African Journal of Biotechnology

Volume 15 Number 52, 28 December 2016

ISSN 1684-5315

Academic Journals

ABOUT AJB

The African Journal of Biotechnology (AJB) (ISSN 1684-5315) is published weekly (one volume per year) by Academic Journals.

African Journal of Biotechnology (AJB), a new broad-based journal, is an open access journal that was founded on two key tenets: To publish the most exciting research in all areas of applied biochemistry, industrial microbiology, molecular biology, genomics and proteomics, food and agricultural technologies, and metabolic engineering. Secondly, to provide the most rapid turn-around time possible for reviewing and publishing, and to disseminate the articles freely for teaching and reference purposes. All articles published in AJB are peer-reviewed.

Contact Us

Editorial Office: ajb@academicjournals.org

Help Desk: helpdesk@academicjournals.org

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

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

African Journal of Biotechnology

Table of Content: Volume 15 Number 52 28 December. 2016

ARTICLES

academicJournals

Vol. 15(52), pp. 2845-2853, 28 December 2016
DOI: 10.5897/AJB2016.15255
Article Number: 83D99A662168
ISSN 1684-5315

http://www.academicjournals.org/AJB

African Journal of Biotechnology

Full Length Research Paper

Optimizing *Bacillus circulans* Xue-113168 for biofertilizer production and its effects on crops

Shengping Xue[1]*, Liantian Miao[2], Yujie Ma[1], Yan Du[1] and Hongbo Yan[1]

[1]College of Bioscience and Bioengineering, Hebei University of Economics and Business, Shijiazhuang, 050061, China.
[2]Key Laboratory of Microbial Engineering, Chinese Academy of Sciences, 100101, China.

Received 3 February, 2016; Accepted 26 September, 2016

In this study, *Bacillus circulans* Xue-113168 biofertilizer was produced through solid state fermentation processes using food waste and feldspar. Results confirmed that solid state fermentation has considerable advantages compared to complex process (solid-state and bio-bleach). The control of pH, temperature, and humidity effectively led to the formation of 2×10^9 cfu/g spore and dissolution of potassium at a rate of 41.53%. Compound microbial fertilizer (CMF), formulated by humic acid and K_2HPO_4 with biofertilizer, has a quick and durable effect. CMF increases the yield of rapeseeds by 75 to 89%, provides higher vitamin C and reduces nitrate in leaf. Yields of selenium-enriched jujube and jujube increased, respectively, in the CMF compared to the matrix control; rates of anthracnose and rust diseases also decreased. Furthermore, our results showed that CMF improved soil properties, such as organic matter, NPK content from 8.83 to 16.16 kg hm^2, and reduced chemical fertilizer from 25 to 11%, respectively. For convenient medium, robust process and good effect, CMF is suitable for potassium deficiency and undeveloped arable land resources.

Key words: compound microbial fertilizer, *Bacillus circulans* Xue-113168, solid-state fermentation (SSF), process, optimize.

INTRODUCTION

Plant biostimulants contain substance(s) and/or micro-organisms whose function when applied to plants or the rhizosphere is to stimulate natural processes to enhance/benefit nutrient uptake, nutrient efficiency, tolerance to abiotic stress, and crop quality. Biostimulants (Dobbss, 2016; Canellas et al., 2015;Patrick du Jardin, 2015) are ingredient from microbe or others, abiotic stress resistance is promoted, and the effect is not dependent on the nutritional components. Biochemical humic acid are produced from organic by-products and waste residue, waste liquid in organic materials by microbial fermentation and physical and chemical processes (Luciano, 2015). Jujube rust and anthracnose are important diseases that are difficult to control after their occurrence and can cause heavy production loss and low fruit quality. Present research on jujube rust has only dealt with its epidemiology and chemical control. Compound microbial fertilizer (CMF) can palliate Jujube rust and anthracnose, enhance quality attributes of yields, protect and improve soil health by fostering development of beneficial soil microorganisms. Anthracnose and rust disease are soil-borne diseases

*Corresponding author. E-mail: xsp6210@163.com. Tel: +8615373655258. Fax: 86-0311-87655680.

caused by fungi which are difficult to deal with. Zhao et al. (2011) demonstrated how to control *Fusarium* wilt disease in *Cucumis melo*, using bioorganic fertilizer. Plant growth-promoting rhizosphere (PGPR) prevents and controls plant diseases especially soil-borne disease through the followings: competition between the pathogens and space site, the development of antibiotics to inhibit the growth of pathogens and induction of systemic resistance (ISR) of plant (Pacôme et al., 2016). Microbial fertilizers enrich soil fertility (Padmavathiamma et al., 2008), improve fertilizer utilization, inhibit the absorption of nitrate nitrogen, heavy metals, and pesticides by crops, clean up and repair the soil and reduce the occurrence of crop diseases. Biofertilizers could contribute to relieving stresses, that is, energy crisis, scarcity of resources, and environmental pollution (Ho et al., 2010; Basak and Biswas, 2010; Nishanth and Biswas, 2008; Iqbal et al., 2010; Kumar et al., 2010). The application of humic acids, organic wastes and biofertilizers might minimize the pollution caused by chemical fertilizers (Eman, 2008), and the scale of biofertilizers production could be achieved by using a composting mode (Farrell and Jones, 2009).

Optimization of PGPR (Esitken et al., 2010; Kloepper, 2009) by solid state ferment (SSF) in a less-cost and convenient way is urgent. Low water activity in SSF facilitates formation of spore on the solid substrate and the production of large quantities of enzymes, which is different from submerged fermentation (SMF) processes (Barrios-González, 2012). Microbial release of potassium from K-bearing minerals consists of fermentation and bioleach (Lian et al., 2008). This study combines two steps into one.

Furthermore, chitinase and its hydrolysis product have disease resistance effects. Therefore, it is necessary to explore the ability of high dissolving potassium and chitinase activity of strains, its control process, and using it for SSF. In this study, a biological fertilizer, *Bacillus circulans* strain, with a high rate of spore formation and ability to dissolve K and chitinase, was created, making the best use of agricultural and mineral resources (Kloepper, 2009; Zhu et al., 2012). This enhances crop yields, improves the utilization rate of chemical fertilizers and pesticides, and promotes the sustainable development of agricultural industries (Rabish and Keshav, 2013). Thus, the integrated use of PGPR with chitinase and value-added composted organic waste could be highly effective in improving yield.

The present study illustrates the effectiveness of inoculation with PGPR containing chitinase and humic acid in the presence and absence of chemical fertilizer in improving growth, yield and reducing disease under pot and field conditions.

MATERIALS and METHODS

This study screens a large number of available raw materials for a solid substrate through SSF process. The solid substrate does not only supply the nutrients to the anchorage for the cells, but also induces enzymes. The culture medium contains nutrients and the supporting materials. The former are generally flour, while the latter include shrimp shell, spent mushroom substrate, corn bran, potassium feldspar powder, etc. Shrimp shell is rich in chitin for inducing chitinase. K-feldspar is a source of potassium. SMS functions as antibiotics and phytohormone. Flour can be used as nutrients.

K-feldspar was purchased from Lingshou County, Hebei PR, China. The material was ground and then passed through 100 mesh screens. An analysis showed that it contained 10.0% total potassium and insoluble potassium. The shrimp shell was taken down from the South American shrimp. The material was ground and then passed through 16 mesh screens. The analysis showed that it contains 30% protein, 45% ash, and 20% chitin.

SMS of *Pleurotus ostreatus* was obtained from a local market. The material was ground and then passed through 16 mesh screens. The analysis showed that it contained 4.45% organic matter and 1.58% total N. Its C/N was 28/1, water absorption 78 to 80%, and humic acid content 20 to 30%. Corn bran was from the North China Pharmaceutic Corporation, Hebei PR, China. The analysis showed that it contained 11.8% protein, 32% glucose and 11% cellulose.

Potassium dissolving microorganism (KSM)

This K-limited medium is designed to isolate the bacteria that seek to release K from feldspar (Hutchens et al., 2003). *Bacillus circulans* Xue-97316 was isolated from corn rhizosphere soil using K-feldspar as the sole potassium source for the medium (g/L): starch, 5.0; yeast extract, 1.0; $MgSO_4$.$7H_2O$, 0.5; $CaCO_3$, 0.1; $FeCl_3.6H_2O$, 5 mg; pH 7.5). After 1-day incubation at 30°C, *B. circulans* was selected by colony morphology. The single colony looked like a glassy bead. This isolate was originally identified using biochemical and physiological tests. The potassium dissolving rate was determined as = (St-Sc)/It×100%, where St and Sc are the water-soluble potassium in the treatment and matrix, respectively, and It = the total potassium in the treatment.

In order to make the UV light convenient and effective, *B. circulans* strain with a high potassium dissolving rate was bred before using UV mutagenesis and it was screened in a fermentation broth using potassium tetraphenylborate spectrophotometric method. *B. circulans* strains with chitinase activities were previously screened using colloid chitin as a sole C source in a medium. *B. circulans* Xue-113168 was obtained from a series of mutation steps (Figure 1); chitinase and dissolving potassium were deposited as patent strains in the Chinese General Microbiological Culture Collection Center (CGMCC) with the accession number 5155 (Xue, 2013).

Bacillus CGMCC N0.5155 has the ability to dissolve potassium, resist potassium and produce chitinase/chitosanase, etc. The preparation method is as follows: *B. circulans* (potassium bacteria) (*B. circulans* Xue-97316), isolated from maize rhizosphere, in Luancheng, Hebei Province, was put under ultraviolet mutagenesis to obtain 300 single colonies. Then five strains were obtained which have high-throughput screening of 40% potassium solution and 75% sporulation by colony dug block method. After that, under UV mutagenesis, screening with colloidal chitin as sole carbon was done to get three strains whose potassium resistance rate is up to 8%. Afterwards, these strains were respectively placed as follows: in a shake flask of 2.27% K_2O→2.27% K_2O; sterile shake flask→a 4.54 to 5% K_2O; sterile shake flasks→ a 7.5% K_2O; sterile shake flasks → a 10% K_2O; sterile shake flasks→12.5% K_2O; sterile shake flasks→ 15% K_2O containing 12.5 to 15% K_2O tablet isolated from colonies of a single seed, potassium-resistant strains serially passaged 30 times. The mutant bacteria are a high efficient method for bioleaching.

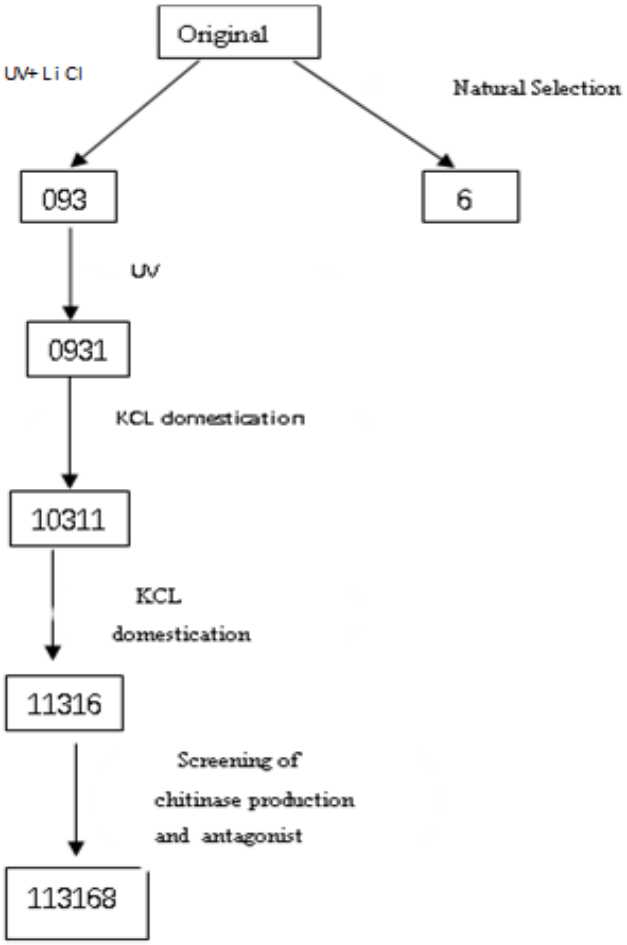


Figure 1. Breeding pedigree of *B. Circulans* Xue 113168.

Preparation of biofertilizer using SSF

The biological fertilizer was made from potassium-dissolving bacteria fermentation; meanwhile, the K releasing course was completed in the process of the preparation. The culture was grown at an initial moisture content of 60 to 65% and 30°C. The SSF was turned over every 48 h for 7 days. The pH was not regulated during the fermentation. Flour and feldspar were used as substrates at 2 and 10% (w/v), respectively, for the SSF with *B. circulans* Xue113168. After 168 h of fermentation, the SSF was autoclaved, maintained overnight and then filtered to detect the soluble potassium in the SSF.

Testing the quality index

Live bacteria counting culture medium are ingrient as follows: (g/L): sucrose (5.0), MgSO4 (1.0), FeCl3 (0.2), yeast extract (0.2), (NH4)2SO4 (0.5), KH2PO4 (1), pH 7.0. The water content was tested using the vacuum oven method. pH was measured using a pH meter in a solid-water ratio (1:1). The CMF was digested with H_2SO_4-H_2O_2. The total N content was determined using the Kjeldahl method; for the total K content, the potassium tetraphenylborate gravimetric method was used and for the total P content, molybdenum antimony anti-colorimetry was used. The organic matter was digested with H_2SO_4-$K_2Cr_2O_7$ and measured using the method of Yeomans and Bremner (1988). To determine humic-C, CMF or SMS was extracted using a mixture of 0.1 M sodium pyrophosphate and. 0.1 M sodium hydroxide at pH 13. The C content of the humic acids was determined using the method of Yeomans and Bremner (1988).

Pot experiment

The experiment was conducted in open air and natural conditions at Hebei to investigate the efficiency of the CMF. The kind of the soil used in the pot experiments is calcareous, with pH 7.8; organic matter, 20.30 g/kg; and total K, 3%. Each pot (20-cm diameter) contained 3 kg of soil. Rapeseed (seven green leaves), obtained from the Hebei Seed Corporation, was used as the inoculant for the experiment in open and natural conditions. The experiments were conducted under six conditions: C1, 65% CMF 3.23 kg/hm^2 + Regular chemical fertilizer 35%; C2, 75% CMF 8.83 kg/hm^2 + Regular chemical fertilizer 25%; C3, 89% CMF 16.16 kg/hm^2 + Regular chemical fertilizer 11%; C4, Sterilized CMF; C5, No fertilizer; C6, 10 kg/hm^2 CH_4N_2O + 20 kg/hm^2, $Ca(H_2PO_4)_2.H_2O$ + 5 kg/hm^2 KCl. Each treatment was performed in quadruplicate.

Field experiments

Field experiments were performed at a Soil and Fertilizer Station in Hebei Province. The experimental layout was a randomized complete block design with four replicates; there was a total of 98 jujube and 0.1332 hm^2 and 0.1385 hm^2 cherry tomato in four different farms in Hebei, China. Three treatments used were: C1, Regular fertilization; C2, Regular fertilization + CMF; C3, Regular fertilization + sterile CMF.

Soil and plant samplings and analyses

Soil samples were dried at room temperature (approximately 25°C) for 2 weeks and then passed through a 2-mm sieve. Soil pH was measured using a pH meter, and 5 g of soil was mixed with 5 ml of distilled water. The available P was extracted with sodium bicarbonate (Oslen et al., 1954), and its concentration was determined using molybdenum antimony anti-colorimetry. The available K in the soil was extracted with 1 M HNO_3, and its concentration was determined using the potassium tetraphenyl-borate gravimetric method.

Soil organic C was determined by oxidizing the organic matter in the soil samples with $K_2Cr_2O_7$ (potassium dichromate) in sulfuric acid (98%) for 30 min and then measuring the concentration of Cr^{3+} formed. Approximately 12 g of a root-free soil sample was individually placed into a 50 ml glass beaker, which were placed into a desiccator (18-dm^3 volume). Forty-five milliliters of ethanol-free $CHCl_3$ was used as a fumigant (22°C, 20 h). The fumigation process was simultaneously started. The non-fumigated soil was shaken (150 rev min^{-1}) for 30 min with 50 ml of 0.5 M K_2SO_4 (mean soil: solution ratio=1:13 (w:v)) and filtered using Whatman paper (No. 42). The fumigated soil was extracted as described earlier after the removal of the $CHCl_3$ from the soil by repeated evacuations (Högberg and Högberg, 2002). The microbial C biomass content was determined using the method of Yeomans and Bremner (1988). Vitamin C content was measured using 2,6- dichloroindophenol titration. The nitrate content was measured using a UV spectrophotometer.

Fungi, Actinomycetes and bacteria in the soil were detected using PDA, Gause No. 1 and Plate Count Agar, respectively.

The total N, total P, and total K contents detected in the strong digested with H_2SO_4-H_2O_2 were determined according to the described methods.

Table 1. The influence of different media on the SSF.

Combination	Corn bran	Spent mushroom substrate	Corn bran + Shrimp shell	Corn bran + Shrimp shell + Spent mushroom substrate
K dissolving rate (%)	2.03±0.21[a]	42.00±1.61[c]	24.09±0.96[b]	43.00±1.72[c]
Living bacteria lg (CFU)	8.02±0.11[a]	10.08±0.13[c]	9.04±0.12[b]	9.16±0.14[b]
Spore formation rate (%)	70.00±3.63[a]	82.62±3.86[b]	80.00±4.02[b]	83.18±3.93[b]

For each column, values not marked with the same letter in superscript are significantly different at $p<0.05$ (Duncan's). For each column, values not marked with the same letter in superscript are significantly different at $p<0.05$ (Duncan's).

Table 2. Results of the orthogonal experiment.

Treatment number	Wheat flour content (%)	$(NH_4)_2SO_4$ content (%)	$MgSO_4$ content (%)	K dissolving rate (%)	Spore concentration lg (CFU)
1	0.5	0.1	0.2	27.58	9.30103
2	0.5	0.5	0.1	22.19	8.30103
3	0.75	0.1	0.1	41.53	9.69897
4	0.75	0.5	0.2	19.85	9.30103

Statistical analyses

The experimental data were subjected to an analysis of variance (ANOVA) using a significance value of $p<0.05$ and Duncan's multiple range test (Duncan's) using SPSS 18.

RESULTS AND DISCUSSION

High dissolving rate of *B. circulans* Xue-113168 and SSF

B. circulans Xue-113168 was isolated from a corn rhizosphere and bred by UV. Its ability to dissolve potassium ores enables *B. circulans* to be applied as a biological fertilizer. Microorganisms that dissolve potassium ores include silicate bacteria, such as *Bacillus mucilaginosus, B. circulans, B. acidophilus*, and fungi (Sheng, 2005; Lian et al., 2008). *B. circulans* Xue-113168 has a high potassium dissolving rate (41%) and also produces chitinase (Xue, 2013).

Potassium dissolving microorganisms (KSM) are able to solubilize 'unavailable' forms of K-bearing minerals, such as micas, illite and k-feldspar, by excreting organic acids that either directly dissolves rock K or chelate silicon ions to bring the K into solution (Sheng, 2005). *B. circulans* Xue-113168 ferments and dissolves K simultaneously. The released soluble K in the fermentation medium was detected. Using potassium feldspar as the substrate of the solid-state fermentation, the detection of spore concentration of 2×10^9 cfu/g, and soluble potassium accounted for 1%. Among the detected isolates, *B. circulans* Xue-113168 displayed the highest K-solubilizing activity, as high as 41.53% after 7days of culture with K-feldspar powder. This cycle is shorter compared to that determined by Lian et al. (2008), who studied SSF and bioleached K-containing minerals for 15 days. One step of SSF adopted in this study has more advantages than the two steps solid-state fermentation combined with bioleaching done by Lian et al. (2008).The mutant bacteria are a high efficient method for bioleaching. Same conclusion was reached by Yingbo et al. (2011).

Utilization of waste as a substrate for SSF of *B. circulans* Xue-113168 for biofertilizer production

The raw materials and formulation were key factors for the SSF and they significantly influenced the overall cost. The formulation of a SMF could be similar to that of SSF according to Barrios-González (2012). However, a SSF medium is typically composed of a solid substrate. As a result, sucrose was replaced with wheat flour for the SSF in this study.

The type and solid state of substance in the SSF medium exert a significant influence on the SSF process, especially the potassium dissolving rate, the amount of live bacteria, and the spore formation rate. The influence of different media on SSF is shown in Table 1.

It can be concluded from Table 2 that the combination of corn bran, shrimp shell, and spent mushroom substrate was the best medium for the SSF. The K-dissolving rate (43.00%) and spore formation rate (83.18%) achieved were the best for all of the groups, and the live bacteria amount 9.16 cfu/g was second only to the spent mushroom substrate group (10.08 cfu/g).

Table 3. Variance analysis of orthogonal experiment.

Resource	III Square sum	df	Mean square	F	Sig.	III Square sum	df	Mean square	F	Sig.
Rectify model	4.03[a]	3	1.34	23.98	0.00	1430.71[a]	3	476.90	2044.95	0.00
Intercept	1319.87	1	1319.87	23542.81	0.00	12813.67	1	12813.67	54944.69	0.00
A	1.28	1	1.28	22.78	0.00	202.28	1	202.28	867.37	0.00
B	2.61	1	2.61	46.52	0.00	878.09	1	878.08	3765.21	0.00
C	0.15	1	0.15	2.64	0.13	350.35	1	350.34	1502.27	0.00
Error	0.67	12	0.06	-	-	2.80	12	0.233	-	-
Total	1324.58	16	-	-	-	14247.182	16	-	-	-
Total corrected	4.71	15	-	-	-	1433.51	15	-	-	-

[a]R -squared = 0.857（Adjusted R-squared = 0.821); [b]R-squared = 0.998 (Adjusted R-squared = 0.998).

