521.1), *Spinacia oleracea* (BAE54520.1), *Brassica juncea* (ACH58397.1), *Solanum cardiophyllum* (AEJ90547.1), *Ipomoea horsfalliae* (ACS71531.1), *Ipomoea alba* (AAP82018.1), *Morus alba* (AEN55613.2) and *Litchi chinensis (*HQ402913.1).

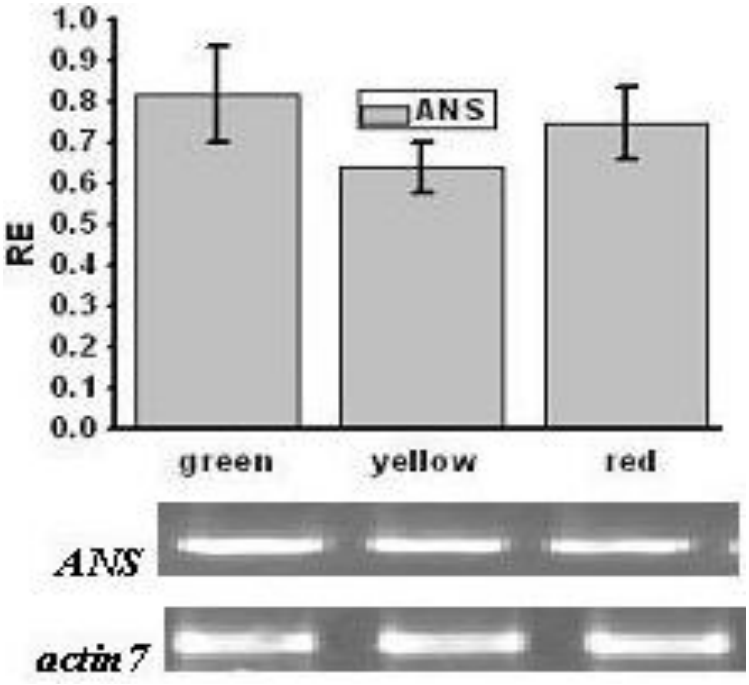

Figure 3. Expression of *ANS* was measured by RT-PCR at three developmental stages. Expression of *ANS* was the highest in the green stage, and the lowest in the yellow stage. Values are the average ±SD of three replicant.

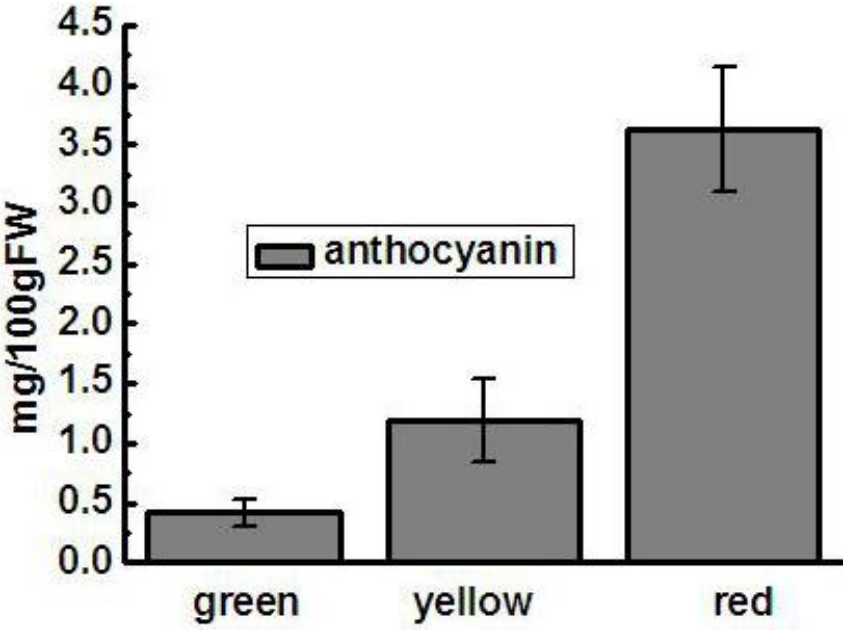

Figure 4. Total anthocyanin concentration in the three stages of mango peel.

anthocyanin in the 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

We wish to thank He Junhu, Zhu Min and Chen Huarui in Tropical Crops Genetic Resources Institute, Chinese Academy of Tropical Agricultural Sciences. This work was supported by “the Fundamental Scientific Research Funds for CATAS-TCGRI” (Project No. 1630032013003), and “The Non-Profit Scientific Research Institution Reform Started Funding Projects for CATAS” (pzs-201225).

