African Journal of Plant Science

Volume 10 Number 4, April 2016
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.

Contact Us

Editorial Office: ajps@academicjournals.org

Help Desk: helpdesk@academicjournals.org

Website: http://www.academicjournals.org/journal/AJPS

Submit manuscript online http://ms.academicjournals.me/

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

Submission of a manuscript implies: that the work described has not been published before (except in the form of an abstract or as part of a published lecture, or thesis) that it is not under consideration for publication elsewhere; that if and when the manuscript is accepted for publication, the authors agree to automatic transfer of the copyright to the publisher.

African Journal of Plant Science

Table of Content: Volume 10 Number 4, April 2016

ARTICLES

academicJournals

Vol. 10(4), pp. 77-83, April 2016
DOI: 10.5897/AJPS2015.1389
Article Number: E31C6ED58265
ISSN 1996-0824

http://www.academicjournals.org/AJPS

African Journal of Plant Science

Full Length Research Paper

Establishment and optimization of high efficiency embryogenic callus induction system in *Dendrobium candidum*

Nada Mohammed Reda Refish[1], Linqing Wang[1], Chunhua Fu1,[2], Xiangping Xu[1], Wenwen Jin[1,2]*, Maoteng Li[1,3] and Longjiang Yu[1,2]

[1]Institute of Resource Biology and Biotechnology, Department of Biotechnology, College of Life Science and Technology, Huazhong University of Science and Technology, Wuhan, 430074, China.
[2]Key Laboratory of Molecular Biophysics of Ministry of Education, Wuhan 430074, China.
[3]Hubei Collaborative Innovation Center for the Characteristic Resources Exploitation of Dabie Mountains, Huanggang 435599, China.

Received 9 January, 2016; Accepted 16 April, 2016

The wild resource of *Dendrobium candidum,* a well-known epiphytic orchid, is limited. Plant cell culture technology is a promising alternative for production of high-value secondary metabolites .In this research, factors affecting the induction, maintenance, and multiplication of callus from protocorm-like bodies (PLBs) of *D. candidum* were systematically studied, and a technical callus inducing system with 100% rate was established which higher than previously reported values. The result showed that 2,4-dichlorophenoxy acetic acid(2,4-D) and 1-naphthalene acetic acid (NAA) could of benefit to the callus induction. The optimized medium for callus induction was MS medium containing 0.5 mg/L 2,4-D and 0.25 NAA or 0.5 mg/L kinetin (KT). The callus clump enrichment method was used for directional screening and cell adaptation on MS medium supplied with plant growth regulators (PGR) such as 5 mg/L NAA and 0.5 mg/L 6-benzyladenine (6-BA) or KT for 30 days per cycle. After 6 to 9 months, *D. candidum* cell lines were obtained and the histological analysis showed that the main differences between callus and PLBs were meristematic cell content and internal cell differentiation degree, and the meristematic cells distributed in the external strip were contributed to the formation of callus. At last, the amount of polysaccharides and total amino acids were compared between the obtained cell lines, tissue culture seedlings and PLBs. The results showed that polysaccharides was 24.67% in callus and higher than that in tissue culture seedlings and PLBs. The amount of total amino acids was 5.94% in callus and higher than that in tissue culture seedlings. So the callus is considered a good choice for the expanding of *D. candidum* medicine sources.

Key words: *Dendrobium candidum,* Callus induction, tissue culture, natural products, plant growth regulators.

INTRODUCTION

Dendrobium candidum Wall. ex Lindl is a species that belongs to the Orchid family and a well-known orchid and epiphytic herb in South and Southeast Asian countries, which has been used as a Chinese traditional herb for over 1500 years (Leung, 2006). Modern pharmacological research has shown that *D. candidum* is an important

Table 1. Effects of single plant growth regulators (PGRs)on callus inductionof *D. candidum.*

Medium	PIC	2,4-D	NAA	Callus induction rate (%)	Callus color	Callus status
M1	0.25			2.1±0.35^{d}*	Brown	Died
M2	0.5			9.4±0.85^{d}	Brown	Died
M3	1.0			0.9±0.28^{d}	Brown	Died
M4		0.5		92.5±3.5^{ab}	Yellow	Growth well
M5		1.0		100^{a}	Yellow	Growth well
M6		1.5		47.5±10.6^{c}	Brown	Died
M7			1.0	27.3±5.9^{d}	Brown	Died
M8			5.0	89.5±6.2^{b}	Yellow	Growth well
M9			10.0	83.2±8.1^{b}	Yellow	Growth well

*All values are means ± SE (n = 3).Mean values in each column followed by the same letter are not significantly different (P < 0.05) by the one-way ANOVA analysis.

candidate for modern drug development because it enhances immunity, exhibits antitumor activity, and other regulatory roles (Li et al., 2009; Luo et al., 2010; Xiao et al., 2011; Xing, 2013; Bian et al., 2002). The production of *D. candidum* in Yunnan was estimated to be lower than 1000 kg in 1998, which cannot meet the market (Huang et al., 1996), the development of *D. candidum* cultivation, is therefore necessary.

Plant cell, tissue, and organ cultures, such as callus, embryo (protocorm-like bodies, PLBs), and tissue culture seedlings, are complementary systems used to enhance the beneficial bioactivities of *D. candidum* (Kolewe et al., 2008). *D. candidum* can be micropropagated using explants, such as PLBs, protocorms, immature seeds, and shoot tips (Paulsen, 2001; Chen and Wang, 2005; Su and Yang, 2006) and there are also some studies focused on the induction, proliferation, and liquid culture of PLBs (Men et al., 2003; Shi and Zhao, 2012; Jasim et al., 2015). There is recently study on the PLBs suspension cultures of *D. candidum* in balloon type bubble bioreactors for the production of biomass and bioactive compounds such as polysaccharides, coumarins, polyphenolics, flavonoids, vitamin C and vitamin E (Cui et al., 2014). Plant cell culture is also a promising alternative for production of high-value bioactive compounds (Rao and Ravishankar, 2002; Ramawat and Merillon, 2008; Rischer et al., 2013). And there are only some report on the callus induction, but it is easy to differentiation into protocorm or PLB (Roy and Banerjee, 2003; Chen and Chang, 2000; Zhao et al., 2008), and there is no research on callus maintain or scale-up process for the production of bioactive compounds from *D. candidum* suspension cultures. The present study established an effective method for embryogenic callus induction and maintain, then the formation and characteristics of the callus were confirmed by anatomy analysis, at last, the amount of polysaccharides and total amino acids among different explants (tissue culture seedlings, callus, and PLBs) of *D. candidum* were compared to determine the most suitable methods for expanding *D. candidum* sources.

MATERIALS AND METHODS

Plant materials and cultivation

Shoot explants were obtained from wild *Dendrobium* plants in Yunnan province (which have been confirmed by a plant taxonmists named Xiang Jun in Huanggang normal university) were maintained on MS medium supplied with 1 mg/L thidiazuron (TDZ), 0.5 mg/L 1-napthalene acetic acid (NAA), and 0.5 mg/L 2,4-dichlorophenoxy acetic acid (2,4-D) at 25°C in the dark.

Callus induction and cell line formation of *D. candidum*

PLBs were used as explants and MS medium as the basic culture medium for callus induction. The effects of picloram, 2,4-D, NAA, kinetin (KT), and 6-benzyladenine (6-BA) and their combinations on callus induction were determined (Tables 1 and 2).Induction was performed in the dark at 25°C. Twenty explants were inoculated for each experiment. The Callus induction rate and Browning intensitywere evaluated after 20 day of culture.