Fermentation was formulated to obtain a higher concentration of spores and potassium dissolving capacity for *B. circulans* Xue-113168. It was initially determined through a single factor experiment. The formulation was further optimized through an orthogonal experiment. The design and variance analysis results of the experiment are shown in Tables 2 and 3.

It can be concluded from Table 3 that the range of $(NH_4)_2SO_4$ concentrations was the most important variable. This suggests that in the *B. circulans* Xue-113168 culture, the effects of the three factors on the potassium dissolving ratio were $(NH_4)_2SO_4$ content > $MgSO_4$ content > wheat flour content; all three factors have significant effect on potassium dissolving ratio ($P<0.05$) and that the optimal conditions were wheat flour content, 0.75%; $(NH_4)_2SO_4$ content, 0.1%; and $MgSO_4$ content, 0.2%.

For the spore concentration, it can be concluded that the ranges of $(NH_4)_2SO_4$ and wheat flour concentrations were larger compared to the $MgSO_4$ concentration. Thus, the effects of the three factors on the spore concentration: $(NH_4)_2SO_4$ content > wheat flour content > $MgSO_4$ content, $MgSO_4$ are not significant on spore concentration ($p>0.05$). Optimal conditions were wheat flour content, 0.75%; $(NH_4)_2SO_4$ content, 0.1%; and $MgSO_4$ content, 0.2%. The contents of $(NH_4)_2\ SO_4$ in the media could affect the growth and metabolites of the *B. circulans* Xue-113168 and the mineral bioweathering. This is consistent with Zhi (2012)'s study. The K-dissolving rates are not different between the SMS and Corn bran + Shrimp shell + SMS in the SSF (Table 1), and the humic acids in the SMS also dissolve the K-feldspar, which is complementary to the action of *B. circulans* Xue-113168.

Controlling the SSF process

Spore formation was affected by humidity and temperature. Controlling the moisture content of the SSF process was performed as follows. During the first step, at the end of the sterilization process, the moisture content was adjusted to approximately 60%. During the second step, after the inoculation, the moisture content was adjusted to 60 to 65%. At the end of the fermentation, the moisture content was 50%. The moisture content was controlled using environmental moisture and temperature, which had three phases. During the prophase, the temperature was 33 to 35°C, whereas, at the metaphase, the temperature dropped to 30 to 33°C. However, it increased to 33 to 35°C during the anaphase.

The fermentation time was also an important parameter. By the time that the spores had formed, the fermentation had typically finished. An appropriate temperature and humidity ensured that the spores were formed. When the fermentation lasted for 4 days, the potassium dissolving rate was 24.09%. After the fermentation time was extended to 7 days, the potassium dissolving rate increased to 41.53%. Lian et al. (2008) spent 25 days on the fermentation: 15 days for SSF and 10 days for biological leaching. In this study, the SSF that simultaneously dissolved potassium required 7 days.

The potassium dissolving process was a synthesized multifaceted result. There is no single gene that encodes for a potassium dissolving ability in silicate bacteria, which is similar to the process of dissolving phosphate. It is well known that there are many differences between SSF and SMF in physiology, such as AW and metabolite. The pH range for *B. circulans* Xue-113168 is 5 to 9, and the temperature range is 28 to 37°C. Moreover, the indispensable moisture required for SSF was low. All the characteristics described earlier make *B. circulans* Xue-113168 suitable for SSF. Sheng (2005) demonstrated that the potassium dissolving rate of *Bacillus edaphicus*, which has been proven to have a low potential for commercial use, is low. Tan (1978) reported that 9 to 28% of the potassium that they measured was released by humic acids. It has been suggested that the potassium dissolving rate (41.53%) of *B. circulans* Xue-113168 may include dissolved potassium from K-feldspar through the

Table 4. Results of the quality index determination experiment.

Item	Effective viable cells (billion/g)	NPK ($g.kg^{-1}$)	Organic matter ($g \cdot kg^{-1}$)	N Nitrogen ($g.kg^{-1}$)	P_2O_5 (%)	Available potassium ($g.kg^{-1}$)	Total K_2O ($g.kg^{-1}$)
NY/T798-2015	0.2	80-250	200	-	-	-	-
CMF	2	150	250	12	38	80	100

Table 5. Effect of different soil treatments on the biochemical characteristics of the rapes during the first year (Values are provided as the mean ± SD).

Condition	Dry weight (%)	NO_3 (mg/kg)	Vit C (mg/kg)	Shoot height (cm)	Phosphorus use efficiency (pue)
C1	1.93 ± 0.13^{b}	78.123 ± 1.69^{a}	21.90 ± 0.17^{a}	10.86 ± 0.13^{b}	0.016^{c}
C2	3.53 ± 0.22^{d}	51.38 ± 0.29^{b}	22.82 ± 0.19^{b}	13.11 ± 0.15^{a}	0.15^{e}
C3	2.52 ± 0.12^{c}	53.78 ± 0.29^{c}	22.49 ± 0.1^{c}	13.12 ± 0.12^{a}	0.25^{f}
C4	2.49 ± 0.17^{c}	86.10 ± 1.58^{d}	14.94 ± 0.14^{d}	10.11 ± 0.10^{d}	-0.013a
C5	1.59 ± 0.11^{a}	87.25 ± 1.48^{d}	13.09 ± 0.15^{e}	9.31 ± 0.47^{c}	0.075^{d}
C6	1.71 ± 0.12^{ab}	91.20 ± 1.58^{e}	14.39 ± 0.11^{f}	10.71 ± 0.26^{b}	0^{b}

For each column, values not marked with the same letter in superscript are significantly different at $p<0.05$ (Duncan's). For each column, values not marked with the same letter in superscript are significantly different at $p<0.05$ (Duncan's).

action of humic acids (Friedrich, 1991).

Pot experiment

The results indicate that the rape yield and soil fertility, such as available N, available P, available K and organic matter, have increased. The soil fertility and rape quality and yield were improved by *B. circulan* Xue-113168 and the matrices. In addition, the environment was also improved. Significant increases in dry shoot weights were observed in the rape when the soil was inoculated with *B. circulans* strain Xue-113168 compared to the soil without inoculum, and different treatments affected the soil fertility. The experimental results for the influences of the different treatments on the soil fertility are shown in Table 5. Vitamin C concentration increased by 52.7% in the 75% CMF for the matrix control; however, the nitrate concentration decreased by 77.5% in the 75% CMF for the chemical fertilizer control. The dry weight of the rape increased by 82.9, 40.08, 41.77, 122, and 106.43% in the 75% CMF treatment for the 65% CMF, 89% CMF, substrate, no fertilizer, and chemical fertilizer soils, respectively (Table 4). The alkaline hydrolyzable N and available P contents in the CMF treatments were higher compared to those of the chemical fertilizer treatment, indicating the benefits of a biofertilizer by supplying and enhancing the release of N and P (Table 5). Alkyl hydrolyzable N in the 75% CMF was significantly increased compared to the other groups. The available P and K in the CMF soil for the control were higher. The K content in the rape in the CMF for control was also higher. It can be concluded from Table 5 that the use of a chemical fertilizer can give some nutrients to crops; however, it also decreased soil fertility in the soil by less beneficial microorganism. The use of CMF not only provided indispensable nutrients for crop growth but also increased the organic matter content in the soil, which enriched the soil quality. Remarkably, the available potassium in the soil was increased 1.64 times in the 89% CMF for the substrate group; however, 65 and 75% CMF could not increase the available potassium in the soil compared to the control. Thus, *B. circulans* Xue-113168 was able to dissolve the insoluble potassium in the K-feldspar and other soil K-minerals when enough dose, such as 89% CMF, was utilized. The dose designs were 3.23, 8.83, and 16.16 kg/hm^2. They reduced the amount of chemical fertilizer used (35, 25, and 11%), and improved the phorphase efficiency of the fertilizer used (1.6, 15, and 25% respectively). PUE refers to the increment in soil P status by applying bio-based fertilizers compared to the increment caused by applying chemical fertilizer (Vaneeckhaute et al., 2016). The microbial analyses showed important differences for all treatments (Table 6). The community structure of the microorganisms was altered by the CMF. The numbers of bacteria in the soil increased 1.35 and 1.57 times in the soils amended with CMF 89 and 75%, respectively, compared to the chemical fertilizer soil. Actinomycetes were not different among the various treatments (Table 7). However, the fungal number was lower in the CMF compared to the control group, which might be the reason for the decreased disease occurrence in the CMF treatment. The organic C content in the biofertilizer group was

Table 6. Some chemical properties of the soils in different treatments after the rape harvest.

Condition	Organic matter (%)	Alkyl hydrolysable N (mg/kg)	Available P (mg/kg)	Available K (mg/kg)
C1	2.97±0.06^{d}	93.21±0.67^{b}	1.63±0.10^{ab}	94.83±0.68^{b}
C2	3.05±0.12^{d}	160.33±1.24^{c}	1.64±0.11^{ab}	157.36±1.12^{a}
C3	3.09±0.17^{d}	86.72±0.59^{a}	2.05±0.16^{c}	421.43±2.98^{c}
C4	2.69±0.08^{c}	84.70±0.57^{d}	1.62±0.12^{ab}	159.42±1.08^{a}
C5	2.43±0.08^{ab}	86.24±0.43^{a}	1.82±0.14^{b}	205.91±1.70^{d}
C6	2.5±0.11^{cb}	82.52±0.73^{e}	1.58±0.12^{a}	246.20±1.57^{e}

The data are expressed as the mean ± standard error. Data in a column with a different letter are significantly different at a Duncan's significance level of 0.05.

Table 7. Effect of different treatments on microorganisms (Lg cfu/g dry soil).

Treatment	Silicate bacteria	Bacteria	Fungi	Actinomycetes	Microbial biomass (mg/kg)
Low-dose group	6.19±0.13^{a}	5.42±0.026^{a}	6.93±0.120^{a}	5.19±0.090^{ab}	793±4.6^{a}
Middle-dose group	6.34±0.090^{a}	5.44±0.089^{a}	6.91±0.100^{a}	5.20±0.100^{ab}	835±6.3^{b}
High-dose group	6.54±0.096^{a}	5.41±0.092^{a}	6.92±0.075^{a}	5.32±0.100^{b}	853±6.0^{c}
Matrix control	5.11±0.092^{b}	5.39±0.095^{a}	7.19±0.086^{b}	5.19±0.110^{ab}	511±3.8^{d}
No fertilizer	4.96±0.970^{b}	5.05±0.099^{b}	7.18±0.080^{b}	5.15±0.070^{a}	408±3.3^{e}
Fertilizer control	4.33±0.091^{c}	5.03±0.080^{b}	7.23±0.083^{b}	5.23±0.080^{ab}	382±2.7^{f}

The data are expressed as the mean ± standard error. Data in a column with a different letter are significantly different at a Duncan's significance level of 0.05.

significantly higher compared to the control group, which is in agreement with the microbial biomass C content.

The silicate bacterial counts in the rape rhizosphere increased 1.21, 0.64, 26.12, 113.6, and 160.5 times in the 89% CMF treatment for the 65% CMF, 75% CMF, substrate, no fertilizer, and chemical fertilizer soil, respectively (Table 6). Inoculation of rape with the biofertilizer reduces the recommended fertilizer level by 30%.

Field trials

Yields of selenium-enriched jujube and jujube increased by 6.19 and 8.4%, respectively in the CMF soils compared to the matrix control (Figure 2). Jujube rust significantly reduced as well as the anthrax for the trees. The fruit abscission rate uniformly decreased with the rosy fruit coloration. The average weight of the fruit was approximately 10.9 g for process 2, whereas the average weight for processes 1 and 3 was 9.8 g. The yields of cherry tomato Jinpeng and East St No. 1 increased by 8.41 and 9.29%, respectively, in the CMF soils for the matrix control. Vigorous plant growth, root development, robust stems and dark green leaves were also observed. Late fruit suffered from less plant disease. A developed root system, dark green leaves, and less mosaic disease were all evident compared to the other two treatments. Fruit coloration and less immature fruit were also observed.

Jujube plant number with anthracnose decreased significantly, and rust disease also significantly reduced. The fruit fall off rate reduced, and fruit coloring uniformity was ruddy. Tomato plants thrived well, root system, stem and leaves developed strongly; leaves were invisibly green. Later, the fruits became large and neat and had equitable coloring; and plant diseases also reduced. The mechanisms are synergy and complementary between microorganisms and soil, microbial and nutrient elements (fertilizers), microbes and crops, by means of interaction between microorganisms. CMF in this study helps to address some of the most important challenges such as anthracnose and rust disease facing green agriculture in coming years.

The efficacy components in the CMF are *B. circulans* Xue-113168, its metabolite, silicon and humic acid and chitin (chitosan). Silicon and other elements were released from K feldspar by the *B. circulans* Xue-113168. Data will be reported later. Jayawardana et al. (2016) also verified the reduction of anthracnose disease using rice hull as a silicon source. The chitin in the shrimp shell and spent mushroom substrate can induce *B. circulans* Xue-113168 chitinase. A similar result was reported by Wang et al. (2006) and Chang et al. (2007).

Bioorganic fertilizers and humates are environmentally friendly and inexpensive because they are composed of recycled wastes such as SMS, shrimp shell and corn bran. Humic acids are the adhesives that combine

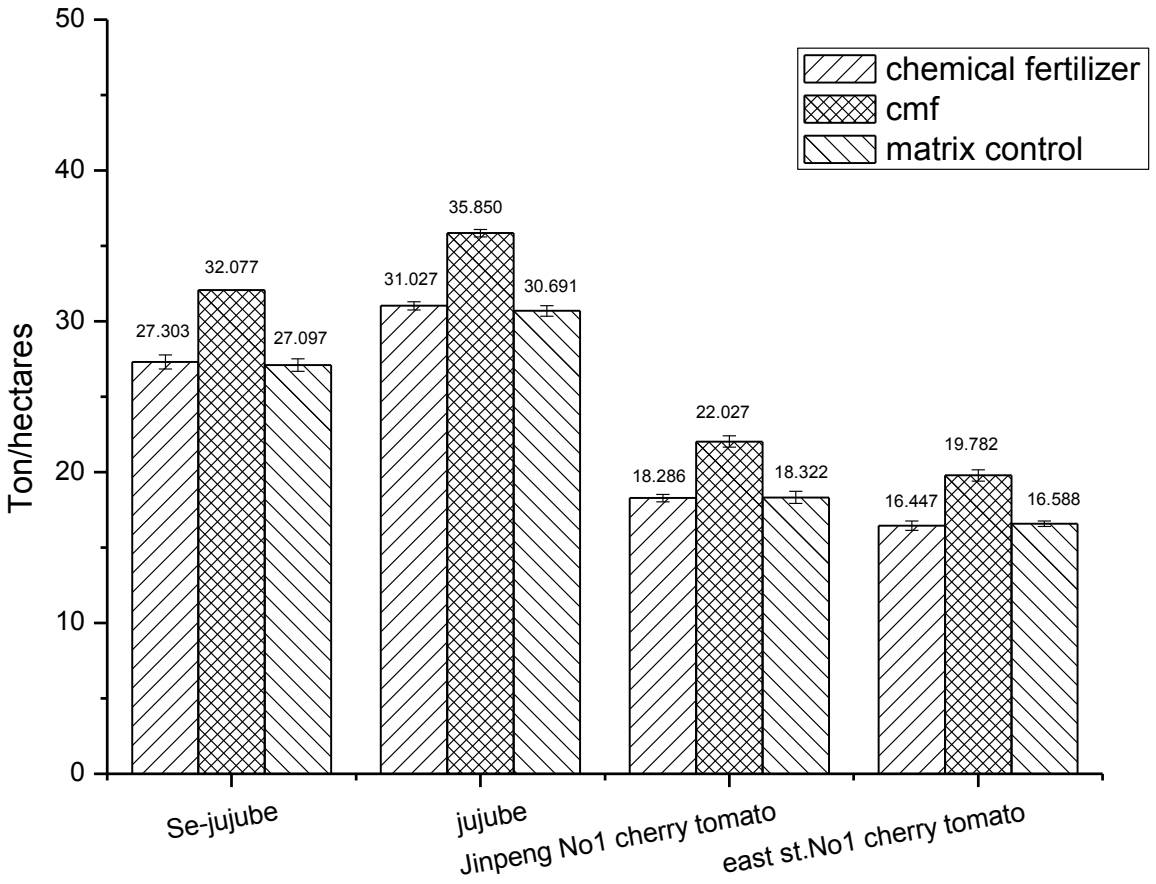


Figure 2. Field test of different treatments on selenium-enriched jujubes, jujubes, and cherry tomatoes. CMF: compound microbial fertilizer matrix control: CMF with *B. circulans* destroyed.

chemical fertilizers with microorganisms. Violante et al. (1999) demonstrated the formation of OH-Al-humate-montmorillonite complexes. However, the interaction among humate, K-feldspar and potassium during SSF or in a compound biofertilizer, which form the K-humate-feldspar complexes, requires further study.

Conclusion

B. circulans Xue-113168 has a 41% potassium dissolving rate and produces Chitinases, induced by chitin, which are in spent mushroom substrate, and shrimp shells. Microorganism combination of Chitinase activity can increase crop yields and lessen the incidence of disease in China's Hebei area. The instance has been reported by Zhao et al. (2011). This study found that the optimal dosage of CMF application was 8.83 kg/hm^2-16.16 kg/hm^2 rapeseed. The study of CMF fertilizer and recycling resource utilization efficiency promote the sustainable development of agriculture industry. The complex formulation and process of CMF is applicable to factories, especially farmers' use on site.

Conflict of interests

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

This work was supported by the Ministry of Science, Republic of China under Grant（2008GA620020）; Education Commission, Hebei Province under Grant(20090431); Funded by the natural fund of Hebei Province (C2015207019).

Abbreviations

CMF, Compound biofertilizer; **SSF,** solid-state fermentation; **PGPR,** plant growth-promoting rhizobacteria.

REFERENCES

Barrios-González J(2012). Solid-state fermentation: Physiology of solid

medium, its molecular basis and applications. Process Biochem. 47:175-185.
Basak BB, Biswas DR (2010). Co-inoculation of potassium solubilizing and nitrogen fixing bacteria on solubilization of waste mica and their effect on growth promotion and nutrient acquisition by a forage crop. Biol. Fertil. Soils 46:641-648.
Canellas LP, Olivares FL, Aguiar NO, Jones DL, Nebbioso A (2015). Humic and fulvic acids as biostimulants in horticulture. Sci. Hortic. 196(30):15-27.
Chang WT, Chen YC, Jao CL (2007). Antifungal activity and enhancement of plant growth by Bacillus cereus grown on shellfish chitin wastes. Bioresour. Technol. 6:1224-1230.
Eman AA, El-Monem A, Saleh MM, Mostafa EA (2008). Minimizing the quantity of mineral nitrogen fertilizers on grapevine by using humic acid, organic and biofertilizers. Res. J. Agric. Biol. Sci. 4:46-50.
Esitken A, Yildiz HE, Ercisli S, Donmez MF, Turan M, Gunes A (2010). Effects of plant growth promoting bacteria (PGPB) on yield, growth and nutrient contents of organically grown strawberry. Sci. Hortic. 124:62-66.
Farrell M, Jones DL (2009). Critical evaluation of municipal solid waste composting and potential compost markets. Bioresour. Technol. 100:4301-4310.
Friedrich S, Platonova NP, Karavaiko GI, Stichel E, Glombitza F (1991). Chemical and microbiological solubilization of silicates. Acta Biotechnol. 3:187-196.
Ho CP, Yuan ST, Jien SH, Hseu ZY (2010). Elucidating the process of co-composting of biosolids and spent activated clay. Bioresour. Technol. 21:8280-8286.
Högberg MN, Högberg P (2002). Extramatrical ectomycorrhizal mycelium contributes one-third of microbial biomass and produces, together with associated roots, half the dissolved organic carbon in a forest soil. New Phytol. 154:791-795.
Hutchens E, Valsami-Jones E, McEldowney S (2003). The role of heterotrophic bacteria in feldspar dissolutionan experimental approach. Mineral. Mag. 67(6):1157-1170.
Iqbal MK, Shafiq T, Ahmed K (2010). Characterization of bulking agents and its effects on physical properties of compost. Bioresour. Technol.101:1913-1919.
Jayawardana RK, Weerahewa D, Saparamadu J (2016). The mechanisms underlying the anthracnose disease reduction by rice hull as a silicon source in capsicum (*Capsicum annuum* L.) grown in simplified hydroponics. Procedia Food Sci. 6:147-150.
Kloepper JW (2009). Plant-microbe interaction in improving fertilizer use efficiency. Appl. Microbiol. Biotechnol. 85(1):1-2.
Kumar R, Verma D, Singh BL, Kumar U (2010). Composting of sugar-cane waste by-products through treatment with microorganisms and subsequent vermicomposting. Bioresour. Technol. 101:6707-6711.
Lian B, Wang B, Pan M, Liu C, Teng HH (2008). Microbial release of potassium from K-bearing minerals by thermophilic fungus Aspergillus fumigates. Geochim Cosmochim Acta 1:87-98.
Nishanth D, Biswas DR (2008). Kinetics of phosphorus and potassium release from rock phosphate and waste mica enriched compost and their effect on yield and nutrient uptake by wheat. Bioresour. Technol. 9: 3342-333.
Olsen SR, Cole CV, Watanabe FS (1954). Estimation of available phosphorus in soils by extraction with sodium bicarbonate. U.S. Dep. of Agric. Circ. No. 939.
Pacôme AN, Nadège AA, Farid B-M, Adolphe A, Lamine B-M(2016). Plant growth promoting rhizobacteria: Beneficial effects for healthy and sustainable agriculture. Afr. J. Biotechnol. 15(27):1452-1463.
Padmavathiamma PK, Li LY, Kumari UR (2008). An experimental study of vermi-biowaste composting for agricultural soil improvement. Bioresour. Technol. 9:1672-1681.
Patrick du Jardin (2015). Plant biostimulants: Definition, concept, main categories and regulation. Sci. Hortic. 196(30):3-14.
Rabish CS, Keshav Singh (2013). Vemicomposts: A alternative biofertilizers for zayad crops. J. Agric. Technol. 9(3):711-726.
Sheng X (2005). Growth promotion and increased potassium uptake of cotton and rape by a potassium releasing strain of Bacillus edaphicus. Soil Biol. Biochem. 37:1918-1922.
Tan KH (1978). Effects of humic and fulvic acids on release of fixed potassium. Geoderma 1:67-74.
Vaneeckhaute C, Janda J, Vanrolleghem PA, Filip MG, Meers E (2016). Phosphorus Use Eficiency of Bio-Based Fertilizers: Bioavailability and Fractionation. Pedosphere 26(3):310-325
Violante A, Arienzo M, Sannino F, Colombo C, Piccolo A, Gianfreda L (1999). Formation and characterization of OH-Al-humate-montmorillonite complexes. Org. Geochem. 30:461-468.
Wang SL, Lin TY, Yen YH, Liao HF, Chen YJ (2006). Bioconversion of shell fish chitin wastes for the production of Bacillus subtilis W-118 chitinase. Carbohydr. Res. 15:2507-2515.
Xue SP (2013). CA. Patent ZL 201110256623.1(in Chinese).
Yeomans JC, Bremner JM (1988). A rapid and precise method for routine determination of organic carbon in soil. Commun. Soil Sci. Plant. Anal. 19:1467-1476.
Yingbo D, Hai L, Han W, Xiaolan M, Kaibin F, Hongwei W (2011). Effects of ultraviolet irradiation on bacteria mutation and bioleaching of low-grade copper tailings. Miner. Eng. 24(8):870-875.
Zhao Q, Dong C, Yang X, Mei X, Ran W, Shen Q, Xu Y (2011). Biocontrol of Fusarium wilt disease for Cucumis melo melon using bio-organic fertilizer. Appl. Soil Ecol. 1:67-75.
Zhi H, Guangyou M, Linyan H, Xiafang S(2012). Feldspar weathering of two potassium --bearing mineral-·solubilizing bacteria. Acta Microbiol. Sin. 52(2):206-213 (in chinese).
Zhu HJ, Sun LF, Zhang YF, Zhang XL, Qiao JJ (2012). Conversion of spent mushroom substrate to biofertilizer using a stress-tolerant phosphate-solubilizing Pichia farinose FL7. Bioresour. Technol. 111:410-416.