REFERENCES

Akihisa T, Tokuda H, Ukiya M, Iizuka M, Schneider S, Ogasawara K, Mukainaka T, Iwatsuki K, Suzuki T, Nishino H (2003). Chalcones, coumarins, and flavanones from the exudate of Angelica keiskei and their chemopreventive effects. Cancer Lett. 201:133-137.

Grotewold E (2006). The genetics and biochemistry of floral pigments. Annu. Rev. Plant Biol. 57:761-780.

Harborne JB, Williams CA (2000). Advances in flavonoid research since 1992. Phytochem. 55:481-504.

Holton TA, Cornish EC (1995). Genetics and Biochemistry of anthocyanin biosynthesis. Plant Cell 7:1070-1083.

Lesnick ML, Chandler VL (1998). Activation of the maize anthocyanin gene a2 is mediated by an element conserved in many anthocyanin promoters. Plant Physiol. 117:437-445.

Mill.) waste. J. Agric. Food Chem. 52:1890-1897.

Mol J, Grotewold E, Koes R (1998). How genes paint flowers and seeds. Trends Plant Sci. 3:212-217.

Parejo I, Viladomat F, Bastida J, Schmeda-Hirschmann G, Burillo J, Codina C (2004). Bioguided isolation and identification of the nonvolatile antioxidant compounds from fennel (*Foeniculum vulgare*

Pool-Zobel BL, Bub A, Schro¨ der N, Rechkemmer G (1999). Anthocyanins are potent antioxidants in model systems but do not reduce endogenous oxidative DNA damage in human colon cells. Eur. J. Nutr. 38:227-234.

Saito K, Kobayashi M, Gong Z, Tanaka Y, Yamazaki M (1999). Direct evidence for anthocyanidin synthase as a 2-oxoglutarate-dependent oxygenase: molecular cloning and functional expression of cDNA from a red forma of Perilla frutescens. Plant J. 17:181-189.

Turnbull JJ, Nagle MJ, Seibel JF, Welford RWD, Grant GH, Schofield CJ (2003). The C-4 stereochemistry of leucocyanidin substrates for anthocyanidin synthase affects product selectivity. Bioorg. Med. Chem. Lett. 13:3853-3857.

Welford RWD, Turnbull JJ, Claridge TDW, Schofield CJ, Prescott AG (2001). Evidence for oxidation at C-3 of the flavonoid C-ring during anthocyanin biosynthesis. Chem. Commun. 12:1828-1829.

Wilmouth RC, Turnbull JJ, Welford RWD, Clifton IJ, Prescott AG, Schofield CJ (2002). Structure and mechanism of anthocyanidin synthase from Arabidopsis thaliana. Structure. 10:93-103.

academicJournals

Vol. 8(3), pp. 153-163, March 2014
DOI: 10.5897/AJPS2014.1164
Article Number: A60BE3246908
ISSN 1996-0824

http://www.academicjournals.org/AJPS

African Journal of Plant Science

Full Length Research Paper

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

D. K. Okello[1*], L.B. Akello[1], P. Tukamuhabwa[2], Odong, T.L[2]., M, Ochwo-Ssemakula[2] , J. Adriko[3] and C. M. Deom[4]

[1]National Semi-Arid Resources Research Institute, P.O Private Bag Soroti, Uganda.
[2]Department of Production, School of Agricultural Sciences, Makerere University, P.O. Box 7062, Kampala, Uganda.
[3]Department of Pathology, University of Georgia, Miller Plant Sciences Building, Athens 30602.
[4]Department of Pathology at the University of Georgia, Miller Plant Sciences Building, Athens 30602.

Received 12 February, 2014; Accepted March 14, 2014

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

*Corresponding author. E-mail: kod143@gmail.com.

Table 1. Regions/districts surveyed.

Region	Districts	Freq	%
North	Arua, Lira, Nwoya, Nebbi, Oyam, Pader	40	34
Central	Lyantonde, Mukono	8	7
East	Bugiri, Bukedea, Busia, Iganga, Jinja, Kaberamaido,Mbale, Soroti, Tororo, Namutumba	42	36
West	Hoima, Isinygiro, Masindi, Mbarara, Rubirizi	28	24
Total		118	100

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 (Murat 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 ICRISAT 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 2. Gender.

