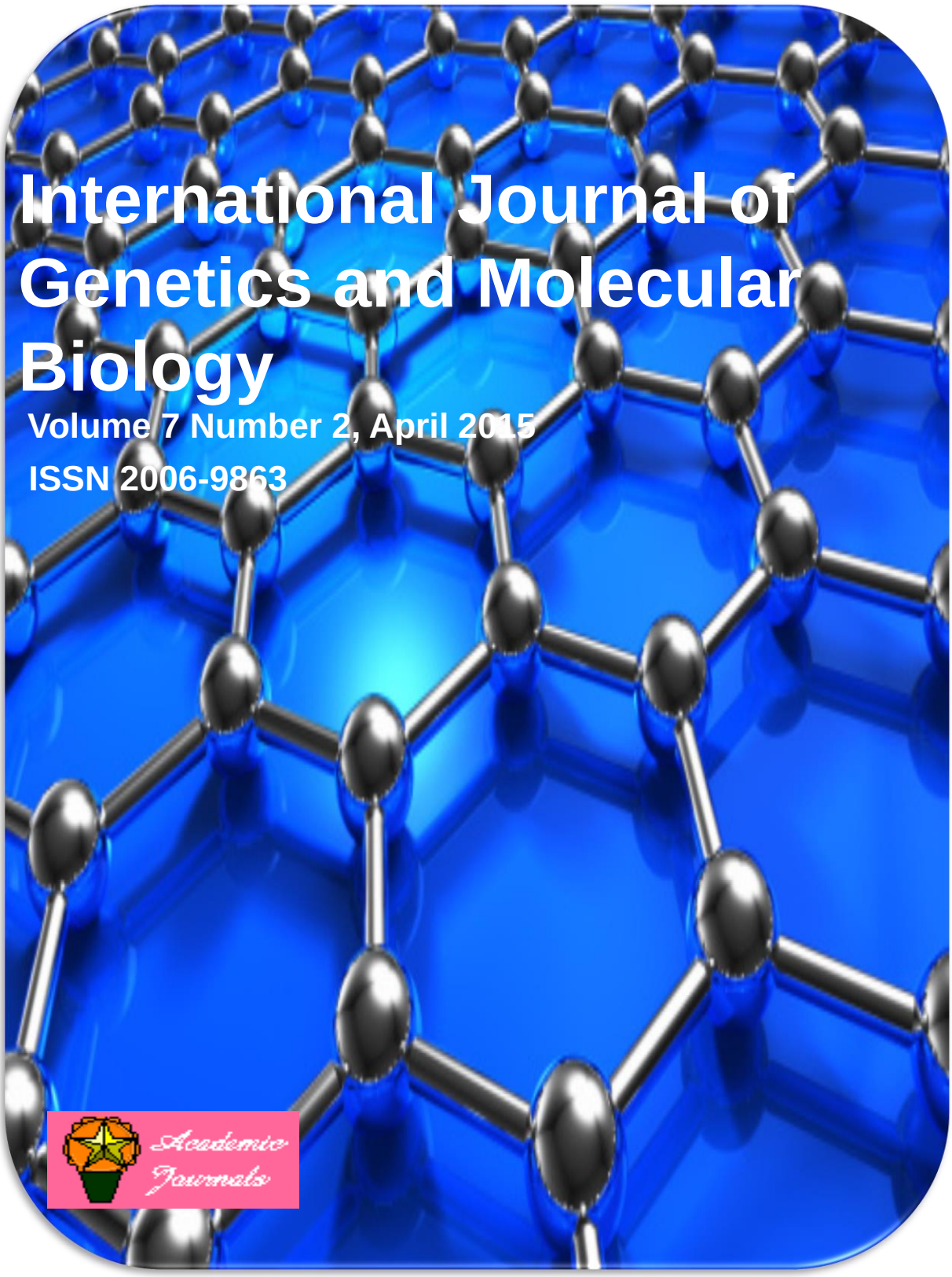

International Journal of Genetics and Molecular Biology

Volume 7 Number 2, April 2015
ISSN 2006-9863

Academic Journals

ABOUT IJGMB

The **International Journal of Genetics and Molecular Biology (IJGMB)** (ISSN 2006-9863) is published Monthly (one volume per year) by Academic Journals.

International Journal of Genetics and Molecular Biology (IJGMB) provides rapid publication (monthly) of articles in all areas of the subject such as DNA and RNA, Influence of risk factors on onset of hyperlipidemia in people with cerebrovascular insult, Study of proteinase activity of Lactobacillus plantarum etc.

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 IJGMB 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 ijgmb@academicjournals.org.

With questions or concerns, please contact the Editorial Office at ijgmb@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 International Journal of Genetics and Molecular Biology 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.