academicJournals

Vol. 15(52), pp. 2854-2863, 28 December 2016
DOI: 10.5897/AJB2016.15589
Article Number: 36C4B9662170
ISSN 1684-5315

http://www.academicjournals.org/AJB

African Journal of Biotechnology

Full Length Research Paper

Effect of solarization with fresh chicken manure on verticillium wilt (*Verticillium dahliae* Klebb) and yield in eggplant

Ismail Cimen[1*] and Mahir Basaran[2]

[1]Plant Protection Department, Faculty of Agriculture, Dicle University, Diyarbakır, Turkey.
[2]The Ministry of Agriculture and Animal Food Directorate of Diyarbakir, Turkey.

Accepted 29 July, 2016; Accepted 24 November, 2016

The present study was carried out to evaluate the effect of reducing wilt disease through the medium of fresh chicken manure (FCM) mixed with soil before solarized and then artificial *Verticillium dahliae* (V.d) inoculation on yield of eggplant (*Solanum melongena* L.) under field conditions. According to the split-plot design, solarization of main plots, sub-plots with fresh chicken manure and *V dahliae* inoculation were established as a mini parcel in experiment with three replications. During solarization, the average temperature values were ensured by means of increasing the fresh chicken manure per unit area recorded 24 times per day (24 h) at intervals of one week. Among the 6 applications form, the highest average temperature was obtained at "12 kg FCM m^{-2} +solarization" application for soil surface, 10, 20 and 30 cm depth. There was increasing 18°C, a temperature according to control (no FCM), and this difference decreased with increasing soil depth, which was calculated as 8°C at 30 cm depth soil. During the day, the highest temperature values were obtained in the middle of the day on the ground, and underground measurements were performed at night.

Key words: Soil solarization, fresh chicken manure, *Verticillium dahliae*, eggplant, yield.

INTRODUCTION

Turkish cuisine is an indispensable vegetable in the Solanaceae family, eggplant, looking hotter temperature than among the vegetables. One of the most important problems affecting the production of eggplant is Verticillium wilt disease caused by *Verticillium dahliae* in soil borne fungal agents. *V. dahliae* is able to infect more than 400 plant species, including annual, herbaceous crops and weeds, as well as fruit, landscape, and ornamental trees, and shrubs (Pegg and Brady, 2002). This problem could be eliminated by soil fumigation with chemical in vegetable cultivation in narrow spaces (Jarvis, 1993) and/or crop rotation of large areas of land (Green, 1967). However, it is known that chemicals have harmful effects on ecology. The most common chemical to be used in fumigation was methyl-bromide. Due to its dangerous effect on ozone layer of the upper

*Corresponding author. E-mail: icimen@dicle.edu.tr

atmosphere, the methyl bromide has been banned since 2005 (Speth, 2004). Today, the search for an alternative method instead of these chemicals has increased. It is possible to use solar energy instead of fumigation, and it is also beneficial to use it (Katan, 1987; Zayed et al., 2013), for a period of one to two months during the hot days of the year by covering the soil with plastic. Soil solarization is a term that refers to disinfestation of soil by the heat generated from trapped solar energy. This method eradicates or reduces weed seed germination (Cimen et al., 2010b; Lalitha et al., 2003; Ozaslan et al., 2015) and some vegetable diseases caused by soil-borne pathogens (Cimen et. al., 2009; Cimen et. al., 2010a) as well; eggplant (Tamietti and Valentino, 2001).

The level of organic matter in the soil is critical to soil fertility. This situation can be eliminated with animal manure to some extent. Chicken manure has long been recognized as perhaps the most desirable of these natural fertilizers because of its high nitrogen content. For growing vegetables, it is not recommended to use fresh manure directly on the growing area because they will burn tender plant roots (Aryantha et al., 2000).

However, when mixed soil in non-cropland areas, combustion of fresh chicken manure with solarization can be used to heat the soil. This study will be an opportunity for intensive vegetable cultivation; especially, so only solarization with transparent polyethylene is not enough even with summer temperatures and cold climate zone. In this study, both for benefiting from nutritious properties of fresh chicken manure and increasing the temperature of the soil together with solarization, it was aimed to enhance yield in eggplant by reducing wilt disease caused by *V. dahliae,* which is the most important problem in eggplant.

MATERIALS AND METHODS

The study was conducted in the field of loamy-clay structure soil in the Faculty of Agriculture, Dicle University, Turkey. For inoculation, inoculum was prepared in the Phytopathology Laboratory, and seedlings in peat were grown in the greenhouse until they reached two- to three-leaf stage on 23 May 2011. The polythene as a cover sheet material was used as 0.02 mm thickness, and also a digital thermometer was used to measure soil temperature for solarization. Eggplant (*Solanum melongena* L. cv. Kemer) was used as a plant material. *Verticillium dahliae* Kleb. isolates were obtained from the Plant Protection Departments of Agricultural Faculties in University of Çukurova and University of Mustafa Kemal. Fresh chicken manure used in this study was taken from "Diyarbakır Gün Tavukçuluk".

According to the split-plot design, solarization of main plots sub-plots with fresh chicken manure and *V dahliae* inoculation has been established as a mini parcel in experiment with three replications. The study involved a total of 36 parcels, and there were 50 plants per plot (that is, a spacing of 40 cm within the row and 70 cm between rows).

The process of soil solarization

After irrigated on July 8, the soil of trial area was deeply tilled with plow on 15 July 2010. According to the experimental design chosen, after fresh chicken manure (FCM) had been delivered as 6 and 12 kg m^{-2} on 4 August 2010 in predetermined parcels, the soil was mixed by using a field cultivator, and then sprinkler irrigation was performed. Also, on 17 August 2010, predetermined main plots were covered with transparent polythene material in 7 × 15 m size. All sides of polythene were put under the soil with a depth of 40 and 50 cm. Nevertheless, in control parcel, nothing was done except soil treatment. Also, on 17 August 2010, predetermined main plots were covered with transparent polythene material in 7 × 15 m size. All sides of polythene were put under the soil with a depth of 40 and 50 cm, but in control parcel, nothing was done except soil treatment.

Determination of soil temperature

Soil temperature (°C) was measured in both solarized and no solarized parcels. For this purpose, 4 wires were installed at 5, 10, 20 and 30 cm depths inside edge of one meter in each main plots. Soil temperature was measured through these wires connected to a digital thermometer. During solarization, these measurements were recorded 24 times per day (24 h) at intervals of one week.

Growing of eggplant seedlings

Eggplant seedlings were grown under controlled conditions. First, peat (turf) used as substrate was autoclaved for sterilization at 121°C for 90 min in the Phytopathology Laboratory. Then, it was filled with standard plastic seed containers (involving 70 cells). Later, eggplant seeds were sown two per cell in the container; seed sown in this medium was moved to the greenhouse, and germination of the seeds was awaited. The eggplant seedlings germinated were reduced one plant per eye, and water was applied to seedlings every two days.

Preparation of inoculum

Firstly, *V. dahliae* isolates were grown on potato dextrose agar plates at room temperature (22 ± 1°C) and then fungus was grown in erlenmeyers (250 ml) containing autoclaved (121°C) wheat (800 g cracked wheat + 200 ml water). The grains were inoculated with *V. dahliae* isolates and incubated at 22 ± 1°C in darkness. This inoculum was kept in a freezer until it was inoculated in experimental plots.

Inoculation of *V. dahliae*, transplanting of eggplant seedlings and cultural practices on experimental plots

V. dahliae inoculum firstly solarized plots and then non-solarized ones were given on May 23, 2011. For this process, prior to the inoculum saturated with wheat grain of 5 g per plant was mixed with river sand, and then this mixture was given on seedlings to be planted in the ground. On the same day, the eggplant seedlings were before transplanted in mini parcels without *V. dahliae* inoculation and then those with inoculation.

Surface irrigation system was applied 27 times during the eggplant season, from the date of seedlings transplanted to the last harvest (October 10, 2011). This was before going through without inoculum plots in order to help prevent the spread of infection.

Especially, in the initial stage of growing eggplant, intensive weeds in without solarized main plots were observed, and these weeds were drawn by hand. Thrips, red spider and flea beetles damage were observed on eggplants. Wettable powder Sulphur was applied to red spider.

The determination of plant mortality and disease severity

In the beginning of the experiment, dead (diminishing) seedlings were renewed and planted in locations where those dead seedlings diminished on June 13, 2011. Then, the percentage of plant mortality was determined by counting all the dead plants stemming from abiotic or biotic reasons on July 4, July 7 and August 3, 2011. These values were transformed to angle value, and variance analyses were conducted by using MSTAT-C programme.

According to discoloration in the stem section taken at the second nodium from the soil surface, the disease incidence was determined in all of the plants after the last harvest had been done. Disease incidence was evaluated as the following scale: 0=no discoloration xylem on trunk sectional area; 1=1-33% discoloration xylem; 2=34-67% discoloration xylem; 3=68-100% discoloration xylem (Buchenauer and Erwin, 1976).

Observation of plant growth

Plant height was measured from the soil level to the terminal bud by using a meter rule. This measurement was performed three times after 45 days of planting to August 8, 2011 on all plants per plot. The mean plant height (cm) was found by dividing the total number of plants per plant in the plot.

Obtaining yield

The first harvest was made on July 22, 2011; two months after eggplant seedlings had been transplanted. Having taken into account the market situation at harvest time, eggplant fruits reaching a length of 20 to 25 cm were harvested by hand, by pulling up or cutting down at the junction of the fruit and the stalk.

RESULTS AND DISCUSSION

Solarization and fresh chicken manure increased ($p \leq 0.05$ and $p \leq 0.01$) plant height, whereas *V. dahliae* inoculation decreased ($p \leq 0.01$) the height of eggplant seedlings planted in land, approximately 6 months after soil solarization. The effect of solarization was not observed on plant mortality, and disease severity but fresh chicken manure decreased them ($p \leq 0.01$) in parallel with its increasing dose. Therefore, the effect of yield was higher than solarization. The total crop yield increased to 45% by solarization. This increase was 2-fold by fresh chicken manure (12 kg FCM m^{-2}) compared with control (no FCM).

Among triple combination, the highest total yield as 40 tons per hectare was obtained in plot (+S +12 kg FCM m^{-2} -Vd) in which 12 kg m^{-1} of fresh chicken manure was mixed before solarization and without inoculation of *V. dahliae*. In 12 combinations, the least total yield as 6 tons ha^{-1} was obtained in “-S -FCM +Vd “. Here seven-fold increase was obtained. When the aim of the study is considered to reach high efficiency in terms of replacing the application of *V. dahliae*, quick possibility of infection is considered "+ S +12 kg FCM m-2 + Vd" combination, and the yield increase was 4.3 fold higher with respect to the control (-S-FCM + Vd).

Effect of solarization on soil temperature

During solarization, having been recorded 24 times per day (24 h) at intervals of one week, the highest temperature values were obtained in the middle of the day on the soil surface. However, the highest temperature values were measured at night under the ground.

In general, the highest average temperature was obtained on soil surface, and soil temperature dropped with the increase in the depth of soil layer in all the six applications. Soil temperature increased by means of supplementing the FCM per unit area in non-solarized parcels by comparison of treatments to controls (without FCM), and this increase jumped by solarization effect. Solarization process after FCM, the soil temperature continued to rise, and this increase "12 kg FCM m^{-2} + solarization" combination reaching a peak (Figure 1).

This application was compared with the control combination plots (without FCM and solarization); the average temperature increase was 18°C on the soil surface, 11.5°C at 10 cm depth, 10°C at 20 cm depth and 9.5°C at depth 30 cm. With this application, according to the solarization alone, average temperature rise was found as 4°C at 30 cm soil depth (Figure 1).

Our findings have also been supported by the findings according to which a 5 to 9°C of temperature difference alone was sufficient solarization in a previous study (Ragon and Vilson, 1985). Especially, also, temperature above ground was higher than that of previous similar studies (Lalitha et al., 2001; Hassing et al., 2004; Benlioglu et al., 2005; Cimen et al., 2010a).

The first possibility that comes into mind is that FCM must have heated the soil because it had undergone its combustion phases in the soil layer, and soil temperature increased further by a combination between it and solarization. It was not coincide with previous research heating the soil related to heat caused by the fresh chicken manure. However, there were similarities between two studies related to the burned chicken manure in the greenhouse (Boz, 2009) and open land (Benlioğlu et al., 2005).

The determination of plant mortality and disease severity

On the first observation, some of the eggplant seedlings transplanted plants in the plots seemed to have had weaker growth, and then they died. Containing three different dates, the percentage of plant mortality as well as their angle values is given in Table 1. Also, index values related to disease incidence by *V. dahliae* have also been added to Table.

The percentage of plant loss values was close to each other in both solarized main plots and without ones, and so were disease index values. However, plant losses or plant mortality was reduced by means of supplementing

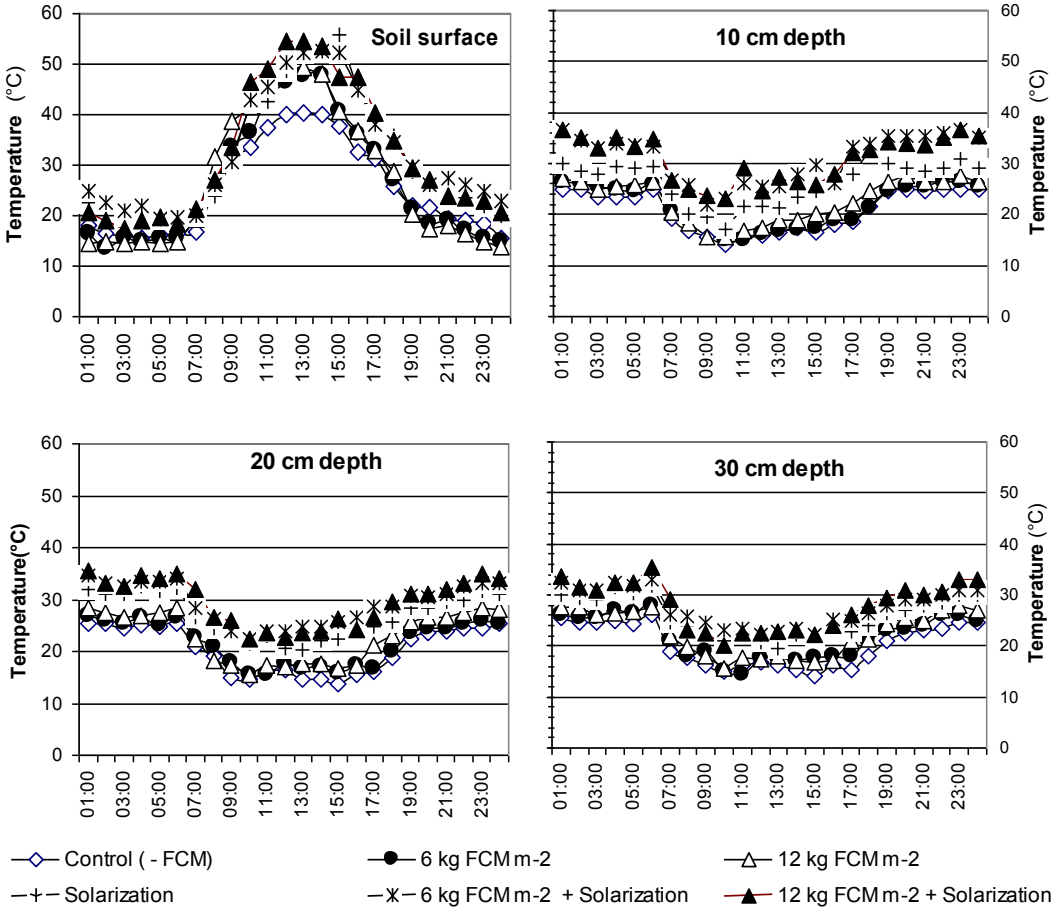


Figure 1. Effect of solarization and together with fresh chicken manure (FCM) on soil temperature (°C) at soil surface, 10, 20 and 30 cm soil depth (2010).

Table 1. Effect of solarization, fresh chicken manure (FCM) and *Verticillium dahliae* on plant mortality with diseases incidence in eggplant (2011).

Treatments	Observation times				
	Plant mortality (number)				Diseases incidence (0-3) (7 December)
	1st count (13 June)	2nd count (4 July)	3rd count (13.July)	4th count (3 Aug)	
Solarization	NS	NS	NS	NS	NS
Non Solarization	27.33(29.43)	31.00(31.84)	31.00(31.88)	32.55(32.93)	0.45
Solarization	25.44(27.96)	30.66(31.74)	30.88(31.97)	31.77(32.59)	0.39
Fresh chicken manure	**	**	**	**	
Non FCM	36.33(36.46)[a]	43.33(40.63)[a]	43.16(40.54)[a]	44.33(41.23)[a]	0.53
6 kg FCM/m^2	31.50(32.80)[a]	31.50(33.00)[b]	31.33(32.89)[b]	33.16(34.12)[b]	0.36
12 kg FCM/m^2	11.33(16.82)[b]	17.66(21.75)[c]	18.33(22.34)[c]	19.00(22.92)[b]	0.36
LSD %1	6.27	7.124	7.083	6.743	
SolX FCM	NS	NS	NS	NS	NS
Inoculation (*V. dahliae*)	**	**	**	**	**
Non V. d	14.44(20.19)[b]	18.88(23.92)[b]	19.22(24.18)	19.77(24.52)	0.07
Ino V. d	38.33(37.19)[a]	42.77(39.66)[a]	42.66(39.67)	44.55(40.99)	0.77
Sol X Ino	NS	NS	NS	NS	NS
FCM X Ino	NS	NS	NS	NS	NS
SolX FCM X Ino	NS	NS	NS	NS	NS

*, ** Significant at 0.05 and 0.01 levels respectively. NS = not significant 0.05 level.

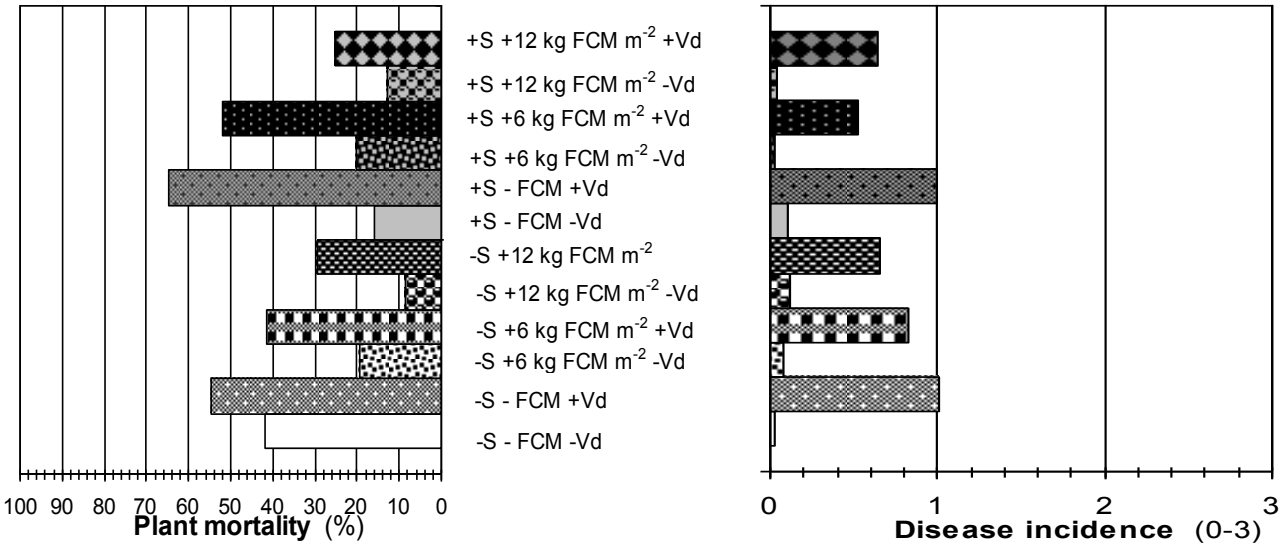


Figure 2. Effect of the triple combination of Solarization, FCM and *V. dahliae* inoculation on plant mortality with diseases incidence in eggplant (2011).