Gender of farmers	%				
	General	North	Central	East	West
Female	52	25	50	61	71
Male	48	75	50	39	29
Total	100	100	100	100	100

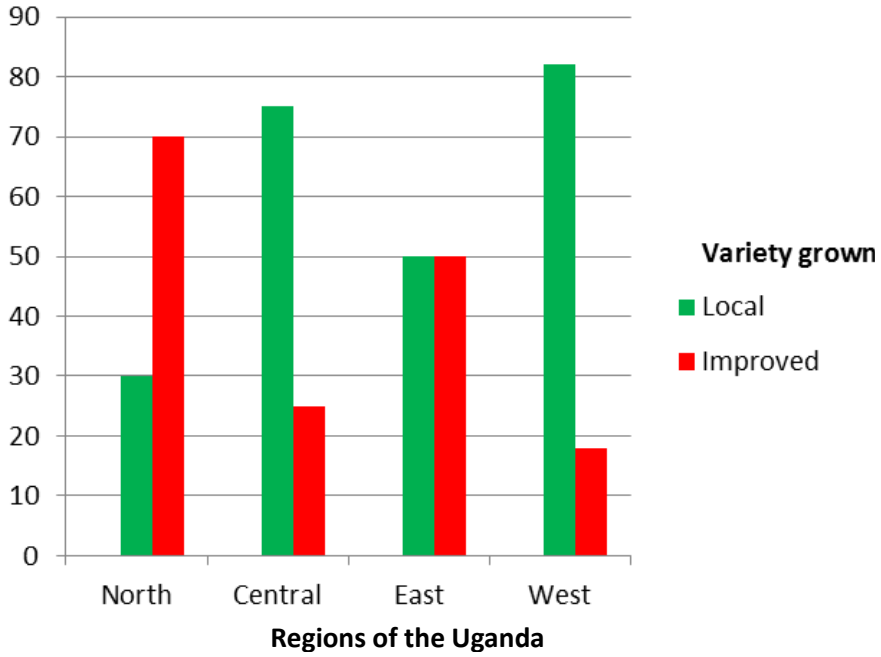

Figure 1. Varieties grown.

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 3. Seed source.

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

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

Table 4. Growth stage.

Growth stage	%				
	General	North	Central	East	West
Past 50% anthesis	62.7	82.5	62.5	74	18
Physiological maturity	37.3	17.5	37.5	26	82
Total	100	100	100	100	100

Table 5. Previous crop.

Percentage	%				
	General	North	Central	East	West
Bananas	1	0	13	0	0
Legumes	18	10	38	19	21
Vegetables	3	2.5	0	0	7
Tubers	30	30	13	50	4
Cotton	3	5	0	0	4
Cereal	36	40	38	24	50
Simsim	4	10	0	2	0
Sunflower	1	0	0	2	0
Virgin land	5	2.5	0	2	14
Total	100	100	100	100	100

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

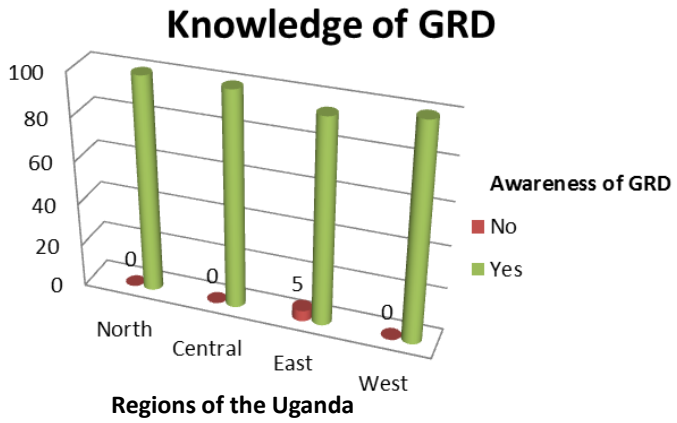

Figure 2. Knowledge of GRD.

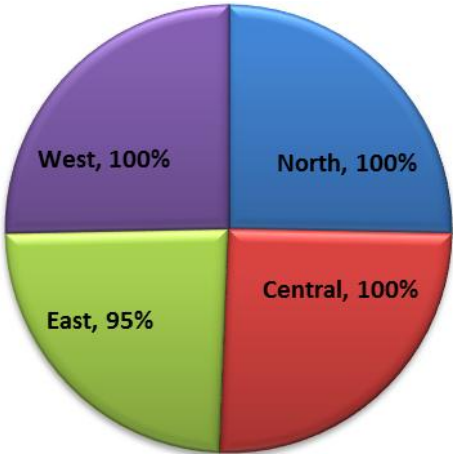

Figure 3. See GRD in the field. The percentage is calculated based on 118 respondents (farmers).