D. candidum cell lines were obtained using the callus clump enrichment method

The well-conditioned buff callus briquette was minced with forceps and inoculated in the subculture medium 1 (SCM1). This process was repeated several times, with the first three to four multiplication cycles for 15 days and multiplication cycles after 3 months were gradually extended to 20 day.

The subculture medium was adjusted to subculture medium 2

*Corresponding author. E-mail: jww@hust.edu.cn. Tel: 86-27-87792264. Fax: 86-27-87792265.

Table 2. Effects of plant growth regulators (PGRs) combinations on callus induction.

Medium	2,4-D	NAA	KT	6BA	Callus formation time (days)	Callus induction rate (%)	Browning intensity	Growth state	Re-differentiation
MC1	0.5		0.25		9	100[a]*	-	Good condition	+
MC2	0.5		0.5		10	100[a]	-	Good condition	+
MC3	1.0		0.25		8	100[a]	+	Grow well	++
MC4	1.0		0.5		7	100[a]	+	Grow well	++
MC5		5.0		0.25	12	100[a]	-	Grow well	++
MC6		5.0		0.5	12	100[a]	-	Grow well	++
MC7		10.0		0.25	14	85.0±7.1[b]	++	Grow slowly	+++
MC8		10.0		0.5	14	87.5±3.5[b]	++	Grow slowly	+++
MC9		5.0	0.25		12	100[a]	-	Grow well	++
MC10		5.0	0.5		12	100[a]	-	Grow well	++
MC11		10.0	0.25		13	85±7.1[b]	++	Grow slowly	+++
MC12		10.0	0.5		13	87.5±10.6[b]	++	Grow slowly	+++

The Callus induction rate and Browning intensity were checked in 20th day after the culturing, and growth state, re-differentiation were checked after 3 months culture. Re-differentiation:"+" means the re-differentiation rate is lower than 10%; "++" means the re-differentiation rate is around 30%, and "+++"means the re-differentiation rate is around 50%. Browning intensity: "-" means no browning; "+" means a little browning; "++" means a heaven browning .* All values are means ±SE (n = 3). Mean values in each column followed by the same letter are not significantly different (P < 0.05) by the one-way ANOVA analysis.

(SCM2), and the multiplication cycle was gradually extended from 20 to 30 day to adapt to the final consumption demand of *D. candidum* cell culture.

SCM1: MS + 0.5 mg/L 2,4-D + 0.5 mg/L KT + 1% agar+3% sucrose
SCM2: MS + 5 mg/L NAA + 0.5 mg/L 6-BA + 1% agar+3% sucrose.

Anatomical analysis

Anatomical analysis of PLBs and callus of *D. candidum* was conducted using paraffin sections according to the methods of Ennajeh et al. (2010) and Zhao et al. (2007). Photographs were obtained using a microscope (AH2, Olympus, Japan).
.

Polysaccharide extraction and analysis

Approximately 0.5 g of samples were collected, dried in an oven at 55°C for 20 h, and then powdered using a mortar. The polysaccharides was obtained by water extraction and ethanol precipitation, and analyzed by phenol-sulfuric acid according to the method of Tong et al. (2015). Anhydrous glucose was used as a standard in determining the total polysaccharides content. From anhydrous glucose stock concentration of 0.09 g /L, several dilutions were made to prepare a series of concentrations at 0.2, 0.4, 0.6, 0.8 and 1.0 mg/mL. A standard curve ($Y=0.0082X+0.0036$.$R^2=0.9992$) was constructed with the optical density at 490 nm against the concentrations of glucose. The total polysaccharides content of the samples were expressed in milligram of glucose equivalent per gram of samples.

Amino acid extraction and analysis

Approximately 0.25 g of samples were dried in an oven at 55°C, powered, and then transferred to tapered plug bottles. About 20 mL of 0.1 mol/L HCl solution was added, and the mixture was ultrasonicated at 30°C for 30 min .The mixture was suctioned, and the pH was adjusted to 7 neutral with 15% hydroxide sodium solution. The samples were then transferred to 100 mL volumetric flasks and adjusted to 100 mL by adding distilled water. Briefly, 4.0 mL of the extract was transferred to a 10 mL tube and added with 1.0 mL of sodium acetate buffer salt solution (pH 6.5) with 1.0 mL of 2% ninhydrin solution. The mixture was heated in a water bath at 100°C for 40 min and cooled for 15 min before adjusting to 50 mL by adding water. Aspartic acid (HPLC≥98%, CAS: 5794-13-8) was used as a standard in determining the total amino acid content. From aspartic acid stock concentration of 0.1014 g /L, several dilutions were made to prepare a series of concentrations at 0.2, 0.4, 0.6, 0.8 and 1.0 mg/mL. A standard curve ($Y=13.93x-0.1799$, $R^2=0.9996$) was constructed with the optical density at 568 nm against the concentrations of aspartic acid. The total amino acid content of the samples were expressed in milligram of aspartic acid equivalent per gram of samples.

Statistical analysis for callus induction and cell line formation

Each treatment was repeated three times, for polysaccharide and for amino acids analysis, each sample were extracted three times and measurements were repeated three times. Data are the mean values of three replicates, and are expressed as mean±standard error (SE). Statistical analysis was performed using one-way ANOVA using an SAS software package.

RESULTS

Effects of different plant growth regulators on the callus induction and cell line formation of *D. candidum*

Since the auxin could benefit to the callus induction in many genus, so the effect of the auxin analogues

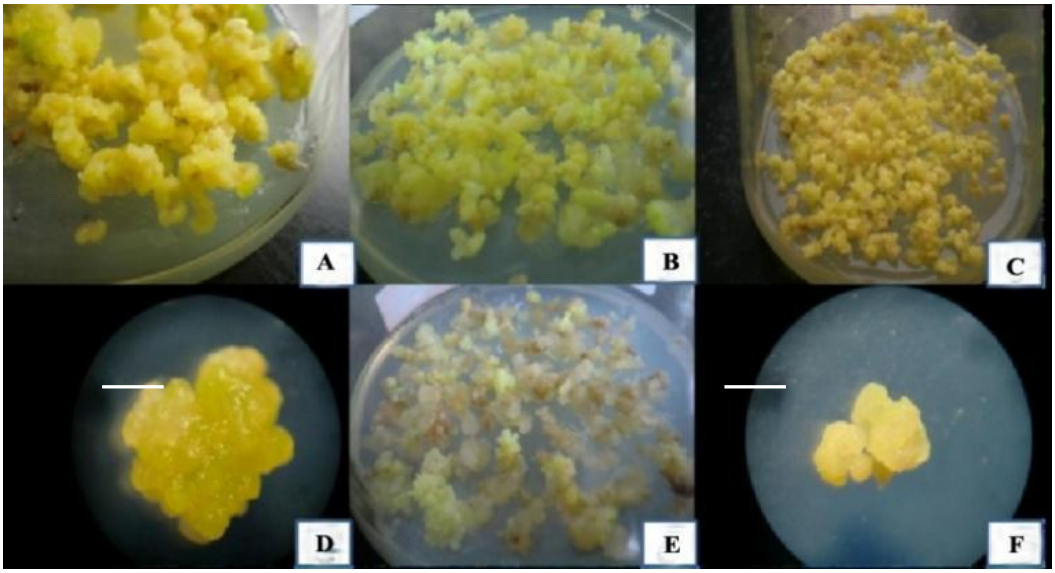

Figure 1. Cell acclimation of *D. candidum*. A, D: Initial subcultured cells; B, E: The differentiated cells during subculture; C,F: Cell lines formed after 3 months on SCM2 medium. The images in D, F were obtained under a stereo microscope at 20×, the bar represent 1 mm.