FCM per unit area in the sub plots, and the results of the statistical analysis were significant (P = 0.01) for each count (four times). As to disease incidence value, there were similar situations in the sub plots although statistical difference was not found to be significant. This value was 0.53 for control (no FCM), whereas it was found as 0.36 in both of FCM doses, and there were approximately 32% decrease in the disease incidence. There was statistically insignificant relationship between the solarization and FCM for plant loss and disease index (Table 1).

The young eggplant seedlings were transplanted into soil that may have been exposed to multi-influence from biotic or abiotic factors. It is possible that the application of solarization may have eradicated or reduced common weeds and soil borne pathogens which interfere with nutrition of seedlings or even lead to their death. Contrary to this possibility, solarization may have caused the eradication or decrease in beneficial microorganisms, such as mycorrhizal fungi in soil. Indeed, interpretations of our findings seem to be consistent with those reported in previous studies (Schreiner at al., 2001; Lalitha at al., 2003).

The inoculation of *V. dahliae* in the early stage of transplanting seedling inhibited their growth, hence leading to the death of some of them at the beginning of the vegetation period. Plant loss was 19.77% at the fourth counting in mini plots without inoculation, while this percentage increased to 44.55% with inoculated ones. According to discoloration in the trunk section of the existent plants after the last harvest, little or no disease index value was determined as 0.07 in plots without inoculation, whereas this disease incidence was determined to be 0.77 in inoculated ones. The results of the statistical analysis were significant (P = 0.01) for both the percentage of plant loss and disease incidence in mini parcels.

The difference between the inoculation of *V. dahliae* with the solarization was statistically insignificant and also similarly, there was no interaction between the inoculation of *V. dahliae* and FCM for plant loss and disease index. There was no statistical significance between the triple combination of Solarization, FCM and *V. dahliae* inoculation for plant loss and disease incidence (Table 1).

At the forth count, the least plant mortality (8.66%) emerged in "-S + 12 kg FCM m^{-2} -Vd" application, in which 12 kg of fresh chicken manure per square meter was given but after then, solarization and inoculation of *V. dahliae* were not applied in between triple combination plots. But as for the most plant loss, there was 64.66% in a combination (+ S - FCM + Vd) that FCM had not been used but solarization and inoculation were applied. According to discoloration in the trunk section of the existing plants after the last harvest, the least and the highest diseases were found as plant loss in the same combinations ("-S + 12 kg FCM m^{-2} -Vd" and "+ S - FCM + Vd") (Figure 2).

It is clear that inoculums must have led to disease or even death, whereas solarization has been ineffective for the plant loss in plots with transplanted seedlings. The young eggplant seedlings must have been died by inoculums mixed in the ground of seedling planting. In fact this interpretation is consistent with previous studies reporting that infection could be achieved by single-site inoculations of roots of eggplant seedlings with microsclerotia of the wilt-causing *V. dahliae* (Bejarano-Alcazar et al., 1999), and that occurrence of Verticillium wilt and its severity increased with increasing soil inoculum level of *V. dahliae* (Grogan et al., 1979;

Table 2. Effect of solarization, fresh chicken manure (FCM) and *Verticillium dahliae* on plant height (cm) in eggplant (2011).

Treatments	1st measure (8 June)	2nd measure (27 June)	3rd measure (11 Aug)
Solarization		*	*
Non Solarization	29.62	48.31	54.38
Solarization	30.68	52.68	61.19
Fresh chicken manure	**	**	**
Non FCM	21.50^{b}	40.64^{c}	49.12^{b}
6 kg FCM/m^2	31.97^{a}	51.95^{b}	60.49^{a}
12 kg FCM/m^2	36.99^{a}	58.89^{a}	63.74^{a}
LSD	5.11	6.30	5.66
SolX FCM	*	*	*
Inoculation (*V. dahliae*)	**	**	**
Non ino. V. d	37.35	58.64	63.33
Ino V. d	22.96	42.35	52.24
Sol X Ino V. d			
FCMX Ino V. d	*	*	*
Non FCM x (-) Ino	27.73^{bc}	47.07^{cd}	53.42^{cd}
Non FCM x (+) Ino	15.27^{d}	34.22^{e}	44.82^{e}
6 kg FCM/m^{2}x (-) Ino	42.73^{a}	65.69^{a}	69.71^{a}
6 kg FCM/m^2) x (+) Ino	21.21^{cd}	38.22^{de}	51.28^{de}
12 kg FCM/m^2) x (-) Ino	41.58^{a}	63.16^{ab}	66.87^{ab}
12 kg FCM/m^2) x (+) Ino	32.39^{b}	54.62^{bc}	60.62^{bc}
LSD	7.22	8.919	8.013
SolX FCM X Ino	NS	NS	NS

*, ** Significant at 0.05 and 0.01 levels respectively. NS = not significant 0.05 level.

Paplomatas et al., 1992; Pullman and DeVay, 1982; Xiao and Subbarao, 1998).

Decreasing seedling losses, the severity of *V. dahliae* infection with artificial inoculum must have been reduced because chicken manure is rich organic manure, stimulating the growth of antagonistic saprophytic microorganisms. This opinion has been supported by the results of a similar study conducted in Greece. According to the aforementioned study, the microbial nature involved in the suppressiveness of a compost amendment against *V. dahliae* was investigated in eggplant seedling in both the nursery and the field, and as a result of study, it was reported that treatments with *F. oxysporum* and *P. fluorescens* strains exhibited reduced disease severity; however, an increase in yield compared to the untreated control was not observed (Malandraki et al., 2008).

Plant growth

Plant height in solarized plots was higher than that in non-solarized ones, and results based on the second and third measurements were significant (P = 0.05). Plant growth also increased by means of supplementing FCM per unit area in the sub plots, and results of measurement in statistical terms were significant (P = 0.01) at three times. There was an interaction between the solarization and FCM in statistical significance (P = 0.05) (Table 2).

As a result of increasing soil temperature thanks to solarization (Figure 1), it must have made a strong effect on growth by means of more nutrients taken up by eggplant because of eradicating or decreasing the soil borne pathogens and weeds. The current findings are also consistent with previous studies (Yucel et al., 2007; Lalitha et al., 2001; Candido et al., 2008; Cimen et al., 2010a). In addition, eggplant growth was accelerated even further by chicken manure due to the fact that it improved soil structure thanks to its contain organic materials.

Artificial inoculation of *V. dahliae* caused decrease in the plant height, and results of all three measurements were significant (P = 0.01). This effect decreased from beginning towards the end of vegetation. From the first to third measurement, these decreases were calculated as follows: 38.52, 27.77 and 17.51% (Table 2).

The plant mortality was the highest, and growth of surviving ones was gradually decreasingly inhibited from the stage of transplanting due to given inoculum. This interpretation is consistent with a previous study on infection that could be achieved by single-site inoculations

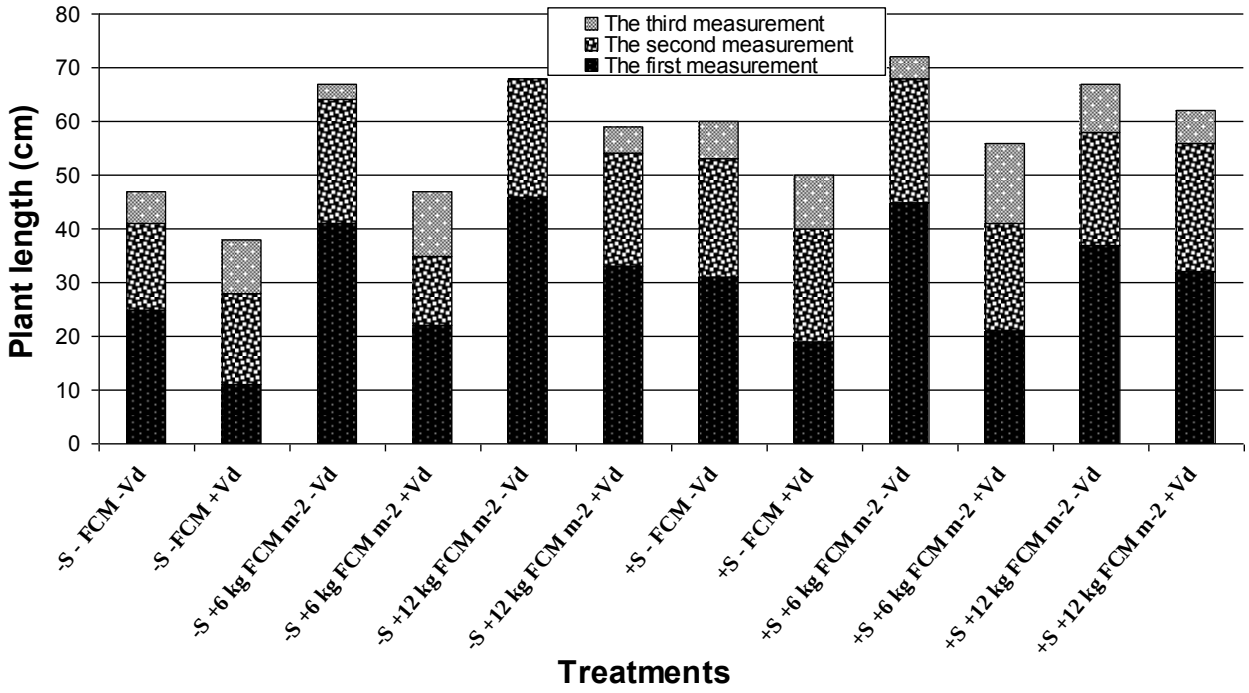


Figure 3. Effect of the triple combination of Solarization, FCM and *V. dahliae* inoculation on plant growth in eggplant; among measurement dates, plant growth as plant height for this triple combination was cumulatively given (First measure, 8 June; Second measure, 27 June; Third measure, 11 August, 2011).

of roots of eggplant seedlings with microsclerotia of the wilt-causing *V. dahliae* (Bejarano-Alcazar at al., 1999). Afterwards, we reached the opinion that plant growth had accelerated owing to reducing of the number plant per plot and increasing the soil temperature.

Interaction between the inoculation of *V. dahliae* together with solarization was statistically insignificant for plant length, whereas its interaction between with FCM was seen and it was significant (P = 0.05) for three measurements. The effect of the one together with solarization, FCM and inoculation of *V. dahliae* on plant growth was insignificant (Table 2). Among measurement dates, plant growth as plant height for this triple combination is cumulatively given in Figure 3. In triple combination plots, the highest plant height was measured as 72. 13 cm at the last measurement in "+S +6 kg FCM m^{-2} -Vd" combination plots where 12 kg of FCM per square meter was given after which solarization applied but there was not inoculation of *V. dahliae*. However, the least plant growth occurred as 38.42 in FCM and solarization non applied but inoculated with *V. dahliae* (-S - FCM +Vd) combination ones (Figure 3).

Yield

For total yield at first 3 harvests, the increasing yield rate was 22% in solarized plots in terms of earliness, as compared to non-solarized ones. This increment trend continued throughout the growing season and resulted in 45% increment at final assessment as total of 9 harvesting (Table 3). Positive effect on plant growth by means of solarization has also reflected yield increase. Hence, repeating the same interpretation is available as well, and results are compatible with those reported by Schreiner et al. (2001) and Lalitha et al. (2003). Growth of common weeds was faster than those of eggplant in non-solarized plots, and they were manually removed or cut off. If this cultural practice was not necessary, the yield would be decreased even more due to inhibiting growth of eggplant in non-solarized parcels as control. For this reason, the effect of solarization on yield could have increased much more according to us. Our findings are consistent with previous studies carried out on eggplant (Tamietti and Valentino, 2001) and for some vegetables (Cimen at al., 2009; Cimen at al., 2010a).

Since chicken manure reduced both plant loss and wilt disease severity caused by *V. dahliae* (Table 1), and also it positively stimulated the plant growth (Table 2), increasing the yield of eggplant. For total yield at first 3 harvests, the highest yield was obtained in a sub plots where FCM had been given as 12 kg km^{-2}, and this rate of yield increase was 2.8-fold greater than the control without FCM. This increment trend continued throughout the growing season and resulted as 2-fold increment at final assessment as a total of 9 harvesting. Both of the results were statistically significant (Table 3). The interaction between solarization and FCM was statistically

Table 3. Effect of solarization, fresh chicken manure (FCM) and *Verticillium dahliae* on yield(ton/ha) in eggplant (2011).

Treatments	Harvest										
	1 (22 Jul)[x]	2 (02 Aug)	3 (12 Aug)	Total of 3 harvest	4 (24 Aug)	5 (06 Sep)	6 (20 Sep)	7 (30 Sep)	8 (14 Oct)	9 (14 Nov)	Total of 9 harvest
Solarization	NS	NS	**	NS	*	*	NS	NS	NS	NS	NS
Non-solarized	0.21	1.43	2.04	3.68	2.99	2.95	2.57	1.88	3.24	0.87	18.18
Solarized	0.18	1.51	2.78	4.47	4.93	4.45	5.25	2.20	3.80	1.18	26.28
FCM		**	**	**	**		**	**	**		**
-FCM	0.07	0.67[b]	1.28[b]	2.02[b]	2.30[b]	3.29	2.03[b]	1.04[b]	2.00[b]	0.77	13.45[b]
6 kg FCM m^{-2}	0.17	1.63[ab]	2.95[a]	4.75[a]	4.85[a]	3.32	4.27[a]	2.67[a]	4.61[a]	1.01	25.48[a]
12 kg FCM m^{-2}	0.34	2.11[a]	3.01[a]	5.46[a]	4.72[a]	4.47	5.42[a]	2.38[ab]	3.93[a]	1.29	27.67[a]
SX FCM	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
V. dahliae ino.	**	**	**	**	**	NS	NS	**	**	*	**
- V. d	0.34	2.41	3.43	6.18	5.65	4.01	4.49	2.66	4.59	1.22	28.80
+ V. d	0.05	0.52	1.39	1.96	2.27	3.38	3.32	1.42	2.44	0.82	15.56
SX Vd ino.	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
FCM X Vd ino.	NS	*	NS	NS	NS	NS	NS	NS	NS	NS	NS
SX FCM x Vd ino.	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

* , ** Significant at 0.05 and 0.01 levels respectively. NS = not significant 0.05 level. [x:] harvest date in 2011.

insignificant for the results of yield. According to the aforementioned study, our finding is supported by a study conducted in Greece (Malandraki et al., 2008).

Inoculation of *V. dahliae* caused a decrease in yield because of both the increase in eggplant seedlings and incidence of disease in surviving ones. Total yield decreased by 46%, and the result was significant at the 1% level.

The interaction among solarization, FCM and inoculation of *V. dahliae* on eggplant yield was insignificant (Table 3), but the numerical values differ considerably among treatments for this triple combination plots (Figure 4).The highest yield was obtained as 40.00 ton ha^{-1} in total yield of harvest for "+S +12 kg FCM m^{-2} -Vd" combination plots where 12 kg of FCM per square meter was given after then solarization applied but there was not inoculation of *V. dahliae*, and this was followed by "+S +6 kg FCM m^{-2} -Vd" treatment as 38.20 ton ha $-^{-1}$. However, the least yield occurred as 6.00-ton ha^{-1} in "-S -FCM +Vd "between triple combinations. The yield increased by more than seven folds for first one as compared to the last one. In that practical application, considering the possibility of infection of *V. dahliae* instead of the first one, that is, with "+S +12 kg FCM m^{-2} +Vd" treatment, this increase was 4.3 folds according to last one (Figure 4). These findings obtained by us in field conditions are consistent with those of earlier studies in controlled environments (Grogan et al., 1979; Paplomatas et al., 1992; Pullman and DeVay, 1982; Xiao and Subbarao, 1998; Bejarano-Alcazar at al., 1999).

Conclusıons

The amount of organic matter in soil is an important factor in soil structure. This problem can be overcome by supplementation of green plant or animal manure to some extent if there is inadequate soil organic matter. The nutritional value of chicken manure is quite higher than others, but it is recommended to have been burnt for growing vegetable. When used as fresh, it can cause burning or be harmful to culture plants. However, on non**-** crop land**,** its stage of combustion can be used for heating of soil, which is mixed before solarization. This situation will be an opportunity for intensive vegetable cultivation even if there is simple transparent polyethylene for solarization in cold-mild climate zones with

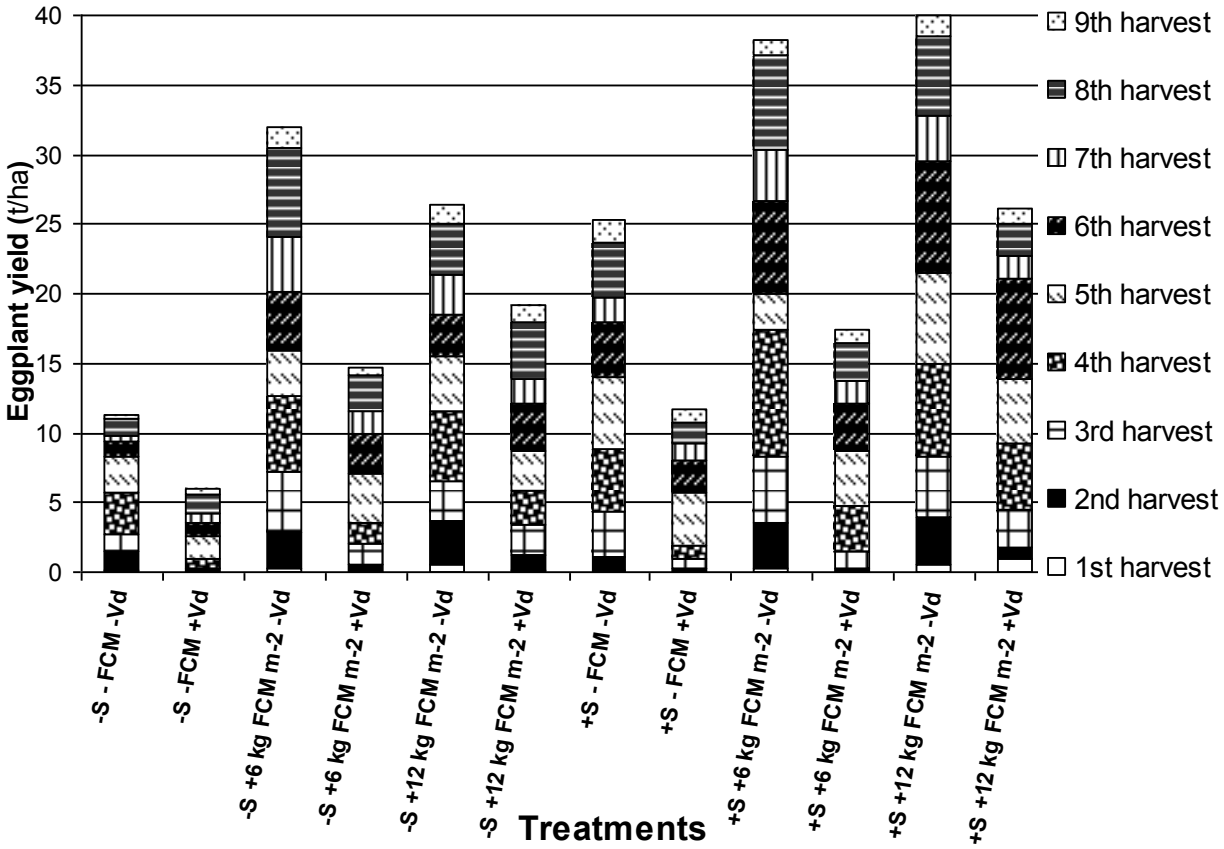


Figure 4. Effect of the triple combination of Solarization, FCM and *V. dahliae* inoculation on yield in eggplant; 9 harvest for this triple combination was cumulatively given (2011).

inadequate summer temperatures. By means of chicken manure with solarization application, even if there have been adequate summer temperatures**,** most of which have high resistance to heat and drought also non-possibility of chemical control, some of soil-borne disease pathogens, nematodes and weed seeds will be possible to eradicate. On the other hand**,** poultry growers will not encounter problems in order to keep the stage of the combustion of this fresh manure in their chicken farming; that is to say, this fresh chicken manure will be better assessed.

Conflicts of Interests

The authors have not declared any conflict of interests.

ACKNOWLEDGMENT

This research was part of a project that was financially supported by Research Foundation of Dicle University (10-ZF-137). The author extends their thanks to Erhan Akalın (Gün Tavukçuluk İşletmesi, Diyarbakır) for providing fresh chicken manure.

REFERENCES

Aryantha IP, Cross R, Guest DI (2000). Suppression of *Phytophthora cinnamomi* in Potting Mixes Amended with Uncomposted and Composted Animal Manures. Phytopathology 90(7):775-782.