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.

Rosette symptom types	%				
	General	North	Central	East	West
Green	95	97.5	100	98	86
Yellow	5	2.5	0	2	14
Total	100	100	100	100	100

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

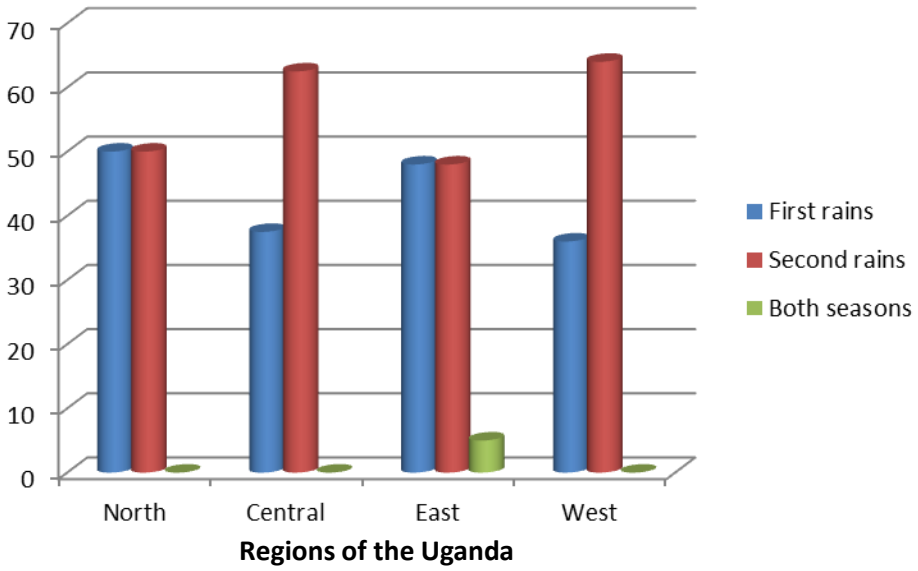

Figure 5. Season GRD is common.

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.

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

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

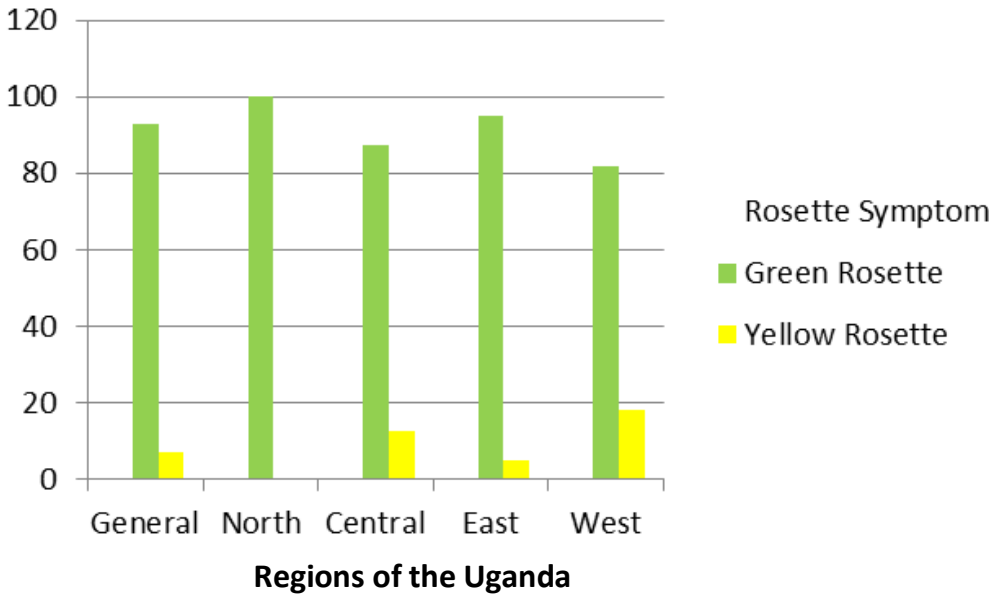

Figure 7. Predominant GRD symptom type observed in the field by the team.