International Journal of Genetics and Molecular Biology

Table of Contents: Volume 7 Number 2 April, 2015

ARTICLES

academicJournals

Vol. 7(2), pp. 8-14, April, 2015
DOI: 10.5897/IJGMB2015.0108
Article Number: B8D441352705
ISSN 2006-9863

http://www.academicjournals.org/IJGMB

International Journal of Genetics and Molecular Biology

Full Length Research Paper

Effects of cytokinin types and their concentration on *in vitro* shoot induction and multiplication of korarima

Rahiel Hagos[1] and Hailay Gebremdhin[2]*

[1]Department of Dry Land Crop and Horticultural Sciences, Mekelle University, P. O. Box 231, Mekelle, Ethiopia.
[2]Department of Plant Science, Debre Berhan University, P. O. Box 445, Debre Berhan, Ethiopia.

Received 18 January, 2014; Accepted 10 April, 2015

Korarima (*Aframomum corrorima* (Braun) P.C.M. Jansen) is a herbaceous perennial plant that belongs to the family Zingiberaceae in which lack of a steady supply of quality planting material is one of the bottlenecks for the exploitation of the export potential. Thus, the use of micropropagation is suggested to alleviate these problems. In micropropagation, *in vitro* seed germination and multiplication of hypocotyls/shoot tips to regenerate shoots is preferred because it minimizes sterilization cost. So far, there was no available efficient protocol optimization on *in vitro* propagation of korarima in Ethiopia. Therefore, an experiment was conducted to determine the optimum concentration of Kinetin (KIN) and N-6-benzyladenine (BA) on *in vitro* regenerating of korarima explants from hypocotyls/shoot tips. Multiplied shoots were subcultured on hormone free medium for four weeks to avoid carryover effect. The different concentrations of KIN (0, 0.5, 1.0, 1.5 and 2.0 mg/l) and/or BA (0, 1.5, 3.0, 4.5 and 6.0 mg/l) were arranged in randomized complete design (CRD) with four replications. Results indicate that medium containing 6.0 mg/l of BA alone was found to be optimum for shoot induction after six weeks. High level of BA alone was found the best for excess shoot proliferation, but high level of KIN mostly enhances longer shoots and regenerates minor roots. Generally, it can be concluded that Korarima can successfully induce shoot from hypocotyls that originated from seeds without any contamination on the initial inoculation of the explants and higher concentration of BA (6.0 mg/l) could be used to obtain desirable shoot regeneration of korarima.

Key words: Cytokinins, N-6-benzyladenine (BA), hypocotyls, kinetin (KIN), shoot induction, shoot Multiplication, *in vitro.*

INTRODUCTION

Korarima (*Aframomum corrorima* (Braun) P.C.M. Jansen), which is native to Ethiopia, is one of the renowned spices and medicinal plants of the family *Zingiberaceae*. It is a herbaceous, perennial and aromatic species classified under the monocotyledonous crops. Morphologically, the plant resembles Indian cardamom (*Elettaria cardamomum*) and consists of an underground rhizome, a pseudostem and several broad leaves (Eyob, 2009). Mature korarima

*Corresponding author. E-mail: hailushgm@yahoo.com

plants can reach a height of 1-2 m and set seeds after 3-5 years of planting, depending on the planting materials used and it continues to bear seeds for several years. Different plant parts of korarima (seeds, pods, leaves and flowers) are locally used in traditional medicine to treat humans and cattle as well. Since its rhizomes and leaves spread well on the ground, korarima plants could also serve a lot for soil and water conservation, especially by covering and protecting the soil from erosion and drying all year round in the mountainous areas where the crop is commonly cultivated (Eyob et al., 2008).

The production and productivity of korarima is continuously decreasing mainly due to absence of expansion of their cultivated areas and destruction of their forest natural habitat, which had been and still is the major source of korarima production. In line with this, results from a recently undertaken formal survey carried out in parts of Southern Ethiopia, attempts of the Ethiopian government to motivate farmers to expand their korarima plantations were not successful due to the associated varied production constraints thereof. This mainly includes lack of improved korarima varieties with high yielding potential and product quality, together with suitable agronomic practices, like best techniques of propagation (Jansen, 2002; Eyob, 2009).

Korarima can be propagated either by seed or by cutting of its clumps, though the latter is by far the most common method, as it yields earlier and ensures a true-to-type propagation than the former. During *in vivo* propagation of korarima, the suitable propagation technique is by using seed (Eyob, 2009). However, vegetative propagation through cuttings results in the destruction of the productive garden, on top of the commonly associated shortage of planting materials to cover wider areas of land. Consequently, seed propagation of korarima is undertaken to cover large areas of land retaining the mother productive stand intact, however, it is essential to give the utmost care while preparing the seeds (Endashaw, 2007). Nevertheless, the slow seed germination and growth of the subsequent seedlings are concerns of korarima growers. The germination of korarima seeds faces certain problems due to the presence of dormancy, possibly associated with its hard seed coat like were reported on seeds of *Elettaria* species (Sulikeri and Kololgi, 1977) and presence of low food reserve in the seed endosperm. More importantly, as korarima is mainly propagated through the vegetative means using one year old rhizomes, the need for bulk of rhizomes as planting materials and slow multiplication rate of the rhizomes is a serious bottleneck observed. Also the destructive harvesting of the rhizomes associated with vegetative propagation is a serious threat as there is always the possibility of losing the mother plant during this process. The problem becomes much more aggravated when one has to transport the rhizome clumps to distant areas due to their perishable nature and relatively costly transportation. Susceptibility to unknown diseases is also among the major problems faced by korarima growers using the conventional vegetative propagation (Tefera and Wannakrairoj, 2004; Eyob, 2009).