Bejarano-Alcazar J, Termorshuizen AJ, Jimenez-Diaz RM (1999). Single-site root inoculations on eggplant with microsclerotia of *Verticillium dahliae.* Phytoparasitica 27(4):279-289.

Benlioğlu S, Boz O, Yıldız A, Kavalcı G, Benlioğlu K (2005). Alternative Soil Solarization Treatments for the Control of Soil-borne Diseases and Weeds of Strawberry in the Western Anatolia of Turkey. J. Phytopathol. 153:423-430.

Boz O (2009). Effects of olive processing waste, chicken manure and Dazomet on weeds with or without soil solarisation. Afr. J. Biotechnol. 8(19):4946-4952.

Buchenauer HD, Erwin C (1976). Effect of the plant growth retardant Pydanon on Verticillium wilt of cotton and tomato. Phytopathology 49:68-72.

Candido V, D'addabbo T, Basile M, Castronuovo D, Miccolis V (2008). Greenhouse soil solarization effect on weeds, nematodes and yield of tomato and melon. Agron. Sustain. Dev. 28:221-230.

Cimen I, Pirinc V, Sagir A (2010a). Determination of long-term effects of consecutive effective soil solarization with vesicular arbuscular mycorrhizal (VAM) on white rot disease (Sclerotium cepivorum Berk.) and yield of onion. Res. Crops 11(1):109-117.

Cimen I, Turgay B, Pirinç V (2010b). Effect of solarization and vesicular arbuscular mychorrizal on weed density and yield of lettuce (*Lactuca sativa* L.) in autumn season. Afr. J. Biotechnol. 9(24):3520-3526.

Cimen I, Pirinç V, Sagır A, Akpinar C, Guzel S (2009). Effects of solarization and vesicular arbuscular mycorrhizal fungus (VAM) on phytopthora blight (*Phytophthora capsici* leonian) and yield in pepper.

Afr. J. Biotechnol. 8(19):4884-4894.
Green RJ (1967). Control of verticillium wilt of peppermint by crop rotation sequences. Plant Dis. Rep. 51:449-453.
Grogan RG, Ioannou N, Schneider RW, Sall MA, Kimble KA (1979). Verticillium wilt on resistant tomato cultivars in California: Virulence of isolates from plants and soil and relationship of inoculum density to disease incidence. Phytopathology 69:1176-1180.
Hassing JE, Motsenbocker CA, Monlezun CJ (2004). Agroeconomic Effect of Soil Solarization of Fall-Planted Lettuce. Sci. Hortic. 101:223-233.
Jarvis WR (1993). Managing diseases in greenhouse crops. American Phytopathological Society Press, St. Paul Minnesota.
Katan J (1987). Soil Solarization John Willey and Sons, Inc, London, Pp. 77-105.
Lalitha BS, Nagaraj KH, Anand TN (2001). Effect of soil solarization on weed dynamics and yield of groundnut-tomato sequence. Mysore J. Agric. Sci. 35:226-231.
Lalitha BS, Nanjappa HV, Ramachandrappa BK (2003). Effect of Soil Solarization on Soil Microbial Population and the Germination of Weed Seeds in the Soil. J. Ecobiol. 15:169-173.
Malandraki I, Tjamos SE, Pantelides I, Paplomatas EJ (2008). Thermal inactivation of compost suppressiveness implicates possible biological factors in disease management. Biol. Control 44:180-187.
Ozaslan C, Cimen I, Kizmaz MZ, Pirinc V, Kara A (2015). Determination of long-term effects of consecutive effective fresh chicken manure with solarization and verticillium wilt (*Verticillium dahlia* Klebb) on weed and its control in eggplant. Afr. J. Biotechnol. 14(19):1614-1623.
Paplomatas EJ, Basset DM, Broome JC, DeVay JE (1992). Incidence of Verticillium wilt and yield losses of cotton cultivars (*Gossypium hirsutum*) based on soil inoculum density of *Verticillium dahliae.* Phytopathology 82:1417-1420.
Pegg GF, Brady BL (2002). Verticillium wilts. CABI Publishing, New York.
Pullman GS, DeVay JE (1982). Epidemiology of Verticillium wilt of cotton. A relationship between inoculum density and disease progression. Phytopathology 72:549-554.
Ragon D, Vilson JB (1985). Control of Weeds, Nematodes and Soil-Borne Pathogens by Soil Solarization. Alafua Agric. Bull. 13(1):13-20.
Schreiner RP, Ivors KL, Pinkerton JN (2001). Soil solarization reduces arbuscular mycorrhizal fungi as a consequence of weed suppression. Mycorrhiza 11(6):265-311.
Speth JG (2004). Red Sky at Morning: America and the Crisis of the Global Environment, New Haven: Yale University Press, 95.
Tamietti G, Valentino D (2001). Soil solarızatıon: A useful tool for control of vertıcıllıum wılt and weeds in eggplant crops under plastıc in the po valley. J. Plant Pathol. 83(3):173-180.
Xiao CL, Subbarao KV (1998). Relationship between *Verticillium dahliae* inoculum density and wilt incidence, severity and growth of cauliflower. Phytopathology 88:1108-1115.
Yucel S, Ozarslandan A, Colak A, Ay T, Can C (2007). Effect of solarization and fumigant applications on soilborne pathogens and root-knot nematodes in greenhouse grown tomato in Turkey. Phytoparasitica 35:450-456.
Zayed MS, Hassanein MKK, Nahed HE, Abdallah MMF (2013). Productivity of pepper crop (*Capsicum annuum* L.) as affected by organic fertilizer, soil solarization, and endomycorrhizae. Ann. Agric. Sci. 58(2):131-137.

academicJournals

Vol. 15(52), pp. 2864-2873, 28 December 2016
DOI: 10.5897/AJB2016.15713
Article Number: 44297A562173
ISSN 1684-5315

http://www.academicjournals.org/AJB

African Journal of Biotechnology

Full Length Research Paper

Nucellar embryogenesis and plantlet regeneration in monoembryonic and polyembryonic mango (*Mangifera indica* L.) cultivars

Manish Shukla, Khair Tuwair Al-Busaidi*, Ghaya Sultan Al-Blushi, Abdullah Hamed Al-Burashdi, Hamoud Darwish Al-Hasani, Mohammed Hamed Al-Jabri and Basem Saif AL-Kalbani

Plant Production Research Centre, Fruit Crop Research Section, Directorate General of Agriculture and Livestock Research, Ministry of Agriculture and Fisheries, P. O. Box 50, Seeb, Postal Code 121, Sultanate of Oman.

Received 5 October, 2016; Accepted 16 December, 2016

Biotic and abiotic stress particularly fungal diseases and salinity are major challenges facing mango cultivations in Oman. Micropropagation technique for multiplying disease resistant and salinity tolerant elite cultivars could be utilized to replace dead and infected plants in mango orchards but standardize *in-vitro* regeneration protocol via somatic embryogenesis is prerequisite. Nucellar tissues from immature mango fruits of monoembryonic cultivars Alphonso, Amrapali, Dashehari and Zafran, and polyembryonic cultivars Carabao and Turpentine were used as explants to induce somatic embryogenesis plantlets. Gamborg's B5 macronutrients, Murashige and Skoog micronutrients, iron source, vitamins and organics were used as standard basal media for all types of media used at each stage of somatic embryo development and regeneration. Induction medium 2 containing 2 mg/l 2,4-Dichlorophenoxyacetic acid and 0.5 mg/l 6-Benzylaminopurine were induced highest percentage of primary somatic embryos for Alphonso (22.08%) while induction medium 3 having 1 mg/l 2,4-Dichlorophenoxyacetic acid with sucrose 60 gm/l and induction medium 1 containing 1 mg/l 2,4-Dichlorophenoxyacetic acid and 0.25 mg/l 6-Benzylaminopurine induced highest percentage of primary somatic embryos in Carabao (29.17%) and Turpentine (42.71%) respectively. Maximum somatic embryo germination were achieved in germination medium 2 containing 0.1 mg/l Indole-3-acetic acid and 0.5 mg/l Gibberellic acid for Alphonso (7.34%) and Turpentine (3.34%) while for Carabao (18.59%) in germination medium 1 which does not contain any plant growth regulators. Germinated plantlets are surviving well in ex-vitro conditions after 4 months of transfer to greenhouse and survival rate of 66.66% for Alphonso, 26.68% for Carabao and 49.16% for Turpentine was obtained.

Key words: Mango, nucellar embryogenesis, monoembryonic, polyembryonic, somatic embryo, germination, survival rate.

INTRODUCTION

Mango (*Mangifera indica* L.) a popular fruit of tropical and subtropical region belongs to the dicotyledonous family

Anacardiaceae. It is widely cultivated in this region due to its delicious taste, high nutritive value, varietal diversity and higher demand for food processing industry in many parts of the world. Mango was introduced to the Sultanate of Oman from the Indian subcontinent and East Africa over hundreds of years ago. Now, it is fourth most important fruit crop after date palm (*Phoenix dactylifera* L.), banana (*Musa* spp) and lime (*Citrus aurantifolia* Swingle) in terms of area (148,514 ha) and production (14257 ton) (MAF, 2015). Mango cultivars are either monoembryonic or Indian type, or polyembryonic or Southeast Asia type (Mukherjee and Litz, 1977). Seedlings of polyembryonic cultivars are true-to-type unlike monoembryonic cultivars which are genetically not homogenous. Evaluation of mango genotypes resulted into the recommendation of 16 good quality and high yielding cultivars for cultivation in Oman and majority of them are monoembryonic such as Alphonso, Amrapali, Dashehari, and Zafran of Indian cultivars (MAF, 1989; 1990, 1991, 1992). These cultivars are semi-vigorous with semi erect tree shape and early ripening (April to mid-may) with average yield ranged from 45 to 100 kg /tree and fruit quality of 17% total soluble solids (MAF, 1989, 1990). Monoembryonic cultivars are mainly propagated by vegetative method as in Ambalavi, where de-novo adventives embryony is lacking in the nucellus of ovules to get true-to-type plant (Chaturvedi et al., 2004). Grafting and air layering are still a popular practice in propagation of mango monoembryonic cultivars worldwide which is expensive and time-consuming. In Oman, heterozygous rootstock seedlings of local strain named "Omani mango" have been used to propagate recommended monoembryonic mango cultivars resulting in non-uniform trees.

The most important challenge for mango growers in Oman is mango sudden decline disease, caused by the fungus *Ceratocystis fimbriata*, introduced recently and destroyed about 60% of the mango orchards (Al Adawi et al., 2003, 2006; Al Subhi et al., 2006; Galdino et al., 2016). Ministry of Agriculture and Fisheries has also introduced polyembryonic cultivars considering resistant to mango sudden decline disease but the problem was not resolved effectively. Therefore, there is an imperative to find an effective and expeditious method of mango propagation such as *in vitro* regeneration via nucellar organogenesis (Hartmann et al., 1997). Nucellar tissues from ovules of immature mango fruits were used to induce nucellar embryogenesis and this is a widely accepted micropropagation technique for mango tissue culture to obtain true-to-type plants. Somatic embryo formation and plantlet regeneration can be achieved from induced callus of nucellar explants of mango fruits (Chaturvedi et al., 2004; Laxmi et al., 1999). This technique has been used successfully by many researchers (Al-Busaidi et al., 2016; Chaturvedi et al., 2004; Lad et al., 1997; Litz et al., 1984; Malabadi et al., 2011; Nower, 2013; Pateña and Barba, 2011) to multiply monoembryonic and polyembryonic mango cultivars but the response is genotype dependent (Ara et al., 2000; Litz, 1984; Litz et al., 1982). Standardize protocol for somatic embryogenesis and plantlet regeneration is foremost required to obtain true-to-type and disease free mango plantlets in large numbers. Therefore, the present study aims to develop an efficient and reliable protocol for nucellar embryogenesis and plantlet regeneration for *M. indica* cultivars Alphonso, Amrapali, Dashehari, Zafran, Carabao and Turpentine.

MATERIALS AND METHODS

Explants source and culture medium

Nucellar tissues from ovular halves of immature mango fruits are used as explants for callus initiation and primary somatic embryo induction for developing tissue culture protocol of Mango (*M. indica* L.) monoembryonic cultivars of Alphonso, Amrapali, Dashehari and Zafran and polyembryonic cultivar of Carabao and Turpentine. Immature mango fruits of size 2.0 to 3.5 cm (small size) and 3.5 to 5.0 cm (medium size) in length (approx. 30 to 40 days of pollination) were collected from *Mangifera indica* L. cv Alphonso, Amrapali, Dashehari, Zafran, Carabao and Turpentine (but only small size fruits for Turpentine) trees from mango gene bank at Wadi Hebi Research Farm, Ministry of Agriculture and Fisheries, Sultanate of Oman. Processing of fruits and all the subsequent experiments were conducted at the Tissue Culture Unit, Directorate General of Agriculture and Livestock Research, Ar Rumais, Oman during the year 2014 to 2016. Mango fruits of two sizes were processed separately and cultured on same day after collection. Fruits were washed thoroughly with soap and running tap water for few minutes. Then surface sterilization was done by sequential treatment with 70% ethanol (v/v) (10 min), 0.1% $HgCl_2$ (w/v) (5 min) and finally rinsed with sterile double distilled water 3 to 4 times under laminar flow hood (Al-Busaidi et al., 2016). Culture media with different compositions of salts, organics and plant growth regulators (Table 1) were evaluated for each stage of nucellar embryogenesis and plantlets regeneration to achieve tissue culture raised mango plantlets in 6 mango cultivars. Modified basal media composition containing Gamborg's B5 macronutrients (Gamborg, 1970), MS micronutrients, Fe-EDTA, vitamins and organics (Murashige and Skoog, 1962) were used in all different media types, that is, induction media (IND), proliferation media (P), maturation media (M) and germination media (GM) for somatic embryo induction, proliferation, maturation and germination stages respectively. Various concentrations of plant growth regulators, L-glutamine, malt extract, L-ascorbic acid, PVP (Polyvinylpyrrolidone), sucrose, phytagel and activated charcoal were also added along with basal media in different media types (IND1, IND2, IND3, P, M, GM1, GM2, GM3, GM4 and GM5) based on their requirements at different developmental stages of somatic embryogenesis (Table 1). pH was adjusted to 5.8 and solidifying agent phytagel was added at concentration 2.5 gm/l in all media types just before sterilization of media.

*Corresponding author. E-mail: abouanfalalbusaidi@yahoo.com. Tel: +968 26893131.

Table 1. Media compositions for nucellar embryogenesis and plant regeneration in 6 Mango cultivars viz. Alphonso, Amrapali, Dashehari, Zafran, Carabao and Turpentine.

Components	Induction media			Proliferation medium	Maturation medium	Germination media				
	IND1	IND2	IND3	P	M	GM1	GM2	GM3	GM4	GM5
B5 Macronutrients	As formulated	As formulated	As formulated	As formulated	As formulated	As formulated	As formulated	As formulated	As formulated	Half strength
MS Micronutrients	As formulated	As formulated	As formulated	As formulated	As formulated	As formulated	As formulated	As formulated	As formulated	As formulated
MS Fe-EDTA	As formulated	As formulated	As formulated	As formulated	As formulated	As formulated	As formulated	As formulated	As formulated	As formulated
MS Vitamins	As formulated	As formulated	As formulated	As formulated	As formulated	As formulated	As formulated	As formulated	As formulated	As formulated
2,4-D (mg/l)	1	2	1	-	-	-	-	-	-	-
BAP (mg/l)	0.25 l	0.50	-	-	-	-	-	0.5 l	-	-
IAA	-	-	-	-	100 µg/l	-	0.1 mg/l	0.1 mg/l	0.1 mg/l	-
NAA	-	-	-	-	-	-	-	-	-	-
Kinetin	-	-	-	-	-	-	-	-	-	-
GA3 (mg/l)	-	-	-	-	-	-	0.5	0.5	-	-
ABA	-	-	-	-	100 µg/l	-	-	-	-	-
PEG (mg/l)	-	-	-	-	100	-	-	-	-	-
L-Glutamine (mg/l)	400	400	400	400	-	400	400	400	-	400
Malt extract (mg/l)	500	500	500	500	-	500	500	500	500	500
L-Ascorbic acid (mg/l)	100	150	100	100	-	-	-	-	-	-
PVP (mg/l)	100	50	100	100	-	-	-	-	-	-
Sucrose (g/l)	30	30	60	30	30	20	20	20	20	40
Phytagel (mg/l)	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Activated charcoal (mg/l)	-	-	-	-	-	50	50	50	50	-
pH	5.8	5.8	5.8	5.8	5.8	5.8	5.8	5.8	5.8	5.8

2,4-D, 2,4-Dichlorophenoxyacetic acid; ABA, Abscisic acid; BAP, 6-Benzylaminopurine; GA3, Gibberellic acid; IAA, Indole-3-acetic acid; NAA, 1-Naphthaleneacetic acid; PEG, *Polyethylene glycol;* PVP, Polyvinylpyrrolidone; MS, Murashige and Skoog media; B5, Gamborg's media; IND, Induction media; P, Proliferation media; M, Maturation media; GM, Germination media.

Callus induction, somatic embryo production and proliferation

The sterilized immature mango fruits of both small and medium size from all 6 cultivars were opened under laminar flow hood and intact ovule cut longitudinally into two halves. After removing the zygotic embryo part carefully, the ovular halves having nucellar tissues were cultured in GA-7 Magenta plant culture vessels containing induction media IND1, IND2 and IND3 under the aseptic conditions and incubated at 25 ± 2°C, 60% relative humidity (RH) in dark conditions for induction of somatic embryogenesis. Nucellar tissues from ovules of immature mango fruits are preferred explants source to induce nucellar embryogenesis and *in-vitro* regeneration for monoembryonic and polyembryonic mango cultivars (Chaturvedi et al., 2004; Laxmi et al., 1999; Malabadi et al., 2011). Based on previous reports and our preliminary experiments (Al-Busaidi et al., 2016) three different media compositions were used in this study to evaluate the response of mango cultivars and size of explants to different media compositions. Frequent sub-culturing was performed initially to reduce the explants browning due to phenolics exudation. Explants browning in post culture stages are one of the major hurdles in mango tissue culture experiments and several strategies were developed for prevention of explants browning effectively. L-Ascorbic

acid (100 mg/l) and 0.3% PVP were added in the induction (IND1, IND2 and IND3) and proliferation (P) media to control explants browning, along with frequent sub-culturing of explants initially and incubation of nucellar cultures in dark conditions to minimize the effects of phenolic exudations (Ara et al., 2000; Litz, 2003).

The numbers of explants showing callus initiation, pro-embryonic calli (PEC) and primary somatic embryos (PSEs) production were recorded after 60, 90, 120 and 150 days of culture from both size fruit explants of each cultivar and percentage callusing and PSE induction was calculated. PEC and initial globular shaped PSEs form all 6 cultivars were transferred to proliferation media (P) for proliferation. Primary somatic embryos (PSEs) induced in different induction media were transferred to proliferation media (P) for further proliferation and multiplication to produce large number of somatic embryos for maturation and germination. Production of large number of somatic embryos (SEs) from primary somatic embryo explants is called proliferation of somatic embryos. PSEs were sub-cultured onto proliferation media (P) to induce and production of secondary somatic embryos.

Maturation and germination of somatic embryos

Proliferated somatic embryos of heart stage, torpedo stage and cotyledonary stage were transferred to maturation media (M) (Table 1) for maturation. Maturation of somatic embryos is an important step to establish bipolarity in globular somatic embryos, minimize the fasciation and synchronization in the development of somatic embryos (Krishna and Singh, 2007; Singh et al., 2001; Thomas, 1999). The cultures at maturation stage were incubated for 4 weeks at 25 ± 2°C, 55 to 0 % RH in dark. Matured cotyledonary stage somatic embryos were transferred to different germination media GM1, GM2, GM3, GM4 and GM5 (Table 1) for germination of somatic embryos and plantlets formation. The cultures in germination media were incubated at 25±2°C temperature, 55 to 60% RH and 16 h photoperiod with 40 $mmolm^2S^{-1}$ light intensity (Al-Busaidi et al., 2016). Numbers of germinated somatic embryos (visible leaves, shoots and roots) were counted after 6 weeks of transfer to germination media and percentage germination was calculated for each germination media. Somatic embryos were considered to be germinated when plantlets have been formed and well developed shoots, roots and green leaves are visible and it is also important to mention that germinated embryos are considered as plantlets in this study.

Acclimatization and hardening

Germinated mango plantlets of different cultivars were transplanted into plastic plant trays containing mixture of sand, peatmoss and perlite in 1:1:0.5 ratios and transferred to the green house for acclimatization and hardening. These plantlets were irrigated on alternate days and covered with transparent polyethylene sheets initially for one month to maintain high humidity and low transpiration. Polythene covers were removed after one month and plants were transferred to medium size black plastic pots (30 × 15 cm dimensions) individually in the same sand mixture and nutrients and water were added by fertigation. Total number of mango plantlets transferred to the green house, their growth conditions and number of plants survived after each month of transfer were recorded for each cultivar to calculate the survival percentage in green house conditions.

Statistical analysis

All the experiments were performed as a completely randomized design (CRD). Each experiment was conducted minimum three times with four replicates and minimum 20 explants or 40 somatic embryos in each replicate. All the collected data were tabulated using Microsoft Excel software 2010 and statistically analyzed by the software GenStat Release 11.1 (VSN International, Hemel Hempstead, UK). Analysis of variance (ANOVA) was done for data analysis using GenStat Release 11.1 and significant differences between treatments were determined on the basis of Duncan's multiple range test at $p<0.05$.