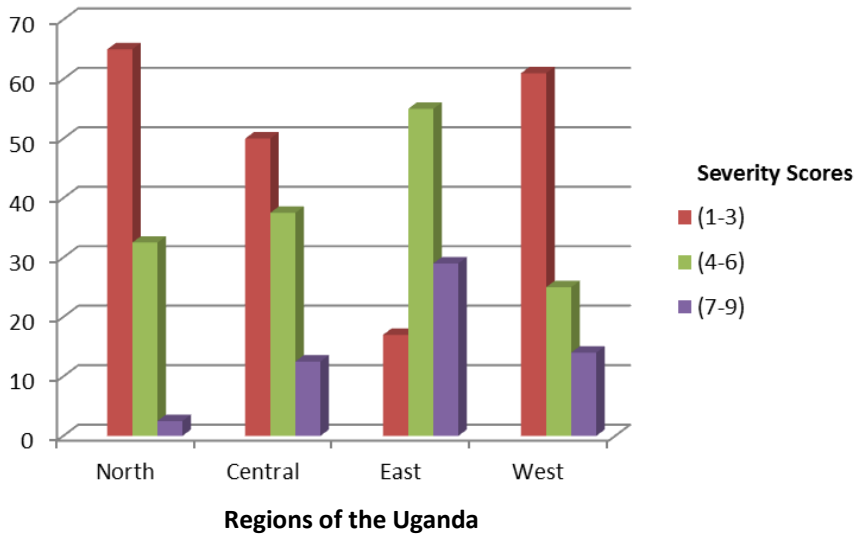

Figure 8. GRD severity. Resistant = (1-3); moderate resistant = (4-6); susceptible = (7-9).

Table 8. Analysis of association between the presence of rosette in the field, knowledge of rosette and other factors.

Row factor	Column factor	Pearson chi-square value, degree of freedom	P-value	Significant association
Presence/absence of Rosette in the field	Management of Rosette	61.73 df =10	<0.001	Yes
	Source of seed	8.08 df = 8	0.426	No
	Variety grown	2.67 df =3	0.445	No
	Previous crop in the field	15.42 df=22	0.843	No
	Gender	1.90 df =1	0.168	No
	Knowledge of Rosette disease	0.04 df =1	0.851	No
	Stage of crop growth	1.21 df =2	0.546	No
	Variety grown	2.67 df =3	0.445	No
	Season	0.05 df =2	0.975	No
	Most common rosette symptom	0.11 df =1	0.741	No
Knowledge of rosette disease	Previous crop in the field	8.40 df =22	0.996	No
	Management of Rosette disease	2.82 df =10	0.985	No
	Gender	1.90 df =1	0.168	No
	Source of seeds	4.96 df =8	0.762	No
	Variety grown	0.11 df =3	0.991	No

grown and as well as the gender of the farmer.