picloram, 2,4-D,NAA on the callus induction of *D. candidum* were examined. The results showed that PLB cultured on the medium containing picloram are all died, and callus could be induced on the medium containing 2,4-D (0.5 and 1.0 mg/L) with high induced rate above 90%, or NAA (5 and 10 mg/L) with a induced rate around 80 to 90% (Table 1). But the calli induced on the MS medium supplied with 2,4-D alone gradually became brown. Then the effects of MS medium supplied with 2,4-D ,NAA and cytokinin on the callus induction were further carried out. The results showed that the supply of cytokinin could benefit to the callus inductionand 2,4-D-treated group showed visible calli within 7 to 10 day. On day 20, MC1, MC2, MC3, and MC4 media containing 2,4-D and KT obtained 100% callus induction rate. The callus induced on MC1 and MC2 medium containing 0.5 mg/L 2,4-D showed no browning, and were in good conditions with slight re-differentiation rate when transferred to sub-cultured medium SCM1 to form cell line. The callus appeared a slight browning on MC3 and MC4 medium containing 1.0 mg/L 2,4-D, and grow well with middle re-differentiation rate on SCM1 medium. The callus mass gradually appeared after 12 to 14 day culture on medium containing NAA and 6-BA or KT. The callus induction rate on medium (MC5, MC6, MC9, MC10) containing 5 mg/L NAA was also 100%, the calli presented a pale yellow color with almost no browning, and grow well with no re-differentiation on SCM1 medium. The calli induction rate were around 85% on medium (MC7, MC8, MC11, MC12) containing 10 mg/L NAA, the calli showed some browning, and grow slowly with a heavy browning.

As differentiation into seedlings easily occurs during cell line establishment for *D. candidum*, culture conditions must be controlled during domestication. The newly induced callus of *D. candidum* presented a dense structure with large clumps (Figure 1A and D). Some callus became brown and necrotic, whereas the other parts appeared to be differentiated during subculture (Figure 1B and E). A strong tendency toward callus differentiation was observed in the initial subculture. This finding indicated that short subculture periods (15 day) were necessary in the first three to four cycles. Clumps were crushed to granules during sub-inoculation. After 3 months of continuous screening and domestication on medium SCM1, the texture of the callus became visibly loose, granules became stronger and more evident, and the re-differentiation tendency gradually weakened on medium SCM2 (Figure 1C and F).

Comparison of anatomical characteristics between callus and PLBs of *D. candidum*

Callus and PLBs were selected for paraffin sectioning. The results showed the initial PLB contained a small number of meristematic cells distributed in a strip in the near surface zone of tissues, whereas the internal part was composed of non-meristematic cells with few inclusions and large particle sizes (Figures 2A to C). Callus was mainly composed of square or circular meristematic cells with strong division ability and large nuclei. Some cells do division and present a dual-core state (Figures 2D to F). That suggested the main differences between callus and PLBs were meristematic cell content and internal cell differentiation degree, and the meristematic cells distributed in the external strip

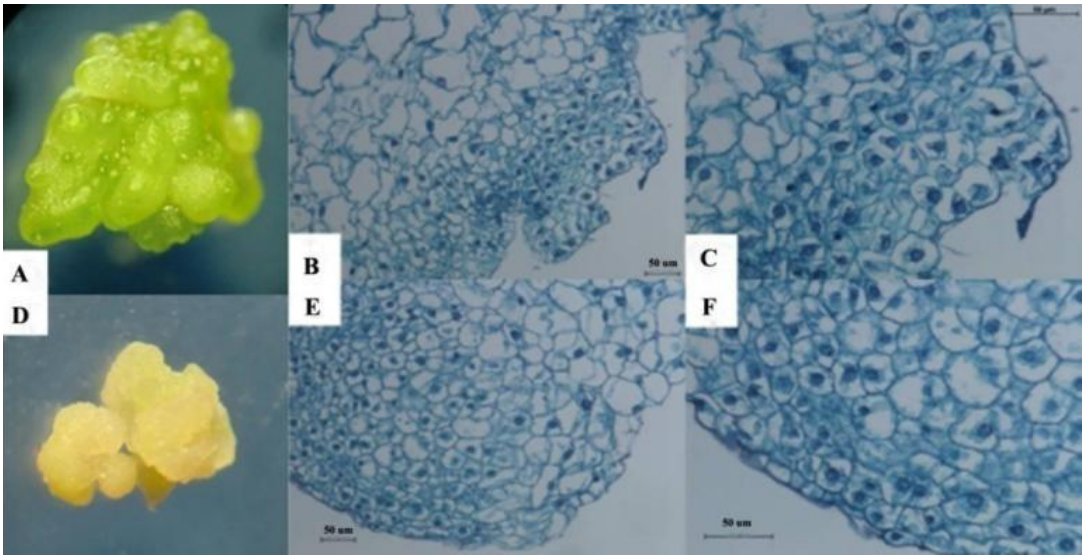

Figure 2. Comparison of the microstructure of protocorm-like bodies(A–C) and callus (D–F) of *D. candidum*.

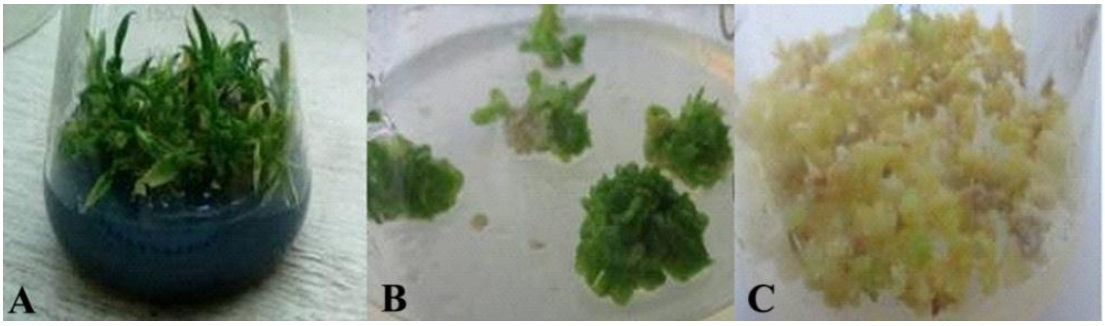

Figure 3. Three different *in vitro* cultures of *D. candidum.* A. Tissue culture seedlings; B. protocorm-like bodies (PLB); and *C. callus.*

Table 3. Polysaccharides and Amino acid compounds content in different *D. candidum* cultures.

Parameter	Polysaccharides content (%)	Amino acid content (%)
Callus	24.67±0.102[a]*	5.94±0.490[b]
Protocorm-like bodies (PLB)	17.01±0.716[b]	14.06±0.104[a]
Tissue culture seedlings	13.80±0.602[c]	3.34±0.180[c]

*All values are means ± standard error SE (n = 3). Mean values in each column followed by the same letter are not significantly different (P < 0.05) by the one-way ANOVA analysis.

were contributed to the formation of callus.

Quality analysis of different kinds of tissue cultures from *D. candidum*

The major bioactive compounds of *D. candidum* are polysaccharides (Liu et al., 1988; Yi et al., 1999). The main active compounds (polysaccharides and amino acid) were compared among different *in vitro* cultures (tissue culture seedling, callus, and PLBs) of *D. candidum* (Figure 3) to evaluate the possibility of producing useful plant bioactivities using plant cell culture (Table 3). The results showed that polysaccharide content was significantly higher (24.67%) in callus than that in the PLBs and tissue culture seedlings (P < 0.05). Another

major compounds of total amino acids were also checked, the amount of total amino acids was 5.94±0.490% in callus and higher than that in tissue culture seedlings, but lower than that in the PLBs (Table 3).