Therefore, enhancement of korarima seed germination and multiplication is essential in propagation and breeding program, as well as for testing and using available germplasm. Particularly, proper tissue culture procedures, which assure successful and efficient propagation from seedling, need to be developed. To date, only two tissue culture studies have been reported by using of either lateral buds from rhizomes of the Jimma local cultivar (Tefera and Wannakrairoj, 2004) or by using pretreated seeds of cultivar Mume collected from the southern part of the country (Eyob, 2009).

Even though korarima has much advantage, lack of a steady supply of quality planting material is one of the bottlenecks for the exploitation of the export potential of korarima in Ethiopia. However, only limited efforts have been made so far to improve the crop using traditional and modern biotechnological approaches. Thus use of micropropagation is suggested to alleviate the problem of shortage of planting materials. *In vitro* seed germination and multiplication of hypocotyls/shoot tips to regenerate shoots is preferred because it minimizes sterilization cost. So far there was no available efficient protocol optimization on *in vitro* propagation of korarima. Hence, this study was initiated to come up with a suitable *in vitro* propagation protocol for the local Jimma cultivar in the absence of coconut water (CW) and Imazalil (IMA), which gave the best result when used as media components by Tefera and Wannakrairoj (2006), but that are not readily available at the local market. In addition, inclusion of CW and TDZ to the culture medium resulted in reduction of both number of roots, root length and shoot length. Therefore, the present study was envisaged to focus on optimizing and/or developing an efficient protocol for the *in vitro* regeneration of korarima from pretreated seeds/hypocotyls of Korarima; Jimma local cultivar.

MATERIALS AND METHODS

Treatments and experimental design

The establishment of contamination free seeds (capsules) of Jimma local cultivar, cultivated around the Jimma located areas was selected from the Jimma Agricultural Research Center (JARC), which was apparent disease-free planting material and fits agronomically. Well ripened fresh capsules (red capsules) were selected and then used as a starting material for the subsequent experiments. Extracted seeds were treated with 50% H_2SO_4 for 60 min and soaked in 250 mg/l of gibberillic acid (GA_3) for 24 and 48 hrs before sterilization and then sterilized by 3.0g/l Kocid for 30 min, 70% ethyl alcohol for 3 min and 25% NaOCl + 2-drop of Tween-80 for 15 min during sterilization.

Every sterilization steps were washed by distilled water. Plant growth regulators (PGR) used for the experiment were N-6-benzyladenine (BA) and Kinetin (KIN). To identify the best combinations of these hormones for shoot induction and multiplication, various concentrations of KIN (0.0, 0.5, 1.0, 1.5, and 2.0 mg/l) and/or BA (0.0, 1.5, 3.0, 4.5 and 6.0 mg/l) were treated with four

replications of each treatment.

Preparation of growth culture conditions

Culture media were prepared by taking the recommended amounts of MS (Murashige and Skoog, 1962) stock solutions supplemented with 3% (w/v) sucrose as a carbon source, and 0.7% (w/v) agar (for shoot multiplication) and 0.8% (w/v) agar (for root induction) as solidifying agents. In each experiment, the desired concentrations and types of PGR were added before autoclaving and 40 ml of the respective medium was poured into 350 ml jams jar and covered with a cap for shoot multiplication. After mixing up all media components together with the combined PGR and adjusting the volume, the pH of the culture medium was adjusted to 5.70 with either 1% N HCl or 1% N NaOH. Later, the respective solidifying agent (agar) was added into the medium. Medium was autoclaved at 15 pounds per square inch (psi) that is 121°C for 20 min. Autoclaved media was allowed to cool in sterile environment after which it was ready for use. Finally, all the autoclaved culture media were retained in the media room for a maximum of three days prior to use.

Plant material, surface disinfection and inoculation of explants

Fresh capsules (seeds) (2–5 mm in diameter) of Korarima were obtained at the peak harvestable stage of capsules and were collected from apparent disease free growing parents. The capsules were collected thoroughly from the agronomically desirable parents and harvested fresh capsules seeds were extracted and then sterilized before culturing on the prepared germinated media. Then seeds were washed five times immediately with tap water at least for 15 min and then they were kept (soaked) under 3.0 g/l Kocid [$Cu(OH)_2$] for 30 min, and rinsed five times with sterilized distilled water. The rinsed seeds were also soaked in 50% of H_2SO_4 for 60 min then seeds were crushed by hands using nylon cloth and immediately soaked in 250 mg/l of GA_3 for 24 and 48 h. Finally, they were washed with sterilized distilled water for 20 min and more scales were then removed, followed by washing with detergents.