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

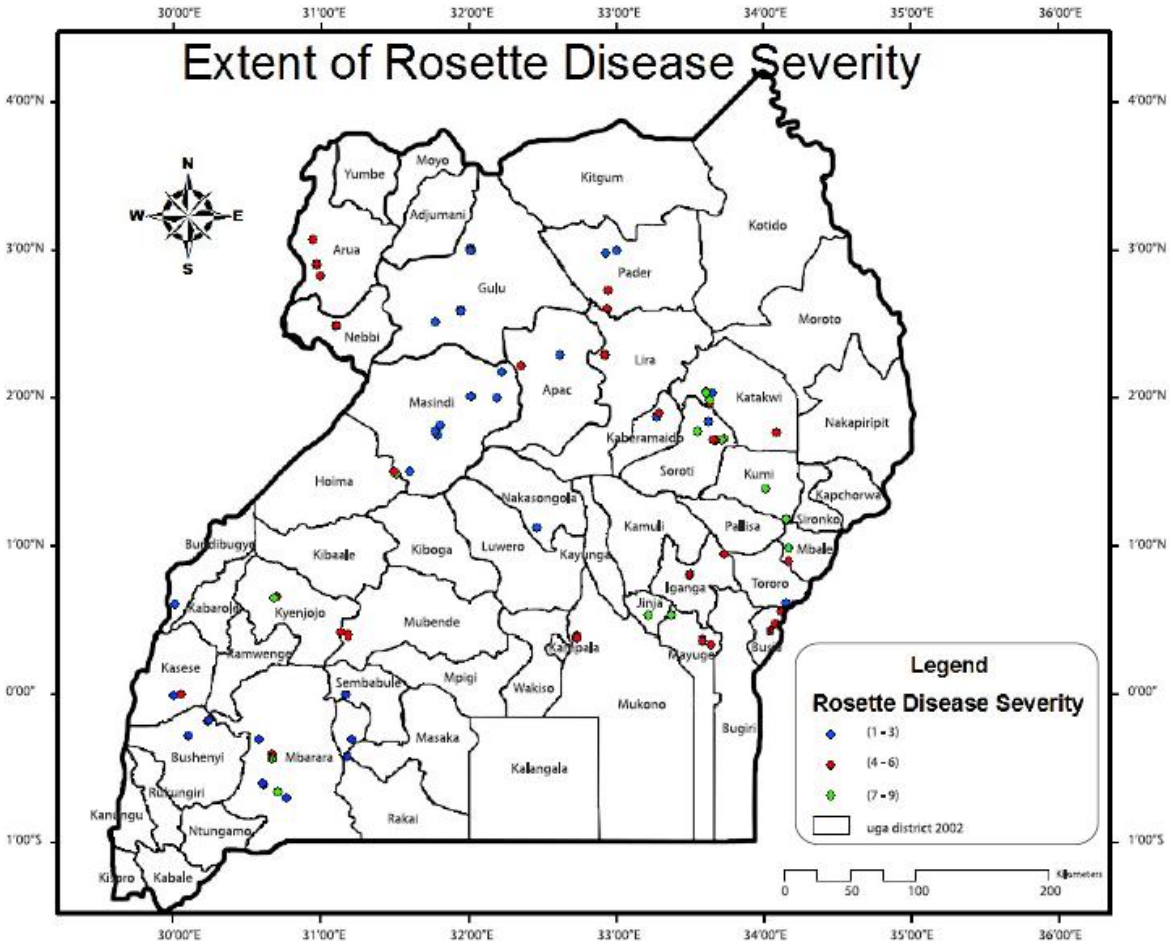

Figure 9. The GRD severity distribution.

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 Ikilki 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 predominates. 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 sat 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 eco-niches are thus 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

The authors are grateful for the financial support from Uganda Millennium Science Initiative (MSI) and the Peanut Collaborative Research Support Program of USAID for funding this study.

REFERENCES

Deom CM, Naidu RA, Chiyembekeza AJ, Ntare BR, Subrahmanyam P (2000). Sequence diversity within the three agents of groundnut rosette disease. Phytopathol. 90:214-219

Hayes RT (1932). Groundnut rosette disease in Gambia. Trop. Agric. 9:211-217.

Hull R (1964). Spread of Groundnut rosette virus by Aphis craccivora (Koch). Nature (Lond) 202:213-214.

Hull R, Adams AN (1968). Groundnut rosette and its assistor virus. Ann. Appl. Biol. 62:139-145.

Kaaya NA, Eigel W, Harris C (2006). Peanut Aflatoxin Levels on Farms and in Markets of Uganda. Peanut Sci. 33:68-75.

Misari SM, Ibrahim JM, Demski JMW, Ansa AO, Kuhn CW, Casper R, Breye E (1988). Aphid transmission of the viruses causing chlorotic rosette and green rosette diseases of peanut in Nigeria. Plant Dis. 72:250-253

Murant AF, Kumar IK (1990). Different variants of the satellite RNA of Groundnut rosette virus are responsible for the chlorotic and green forms of groundnut rosette disease. Ann. Appl. Biol. 117:85-92.

Murant AF, Rajeshwari R, Robinson DJ, Raschke JH (1988). A satellite RNA of groundnut rosette virus that is largely responsible for symptoms of groundnut rosette disease. J. Gen. Virol. 69:1479-1486.

Naidu RA, Bottenberg H, Subrahmanyam P, Kimmins F, Robinson DJ, and Thresh JM (1998). Epidemiology of groundnut rosette virus disease: Current status and future research needs. Ann. Appl. Biol. 132:525-548.

Naidu RA, Kimmins FM, Deom CM, Subrahmanyam P, Chiyeubekeza AJ, van der Merwe PJA (1999). Groundnut Rosette: A virus disease affecting groundnut production in sub-Saharan Africa. Plant Dis. 83:700-709.

Okello DK, Biruma M, Deom CM (2010). Overview of Groundnut research in Uganda: Past, Present and Future. Afr. J. Biotechnol. 9(39): 6448-6459, 27 September, 2010. Available online at http://www.academicjournals.org/AJB. ISSN 1684-5315 © 2010 Academic Journals

Okello DK, Monyo E, Deom CM, Ininda J, Oloka HK (2013). Groundnuts production guide for Uganda: Recommended practices for farmers. National Agricultural Research Organisation, Entebbe. ISBN: 978-9970-401-06-2

Olorunju PE, Ntare BR, Pande S, Reddy SV (2001). Additional sources of resistance to groundnut rosette disease in groundnut germplasm and breeding lines. Ann. Appl. Biol. 159:259-268.