DISCUSSION

In the present research, factors affecting the induction, maintenance, and multiplication of callus of *D. candidum* were systematically studied. An efficient method was established for callus induction and cell line construction and maintenance, and major bioactive compounds were compared among different *in vitro* cultures (tissue culture seedling, callus, and PLBs) of *D. candidum*. The results showed that calli exhibit the highest potential as an alternative medicine source. But for the plant cell culture, plant tissue de-differentiation is the key to callus formation and the induction efficiency was affected by several factors such as explant, plant growth regulators (Tao et al., 2011; Prakash et al., 1996; Nayak et al., 2002). In the de-differentiation induction of *D. officinale*, the highest callus induction rate reached 82% and was achieved using seed-induced PLBs as explants (Zhao et al., 2008). The highest callus induction rate reached 86.67% when tender stem segments were used as explants (Wang and Liang, 2010), and there is a novel study illustrate shedding light on the effect of various plant growth regulator 2,4-DandNAAon callus induction of *ricinus commun*. Callus culture was initiated from cotyledonary leaf and root segments explants from in vitro *Ricinus communis* L seedling. The results showed that 2,4-D and NAA effecting the callus agitation, but 2,4-D was proved to be more efficiency for induction of callus. Individual treatment of NAA reveals low effectiveness for callus induction (Khadiga et al., 2015). In the current study, PLBs (which was defined as somatic embryos in orchids species, Lee et al., 2013) was used as explants and the callus induction rate reached 100% under the optimized condition which was MS medium containing 0.5 mg/L 2,4-D and 0.25 NAA or 0.5 mg/L kinetin (KT). The callus clump enrichment method was used for directional screening and cell adaptation on MS medium supplied with plant growth regulators (PGR) such as 5 mg/L NAA and 0.5 mg/L 6-benzyladenine (6-BA) or KT for 30 days per cycle. While in another study for *Dendrobium Broga* Giant, the percentage of PLBs induced was the highest on half strength MS semi-solid medium supplemented with 1.0 mg/L BAP and 0.5 mg/L NAA. The highest proliferation rate of 8.7% of PLBs was obtained in 1.0 mg/L BAP and 0.5 mg/LNAA (Jasim et al., 2015).

But the establishment of *D. candidum* cell line is challenging, and requires a long period of acclimation. At the beginning of subculture, *D. candidum* cells exhibit a strong ability to re-differentiate and gradually gain the ability to maintain the dedifferentiation state. Plant growth regulator is the key factor to regulate the cell differentiation state. The early subculture cycle of *D. candidum* cell lines is insufficient to maintain the hormone levels in the culture medium above the threshold and thus cannot maintain the cell dedifferentiation state. Meanwhile, plant cell differentiation is controlled by the position effect, changing the cell position appropriately in the critical period before cell division could change cell development mode (Wolpert, 1969). In the subculture of *D. candidum*, cell granulation could be induced by changing the cell position by using tweezers to constantly mince callus.

Polysaccharides are the main components of *D. candidum*, and polysaccharide content is positively correlated with physiological function. Hence, the content of polysaccharides should be the widely accepted evaluation criterion for *D. candidum* and its use as a medicine source. Controlled cell culture conditions are conducive to the implementation of good manufacturing practices and short production cycles to generate medicines from *D. candidum*. Plant cultivation for medicinal purposes will also minimize wild collection, thus supporting plant conservation in the wild. However, total polysaccharide content of cultivated plants must be comparable with the wild plantlets from Yunnan, in which the total polysaccharide ranges from 18 to 46% (Jiang et al., 2014). In the current study, we found that calli possessed the highest polysaccharide content of 24.67%, and exhibit the highest potential as an alternative medicine source for *D. candidum*.

Conclusions

An effective method was established for embryogenic callus induction and maintenance of *D. candidum*. The proposed method included PLB induction under the optimized condition, callus induction and growth, and callus maintenance. The callus induction rate under the optimized conditions was 100% and higher than previously reported values. And the major bioactive compounds of polysaccharidesin callus are higher than in tissue culture seedling and protocorm-like bodies. Therefore, callus maybe a suitable material for utilization of *D. candidum* as a medicine source.

Conflict of interests

The authors hereby declare that no conflict of interest exists among them.

ACKNOWLEDGEMENTS

This work has been supported by the National Natural

Science Foundation of China (31270342), National “11th Five-Year Plan” to Support Science and Technology Project of China (2008BAI63B04). The authors are grateful to the Analytical and Testing Center, Huazhong University of Science and Technology.

Abbreviations

6-BA, 6-benzyladenine,2,4-D 2,4-dichlorophenoxyacetic acid; **Kin,** kinetin; **MS,** Murashige and Skoog (1962) medium; **NAA,** Naphthaleneacetic acid; **PLBs,** Protocorm-like bodies; **TDZ,** thidiazuron; **PIC,** picloram.

REFERENCES

Bian HW, Wang JH, Lin WQ, Han N, Zhu MY (2002). Accumulation of soluble sugars, heat-stable proteins and dehydrins in cryopreservation protocorm-like bodies of *Dendrobium candidum* by the air-drying method. J. Plant Physiol. 159:1139-1145.

Chen JT, Chang WC (2000). Efficient plant regeneration through somatic embryogenesis from callus cultures of Oncidium (*Orchidaceae*). Plant Sci. 160(1):87-93.

Chen YG, Wang HY (2005). Advance on the chemical and pharmacological studies on plants of Flickingeria genus. Lishizhen Med. Mater. Med. Res. 16:725-727.

Cui HY, Murthy HN, Moh SH, Cui YY, Lee EJ, Paek KY (2014). Production of biomass and bioactive compounds in protocormcultures of *Dendrobium candidum* Wall ex Lindl. using balloontype bubble bioreactors. Ind. Crops Prod. 53:28-33.

Ennajeh M, Vadel AM, Cochard H, Khemira H(2010).Comparative impacts of water stress on the leaf anatomy of a droughtresistant and a drought sensitive olive cultivar. J. Hortic. Sci. Biotechnol. 85(4):289-294.

Huang MQ, Cai TY, Liu QL (1996). Effects of polysaccharides from *Dendrobium candidium* on white bloodcells and lymph cell moving inhibition factor of mice. Nat. Prod. Res. Dev. 8(3):39-41.

Jasim U, Pavallekoodi G, Latiffah Z, Chew BL, Sreeramanan S (2015). Theeffect of different growth media,carbon source and PGRs on *Dendrobium* Broga Giant orchids protocorm like bodies PLBs proliferation supported with SEM and TEM analysis, School of Biological Sciences, Universiti Sains Malaysia, Georgetown, 11800 Penang, Malaysia.

Jiang W, Jiang B, Mantri N ,Wu Z, Mao L, Lu H, Tao Z (2014).Comparative Eco physiology analysis of photosynthesis, biomass allocation, polysaccharide and alkaloid content in three *Dendrobium candidum* cultivars . Plant Omics. 7(2):117-122

Khadiga GAE, Magda MA, Moawiya KMN (2015). Effect of Explants and Plant Growth Regulators onCallus Induction in *Ricinus communis* L, Department of Biology and Biotechnology, Faculty of Science and Technology. AL Neelain University, SUDAN, Commission of Biotechnology and Genetic Engineering, National Centre for Research, Sudan.

Kolewe ME, Gaurav V, Roberts SC (2008).Pharmaceutically active natural product synthesis and supply via plant cell culture technology. Mol. Pharm. J. 5(2):243-256.

Lee Y, Hsu ST, Yeung EC (2013). Orchid protocorm-like bodies are somatic embryos. Am. J. Bot. 100(11):2121-2131.