Inside the laminar air-flow cabinet, seeds were rinsed with 70% ethanol for 3 min; followed by one-step surface sterilization using 25% sodium hypochlorite so called commercial bleach (which contains 5.1% active chlorine) mixed with two drop (2.0 ml/l) of Tween-80 (wetting agent) by vigorous shaking for 15 min. Then, the seeds were washed five times using sterilized distilled water and were further trimmed to remove dead seed coat and sulfuric acid affected scales. Before culturing, the explants (sterilized seeds) were soaked in sterile distilled water for 20 min to remove traces of chlorine and other remnants in the seed.

The surface sterilized seeds were inoculated into different liquid MS basal media strengths in an aseptic condition that contained full MS (FMS), half MS, one-fourth MS, one-eighth MS and free water as a control. 20 ml of each liquid media was dispensed into 100 ml baby food jar embedded with cotton pad and were adjusted to pH of 5.70 prior to autoclaving. On top of this, solid one-fourth MS medium was also prepared for germination and it followed the above procedures except with the addition of 7.0 g/l agar. Each germination media strength was inoculated by seeds that emerged from a single capsule.

Effect of cytokinins on shoot induction and multiplication

Induced shoot tips obtained from hypocotyle explants were cultured on MS medium fortified with 1.0 and 3.0 mg/l of KIN and BA in combination for further shoot induction and multiplication, according to Balachandran et al. (1990), Sanghamitra (2000) and Tefera and Wannakrairoj (2004). Then after culturing on hormone free media for one month, new regenerated shoots were cultured on fresh medium supplemented with different hormonal combinations of KIN and BA in jams jars containing 40 ml of MS media with different concentration of each hormone. In each experimental unit, three shoots (1.5-2.0 cm) were cultured per jar/replication with a total observation unit of nine explants per treatment. The experiment was repeated two times, though only data from the last one was used for statistical analysis. Cultures were incubated in the culture room at a constant temperature of 25 ± 2°C and relative humidity of 50-60%, under cool white fluorescent light of 28 µmol m^{-2} s^{-1} (1500-2000 lux) photosynthetic photon flux density with 16 hper day photoperiod for six weeks. After six weeks, data were recorded from individual emerged new shoots obtained from each cultured shoots and subsequently cultured on free hormone media.

Data analysis

Mean values of the parameters were subjected to analysis of variance (one-way ANOVA and two-way ANOVA) using the SAS software packages (SAS, 2008, version 9.2). The significant differences among treatments were compared using Least Significance Difference (LSD) for the one-way ANOVA and Ryan-Einot-Gabriel-Welch range test (REGWQ) for the treatment combinations of the two-way ANOVA at a 5% of probability level.

RESULTS AND DISCUSSION

Selection of shoot tips for the subsequent experiments from *in vitro* germinated seeds

In order to break seed dormancy, seeds were soaked in 50% of H_2SO_4 for 60 min and in 250 mg/l of GA_3 for one to two days. Seeds were not germinated in all media strengths except one-fourth MS strength liquid and solid media. Germination was started after 24 days and the germination percentage was 66.7% (data not shown). Therefore, one-fourth MS medium strength was more preferable for seed germination of korarima and the use of a single capsule for a given basal MS medium strength was also important to obtain uniformly emerged hypocotyls than mixed seeds. Seeds soaked on GA_3 for 1 to 2 days were best for breaking of the dormancy and encouraged seeds to start their germination within 24 days, but they had erratic seed germination. Hypocotyls emerged from the one-fourth solid MS media and treated in 250 mg/l GA_3 for 48 h were highly elongated (thinner) and abnormal in their performance than one-fourth liquid MS medium treated for 24 h (Figure 1).

Seeds placed in sterile cotton pad soaked with distilled water have the ability to germinate better than FMS, half MS and one-eighth MS but it took longest mean days (for more than five months). On top of this, emerged hypocotyls from free distilled water were highly sensitive to bacterial contamination as compared to hypocotyls emerged from one-fourth MS medium. Liquid media including sterile cotton pad was better than solid media for facilitating the imbibitions of water to induce enzymatic activities of the seed and minimize the contamination during inoculation of the hypocotyls in test tubes for further shoot induction