RESULTS AND DISCUSSION

Effect of explants size on callus and primary somatic embryo induction in mango cultivars

Nucellar tissues from all 6 mango cultivars were callused and produced primary somatic embryos variously in different induction media. Although data for number of explants callused and number of explants produced primary somatic embryos (PSEs) were recorded for 60, 90, 120 and 150 days old cultures in induction media but highest callusing was observed in 90 days old culture and highest PSE induction was observed in 120 days old culture in both fruit size explants of all 6 mango cultivars studied (data not shown). Data from Tables 2 and 3 clearly indicates that maximum callusing and PSE induction was observed mainly in small size fruit (2.0 to 3.5 cm) explants in all 6 cultivars. Higher percentage of callus initiation was observed in small size fruit explants of Alphonso (43.4%), Dashehari (32.64%), Carabao (40.97%) and Turpentine (42.36%) while Amrapali (70.83%) and Zafran (36.81%) recorded maximum callusing percentage in medium size fruit (3.5 to 5.0 cm) explants after 90 days of culture in induction media (Table 2). Although higher callus initiation was observed in medium size explants of Amrapali and Zafran but these calluses were non-embryogenic in nature which does not form any primary somatic embryos (Tables 2 and 3).

Nucellar explants from small size fruits were performed extremely well in induction of PSEs in almost all cultivars except Dashehari, while medium size fruit explants of Carabao also induced the formation of PSEs in less numbers. Highest percentage of PSE induction was observed in Turpentine small size fruits explant (28.82%) while Carabao and Alphonso small size fruits explant induced 25 and 15.35% PSEs respectively (Table 3). Very less PSE induction was recorded in Amrapali and Zafran small size fruit explants while almost none of the explants induced PSE formation in Dashehari.

Induction of callusing was recorded in both small and medium size fruits nucellar explants of all 6 cultivars of mango but significantly ($P\geq0.05$) higher number of small size explants produced PSEs only in monoembryonic cultivar Alphonso and polyembryonic cultivars Carabao and Turpentine. Chaturvedi et al. (2004) reported 50% of explants showed induction of nucellar embryogenesis from nucellus tissue of young fruits of size 2.5 cm of monoembryonic cv Ambalavi and Malabadi et al. (2011) achieved induction of somatic embryos from nucellar tissues of 3.0 to 4.0 cm long immature mango fruits of cultivar Ratnagiri. Higher callusing percentage does not

Table 2. Effect of mango cultivars, fruit size and media composition on callus induction.

% Callus induction								
Fruit size (B)	**Small size fruit (2.0-3.5 cm**				**Medium size fruit (3.5-5.0 cm)**			
Media (A) Cultivar (C)	**IND1**	**IND2**	**IND3**	**Mean**	**IND1**	**IND2**	**IND3**	**Mean**
Alphonso	59.37^{abcd}	45.83^{abcdefgh}	25.00^{defgh}	43.4^{NS}	12.50^{h}	37.50^{bcdefgh}	25.00^{defgh}	25.00^{b}
Amrapali	31.25^{cdefgh}	46.87^{abcdefgh}	50.00^{abcdefg}	42.71	79.17^{a}	62.50^{abc}	70.83^{ab}	70.83^{a}
Dashehari	33.33^{cdefgh}	39.58^{bcdefgh}	25.00^{defgh}	32.64	32.50^{cdefgh}	15.62^{gh}	43.75 ^{bcdefgh}	30.62^{b}
Zafran	32.29^{cdefgh}	16.67fgh	44.37^{bcdefgh}	31.11	29.17 ^{cdefgh}	43.73 ^{bcdefgh}	37.50^{bcdefgh}	36.81^{b}
Carabao	39.58^{bcdefgh}	31.25^{cdefgh}	52.08^{abcdef}	40.97	21.87^{efgh}	15.62^{gh}	16.67^{fgh}	18.05^{b}
Turpentine	54.17^{abcde}	31.25 ^{cdefgh}	41.67^{bcdefgh}	42.36	-	-	-	-

Means within column followed by the same letter(s) were not significantly different according to Duncan's multiple range test at 5% level.

Table 3. Effect of mango cultivars, fruit size and media composition on primary somatic embryos (PSE) induction.

% Primary somatic embryo (PSE) induction								
Fruit size (B)	**% Primary somatic embryo (PSE)**				**Medium size fruit (3.5-5.0 cm)**			
Media (A) cultivar (C)	**IND1**	**IND2**	**IND3**	**Mean**	**IND1**	**IND2**	**IND3**	**Mean**
Alphonso	15.63^{cdefg}	22.08^{bcd}	8.33^{defg}	15.35^{b}	12.50^{cdefg}	0.00^{g}	0.00^{g}	4.17^{b}
Amrapali	0.00^{g}	6.25^{defg}	8.33^{defg}	4.86^{c}	0.00^{g}	0.00^{g}	3.13^{fg}	1.04^{b}
Dashehari	0.00^{g}	0.00^{g}	0.00^{g}	0.00^{c}	3.13^{fg}	0.00^{g}	0.00^{g}	1.04^{b}
Zafran	7.29^{defg}	2.08^{fg}	0.00^{g}	3.13^{c}	0.00^{g}	4.17^{efg}	0.00^{g}	1.39^{b}
Carabao	27.08^{bc}	18.75^{cdef}	29.17^{abc}	25.00^{a}	6.25^{defg}	9.38^{defg}	16.67^{cdefg}	10.76^{a}
Turpentine	42.71^{a}	22.92^{bcd}	20.83^{cde}	28.82^{a}	-	-	-	-

Means within column followed by the same letter(s) were not significantly different according to Duncan's multiple range test at 5% level.

correspond with higher PEC initiation or PSE formation also, because they may be non-embryogenic callus. Significantly higher percentage of PSE formation was observed only in 3 cultivars, that is, Turpentine, Carabao and Alphonso.

The results of the present study indicate that explants of small size fruits gave significantly the highest number of callused explants and primary somatic embryos compared to medium size (Table 2). In another study, nucellar tissues from immature mango fruit of size 2.5 to 4.5 cm long were used to induce somatic embryogenesis in cultivar Alphonso (Deore et al., 2000) while rapid production of somatic embryos with normal morphology and germination were achieved in monoembryonic mango cultivars Alphonso, Mundan and Baneshan from the nucellar explants (Jana et al., 1994). An interaction between explants size and cultivars significantly affected on the number of explants callused. It is distinct from our results that small size explants was more favorable for callusing than medium size and also revealed that % PSE induction significantly affected with size of explants as well as genotype.

Effect of media composition on callus and primary somatic embryo induction from nucellar explants in mango cultivars

Percent callus and primary somatic embryo (PSE) induction data from Tables 2 and 3 distinctively indicates that induction media 1 (IND1) and 3 (IND3) worked well for callus initiation and PSE induction in both mono and polyembryonic cultivars. Maximum callusing was observed either in media IND1 or IND3 in most of mango cultivars studied in both small and medium size fruit explants (Table 2). Highest callus initiation in Alphonso (59.37%) and Turpentine (54.17%) was recorded in media IND1 for small size fruit explants while highest callus initiation in Zafran (44.37%) and Carabao (52.08%) was observed in media IND3 (Table 2). Maximum callus initiation for Amrapali (79.17%) and Dashehari (43.75%) was observed in media IND1 and IND3 respectively for medium size fruit explants but this callus were non-embryogenic in nature. Induction of PSE formation was ascertained mostly in media IND1 as in case of Alphonso, Zafran, Carabao and Turpentine and IND3 as in case of Amrapali and Carabao. Maximum PSE induction for Alphonso (22.08%) was recorded in media IND2 while maximum PSE induction for Carabao (29.17%) and Turpentine (42.71%) was recorded in media IND3 and IND1 respectively (Table 3 and Figure 1). None or less PSE induction was recorded for Dashehari and Amrapali cultivar in medium size fruit explants and Carabao only induced PSE in media IND3.

Either 1 mg/l 2,4-D alone (IND3) or in combination with 0.25 mg/l BAP (IND1) was sufficient for producing embryogenic callus and induction of PSEs in most of the

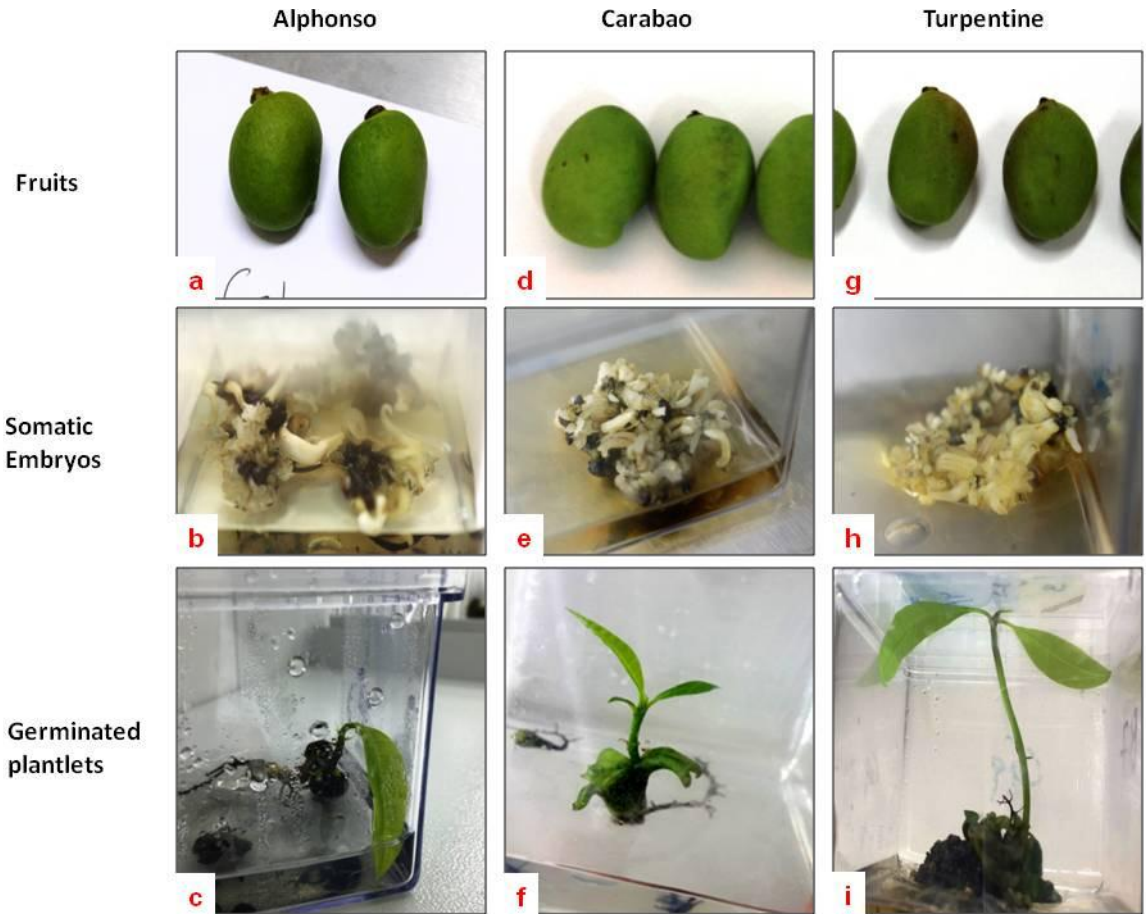


Figure 1. Somatic embryogenesis in Alphonso (a, b, c), Carabao (d, e, f,) and Turpentine (g,h,i).

cultivars studied here. The effect of an interaction between media and cultivar has significantly (P≥0.05) affected on number of explants callused and primary somatic embryo induction (Tables 2 and 3).

Induction media 1 (IND1) having 1 mg/l 2,4-D, 0.25 mg/l BAP, 400 mg/l L-Glutamine and 500 mg/l malt extract along with basal media components induced higher callusing and PSE induction for polyembryonic cultivar Carabao in our studies, but Pateña and Barba (2011) reported PSE induction ranged 16 to 86% in their experiments on Carabao when 0.5 mg/l 2,4-D, 100 ml/l coconut water, 6% sucrose with basal media components used for induction and proliferation of primary somatic embryos which is also dependent on the strain, collection time and tree source.

The data in the present study indicates that primary somatic embryo induction (% PSE) significantly affected by media compositions and mango genotypes as an interaction effect for these two factors (Table 3). Aside of media compositions, size of explants used affected significantly on number of callused explants and PSE induction in different mango cultivars. In another study, on monoembryonic cultivars of Amrapali and Chausa, Ara et al. (2000) observed that 2, 4-D 1 mg/l with basal media components and 6% sucrose was sufficient to stimulate the induction of callus and pro-embryonic calli (PEC) from nucellar tissues explants, while 4.52 µM 2,4-D and 2.27 µM thidiazuron (TDZ) with MS as the basal medium were able to induce somatic embryo formation in Ratnagiri cultivar (Malabadi et al., 2011). This has been well established that plant growth regulator 2,4-D is an essential requirement for primary somatic embryo induction in monoembryonic and polyembryonic mango cultivars, but prolonged presence of 2,4-D in induction media inhibits the proliferation and further growth of somatic embryos beyond globular stage (Krishna and Singh, 2007). Optimum 2,4-D and BAP concentration, malt extract and L-glutamine along with basal media are essential components in improving the embryogenic response and primary somatic embryo induction from nucellar explants. In another study, embryogenic callus induction was obtained from nucellar explants of Alphonso in modified MS medium containing 1 mg/l 2,4-D, 400 mg/l glutamine, 100 mg/l ascorbic acid, 500 mg/l PVP, 60 g/l sucrose and 2.5 g/l Phytagel and the somatic embryos were developed in same medium without 2,4-D

Table 4. Effect of media composition on somatic embryo (SE) germination in different mango cultivars from small size fruit explants.

Media (A) cultivar (C)	Number of SEs germinated						% SEs germination					
	GM1	GM2	GM3	GM4	GM5	Mean	GM1	GM2	GM3	GM4	GM5	Mean
Alphonso	2.67^{cd}	1.67^{cd}	0.00^{d}	2.00^{cd}	2.67^{cd}	1.80^{b}	2.23^{d}	7.34^{bc}	0.00^{d}	2.03^{d}	3.94^{cd}	3.11^{b}
Carabao	19.67^{a}	1.33^{cd}	0.33^{d}	9.67^{b}	8.00^{bc}	7.8^{a}	18.59^{a}	10.85^{b}	1.39^{d}	10.34^{b}	15.83^{a}	11.40^{a}
Turpentine	1.33^{cd}	2.67^{cd}	0.67^{d}	3.67^{bcd}	2.33^{cd}	2.13^{b}	0.87^{d}	3.34^{cd}	2.08^{d}	2.04^{d}	1.66^{d}	2.00^{b}
Mean	7.89^{a}	1.89^{bc}	0.33^{c}	5.11^{ab}	4.33^{ab}	-	7.23^{a}	7.18^{a}	1.16^{c}	4.80^{a}	7.14^{ab}	-

Means within column followed by the same letter(s) were not significantly different according to Duncan's multiple range test at 5% level.

(Deore et al., 2000) while embryogenic nucellar cultures were established successfully for Carabao in induction media containing 4.5 µM 2,4-D (minimum 28 days of treatment), 400 mg/l glutamine, 60 g/l sucrose, 2 g/l gellan gum and basal media B5 major salts, MS minor salts and organics (Lad et al., 1997). Efficient somatic embryogenesis and different stages of somatic embryos has also been reported in five polyembryonic cultivars, that is, Chino, Sabre, Omo, Heart and Turpentine N2-17-2 from nucellar cultures in a liquid media containing 20% coconut water by Litz et al. (1982). Modified basal media with other essential components was used by Ara et al. (2000) and Pateña et al. (2002) for induction of somatic embryogenesis and *in-vitro* regeneration for Amraplai and Carabao cultivar respectively. Varying concentration of different plant growth regulators along with modified basal media and other essential components such as sucrose and phytagel are required at each stage of somatic embryogenesis and plant regeneration as also reported by Chaturvedi et al. (2004) and Al-Busaidi et al. (2016).

It was also observed from our results that low concentration of BAP (0.5 mg/l) and withdrawal of 2,4-D from proliferation media was an important step for getting large numbers of somatic embryos in lesser time and also conversion of PEC and globular somatic embryos into heart shaped embryos and early cotyledonary stage somatic embryos, and similarly. Ara et al. (2000) also found in their studies on monoembryonic cultivars Amrapali and Chausa that the presence of 2,4-D in the medium inhibited the further progression of somatic embryo development. It is obvious by the results that the effectiveness and the positive role of the media compositions on mango callus initiation and PSE formation largely dependent on the size of the explants used, which confirms the effect of the explants size on *in-vitro* regeneration of mango genotypes.

Effect of media composition on somatic embryo germination in mango cultivars

Data in Table 4 present the effect of media composition on germination of somatic embryos in 3 different mango cultivars. Germination of matured cotyledonary stage SEs was observed only in cultures from small size fruit explants in 3 cultivars, that is, Alphonso, Carabao and Turpentine (Figure 1). SE induction and proliferation was also recorded in few cultures containing explants of medium size fruits of Alphonso, Amrapali, Dashehari, Zafran and Carabao, but these SEs were not germinated in any of the germination media except Carabao which was germinated in GM1 (data not shown). Higher percentage germination of SEs was recorded in germination media 2 (GM2) for Alphonso (7.34%), GM1 for Carabao (18.59%) and GM2 for Turpentine (3.34%) (Table 4). Germination media 5 (GM5) also stimulated the germination of SEs of Alphonso and Carabao in higher number. Germination media 2 (GM2) containing 0.1 mg/l IAA and 0.5 mg/l GA3 along with basal media has shown significantly higher percent germination of SEs of Alphonso and Turpentine while Carabao recorded significantly higher germination percentage in GM1 (18.59%) and GM5 (15.83%) media (Table 4), where GM1 did not contain any plant growth regulator and GM5 contains 0.5 mg/l NAA, 2.5 mg/l kinetin and 1 mg/l GA3 along with half strength B5 in basal media and other components (Table 1). In another study, 1 mg/l GA3 in a liquid medium containing half strength B5 macrosalts was used for germination of matured somatic embryos in Amrapali and Chausa by Ara et al. (2000). In general, germination media GM2 and GM5 had shown a good response for germination of SEs in monoembryonic cultivar Alphonso and polyembryonic cultivar Turpentine but germination of SEs for Carabao was the best in a germination media GM1 which did not contain any plant growth regulator.

Sucrose concentration has been reduced to 20 g/l in germination media GM1 to GM4 as reported by Litz (2003) and Laxmi et al. (1999), who suggested that reduced sugar concentration is important for germination and plantlet formation in mango tissue culture. In our study, significantly ($P \geq 0.05$) higher germination rate (18.59%) for Carabao was recorded in GM1 media without any plant growth regulators (PGR) which indicates that somatic embryo germination and plantlet formation might be achieved without any PGR, only in presence of basal media components with L-glutamine and malt extract. Similarly, Pateña and Barba (2011) were able to regenerate plantlets of Carabao in mango

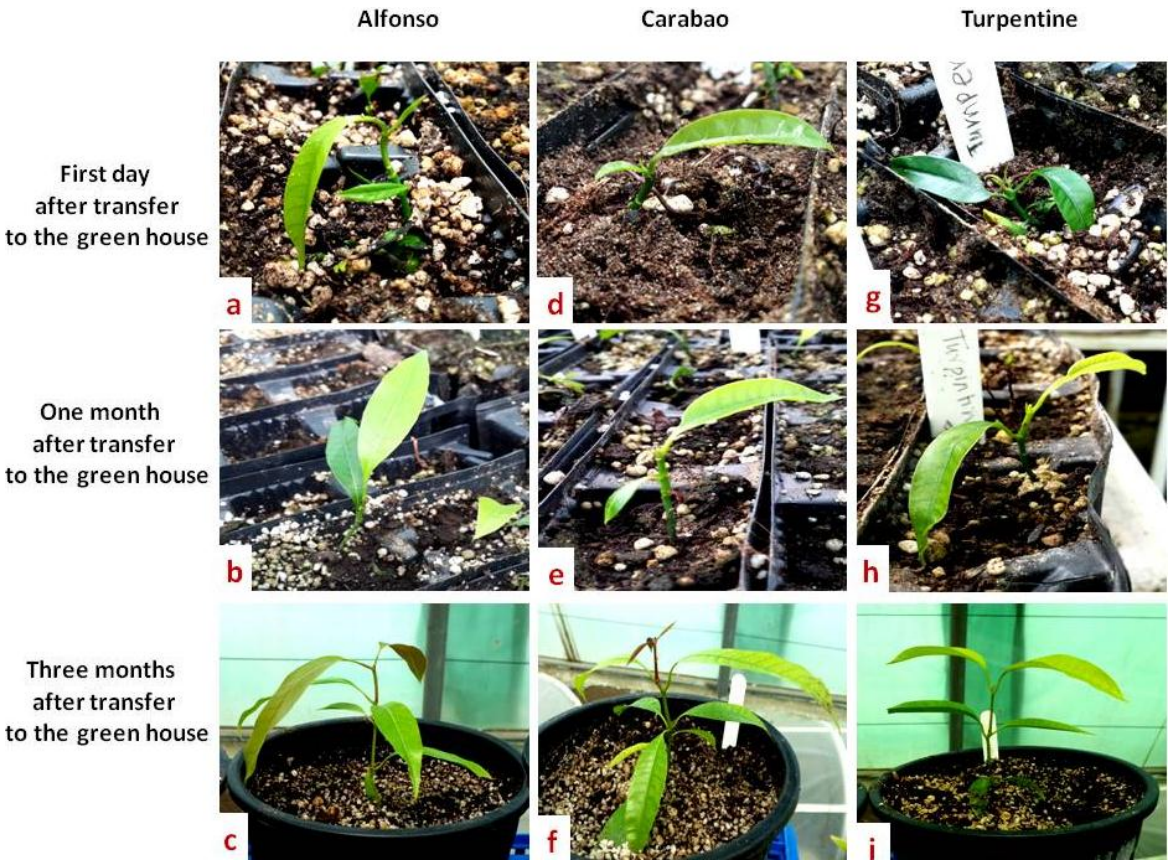


Figure 2. Hardening of tissue culture raised mango plantlets in green house Alphonso (a, b, c), Carabao (d, e, f,) and Turpentine (g,h,i).

medium for plantlet regeneration (MMPR) without any PGR as well, while Malabadi et al. (2011) succeeded in germination of somatic embryo in Ratnagiri cultivar in half strength MS basal media without any PGRs.