Leung PC (2006). Good agricultural practice-GAP: Does it ensure a perfect supply of medicinal herbs for research and drug development? In: Leung PC, Fong HHS, Xue CC (eds.) Current review of Chinese medicine: quality control of herbs and herbal materials, World Scientific Publishing Co. Pte. Ltd, Singapore. pp. 27-55.

Li Y, Wang CL, Wang YJ, Guo SX, Yang JS, Chen XM, Xiao PG (2009). Three new bibenzyl derivativesfrom*Dendrobium candidum*. Chem. Pharm. Bull. 57:218-219.

Liu RJ, Meng AD, Deng XQ, Li YR (1988).Seedling rapid propagation of *Dendrobium candidum*. Acta Pharm. 23:636-640.

Luo AX, He XJ, Zhou SD, Fan YJ, Luo AS , Chun Z (2010).Purification, compositionanalysis and antioxidant activity of the polysaccharides. Carbohydr. Polym. 79:1014-1019.

Men S, Ming X, Liu R, Wei C, Li Y (2003). Agrobacterium-mediated transformation of a *Dendrobium* orchid. Plant Cell Tissue Organ Cult. 75(1):63-71.

Nayak NR, Sahoo S, Patnaik S, Rath SP (2002).Establishment of thin crosssection (TCS) culture method for rapid micropropagation of *Cymbidiuma loifolium* (L.) Sw. and Dendrobium nobile Lindl. (Orchidaceae). Sci. Hortic. 94:107-116.

Paulsen BS (2001). Plant polysaccharides with immune stimulatory activities. Curr. Organ. Chem. 5:939-950.

Prakash L, Lee CL, Loh CS, Goh CJ (1996). *In Vitro* Propagation of Commercial Orchids: An assessment of Current Methodologies and Development of a Novel Approach-Thin Cross-Section Culture, Oxford and IBH Publishing Co. Pvt. Ltd., New Delhi. pp. 42-49.

Ramawat K, Merillon S (2008). Secondary metabolite production from plant cell cultures: the success stories of rosmarininc acid. Plant Cell Tissue Organ Cult. 16:85-100.

Rao RS, Ravishankar GA (2002). Plant tissue cultures: chemical factories of secondary metabolites. Biotechnol. Adv. 20:101-153.

Rischer H, Hakkinen ST, Ritala A, Seppanen-Laakso T, Miralpeix B, Capell T, Christou P, Oksman-Caldentey K (2013) Plant Cells as Pharmaceutical Factories. Curr. Pharm. Des. 19:5640-5660.

Roy J, Banerjee N (2003).Induction of callus and plant regeneration from Shoot tip explants of *Dendrobium* firmbriatum Lindl. var. oculatum Hk.f. Sci. Hortic. 97:333-340.

Shi J, Zhao R (2012). Study on the liquid suspension culture of *Dendrobium candidum* Wall. ex Lindl. seeds. Med. Plant. 3(4):33-35.

Su H, Yang Y (2006). State quo of *Dendrobium* spp. resources of Nabanhe Nature Reserve and counter measures for protection. For. Invent Plan 31:100-102.

Tao J, Liqin Y, Zhao D (2011). Effects of plant growthregulators on In vitro propagation of Cymbidium faberi Rolfe. Afr. J. Biotechnol. 10(69):15639-15646.

Tong H, Mao D, Zhai M, Zhang Z, Sun G, Jiang G (2015).Macrophage activation induced by the polysaccharides isolated from the roots of Sanguisorba officinalis. Pharm. Biol. 53(10):1511-1515.

Wang LP, Liang SY (2010). Study on the Protocorm-like bodies induction and propagation of Dendrobium. Chin. Agric. Sci. Bull. 26(1):265-268.

Wolpert L (1969). Positional information and the spatial pattern of cellular differentiation. J. Theor. Biol. 25(1):1-47.

Xiao L, Ng TB, Feng YB, Yao T, Wong JH, Yao RM, Li L ,Mo FZ, Xiao Y, Shaw PC, Li ZM, Sze SC, Zhang KY(2011). *Dendrobium candidum* extract increases the expressionof aquaporin-5 in labial glands from patients with Sj¨ogren’syndrome. Phytomedicine 18:194-198.

Xing X, Cui SW, Nie S, Phillips GO, Goff HD, Wang Q (2013). A review isolation process, structural, characteristics, and bioactivities of water-soluble polysaccharides from *Dendrobium* plants. Bioact. Carbohydr. Diet. Fibre 1:131-147.

Yi HF, Qi XJ, Zhou WT, Yue H(1999). Prospect seedling rapid development, ex vitro establishment of *Dendrobium candidum* and determination of polysaccharide. Chin. Tradit. Patent. Med. 21:658-659.

Zhao P, Wang W, Feng FS, Wu F, Yang ZQ, Wang WJ (2007). High frequencyshoot regeneration through transverse thin cell layerculture in *Dendrobium Candidum*Wall. Ex Lindl. Plant Cell Tissue Organ Cult. 90:131-139.

Zhao P, Wu F, Feng FS (2008).Protocorm-like body (PLB) formation and plant regeneration from the callus culture of *Dendrobium candidum* Wall ex Lindl. In Vitro Cell Dev. Biol. Plant 44(3):178-185.

academicJournals

Vol. 10(4), pp. 84-88, April 2016
DOI: 10.5897/AJPS2015.1343
Article Number: 1C563BE58268
ISSN 1996-0824

http://www.academicjournals.org/AJPS

African Journal of Plant Science

Full Length Research Paper

Genotypic variability estimates of agronomic traits in secondary triploid banana 'Matooke' (*Musa sp.*, AAA-EA) hybrids

Reuben Tendo Ssali*, Alex Barekye, Henry Buregeya, Rockefeller Erima, Priver Namanya and Jerome Kubiriba

National Agricultural Research Organisation, Uganda.

Received 11 August, 2015; Accepted 2 April, 2016

Effective selection of hybrids for a trait is based on the extent of variation and heritability. This study examined yield parameters of secondary triploid 'Matooke' hybrids and the extent of their genetic diversity based on the traits evaluated. Eleven genotypes, including nine 'Matooke' hybrids and two landraces were evaluated for 12 characters in a preliminary yield trial (PYT) over three crop cycles. Plant height, bunch weight, number of standing leaves at flowering and the youngest leaf spotted with black Sigatoka symptoms showed significant interaction between genotype and crop cycle. While characters such as pseudo stem girth, number of days for fruit filling, number of hands, number of fingers on the second hand and the fruit length showed stable differences amongst these genotypes. The genotypic coefficient of variation for the characters ranged from 7.6% (finger length) to 33.5% (bunch weight); with moderate heritability estimates varying from 13.5% (pseudostem girth) to 67% (plant height). Bunch weight showed strong positive correlation with number of hands, number of fingers on the second hand, the fruit length and pseudostem girth. These results imply that breeders could select for some of these variable and ratoon stable traits in a single cycle of early evaluation trial, subsequently reducing costs, time and space in field testing.