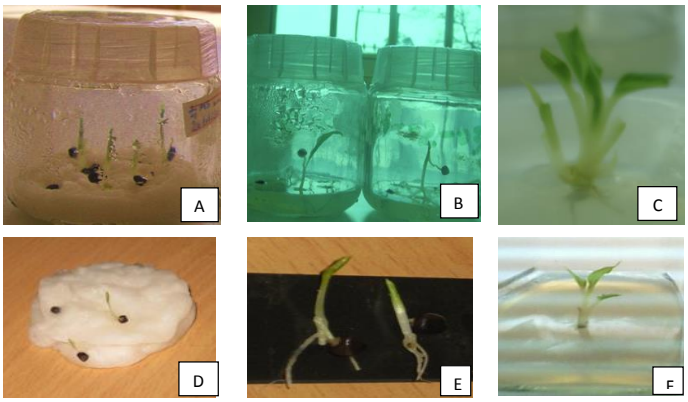

Figure 1. Seed germination of korarima in MS liquid and solid media. A) Hypocotyls emerged from the culturing of korarima seeds in one-fourth MS liquid media. B) Hypocotyls emerged on solid one-fourth MS media; C and F) Inoculation of hypocotyls on induction media; D and E) Hypocotyls generated from free distilled water.

Table 1. Mean and probabilities of the independent effect of KIN and BA on korarima shoot growth after six weeks of culture.

Treatment		Mean (main effect)	
		No. of days to shoot Ind.	No. of leaves
KIN (mg/l)	0	18.07[a]	12.6[a]
	0.5	16.21[ab]	13.6[a]
	1.0	15.3[b]	12.2[a]
	1.5	17.27[ab]	12.47[a]
	2.0	18.2[a]	11.47[a]
BA (mg/l)	0	17.80[a]	9.8[b]
	1.5	16.40[a]	15.2[a]
	3.0	16.87[a]	13.8[a]
	4.5	17.67[a]	11.67[ab]
	6.0	16.33[a]	11.87[ab]
	KIN	0.005***	0.650 [NS]
	BA	0.268 [NS]	0.003***
	KIN*BA	0.073 [NS]	0.193 [NS]
	CV(%)	13.57	30.38

*** = highly significant difference at 1% probability level and * significance at 5% probability level (REGWQ). Means within a column followed by the same letter are not significantly different at P < 0.05 level of significance (REGWQ). No, number; Prob., probability; Ind., induction; NS, non significance.

and multiplication. After eight weeks, hypocotyls (cotyledonary nodal segments) that emerged from the seed culture was taken and cultured in test tube containing MS medium fortified with 1.0 mg/l of KIN and 3.0 mg/l BA according to their germination date for further shoot proliferation. Hypocotyls were also subcultured every month on the same medium of KIN and BA until the desired multiple shoots were obtained for the subsequent experiments.

Effects of different concentrations of KIN, BA and their combination on shoot induction and multiplication of stage of korarima

Number of days to shoot induction

As shown in Table 1, KIN alone results in a high significant difference on number of days to shoot induction (P = 0.005), but both the interaction effects of BA and KIN nor KIN alone significantly affected the number of days to shoot induction. Hence, 1.0 mg/l of KIN was found to have the shortest number of days to shoot induction but it had no significant difference with 0.5 and 1.5 mg/l treatment levels of KIN. The highest number of days to shoot induction was observed in 2.0 mg/l of KIN but there was no significant difference with the PGR free medium.

Number of shoots

Number of shoots was not statistically different among the treatment combinations of KIN and BA, except with the treatment combination of PGR-free medium, 0.5, 1.0, 1.5 and 2.0 mg/l of KIN alone, 1.5 mg/l of BA alone, 0.5 + 1.5, 1.0 + 1.5 and 2.0 + 3.0 mg/l of KIN and BA in combination, respectively and was significantly different from the other treatment combinations (Table 2). But, the best medium for shoot multiplication was obtained on 6.0 mg/l of BA alone (which is similar with findings of Kochuthressia et al. (2010)) and the combination of 1.5 mg/l of KIN with 3.0 mg/l of BA, had a good response to regenerate the largest number of shoots with an average number of 10.33 and 9.67 shoots per plantlet, respectively. But the combination of 1.5 mg/l of KIN with 3.0 mg/l of BA was not significantly different from the combination of 0.5 mg/l of KIN and 3.0 mg/l of BA as well as 3.0 mg/l of BA alone in number of shoots.

The lowest number of shoot multiplication (3.33 in average) was observed at higher concentration of KIN (2.0 mg/l), which is not similar with the findings of Kochuthressia et al. (2010) in which 6.4 shoots/explant regenerated on red ginger (*Alpinia purpurata)* and the PGR free MS medium was used as a control also induced shoots at a rate of 4.33 shoots per explants. The result from the present study on the shoot multiplication of korarima (Jimma local cultivar) agrees with those of Sanghamitra (2000) and Naz et al. (2009) on shoot multiplication of turmeric with high concentration of BA (5.0 mg/l) and Kavyashree (2009) on ginger var. Varada with high concentration of BAP (17.76 µM). But it had no significance different with other treatment combinations, and hence as the concentration of KIN increases to 2.0 mg/l and BA at zero, the average numbers of shoots per plantlet become lowest. In general, the sole use of BA in

Table 2. Effects of different concentration of KIN and BA in shoot induction and multiplication after six weeks of culture.