Olorunju PE, Khun CW, Demski JW, Misari SM, Ansa OA (1991). Disease reactions and yield performance of peanut genotypes grown under groundnut rosette and rosette-free field environments. Plant Dis. 75:1269-1273.

Roossinck MJ (1997). Mechanism of plant virus evolution. Ann. Rev. Phytopathol. 35:191-209.

Savage GP, Keenan JI (1994). The composition and nutritive value of groundnut kernels. Pages 173-21 in: The Groundnut crop: Scientific basis for improvement. J. Smart, ed. Chapman and Hall, London.

Smartt J (1961). The diseases of groundnuts in Northern Rhodesia. Empire J. Exper. Agric. 29:79-87.

Storey HH, Bottomley AM (1928). Rosette disease of the peanut (*Arachis hypogaea* L.). Ann. Appl. Biol. 15:26-45.

Storey HH (1935). Virus Disease of East African Plants: III Rosette Disease of Groundnuts. East Afr. Agric. J. 1:206-211.

Subrahmanyam P, Hildebrand GL, Naidu RA, Reddy LJ, Singh AK (1998). Sources of resistance to groundnut rosette disease in global groundnut germplasm. Ann. Appl. Biol. 132:473-485.

van der Merwe PJA, Subrahmanyam P (1997) Screening of rosette

resistant short-duration groundnut breeding lines for yield and other characteristics. Int. Arachis. Newsl .17:23-24.
Waliyar F, Kumar PL, Ntare BR, Monyo E, Nigam SN, Reddy AS, Osiru M, Diallo AT (2007). A Century of Research on Groundnut Rosette Disease and its Management. Information Bulletin no. 75. Patancheru 502 324, Andhra Pradesh, India: International Crops Research Institute for the Semi-Arid Tropics. 40pp. ISBN 978-92-9066-501-4. Order code: IBE 075.
Wangai AW, Pappu SS, Pappu HR, Okoko N, Deom CM, Naidu RA (1999). First report of the green rosette variant of groundnut rosette disease in Kenya. Plant Dis. 83:782.
Yayock JY, Rossell HW, Harkness C (1976) A review of the 1975 groundnut rosette epidemic in Nigeria. Paper presented at the African Groundnut Council Symposium.

academicJournals

Vol. 8(3), pp. 164-166, March 2014
DOI: 10.5897/AJPS2013.1129
Article Number: 321DD2C46909
ISSN 1996-0824

http://www.academicjournals.org/AJPS

African Journal of Plant Science

Short Communication

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

Precious C. Nwachukwu and Olutayo M. Adedokun*

Department of Crop and Soil Science, Faculty of Agriculture, University of Port-Harcourt, East-West Road, Choba, P.M.B. 5323, River State, Nigeria.

Received 8 November, 2013; Accepted 27 March, 2014

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 (Afieroho 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 sub-strates, could both assist in solving afore mentioned

*Corresponding author. E-mail: olutayo.adedokun@gmail.com.

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

Treatment	Mycelia initiation (day)	Full mycelia run (day)	Sclerotia harvest (day)	Number of Sclerotia	Sclerotia fresh weight(g)
Sawdust	3.50[a]	42.00[ac]	117.00[a]	1.00[a]	4.25[a]
Sawdust and paper	4.00[a]	43.00[a]	87.80[a]	1.00[a]	4.25[a]
Paper and pumpkin	4.75[a]	29.00[b]	104.00[a]	1.00[a]	9.50[a]
LSD	1.9	11.8	81.0	0.7	5.3

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.

Sample	Protein (%)	Crude fiber (%)	Moisture (%)
Sawdust	10.063	9.639	57.778
Sawdust and Paper	8.75	38.02	41.270
Paper and Pumpkin	9.19	32.219	45.345

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 sub-strates.

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 (Rumuosi), fluted pumpkin stem was obtained from a close-by market (Choba), while mushroom spawns established on waste cotton were from Bezaleel 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.

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 ramify-cation 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.

REFERENCES

Adedokun OM, Fasidi IO, Ayodele VI (2003). Spawn production and growth of *Pleurotus tuber-regium* (Fries) Singers on agricultural wastes. Biosci. Res. Commun. 15 (6):437-444. BRC 2002014/15604.