Key words: Genetic variation, heritability, expected genetic gain, selection, *Musa sp.*

INTRODUCTION

Banana (*Musa* sp.) is the eighth most important global food commodity after maize, wheat, rice, potato, cassava, soybean and barley (FAOSTAT, 2013). It is grown in more than 100 countries, with an annual production of around 150 million metric tonnes. More than 30 million people in the East African region depend on banana, mainly grown by smallholder farmers. About 80% of bananas grown in the region are the east African highland banana (*Musa* sp., AAA-EA) type, locally known as 'Matooke'. Annual 'matooke' production in Uganda is estimated to be 10 million tons valued at USD 534 million (Kalyebara et al., 2006; Nyombi, 2013). Many rural

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

communities earn income from sale of bananas. Bananas are perennial, with broad leaves which provide soil cover and a broad root network which maintains soil structure. The banana cropping system, therefore, is also an important component of sustainable environmental management. It is generally tolerant to long dry spells and can grow in a wide range of environments and farming systems and saves on labour for opening land seasonally. Because of the importance of this crop, many of the regional countries have considered banana a high research and development priority. However, average productivity has remained low (<30% of attainable), due to a complex of production constraints, including banana weevil (*Cosmopolites sordidus*, (Germer)), nematodes (*e. g. Radopholus similis* (Cobb) Thorne)), black Sigatoka *(Mycosphaerellafijiensis* (Morelet)),Fusarium wilt *(Fusarium oxysporum* f. sp. *Cubense* (E.F. Smith) Snyder & Hansen)), banana bacterial wilt (*Xanthomonas campestris* pv*. musacerum*) and more recently drought stress (Tushemereirwe et al., 2003, Viljoen, 2010). Farmers manage some constraints through cultural control, many of which are effective in keeping the pest and disease pressures below threshold levels. However, cultural control involves manipulation of environment of the plant host and the parasite. It is a continuous and tedious process for the farmers and can only work in the short run. Use of resistance is more effective and durable option for the management of banana problems (Jones, 2000).

Genetic improvement of bananas to produce cultivars with host plant resistance and other desirable agronomic traits is complicated by low genetic variability, polyploidy nature and the low levels of female and /or male fertility in most widely grown triploid clones (Rowe, 1984; Hilber, 1997; Tezenas du Montcel et al., 1996; Pillay et al., 2002; Ssebuliba et al. 2006). Currently 'Matooke' breeding requires many years of field-testing for several rounds of selection including evaluation for agronomic performance in early evaluation trials (EET) (based on individuals), selected hybrids are further evaluated for pest/disease response, yield and consumer acceptability in the preliminary yield trials (PYT). Promising hybrids from the PYTs are advanced for participatory on-farm evaluation and multi-location evaluation (Ssebuliba et al. 2006; Ssali et al., 2010). An evaluation of the components of variation and heritability among characters will facilitate improvement of this crop by plant breeders. Knowledge of the genetic variability available among and between genotypes of 'Matooke' hybrids is needed as a guide for breeders and other scientist working on the improvement of this crop. Therefore this study was undertaken to evaluate components of genetic variance and to estimate the heritability between the various yield components of 'Matooke' hybrids.

MATERIALS AND METHODS

The experiment was established in October 2010 at National Agricultural Research Laboratories, NARL. NARL is located at 0°25'N, 32°32'E, 1190 m above sea level, 13 km north of Kampala. Mean annual rainfall is about 1190 mm per year with bimodal distribution, the two rainy seasons span from March to June and September to December. Average daily temperatures are 16°C minimum and 29°C maximum. These conditions are generally representative of major banana growing areas of Central Uganda region.

Table 1. Origin of the genotypes evaluated at the National Agricultural Research Laboratories at Kawanda.

Genotype	Pedigree	
	Female	Male
12468S1	917K-2	SH3217
3123K2	246K-1	TMB2X7197-2
12748S1	660K-1	TMB2X8075-7
3192K2	917K-2	TMB2x8075-7
3637K13	222K-1	TMB2X7197-2
4360K3	222K-1	Calcutta 4
3626K1	401k-2	SH3217
3068K1	376K-7	Calcutta 4
3639K2	917K-2	TMB2X8075-7
Km 5	Landrace	
Mbwazirume	Landrace	

Eleven cultivars were assigned in a randomised complete block design for the experiment. The test materials included nine 'Matooke' hybrids developed by the National Banana research Programme, NARO and two control varieties ('Yangambi Km-5' and 'Mbwazirume'). Details on the hybrids and parents are presented in Table 1. Forty eight (48) tissue culture plants for each genotype were planted in four replicates for 3 cropping cycles. The plants were spaced at 3m × 3m and organic manure was applied at planting and regular banana management was followed (Tushemereirwe et al., 2003).

Data was collected on the flowering dates used to compute days to flowering (DTF) and number of standing leaves at flowering (NSL). Harvest date was used to compute Days from flowering to harvest (DFF), number of leaves at harvest, bunch weight (Bwt, kg^{-1}), number of clusters (HDS), number of fingers on the 2nd cluster (FHND2), finger length (FL), finger circumference (FC), youngest leaf spotted at flowering (YLS), plant height at flowering (HT), and girth at 100 cm (G) and the number of leaves at harvest (Lhar). The crop cycling index (CCI=HTS/PHT) was computed from the height of the tallest sucker and the height of the mother plant at flowering.

Statistical analysis

The statistical analysis was performed using the SAS package (SAS Institute Inc., 2004). The generalised linear model (GLM) was used for the analysis of variance. Let Y_i be the average of the measure of a given character of the *i*th genotype for i = 1,...,11 from the experimental group. The linear model for Y_i is:

$$Y_{ij} = \mu+\alpha_i+c_j+\varepsilon_{ij} \quad (1)$$

Where μ is the grand mean, α_i is the genetic effect of the *i*th genotype expressed as deviation from the mean, c_j is the crop cycle and ε_{ij} is the environmental error for the *i*th genotype and the *j*th crop cycle.

The observed variance or phenotypic variance (V_p) of a character

Table 2. Analysis of variance- mean squares for analysis of characters in triploid 'Matooke' hybrids.

Trait	Mean squares			
	Genotype	Cycle	Genotype × Cycle	Error
Plant height (cm)	29047.73*	236098.09*	2663.42*	1083.81
Pseudostem girth at 100 cm (cm)	906.86*	6702.06*	321.91	313.03
Crop cycling index	0.11	0.22	0.06	0.07
Days to fruit filling	2698.49*	3037.42*	570.36	453.19
Bunch weight (Kg)	203.77*	364.43*	26.22*	16.17
No. Hands	7.31*	10.43*	1.24	1.41
No. of fingers on the 2nd hand	37.24*	107.13*	7.13	7.53
Finger length (cm)	23.78*	110.05*	4.98	4.84
Finger circumferences (cm)	20.99	1.09	53.83	140.44
Number of standing leaves	25.07*	24.05*	6.97*	2.51
Youngets leaf spotted	43.50*	17.21	13.30*	7.81
number of leaves at harvest	2.83	0.52	1.16	1.84

*Significant at 5% probability level.

comprises the genotypic variance (V_g) and the environment variance (V_e). This relationship could be expressed symbolically as:

$$V_p = V_g + V_e \quad (2)$$

These components of variance Vg = genotypic variance, Vp = phenotypic variance and Ve = enviromental variance were estimated using the following formula (Wricke and Weber, 1986):

$$Vg = (MSG - MSE / r) \quad (3)$$
$$Vp = (MSG / r)$$
$$Ve = (MSE / r)$$

Where MSG, MSE and r are the mean squares of genotypes, mean squares of error and number of replication, respectively (Baye, 2002). Broad sense heritability (h2) was estimated as a ratio of genetic variance (Vg) and phenotypic variance (Vp) specified by Singh and Chaudhary (1985):

$$h2 = (Vg/Vp) \times 100 \quad (4)$$

Genotypic and phenotypic coefficients of variation were calculated according to the method suggested by Johnson et al. (1955).

$$GCV = (\text{Genotypic variance} / \text{mean value of the trait}) \times 100 \quad (5)$$

$$PCV = (\text{Phenotypic variance} / \text{mean value of the trait}) \times 100 \quad (6)$$

Genotypic correlation coefficients among the traits of 11 banana genotypes were computed using the Proc Corr procedure in SAS.