KIN (mg/l)	BA (mg/l)	NS /explants	SL/explants (cm)	SFW (mg)	SDW (mg)
0	0	4.33^{hgf}	3.11^{a}	390^{ba}	30^{ba}
0	1.5	6.00^{c-h}	2.65^{abc}	413^{ba}	33^{ba}
0	3	9.33^{abc}	2.62^{abc}	333^{ba}	27^{ba}
0	4.5	8.33^{a-d}	1.9^{bc}	290^{ba}	21^{ba}
0	6	10.33^{a}	2.086^{abc}	567^{a}	42^{ba}
0.5	0	4.0^{hg}	3.10^{a}	267^{ba}	23^{ba}
0.5	1.5	5.33^{d-h}	2.52^{abc}	310^{ba}	23^{ba}
0.5	3	9.00^{abc}	2.35^{bac}	390^{ba}	29^{ba}
0.5	4.5	8.33^{a-d}	1.82^{abc}	370^{ba}	28^{ba}
0.5	6	8.33^{a-d}	1.82^{c}	290^{ba}	22^{ba}
1	0	5.75^{c-h}	2.89^{ab}	500^{ba}	39.6^{ba}
1	1.5	6.33^{b-h}	2.257^{abc}	550^{a}	50^{a}
1	3	8.33^{a-d}	2.253^{abc}	177^{b}	16.7^{b}
1	4.5	7.33^{a-g}	2.367^{abc}	357^{ba}	34.7^{ba}
1	6	8.67^{a-d}	2.053^{bc}	353^{ba}	34.7^{ba}
1.5	0	5.33^{d-h}	2.10^{abc}	487^{ba}	30^{ba}
1.5	1.5	8.00^{a-e}	2.21^{bac}	413^{ba}	24^{ba}
1.5	3	9.67^{ab}	1.88^{bc}	430^{ba}	26.7^{ba}
1.5	4.5	8.00^{a-e}	1.756^{c}	377^{ba}	32.3^{ba}
1.5	6	9.00^{a-d}	2.077^{abc}	373^{ba}	33.3^{ba}
2	0	3.33^{h}	2.383^{abc}	477^{ba}	45^{ba}
2	1.5	8.33^{a-d}	1.843^{bc}	510^{ab}	31^{ba}
2	3	4.67^{e-h}	1.633^{c}	483^{ba}	43^{ba}
2	4.5	7.33^{a-g}	1.836^{bc}	320^{ba}	30^{ba}
2	6	7.67^{a-f}	1.820^{c}	233^{ba}	16.7^{b}
Means		7.24	2.21	0.386	0.0307
CV (%)		13.15	13.13	30.06	31.24
SE (±)		0.549	0.168	0.067	0.0055
SE mean (±)		0.21919			
KIN		0.0001***	0.0001***	0.243^{NS}	0.062^{NS}
Prob. BA		<.0001***	0.0001***	0.108^{NS}	0.537^{NS}
KIN*BA		<.0001***	0.0124*	0.009^{***}	0.003^{***}

*** = highly significant difference at 1% probability level and * significance at 5% probability level (REGWQ). Treatment mean values with the same letter in a column are not significantly different at P < 0.05 level of significance (REGWQ). NS, non significance; SE, standard error, CV (%), coefficient of variance; Prob., probability; NS/explants, mean number of shoots per explants; SL/explants, mean shoot length per explants; FW, mean shoot fresh weight, DW- mean shoot dry weight.

shoot induction and multiplication medium is applicable for better multiplication of shoots than it is combined with KIN and it is similar with the results of Nayak (2000), 5 mg/l BA in *C. aromatica* Salisb and Sharma and Singh (1995), 8 mg/l BA in ginger enhanced microrhizome production.

Number of leaves

The number of leaves was not affected by the concentration of KIN alone (P = 0.65) and their interactions (KIN*BA) (P = 0.19), but BA alone highly significantly affected the number of leaves at P = 0.0033 (Table 2). The highest number of leaves was found on 1.5 mg/l of BA and it was not significantly different with the three concentrations of BA (3.0, 4.5 and 6 mg/l), but the lowest number of leaves was observed on PGR-free medium.

Shoot length

Both KIN and BA alone highly significantly affected the shoot length with P≤.0001, but their interaction (KIN*BA) was affected significantly (P = 0.01). Hence the PGR-free medium had the longest mean shoot length (3.11 cm) followed by 0.5 mg/l of KIN alone (3.10 cm) than other