The results in this study revealed that there is a significant interaction (P≥0.05) effect between germination media and mango cultivars on the number of somatic embryos (SEs) germinated (Table 4). Matured cotyledonary stage somatic embryos have taken about 6 weeks for germination and first true mango plantlet (having well developed shoot, leaves and roots) was formed after approximately 8 months of explants culture.

Hardening and *ex-vitro* survival of germinated mango plantlets

Germinated plantlets of Alphonso, Carabao and Turpentine performed steady growth and better survival rate after one month of transfer to the greenhouse (Figure 2 and Table 5). Few of the plants of all 3 cultivars did not survive more than one month of transfer to the green house due to robust environmental conditions but after first one month, all plants were performed better in growth parameters and survived largely showing acclimatized to the new environmental conditions (Figure 2). About 67% plantlets of Alphonso, 27% of Carabao and 50% of Turpentine were survived more than 3 months after transfer to the greenhouse conditions (Table 5). All these plants are still surviving and growing well even after 6 months of transfer to the greenhouse. High survival rate for Alphonso cultivar in this study is promising compared to the previous reports about survival rate of other tissue culture raised mango plants in *ex-vitro* conditions such as 50% survival rate for Amrapali and no survival for Chausa was reported by Ara et al. (2000). About 70% transplant success was achieved by Chaturvedi et al. (2004) for a monoembryonic cv Ambalavi and these plantlets were survived more than 4 months. About 50% survival rate for Turpentine is still good but survival rate for Carabao (27%) is required to be improved and even better acclimatization strategy to be explored for Carabao. Pateña and Barba (2011) successfully transplanted tissue culture raised *ex-vitro* grafted plantlets of Carabao in field after 1.5 years of acclimatization in greenhouse conditions and only 14.3 to 50% survival rate achieved for these *ex-vitro* grafted plantlets in green house.

Table 5. Hardening and *ex-vitro* survival of tissue culture raised mango plants.

Cultivars	Batch No.	No. of plants transferred to green house for hardening	No. of plants survived after 1 month of transfer	No. of plants survived after 3 months of transfer	% plants survived after 3 months of transfer	Mean % survival after 3 months of transfer
Alphonso	Batch 1	12	7	7	58.33	66.66
	Batch 2	6	5	4	66.66	
	Batch 3	8	6	6	75.00	
Carabao	Batch 1	28	2	2	7.14	26.68
	Batch 2	16	7	5	31.25	
	Batch 3	12	6	5	41.66	
Turpentine	Batch 1	8	5	3	37.50	49.16
	Batch 2	6	5	3	50.00	
	Batch 3	5	4	3	60.00	

In this study, tissue culture raised mango plants were produced successfully for 3 cultivars Alphonso, Carabao and Turpentine from nucellar explants of small size fruits within 8 months of time and this is the first report where tissue culture mango plantlets were developed for cultivars Alphonso and Turpentine through nucellar embryogenesis.

Conclusions

Tissue culture raised mango plants were successfully developed for Alphonso, Carabao and Turpentine cultivars through nucellar embryogenesis. Primary somatic embryos were produced and proliferated largely in Alphonso, Carabao and Turpentine small fruit size explants. Some primary somatic embryo induction was also noticed in Amrapali and Zafran but these somatic embryos were unable to germinate. Gamborg's B5 macronutrients, MS micronutrients, iron source and vitamins, and organics were used as basal media at each stage of development. Induction media containing 1 mg/l 2, 4-D alone or in combination with 0.25 mg/l BAP along with 400 mg/l L-glutamine, 500 mg/l malt extract and essential basal media components are the best for obtaining embryogenic callus and somatic embryos in most of the cultivars while 2 mg/l 2,4-D and 0.50 mg/l BAP worked well for inducing somatic embryo formation in Alphonso. Germination of matured somatic embryos were achieved in germination media without any plant growth regulators or only 0.1 mg/l IAA and 0.5 mg/l GA3 along with basal media in Alphonso, Carabao and Turpentine. Better acclimatization strategy and higher survival rate has also been accomplished in Alphonso, Carabao and Turpentine cultivars. An efficient somatic embryogenesis and plantlet regeneration system was developed from nucellar explants by using modified basal media and different concentration of plant growth regulators at each stage in 3 mango cultivars i.e. Alphonso, Carabao and Turpentine. Further studies to increase germination percentage and a strategy for better acclimatization are required to use this protocol for mass propagation of these 3 mango cultivars.

Conflicts of Interests

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

This study was supported and funded by the Agriculture and Fisheries Development Fund, Ministry of Agriculture and Fisheries, the Sultanate of Oman. The authors would like to thank Mr. Hilal Al-Mawali for his assistance in conducting these experiments.

REFERENCES

Al Adawi AO, Deadman ML, Al Rawahi AK, Al Maqbali YM, Al Jahwari1AA, Al Saadi B A, Al Amri IS, Wingfield MJ (2006). A etiology and causal agents of mango sudden decline disease in the Sultanate of Oman. Eur. J. Plant Pathol. 116:247-254.

Al Adawi AO, Deadman ML, Al Rawahi AK, Khan AJ, Al Maqbali YM (2003). *Diplodia theobromae* associated with sudden decline of mango in the Sultanate of Oman. Plant Pathol. 52(3):419-419.

Al Subhi AM, Al Adawi AO, vanWyk M, Deadman ML, Wingfield MJ (2006). *Ceratocystis omanensis*, a new species from diseased mango trees in Oman. Mycol. Res. 110:237-245.

Al-Busaidi KT, Shukla M, Al-Burashdi AH, Al-Blushi GS, Al-Jabri MH, Al-Kalbani BS, Al-Hasani HD (2016). *In vitro* regeneration of Mango (*Mangifera indica* L.) cv. Baramasi through nucellar embryogenesis. J. Hortic. For. 8(5):37-43.

Ara H, Jaiswal U, Jaiswal VS (2000). Somatic embryogenesis and plantlet regeneration in Amrapali and Chausa cultivars of mango (*Mangifera indica* L.). Curr. Sci. 78(2):164-169.

Chaturvedi HC, Agnihotri S, Sharma M, Sharma AK, Jain M, Gupta P, Kidwai NR (2004). Induced nucellar embryogenesis in vitro for clonal multiplication of *Mangifera indica* L. var. Ambalavi: A dwarfing rootstock. Indian J. Biotechnol. 3(2):221-228.

Deore AC, Chavan SS, Ranade SS, Deshpande RS, Dhonukshe BL (2000). Factors affecting in vitro somatic embryogenesis from nucellar tissues of Alphonso mango. Ann. Plant Physiol. 14(2):182-186.

Galdino TVdS, Kumar S, Oliveira LSS, Alfenas AC, Neven LG, Al-Sadi AM, Picanço MC (2016). Mapping Global Potential Risk of Mango Sudden Decline Disease Caused by *Ceratocystis fimbriata*. PLoS One 11(7):e0159450.

Gamborg OL (1970). The effects of amino acids and ammonium on the growth of plant cells in suspension culture. Plant Physiol. 45(4):372-375.

Hartmann HT, Kester DE, Davies FT, Geneve RL (1997). Plant propagation: principles and practices (No. Ed. 6). Prentice-Hall Inc.

Jana MM, Nadgauda RS, Rajmohan K, Mascarenhas AF (1994). Rapid somatic embryogenesis from the nucelli of mono embryonic mango varieties. In Vitro Cell. Dev. Biol. Plant 30(1):55-57.

Krishna H, Singh SK (2007). Biotechnological advances in mango (*Mangifera indica* L.) and their future implication in crop improvement—a review. Biotechnol. Adv. 25(3):223-243.

Lad BL, Jayasankar S, Pliego-Alfaro F, Moon PA, Litz RE (1997). Temporal effect of 2, 4-D on induction of embryogenic nuclellar cultures and somatic embryo development of 'Carabao'mango. In Vitro Cell. Dev. Biol. Plant 33(4):253-257.

Laxmi DV, Sharma HC, Kirti PB, Mohan ML (1999). Somatic embryogenesis in mango (*Mangifera indica* L.) cv. Amrapali. Curr. Sci. 77(10):1355-1358.

Litz RE (1984). In vitro somatic embryogenesis from nucellar callus of monoembryonic mango. HortScience 19(5):715-717.

Litz RE (2003). In vitro regeneration and transformation of mango. In: Jaiwal K., Singh R. P., editors. Plant Genetic Engineering. LLC: Sci. Tech. Publishers, pp. 23-40.

Litz RE, Knight RJ, Gazit S (1984). In vitro somatic embryogenesis from *Mangifera indica* L. callus. Sci. Hortic. 22(3):233-240.

Litz RE, Knight RL, Gazit S (1982). Somatic embryos from culture ovules of poly embryonic *Mangifera indica L.* Plant cell Rep. 1(6):264-266

MAF (1989). Annual Agriculture research Report. Ministry of Agriculture and Livestock research Ministry of Agriculture and Fisheries.

MAF (1990). Annual Agriculture research Report. Ministry of Agriculture and Livestock research Ministry of Agriculture and Fisheries.

MAF (1991). Annual Agriculture research Report. Ministry of Agriculture and Livestock research Ministry of Agriculture and Fisheries.

MAF (1992). Annual Agriculture research Report. Ministry of Agriculture and Livestock research Ministry of Agriculture and Fisheries.

MAF (2015). Agricultural Statistics, Ministry of Agriculture and Fisheries, Sultanate of Oman.

Malabadi RB, Teixeira da Silva JA, Nataraja K, Vijaya Kumar S, Mulgund GS (2011). Induction of somatic embryogenesis in mango (*Mangifera indica* L.). Int. J. Biol. Technol. 2(2):12-18.

Mukherjee SK, Litz RE (1997). The Mango: botany, production and uses. CABI.

Murashige T, Skoog F (1962). A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiol. Plant. 15(3):473-497.

Nower AA (2013). *In vitro* production of somatic embryos from nucellus of mango (*Mangifera indica* L.). Life Sci. J. 10(2):1164-1174.

Pateña LF, Barba RC (2011). The development of techniques for tissue culture of mango (*Mangifera indica* L.) var. Carabao and successful transfer of *ex-vitro* grafted plants to soil and the field. In Vitro Cell. Dev. Biol. Plant 47(5):629-636.

Pateña LF, Carlos-Refuerzo LR, Barba RC (2002). Somatic embryogenesis and plantlet regeneration in mango (*Mangifera indica* L.). In Vitro Cell. Dev. Biol. Plant 38(2):173-177.

Singh SK, Sharma HC, Goswami AM, Singh SP (2001). Abscisic acid aided normal somatic embryogenesis from nucellar tissue in mango cv. Amrapali. In. Rai M, Kumar S, Singh R, Prasad VSRK, editors. Recent Trends in Horticultural Research, pp.161-164.

Thomas P (1999). Somatic embryogenesis and plantlet regeneration from nucellar tissue of mono embryonic mango. J. Hortic. Sci. Biotechnol. 74(1):35-39.

academicJournals

Vol. 15(52), pp. 2874-2897, 28 December 2016
DOI: 10.5897/AJB2016.15464
Article Number: 528FF4562176
ISSN 1684-5315

http://www.academicjournals.org/AJB

African Journal of Biotechnology

Full Length Research Paper

Genetic diversity and population structure of common bean (*Phaseolus vulgaris* L) germplasm of Ethiopia as revealed by microsatellite markers

Zelalem Fisseha[1,2], Kassahun Tesfaye[1], Kifle Dagne[1], Matthew W. Blair[3], Jagger Harvey[4], Martina Kyallo[4], Paul Gepts[5*]

[1]Addis Ababa University, Department of Microbial, Cellular, and Molecular Biology, P.O.Box 1176, Addis Ababa, Ethiopia.
[2]Department of Dryland Crops, College of Dryland Agriculutre, Jigjiga University, Crop Research Directorate, Somali Region Pastoral and Agro-pastoral Research Institute (SoRPARI), P.O.Box 398, 1020, Jijiga, Ethiopia.
[3]Department of Agricultural and Environmental Sciences, Tennessee State University, Nashville, TN 37209, United States of America.
[4]Biosciences Eastern and Central Africa (BecA), ILRI, Nairobi, Kenya.
[5]Department of Plant Sciences / MS1, University of California, 1 Shields Avenue, Davis, CA 95616, USA.

Accepted 13 May, 2016; Accepted 9 December, 2016

The Ethiopian genetic center is considered to be one of the secondary centers of diversity for the common bean. This study was conducted to characterize the distribution of genetic diversity between and within ecological/geographical regions of Ethiopia. A germplasm sample of 116 landrace accessions was developed, which represented different common bean production ecologies and seed types common in the country. This sample was then analyzed with 24 simple sequence repeat (SSR) markers to assess the genetic diversity within and between common bean landraces, classifying them based on SSR clustering, and determining relationships between genetic and agroecological diversity. Representatives of both Andean and Mesoamerican gene pools were identified by STRUCTURE software analysis, as well as a high proportion of hybrid accessions as evidenced by a STRUCTURE K = 2 preset. At the optimum K = 5 preset value, mixed membership of Andean and Mesoamerican genotypes in some of the clusters was also seen, which supported previous findings. Cluster analyses, principal coordinate analysis, and analysis of molecular variance all indicated clustering of accessions from different collection sites, accompanied by high gene flow levels, highlighting the significant exchange of planting materials among farmers in different growing regions in the country. Values of allelic diversity were comparable to those reported in previous similar studies, showcasing the high genetic diversity in the landrace germplasm studied. Moreover, the distribution of genetic diversity across various bean-growing population groups in contrasting geographical/ecological population groups suggests elevated but underutilized potential of Ethiopian germplasm in common bean breeding. In summary, this study demonstrated the geographical, as well as gene pool diversity in common bean germplasm of Ethiopia. This substantial diversity, in turn, should be utilized in future common bean breeding and conservation endeavors in the nation.

Key words: Hybridity, simple sequence repeat, microsatellite, structure, seed exchange, gene flow.

INTRODUCTION

Common bean is the most widely consumed legume species of the genus *Phaseolus* (Freytag and Debouck, 2002). It is a pulse crop used since pre-Columbian times in the Americas and, since the 16th century, in other regions of the world (Gepts et al., 2008). It is a true diploid (2n = 2x = 22) with a small genome (580 Mbp; Broughton et al., 2003). Originating in the Neotropics, common bean was domesticated in Mesoamerica and the Andes (Gepts and Bliss, 1986; Gepts, 1988). The crop has high diversity that is broadly classified into six or seven domesticated races distributed into two gene pools (Singh et al., 1991a, b, c; Blair et al., 2007, 2010b; Pallottini et al., 2004; Kwak and Gepts, 2009; Kwak et al., 2012). The crop is a major legume in Eastern and Southern Africa, occupying more than 4 million ha annually and providing food for ≥100 million people (Wortmann et al., 1998; Fisseha, 2015). Of the total production in sub-Saharan Africa of over 3.5 million MT, 62% is in Eastern and Central African countries (Wortmann et al., 1998; Fisseha, 2015). Common bean became established with the African-European trade, even before the widespread era of colonization (Allen and Edje, 1990; Asfaw et al., 2009). Historical accounts show that common bean was introduced to Ethiopia in the 16th century by Portuguese traders and rapidly became an important component of the diet there (Assefa, 1985; Fisseha, 2015). Ever since the introduction of common bean into Ethiopia, farmers have developed farming practices adapted to local conditions by preservation and exploitation of useful alleles, which have resulted in a range of morphologically diverse landraces (Sperling, 2001). Moreover, recent efforts of the national bean-breeding program in Ethiopia have targeted improvement of on-farm common bean productivity and have benefited since the 1980's from continuous introduction of new germplasm from different parts of the world (Fisseha, 2015).

Today, Ethiopia is among the major bean producers in Sub-Saharan Africa (Wortmann et al., 1998). However, the national bean yield still lags behind the global average (Fisseha, 2015). This can be attributed partially to the low yielding capacity of cultivars under use (Assefa, 1990; Fisseha, 2015). To this end, it is essential to tap the potential of landrace genetic resources in order to introgress novel genes of adaptation, resistance to diseases and pests, and tolerance to abiotic stresses. According to Hornakova et al. (2003), landraces grown by small farmers are rich sources of valuable genes.

East Africa is a secondary center of diversity for common beans, due to the wide range of landraces there (Martin and Adams, 1987; Asfaw et al., 2009, Blair et al., 2010b). Understanding the patterns and levels of genetic diversity of bean landraces and cultivars can shed light on potential adaptation and direction and level of gene flow, and eventually help bean breeding and conservation in Ethiopia. Hence, this research project was undertaken with the principal goal of evaluating the genetic diversity within and between common bean landraces, to classify genotypes based on clustering and to understand the distribution of genetic diversity between and within ecological/geographical regions of Ethiopia.

MATERIALS AND METHODS

Plant materials

A total of 116 landrace accessions collected from a range of common bean production agro-ecologies in Ethiopia, four Ethiopian cultivars, three Kenyan cultivars, and two other cultivars, used as control genotypes for the Andean and Mesoamerican gene pools, respectively, were grown in August, 2012, in a greenhouse in the Biosciences Eastern and Central Africa (BecA-ILRI) hub in Nairobi, Kenya, for DNA extraction and analysis. The Ethiopian accessions were sampled from potential bean growing areas in the country (Supplementary Table 1 and Figure 1). The seeds of the control and commercial cultivars were acquired from the Ethiopian National Bean Research Project, based at Melkassa Agricultural Research Center, Adama, Ethiopia. The landrace accessions were provided by the Gene Bank of the Ethiopian Biodiversity Institute (EIB). A total of ten plants per each accession were planted in a single row in the screen house of BecA-ILRI hub, Nairobi, Kenya in August, 2012 for DNA extraction.

Genomic DNA extraction

For the molecular diversity assessment, total genomic DNA for each accession was isolated from a bulked leaf tissue sample of one week old plants from five randomly selected plants per accession using cetyltrimethylammonium bromide (CTAB) method (Doyle and Doyle, 1987) with some minor modifications, as described in supplementary part 1. However, 47 accessions did not produce enough genomic DNA, probably due to poor leaf sample qualities, which, in turn, imposed the need to repeat DNA extraction from the same, using the Zymoplant seed DNA extraction kit (descriptions on the protocol are presented in Supplementary Part 2).

Microsatellite amplification

Twenty-four (24) microsatellite markers from all the 11 linkage groups were selected based on their Polymorphic Information Content (PIC) values and dispersed map locations (Yu et al., 2000; Pedrosa-Harand et al., 2008; Kwak and Gepts, 2009). Out of the 24 SSR markers, 15 were genomic, and the remaining nine were non-genomic (genic) markers (Supplementary Table 2). Markers were PCR amplified with 6-FAM, NED, PET or VIC 5'-labeled forward primers and unlabeled reverse primers. The primers were run in multiplexes, based on their fluorescence dye and allele size using

*Corresponding author. E-mail: plgepts@ucdavis.edu . Tel: +1-530-752-7743.