RESULTS AND DISCUSSION

Mean squares in the combined analysis of variance for 12 traits were significant for nine of the traits including plant height (HT), bunch weight (Bwt), number of standing leaves at flowering (NSL), the youngest leaf spotted with black Sigatoka symptoms (YLS), pseudo stem girth (G), number of days for fruit filling (DFF), number of fingers on the second hand (FHDS2), number of hands (HDS) and fruit length (FL) (Table 2). Genotypic variance indicates that selection can be successfully applied in this population (Allard, 1960) in terms of these characters. The most important variance components for defining adaptation strategy and yield stability targets are those relating to genotypic and genotype-environment effects (Annicchiarico, 2002). Plant height (HT), bunch weight (Bwt), number of standing leaves at flowering (NSL) and the youngest leaf spotted with black Sigatoka symptoms(YLS) show significant interaction between genotype and crop cycle. While pseudo stem girth (G), number of days for fruit filling (DFF), number of hands (HDS), number of fingers on the second hand (FHDS2) and the fruit length (FL) showed no significant interaction between genotype x crop cycle. Therefore the variable traits under study in 'Matooke' hybrids can be categorised into (a) ratoon unstable agronomic traits-which lack stability of the average effects over crop cycles and (b) ratoon stable agronomic traits-showing stable differences amongst genotypes across the crop cycles.

Difficulties in breeding bananas due to the long generation of the crop (about 18 months to establish from seed to seed), the high cost and space requirements (9 m^2 per mat), it is plausible to select for characters showing stable differences amongst genotypes (Rowe, 1984; Hilber, 1997; Pillay et al., 2002). However, variability is the addition of total hereditary effects from expressed genes as well as the environment. Therefore, the variability is grouped into heritable and non-heritable components with suitable genetic parameters such as genotypic coefficient of variation (GCV%), phenotypic coefficient of variation (PCV%), heritability (h2%) and the expected genetic gain (ega%). These genetic parameters help in the selection of the genotypes for genetic improvement of the crop.

Genetic analysis was made for the nine variable

Table 3. Phenotypic and genotypic components of variability for the agronomic characters of 'Matooke' hybrids.

Trait	Mean	Range	Variance					
			Genotypic	Phenotypic	GCV (%)	PCV (%)	h^2 (%)	*Ega* (%)
Pseudostem girth (cm)	44.9	38.9-52.9	48.7	361.8	15.6	42.4	13.5	11.8
Days to fruit filling	149.1	113.9-161.3	177.3	630.5	8.9	16.8	28.1	9.8
No. Hands	7.0	5.9-8.6	0.5	1.9	10.1	19.7	26.5	10.7
No. of fingers(2nd hand)	17.7	15.3-22.2	2.5	10.0	8.9	17.9	25.0	9.2
Finger length (cm)	16.4	14.9-20.6	1.6	6.4	7.6	15.4	24.5	7.8
Plant height (cm)	258.0	227.6 -309.0	2198.7	3282.5	18.2	22.2	67.0	30.6
Bunch weight (Kg)	11.5	5.9-19.9	14.8	31.0	33.5	48.4	47.8	47.6
No. of standing leaves	9.7	8.5-10.8	1.5	4.0	12.6	20.6	37.5	15.9
Youngest leaf spotted	7.9	6.3-9.2	2.5	10.3	20.0	40.6	24.4	20.4

^{GCV}Genotypic coefficient of variation; ^{PCV}Phenotypic coefficient of variation; h^2 Broad sense heritability; ^{ega}expected genetic advance at 5% selection.

characters between genotypes (Table 3). Phenotypic coefficient of variation (PCV%) was found superior than the genotypic coefficient of variation (GCV%) for all the characters indicating environmental effect on all characters. The phenotypic coefficient of variation ranged from 15.40% (fruit length) to 48.4% (bunch weight). Similarly, genotypic coefficient of variation (GCV%) varied from 7.6% (Finger length) to 33.5% (bunch weight). Heritability (h^2%) estimates were low to moderate, ranging from 13.5% (pseudostem girth) to 67% (plant height). The heritability estimates were moderate for plant height (67%), bunch weight (47.8%) and the number of standing leaves at flowering (37.5%); all of which are ratoon unstable agronomic traits. This means that the potential for genetic improvement was greater in the ratoon unstable agronomic characters than ratoon stable agronomic characters like pseudo stem girth (G), number of days for fruit filling (DFF), number of hands (HDS), number of fingers on the second hand (FHDS2) and the fruit length (FL). Moderate heritability estimates suggested that selection should be delayed to more advance generations for these characters (Teich, 1984; Chaturvedi and Gupta, 1995).

Selection efficiency is related to both the magnitude of heritability and genetic advance (Johnson et al., 1955). The expected genetic advance (ega%) was estimated considering 5% selection from the parent population by the method of Falconer (1976) and Singh and Choudhry (1985). Genetic advance as percent of the mean was moderate to very low for the agronomic traits, highest in bunch weight (47.6%) and lowest in finger length (7.8%). Low genetic advance indicates slight changes of improvement of traits in subsequent generations (Teich, 1984; Chaturvedi and Gupta, 1995). Bunch weight is one of the most preferred yield aspect of bananas; it is directly related to food security and economic returns for banana growing households. Large bunch size, good taste and crop maturation time are some of the key selection attributes by banana farmers in Uganda (Gold et al., 2002, Akankwasa et al., 2013). Despite the importance of the large bunch size to banana farmers, our results show that selection for bunch weight in 'Matooke' hybrids would be difficult due to the lack of stability of the average effects over crop cycles.

Correlation estimates are helpful in determining the components of a complex trait, such as yield, being the complex outcome of different characters; they do not provide an exact picture of the relative importance of direct and indirect influences of each of the components characters towards this trait (Bhatt, 1973). Correlation between traits was done to determine whether selection for one trait will have an effect on another. Simple correlation coefficient for the nine variable characters is shown in Table 4. Bunch weight showed strong positive correlation with number of hands (HDS), number of fingers on the second hand (FHD2), the fruit length (FL), pseudostem girth (G), plant height (Ht) and the number of standing leaves at flowering (NSL). Therefore, selection for these characters may increase bunch weight as a correlated response. This enables early selection of superior genotypes with respect to bunch weight. Fruit maturity period (DFF) had negative correlation with the bunch weight and its components. This implies that selection for early maturity is expected to increase banana yields. A high range of variability, heritability, genetic advance and positive correlation coefficient among traits are an excellent tool for improving or selection of genotypes (Akbar et al., 2003). 'Matooke' hybrids '12468S1' and '3123K2' have been selected from the mean separation of the stable traits. These genotypes have significantly more hands (HDS), number of fingers on the second cluster(FHD2), longer fingers (FL) than the local landrace variety 'Mbwazirume'. In addition these genotypes have a comparable Girth (G) to 'Mbwazirume' unlike other genotypes which had either more hands (HDS), number of fingers on the second cluster (FHD2) or longer fingers (FL) but with significantly smaller pseudo stem girth than 'Mbwazirume' (Table 5). It should

Table 4. Simple correlation coefficients among nine traits in 'Matooke' hybrids.

	HT	G	YLS	DFF	BWT	HDS	FHD2	FL
G	0.3878*							
YLS	-0.0001	0.0247						
DFF	-0.1964*	-0.2211*	0.0296					
BWT	0.51847*	0.5379*	0.0346	-0.1188*				
HDS	0.3093*	0.2583*	-0.0168	0.0180	0.6461*			
FHD2	0.3898*	0.3625*	0.0266	-0.0096	0.6172*	0.4933*		
FL	0.5045**	0.5444*	0.0822	-0.1530*	0.7105*	0.3937*	0.4955*	
NSL	0.0943*	0.1219*	0.5571*	0.0976	0.2309*	0.1280*	0.1467*	0.2565*

*Significant at 5% probability level.