Adedokun OM, Ataga AE (2007). Effects of amendments and bio-augmentation of soil polluted with crude oil, automotive gasoline oil and spent engine oil on the growth of cowpea (*Vigna ungiculata* L. Walp). Sci. Res. Essay 2(5):147-149. http://academicjournals.org/journal/SRE/article abstract/75F9B3D12559

Adenipekun CO, Fasidi IO (2005). Bioremediation of oil polluted soil by *Lentinus subnudus*, a Nigerian white rot fungus. Afr. J. Biotechnol. 4(8):796-798. http://www.ajol.info/index.php/ajb/article/view/15184

Afieroho OE, Lawson L, Adedokun OM, Emenyonu N (2013). Antituberculosis and phytochemical Investigation of the Dichloromethane Extract *Pleurotus tuber-regium* (Fries) singer Sclerotium.http://www.irjponline.com/admin/php/uploads/1609_pdf.pdf

Akanbi WB, Adeboye CO, Togun AO, Ogunrinde JO, Adeyeye SA (2007). Growth, herbage and seed yield and quality of *Telfaira occidentalis* as influenced by cassava peel compost and mineral fertilizer. World J. Agric Sci. 3(4):508-516.

Association of Official Analytical Chemistry. (2002). Official method of Analysis 17th Ed. Association of official chemists, Maryland.

Baysal E, Peker H, Yalinkili MK, Temiz A (2003). Cultivation of oyster mushroom on waste paper with some added supplementary materials. Bioresour. Technol. 89:95-97.

Chiejina NV, Olufokunbi IO (2010). Effects of different substrates on the yield and protein content of *Pleurotus tuber-regium*. Afr. J. Biotechnol. 9:1573-1577.

Chitamba J, Dube F, Chiota WM, Handiseni (2012). Evaluation of Substrate Productivity and Market Quality of Oyster Mushroom (*Pleurotus ostreatus*) Grown on Different Substrates. Int. J. Agric. Res. 7:100-106. http://dx.doi.org/10.3923/ijar.2012.100.106

Fasidi IO, Kadiri M (1993). Use of Agricultural Wastes for the Cultivation of *Lentinus subnudus* (*Polyporales Polyporaceae*) in Nigeria. Rev. Biol. Trop. 41:411-415.

Isikhuemhen SO, Lebauer DS (2004). Growing *Pleurotus tuber-regium.* Mushroom World Publication 11:264-274.

Meier E (2013). The Wood Database. http://www.wood-database.com/wood-articles/wood-allergies-and-toxicity/ (Accessed 09 March, 2013).

Mshandete AM, Cuff J (2007). Proximate and Nutrient Composition of Three Types of Indigenous Edible Wild Mushrooms Grown in Tanzania and Their Utilization Prospects. Afr. J. Food Agric. Nutr. Dev. 7:6.

Okhuoya JA, FO 0kogbo (1990). Cultivation of *Pleurotus tuber-regium* (Fries) Singer on Various Farm Wastes. Proc. Okla. Acad. Sci. 71:1-3.

Onuoha CI, Oyibo G, Ebibila J (2009). Cultivation of straw mushroom (Volvariella volvacea) using some Agro-Waste material. J. Am. Sci. 5(5):135-138.

Onyango BO, Palapala VA, Arama P, Wagai SO, Gichimu BM (2011). Suitability of selected supplemented substrates for cultivation of Kenyan native wood ear mushrooms (*Auricularia auricula*). Am. J. Food Technol. 6:395-403. http://dx.doi.org/10.3923/ajft.2011.395.403

Oso BA (1977). Pleurotus tuber-regium from Nigeria. Mycologia LXIX (2):271-279

Sassine YN, Y Ghora, M Kharrat, M Bohme, Abdel-Mawgoud AMR (2005). Waste Paper as an Alternative for Casing Soil in Mushroom (*Agaricus bisporus*) production. J. Appl. Sci. Res.1(3):277-284.

Stamets P (2000). Growing Gourmets and Medicinal Mushrooms. 3rd Ed. Ten Speed Press- Crown Publishing Group a Division of Random House Inc., New York. 635p.

Ukoima HN, Ogbonnaya LO, Arikpo GE, Ikpe FN (2009). Cultivation of mushroom (*Volvariella volvacea*) on various farm wastes in Obubra Local Government of Cross River State, Nigeria. Pak. J. Nutr. 8:1059-1061. http://www.pjbs.org/pjnonline/fin1239.pdf

African Journal of Plant Science

Related Journals Published by Academic Journals

- *International Journal of Plant Physiology and Biochemistry*
- *Journal of Botany and Plant Pathology*
- *African Journal of Food Science*
- *International Journal of Biodiversity and Conservation*
- *Journal of Yeast and Fungal Research*

academicJournals