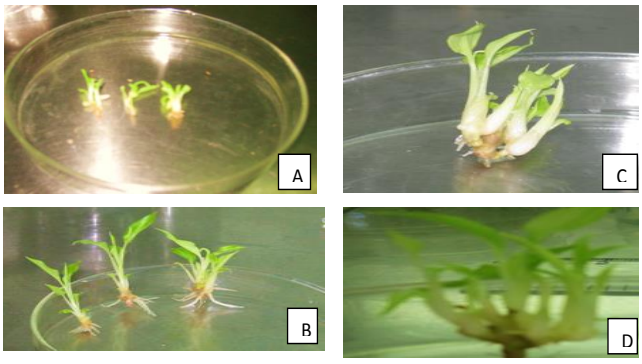

Figure 2. Explants cultured on different cytokinin combinations of KIN and BA. **A)** Explants cultured on KIN (2.0 mg/l) + BA (3.0 mg/l). **B)** Explants cultured on MS + 2.0 mg/l of KIN and regenerates roots**. C)** Explants cultured on KIN (1.0 mg/l) + BA (6.0 mg/l). **D)** Explants cultured on MS + 6.0 mg/l of BA.

treatment combinations, and it had significant difference with 0.5 + 6.0, 1.5 + 3.0, 1.5 + 4.5, 2.0 + 1.5, 2.0 + 3.0, and 2.0 + 6.0 mg/l of KIN and BA treatment combinations. When 2.0 mg/l of KIN combined with the four levels of BA, the shoot length became decreased and had no significant difference among them. But as the concentration of KIN increases from 0.5 to 2.0 mg/l and combined with 6.0 mg/l of BA, there was no significant difference on shoot length (Table 2). Whereas the shortest shoots was obtained on the treatment combination of 2.0 + 3.0 mg/l of KIN and BA (which were reported by Kochuthressia et al. (2010) on red ginger exhibited shoot regeneration rate up to 6.4±0.32 shoots/explants), but it had no significant difference with all treatment combination, except with PGR free medium, 0.5 and 1.0 mg/l of KIN alone, which had a long shoots.

Shoot fresh and dry weight

The interaction of the two factors (KIN*BA) was highly significant (P = 0.009) on shoot fresh and dry weight with P = 0.003, but not their alones (Table 2). In the case of shoot fresh weight, all treatment combinations were not significantly different among themselves. But there was significant difference between 6.0 mg/l of BA alone or 1.0 + 1.5 mg/l or other treatment combinations of KIN and BA in combination with 1.0 + 3.0 mg/l of KIN and BA (which had a lowest shoot fresh weight). On the other side, highest shoot dry weight was obtained on 1.0 + 1.5 mg/l (50 mg per shootlet) of KIN and BA and had significant difference with 1.0 + 3.0 mg/l and 2.0 + 6.0 mg/l (16.7 mg per shootlet) of KIN and BA in combination that is the lowest shoot dry weight was found on them. Generally, there was no significant difference in almost all the treatment combinations, but there was significance different among 1.0 + 1.5 mg/l, 1.0 + 3.0 mg/l and 2.0 + 6.0 mg/l of KIN and BA treatment combinations.

In all, the result shows that, with an increased in concentration of KIN alone in the medium, the number of shoots induced decreased significantly. In this study, higher concentration of KIN alone regenerates roots and longer shoots (Figure 2). On the contrary, BA alone was very responsive to the shoot induction and multiplication but not true for shoot elongation on korarima. The application of 6.0 mg/l of BA alone resulted in significant increased number of shoots, shoot length, shoot fresh weight and dry weight per explant.

This result agrees with works of several authors (Sanghamitra, 2000; Sivakumar and Krishnamurthy, 2000; Tefera and Wannakrairoj, 2004, 2006). In addition, 3.0 mg/l of BA alone was found to be not significantly different from 6.0 mg/l on number of shoots, shoot length, shoot fresh and dry weight. Lastly, from the present study, the combination of BA and KIN developed fewer multiple shoots, which was similar with the results of Purohit and Dave (1996) and Sivakumar and Krishnamurthy (2000) and emerged few roots.

From this study, it can be concluded that, 0.5 mg/l of KIN including MS were more responsive for regeneration of roots and elongation of shoots and also shoots were more vigorous than other treatment combinations in korarima. On the other hand, shoots cultured on 6 mg/l BA including MS, responded more for induction of new microshoots. As the concentration of BA increases throughout the hormonal combination of the medium, the number of shoot induced was increased. All the shootlets regenerated survived (100%) without contamination. However, some physiological senescence like drying on the bottom of the shootlets and tip burn in the shoots cultured with more concentration of KIN were observed.

Conclusion

Generally, different concentration of cytokinins type and concentration influenced the *in vitro* propagation of korarima in shoot induction and multiplication. The use of KIN resulted in significantly lower shoot regeneration as compared to BA. Higher concentrations of BA increased the number of regenerated shoots but decreased shoot length. The application of BA in the medium as compared to KIN stimulates the rate of shoot regeneration and the greatest shoot regeneration was found on media with 6.0 mg/l of BA alone. But at high concentration, it has impacts on destroying plantlets via vitrification andshoot tip necrosis. Therefore, higher concentration of BA (6.0 mg/l) could be used to obtain desirable shoot regeneration of korarima.

Conflict of interest

The authors did not declare any conflict of interest.