Table 5. Means (± Standard error) of agronomic performance of 'Matooke' hybrids for ratoon stable characters.

Genotype	Girth (cm)	DFF (days)	HDS	FHND2	FL(cm)
12468S1	51.4±1.2	148.43±5.6*	8.23±0.3*	18.27±0.9*	20.55±0.7*
12748S1	48.32±6.6	156.56±3.8*	7.90±0.2*	17.90±0.4*	16.59±0.4
3068K1	40.83±1.0*	158.21±4.9*	7.44±0.2*	16.97±0.4	15.61±0.3
3123K2	52.87±1.0	133.94±2.6*	8.58±0.2*	22.21±0.4*	18.41±0.4*
3192K2	40.31±1.0*	156.66±4.4*	5.90±0.2	18.21±0.5*	15.86±0.5
3626K1	42.79±5.6	161.27±3.4*	6.19±0.2	17.52±0.6*	15.87±0.3
3637K13	45.74±0.9	142.09±3.1*	7.11±0.3*	17.97±0.6*	16.21±0.5
3639K2	43.06±1.3	156.33±6.4*	6.10±0.3	17.40±0.5*	15.00±0.6*
4360K3	38.88±1.0*	155.35±2.7*	6.74±0.1	15.78±0.4	14.90±0.4*
Km5	42.10±0.8*	157.22±3.0*	6.41±0.2	18.25±0.6*	15.17±0.5*
Mbwazirume	49.29±0.8	113.90±3.2	6.06±0.1	15.29±0.3	17.23±0.4

*****Within the same column indicates significant different at 5% probability level from landrace-'Mbwazirume' by Dunnett's test.

be noted that all the genotypes take a significantly longer time to mature than the local landrace 'Mbwazirume'.

Bunch weight in 'Matooke' hybrids is moderately heritable and correlated with traits that are least influenced by environment over the cropping cycles like pseudostem girth (G), number of days for fruit filling (DFF), number of hands (HDS), number of fingers on the second hand (FHDS2) and the fruit length (FL). We conclude that the genetic improvement of 'Matooke' for yield could be achieved by selecting for these ratoon-stable traits in early evaluation trials. This implies that by selecting for ratoon-stable agronomic traits, 'Matooke' hybrids can be evaluated in a single cycle in early evaluation trails, subsequently reducing costs, time and space in field testing. Furthermore, these ratoon-stable traits in 'Matooke' can be combined with high density genetic markers to develop genomic selection models for improving 'Matooke' (Ortiz and Swennen, 2014).

Conflict of interests

The authors hereby declare that no conflict of interest exists among them.

ACKNOWLEDGEMENT

This work was supported by the government of Uganda through the priority commodities development research and the Alliance for a Green Revolution in Africa (AGRA), grant No.2009 PASS031.

REFERENCES

Akankwasa K, Ortmann GF, WaleE, Tushemereirwe WK (2013). Farmers' choice among recently developed hybrid banana varieties in Uganda: A multinomial logit analysis. Agrekon 52(2):25-51

Akbar M, Mahmood T, Yaqub M, Anwar M, Ali M, Iqbal N (2003). Variability, correlation and path coefficient studies in summer mustard (Brassica juncea L.). Asian J Plant Sci. 2(9):696-698.

Allard RW (1960). Principles of Plant Breeding. John Wiley and Sons. Inc., New York.

Annicchiarico P (2002). Genotype x Environment Interactions. Challenges and Opportunities for Plant Breeding and Cultivar Recommendations. Series Title: FAO Plant Production and Protection Papers -174. 126p.

Baye T (2002). Genotypic and phenotypic variability in *Vernonia galamensis* germplasm collected from eastern Ethiopia. J. Agric. Sci. 139:161-168.

Bhatt GM (1973). Significance of path coefficient analysis in determining the nature of character association. Euphytica 22:338-343.

Chaturvedi BK, Gupta RR (1995). Selection parameters for some grain and quality attributes in spring wheat (Triticum aestivum L.). Agric. Sci. Dig. Kemal 15(4):186-190.
Falconer DS (1976). Introduction to Quantitative Genetics, 8th ed. Longman, London. P 365.
FAOSTAT (2013). Agricultural Production Statistics Database. Available at: http://faostat3.fao.org/faostat-gateway/go/to/home/E
Gold CS, Kiggundu A, Abera AMK, Karamura D (2002). Selection criteria of Musa cultivars through a farmer participatory appraisal survey in Uganda. Exp. Agric. 38(1):29-38.
Hilber M (1997). Breeding a better banana. IDRC reports archive. Vol. 22, No.1 Ottawa, Canada
Johnson HW, Robinson HF, Comstock RE (1955). Estimation of genetic and environmentalvariability in soybeans. Agron. J. 47:314-318.
Jones DR (2000). History of banana breeding. In: Jones, D.R. (Ed), Diseases of banana, abaca and enset. CAB International, Wallingford, UK, Pp.425-449.
Kalyebara MR, Ragama PE, Kagezi GH, Kubiriba J, Bagamba F, Nankinga KC, Tushemereirwe W. (2006). Economic importance of the banana bacterial wilt in Uganda. Afr. Crop Sci. J. 14:93-103.
Nyombi K (2013). Towards sustainable highland banana production in Uganda: opportunities and challenges. Afr. J. Food Agric. Nutr. Dev. 13:2.
Ortiz R, Swennen R (2014). From crossbreeding to biotechnology-facilitated improvement of banana and plantain. Biotechnol. Adv. 32:158-169.
Pillay M, Hartman J, Tenkouano A (2002). Bananas and plantains: future challenges in Musa breeding In: Crop Improvement, Challenges in the Twenty-First Century. Kang MS (Ed.), Food Products Press, New York. pp. 223-252.
Rowe P (1984) Breeding Bananas And Plantains. Plant Breed. Rev. 2: 135-155.
SAS Institute Inc. (2004). SAS Institute Inc. version 8, Cary, NC. USA.
Singh RK, Choudhry BD (1985). Biometrical Methods in Quantitative Genetic Analysis.Kalynani Publishers, Ludhiana. P 304.
Ssali RT, Nowakunda K, Barekye A, Erima R, Batte M, Tushemereirwe WK (2010). On farm participatory evaluation of East African highland bananas 'EAHB' hybrids (Musaspp). Acta Hortic. 879:585-591
Ssebuliba R, Talengera D, Makumbi D, Namanya P, Tenkouano A, Tushemereirwe W, Pillay M (2006). Reproductive efficiency and breeding potential of East African highland bananas (Musa sp AAA-EA). Field Crops Res. 95:252-255.
Teich AH (1984). Heritability of grain yield, plant height and test weight of a population of winter wheat adapted to Southwestern Ontario. Theor. Appl. Genet. 68:21-23.
Tushemereirwe WK, Kashaija IN, Tinzara W, Nankinga C, New S (2003). Banana Production Manual. Second edition. pp. 28-51.
Viljoen A 2010. Protecting the African banana (Musa spp): prospects and challenges. ISHS Acta Hortic. 879:305-319.
Wricke G, Weber WE (1986). Quantitative genetics and selection in plant breeding. Walter de Gruyter & Co. Berlin, Germany. pp. 28-68.

African Journal of Plant Science

Related Journals Published by Academic Journals

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

academicJournals