REFERENCES

Balachandran SM, Bhat SR, Chandel KPS (1990). In vitro clonal

multiplication of turmeric (Curcuma sp.) and ginger (Zingiber officinale Rosc.). Plant Cell Reports, 8: 521-524. http://dx.doi.org/10.1007/BF00820200

Endashaw B (2007). Study on actual situation of medicinal plants in Ethiopia, Prepared for Japan Association for International Collaboration of Agriculture and Forestry, JAICAF.

Eyob S, Martinsen K, Tsegaye A, Appelgren M, Skrede G (2008). Antioxidant and antimicrobial activities of extract and essential oil of korarima (Aframomum corrorima (Braun) P.C.M. Jansen). Afri. J. Biotechnol. 7(15):2585-2592.

Eyob S (2009). Promotion of seed germination, subsequent seedling growth and in vitro propagation of korarima (Aframomum corrorima (Braun) P. C. M. Jansen). J. Med. Plants Res., 3(9): 652-659.

Jansen P (2002). Aframomum corrorima (Braun) P.C.M. Jansen." Record from Protabase. Oyen,L.P. and Lemmens, R.H. (eds). PROTA (Plant Resources of Tropical Africa /Resources végétales de l'Afrique tropicale. Internet document: http://database.prota.org/PROTAhtml/Aframomum%20corrorima_En.htm, Wageningen, the Netherlands, Accessed on 19 June 2011.

Kambaska K, Santilata S (2009). Effect of plant growth regulator on micropropagation of ginger (Zingiber officinale Rosc.) cv- Suprava and Suruchi. J. Agric. Technol. 5(2): 271-280.

Kavyashree R (2009). An efficient in vitro protocol for clonal multiplication of ginger-var. Varada. Plant Biotechnology, Bangalore University, India. Ind. J. Biotechnol., 8: 328-331.

Kochuthressia KP, Britto SJ, Raj JM, Jaseentha MO, Senthilkumar SR (2010). Efficient regeneration of Alpinia purpurata (Vieill.) K.Schum. plantlets from rhizome bud explants. Intl. Res. J. of Plant Sci., 1(2): 043-047.

Murashige T, Skoog F (1962). A revised medium for rapid growth and bioassays with tobacco tissue culture. Physiologia Plantarum, 15(3):473- 497. http://dx.doi.org/10.1111/j.1399-3054.1962.tb08052.x

Naz S, Ilyas S, Javad S, Ali A (2009). In Vitro Clonal Multiplication and Acclimatization of Different Varieties of Turmeric (Curcuma Longa L.), Sargodha, Sargodha, Pakistan. Pakis. J. Bot., 41(6): 2807-2816.

Nayak S. (2000). In vitro multiplication and microrhizome induction in Curcuma aromatic Salisb. Plant Growth Regul. 32: 41-47. http://dx.doi.org/10.1023/A:1006307316393

Purohit SD, Dave A (1996). Micropropagation of Sterculia urens Roxb. – an endangered tree species. Plant Cell Report, 15:704-706. http://dx.doi.org/10.1007/BF00231929

Sanghamitra N (2000). In vitro multiplication and microrhizome induction in Cucurma aromatica Sallsb. Plant Growth Regulation, 32: 41-42. http://dx.doi.org/10.1023/A:1006307316393

Sharma TR, Singh BM (1995). In vitro microrhizome production in Zingiber officinale Rosc. Plant Cell Rep. 15:274-277. http://dx.doi.org/10.1007/bf00193735

SAS Institute Inc. Statistical Analysis Software, Version 9.2. Cary, North Carolina, USA, 2008.

Sivakumar G, Krishnamurthy KV (2000). Micropropagation of Gloriosa superb L. – an endangered species of Asia and Africa. Current Science, 78:30-32.

Sulikeri GS, Kololgi SD (1977) Seed viability in cardamom (Elettaria cardamomum Maton), Current Research, 6:163-164.

Tefera W, Wannakrairoj S (2004). A micropropagation method for korarima (Aframomum corrorima (Braun) Jansen), Kasetsart University, Kampaengsaen, Thailand. Sci. Asia, 30: 1-7. http://dx.doi.org/10.2306/scienceasia1513-1874.2004.30.001

Tefera W, Wannakrairoj S (2006). Synergistic effects of some plant growth regulators on in vitro shoot proliferation of korarima (Aframomum corrorima (Braun) Jansen). Afri. J. Biotechnol., 5(10): 1894-1901.

International Journal of Genetics and Molecular Biology

Related Journals Published by Academic Journals

- *Biotechnology and Molecular Biology Reviews*
- *African Journal of Biotechnology*
- *African Journal of Microbiology Research*
- *African Journal of Biochemistry Research*
- *Journal of Computational Biology and Bioinformatics Research*
- *International Journal for Biotechnology and Molecular Biology Research*
- *Journal of Developmental Biology and Tissue Engineering*

academicJournals