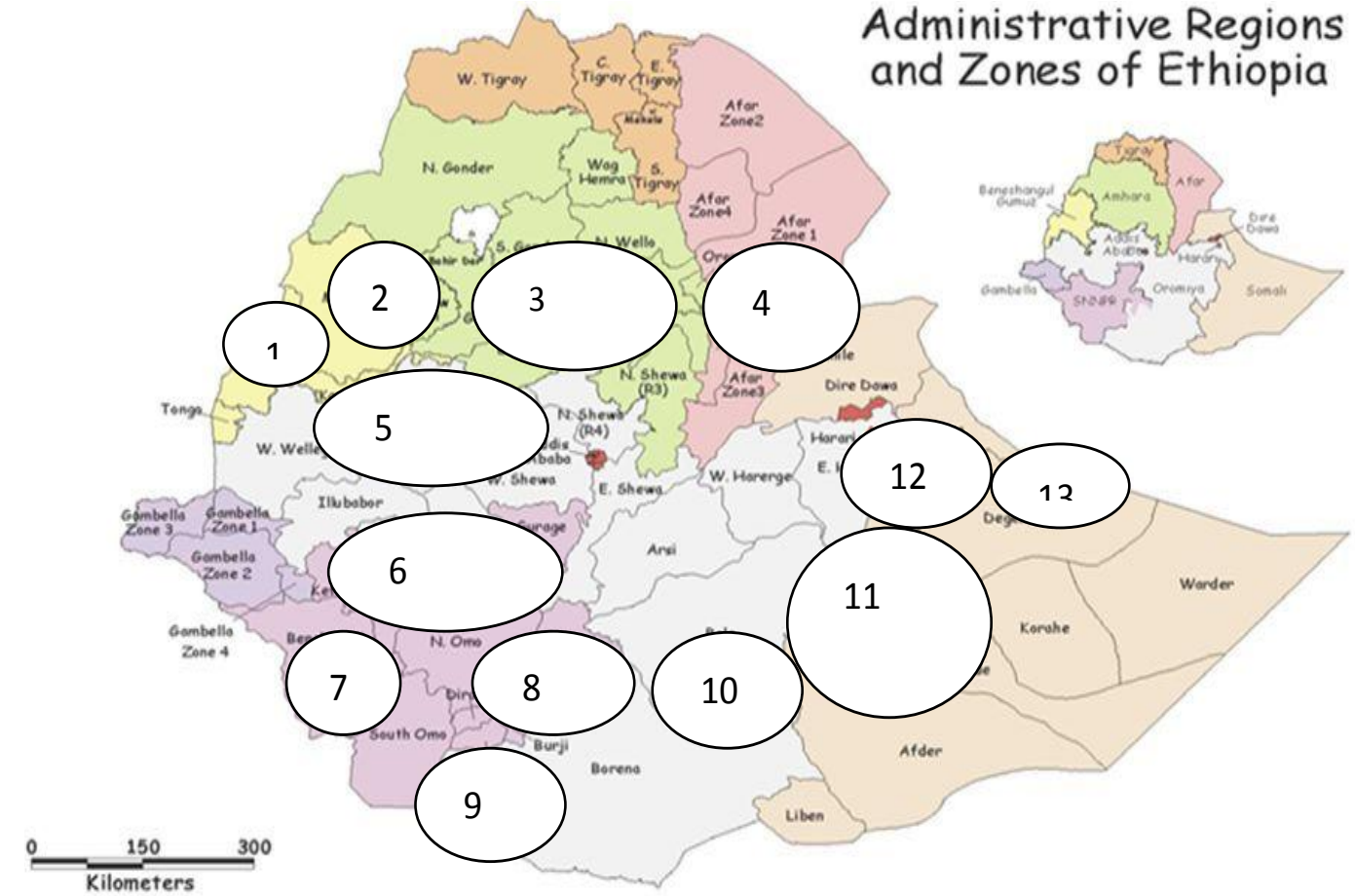


Figure 1. Map showing the collection sites. Key: 1 = Assosa; 2 = Metekel; 3 = Gojam; 4 = North Shewa & South Wello;; 5 = Wellega Gojam; 6 = Jimma and Illubabor; 7=Bench Maji; 8 = North Omo;; 9 = South Omo; 10 = Sidama and Others around; 11 = Bale & Arsi; 12 = East Hararghe;13 = West Hararghe) . The size of the bubbles does not correspond to number of genotypes sampled in each location.

BIONEER ACCUPOWER® Multiplex PCR Premix Kits (Supplementary part 3). Out of the 24 SSR markers, seven were dropped after preliminary evaluation, because they either produced no amplification (BM172 and BMd1) or were monomorphic (BM188, BM183, BMd16, PV-AG001, and PV-AT001). PCR products were run on an ABI PRISM 3730xl fragment analyzer (Applied Biosystems, Foster City, CA, USA) at the BecA-ILRI hub (Sequencing, genotyping, and Oligo unit, SegoLip), and allele sizes were determined by comparing with Genescan LIZ500 size standard using GeneMapper v. 3.7.3.7 software. The observed allele sizes were then adjusted for the discrete allele size using the AlleloBin software (http://test1.icrisat.org/gt-bt/download_allelobin.htm).

SSR genetic diversity analysis

Genalex 6.5b3 (Peakall and Smouse, 2012; http://biology.anu.edu.au/GenAlEx/) was used to calculate genetic diversity parameters, such as genetic distance, number of alleles (N_a); number of effective alleles (N_e); number of private alleles (N_{pa}); observed heterozygosity (H_o); expected heterozygosity (H_e); Shannon's information index (I); analysis of molecular variance (AMoVA); and principal coordinate analysis (PCoA). Genetic associations were determined using the neighbor-joining coefficient with Darwin V. 5.0 (http://darwin.cirad.fr/darwin). Genepop V.4 (Rousset, 2008) and Popgene32 (Yeh et al., 1999) programs were also used to determine genetic diversity, polymorphic loci, gene flow, levels of heterozygosity, fixation index, and F-values,. Finally, PowerMarker v. 3.25 (Liu and Muse, 2005) was used to estimate the number of alleles, polymorphic information content (PIC) values, genetic distance matrices, observed heterozygosity (H_o); and expected heterozygosity (H_e) for each marker across all genotypes and then across genotypes within and between gene pools.

Analysis of population structure

The software program STRUCTURE was run for K values ranging from 2 to 8. Each run was performed using the admixture model and 5,000 replicates for burn-in and 50,000 during the analysis (Pritchard et al., 2000). Evanno et al. (2005) test was performed after 10 simulations per K value. The repeated simulations were conducted for every subpopulation number from K = 2 to K=8 using 5,000 replicates for burn-in and 50,000 replicates according to previous suggestions (Rosenberg et al., 2002; Evanno et al., 2005; Ehrich, 2006). The Δ statistic showed that K = 5 was the optimal number of subpopulations in this analysis (Supplementary Figure 1). This ideal K value presented the highest peak for change in value from and to the previous and subsequent numbers of

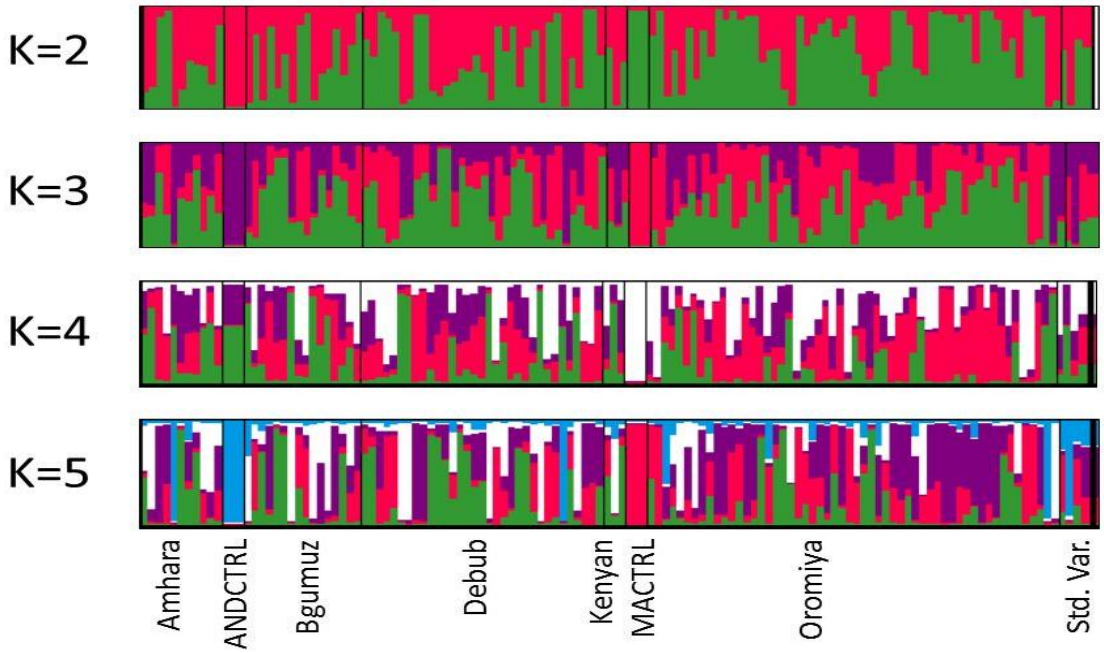


Figure 2. Population structure for 120 common bean accessions from different growing regions of Ethiopia and 3 Kenyan cultivars compared to Andean and Mesoamerican control genotypes at K = 2 to K = 5. Predetermined group names indicated below figure are: Amhara = Genotypes from Amhara Regional State; andectrl = Andean control genotypes; Bgumuz = Genotypes from Benishangul Regional State; Debub = Genotypes from Southern Nations and Nationalities Regional State; Kenyan = Kenyan accessions; MACTRL = Mesoamerican control genotypes; Oromiya = Genotypes from Oromiya Regional State; and Std. Var. = Standard Varieties.

subpopulations, respectively. This showed a gain in precision from subdividing the genotypes into five subpopulations versus any lower or higher numbers of subpopulations. The K=2 analysis was done with a particular interest of distinguishing between Andean and Mesoamerican accessions (Koenig and Gepts, 1989; Kwak and Gepts, 2009). To this end, five independent runs were performed with the admixture model and 5,000 replicates for burn-in and 50,000 replicates during analysis. The clustering in different runs was almost identical (similarity coefficient 0.9914). The run with the lowest likelihood value was selected among the five runs, and the accessions with more than 80% posterior assignment probability in the Mesoamerican cluster were assigned to the Mesoamerican gene pool (and vice versa for the Andean gene pool) (Supplementary Table 3). Lower posterior assignment probability values (that is, between 50 and 80%) may actually indicate hybrids or admixed accessions rather than ‘‘pure’’ accessions (Kwak and Gepts, 2009). Nonetheless, such accessions were included in the K=2 analysis, as they are important in future studies towards shedding light on the population structure of the common bean in Ethiopia, and as baseline information in breeding/improvement programs.

RESULTS

Population structure into the Andean and Mesoamerican gene pools in the common bean germplasm

The population subdivision (as determined by STRUCTURE) (Figure 2), the NJ tree (Figure 3), and the PCoA (Figure 4), showed significant Andean–Mesoamerican gene pool divergence as well as racial differentiation within gene pools. The accessions were assigned to the respective gene pools of origin, as per the methods explained in the “Materials and Methods” for K=2. Consequently, 78 accessions out of the total 125 fell into the Mesoamerican group, whereas the remaining 47 were classified into the Andean group. This classification was based on posterior assignment probabilities p>0.5. This split was generally maintained from K=2 to 3, but broke down for K = 4 and 5 (Figure 2; Supplementary Table 3). The analysis for K = 2 populations showed individual genotypes distributed between the two gene pools, which was congruent with the neighbor-joining and PCoA analyses, which clearly separated the Mesoamerican and Andean gene pools. At K=3, looking jointly into the bar-graphs produced and membership coefficient values, the Mesoamerican gene pool genotypes further separated into two sub-groups but no meaningful interpretation of population structure could be made, while the Andean gene pool genotypes did not show any separation. At K=4, the Mesoamerican accessions further subdivided into two groups with a mild level of admixture but no meaningful interpretation of population structure could be made. At K = 5, the Andean accessions further subdivided into three groups with

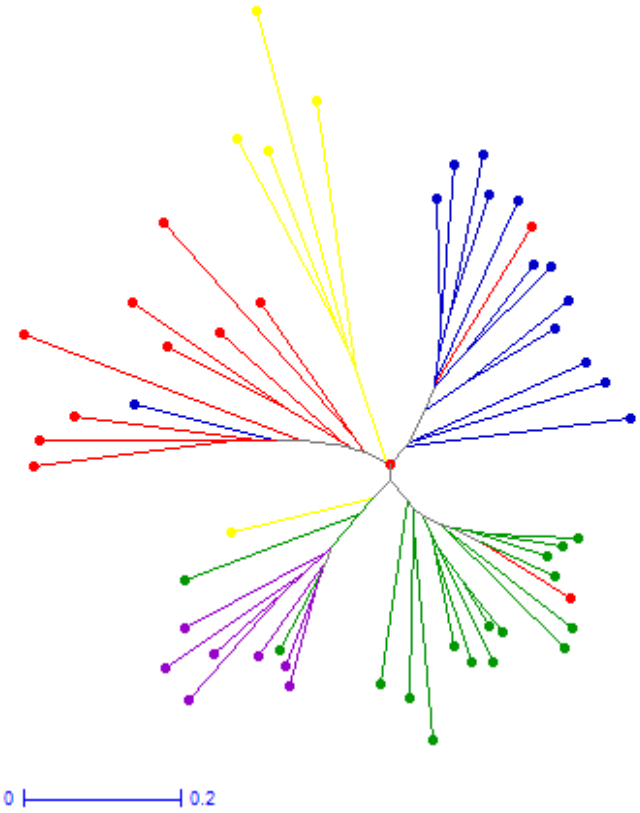


Figure 1. Neighbor-joining dendrogram depicting genetic relationship between common bean accessions from different bean growing populations in Ethiopia with respect to Andean and Mesoamerican control genotypes. Red: Andean Cluster1 (K4); Blue: Andean Cluster2 (K5); Yellow arrows: Andean Control (K1); Green: MA Cluster1 (K2); Purple arrows: MA Control (K3).

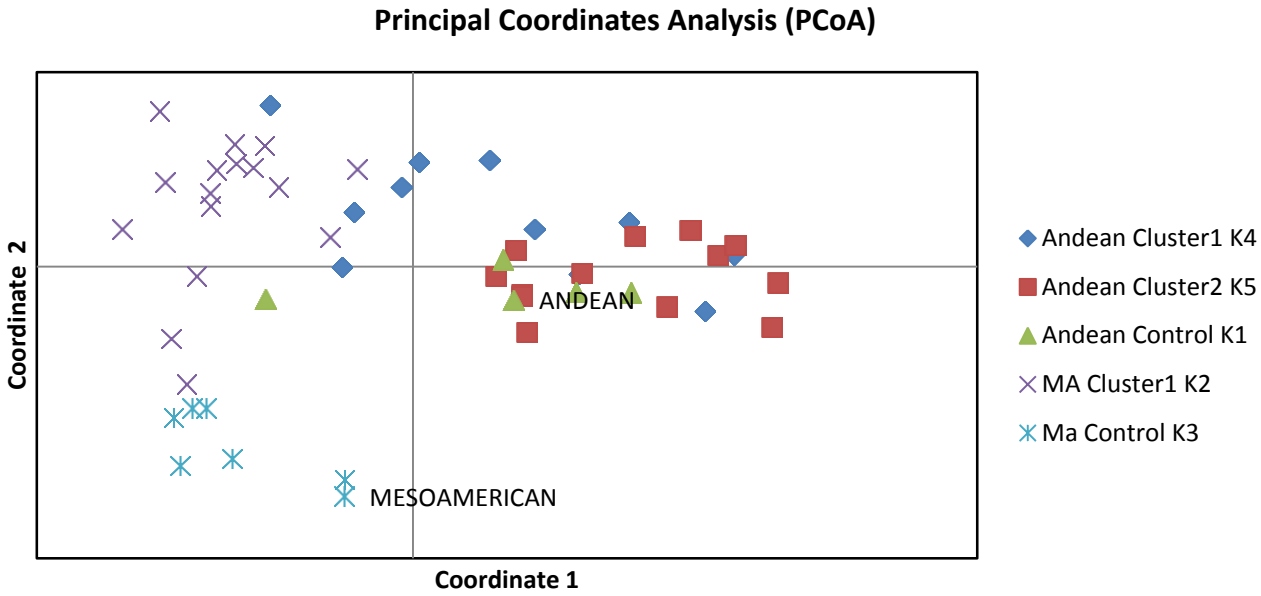


Figure 4. PCoA graph for the 53 accessions from different growing populations in Ethiopia.

Table 1. F_{st} values among five populations identified by STRUCTURE.

K	Andean Cluster 1 (K4)	Andean Cluster 2 (K5)	Andean Control (K1)	Mesoamerican Cluster 1 (K2)	Mesoamerican Control (K3)
5	0.239	0.356	0.547	0.135	0.264

Table 2. Proportion of non-hybrid accessions in K = 5 groups identified by STRUCTURE.

Groups	Total number of accessions	0.8 Cutoff	
		Number of accessions	% from total
Total	125	53	42.4
Mesoamerican	66	23	34.9
Mesoamerican Cluster1 (K2)	41	16	39.0
Mesoamerican control (K3)	25	7	28.0
Andean	59	30	50.9
Andean Cluster1 (K4)	27	11	40.7
Andean Cluster2 (K5)	26	14	53.9
Andean Control (K1)	6	5	83.3

some admixture level, whereas the Mesoamerican accessions did not subdivide further. In the following section, we describe in further details the five groups of K = 5.

Genetic diversity among accessions and cluster groups in STRUCTURE preset K=5

For K=5, the groups were identified as Andean Cluster 1 (K4); Andean Cluster 2 (K5); Andean control (K1); Mesoamerican Cluster 1 (K2) and Mesoamerican control (K3). On average, F_{st} values for Andean populations (K1, K4, and K5) were lower (0.213) compared to those of Mesoamerican populations (K2, and K3) (0.451) (Table 1). We also quantified population admixture for each accession (Figure 2; Supplementary Table 3). The Andean gene pool had a higher proportion of non-hybrid accessions than the Mesoamerican gene pool (51and 35% at the 0.8 cutoff, respectively; Table 2). The proportion of non-hybrid accessions in each K group ranged from 28% (Mesoamerican Controls K3) to 54% (Andean Cluster 2 K5) at the 0.8 cutoff values (Table 2).

The proportions of polymorphic loci were 100% in the Andean Cluster 1 (K4) genotypes; 94% in the Andean cluster 2 (K5), Andean control (K1), and the Mesoamerican cluster 1 (K2); 76% in the Mesoamerican control (K3) (Table 3). On average, the Andean groups had a higher number of alleles (N_a), number of effective alleles (N_e); Shannon Index (I), observed heterozygosity, expected heterozygosity, fixation index, percent of polymorphic loci; genetic distance; and number of private alleles. On the other hand, the Mesoamerican groups had higher hybridity rates than the Andean groups. The highest number of alleles, genetic distance (GD), observed heterozygosity (H_o), hybridity rate (t), and percent of polymorphic loci was recorded for the Andean cluster 1 (K5). The Andean control cluster had the highest Shannon index (I), fixation index (F), number of private alleles (N_{pa}); and number of effective alleles (N_e).

Analysis of Molecular Variance (AMoVA) among accessions and cluster groups in STRUCTURE preset K=5

The AMOVA results showed that 50% of allelic diversity was attributed to individuals within gene pool ($P<0.001$), 31% among individuals in the total population, and the remaining19% was attributed to the diversity among populations (Figure 5). A highly significant genetic differentiation among subpopulations (0.186, $P<0.01$) was observed. Some lower level of gene flow between different cluster of accessions was also reported (that is, 1.1), with higher values among accessions from different Andean gene pool clusters (that is, 1.6) values observed among different Mesoamerican clusters (i.e. 0.3) (Table 4). The average Nei's unbiased genetic distance was higher within each gene pool (0.8), but slightly lower between the Andean and Mesoamerican gene pools (0.7). Within gene pool, the Mesoamerican representatives presented lower genetic distances (0.7) than the Andean gene pool representatives (0.8) (Table 4).

Genetic associations among accessions

Genetic associations among accessions from different

Table 3. Mean SSR diversity for 17 microsatellite loci in five clusters of Ethiopian common bean genotypes.

Parameter	N	Na	Ne	I	H_e	H_o	GD	F	P (%)	N_{pa}	t
Andean Cluster 1 (K4)	11	4.118	2.598	1.032	0.545	0.325	0.304	0.380	100.00	0.154	0.449
Andean Cluster 2 (K5)	14	4.000	2.562	0.990	0.526	0.495	0.286	0.034	94.12	0.211	0.934
Andean Control (K1)	5	3.765	3.007	1.103	0.597	0.382	0.372	0.383	94.12	0.277	0.446
Mean Andean group	-	**3.961**	**2.722**	**1.042**	**0.556**	**0.401**	**0.321**	**0.266**	**96.1**	**0.214**	**0.610**
Mesoamerican Cluster 1 (K2)	16	3.647	2.077	0.819	0.445	0.363	0.229	0.209	94.12	0.174	0.654
Mesoamerican Control (K3)	7	2.412	1.606	0.524	0.298	0.272	0.356	0.067	76.47	0.106	0.874
Mean Mesoamerican group	-	**3.030**	**1.842**	**0.672**	**0.372**	**0.318**	**0.293**	**0.138**	**85.3**	**0.140**	**0.764**
General Mean	**-**	**3.588**	**2.370**	**0.894**	**0.464**	**0.367**		**0.223**	**91.76**	**0.185**	**0.687**

N number of genotypes, N_A number of different alleles, N_E effective number of alleles, N_{PA} number of private alleles, GD gene diversity according to Nei (1978), He expected heterozygosity, H_o observed heterozygosity, I Shannon's information index, F fixation index, t = (1-F)/(1 + F) out-crossing rate, P (%) percent polymorphic loci.

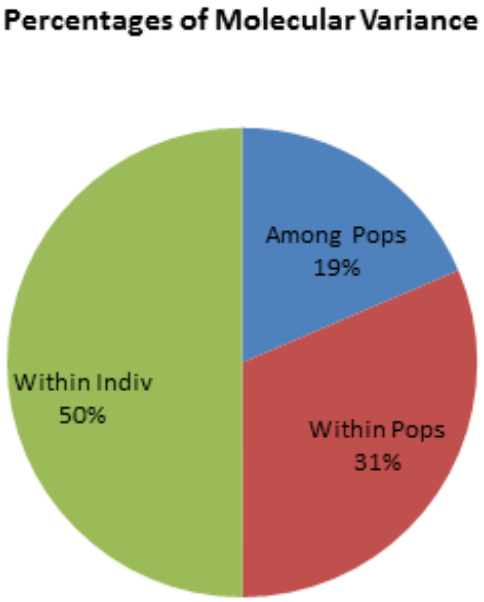


Figure 5. AMOVA pie-chart for the percentage of variation explained among individuals in a population; among populations; and within individuals in all the populations Pops=Populations; Indiv=Individuals.

populations in Ethiopia with respect to Andean and Mesoamerican control genotypes were identified using variation for fluorescent microsatellite markers (Figures 3 and 4). Both the PCoA and Neighbor-Joining graphs indicated the clustering of the bean genotypes into either of the Andean or Mesoamerican control genotypes. In the context of the geographical sample collection sites (Supplementary Table 1), genotypes from the same collection site were often in different clusters and likewise accessions from different collection sites often clustered together (Figure 6), indicating the possibility of gene flow by seeds between sites and regions within Ethiopia.

A principal coordinate analysis (PCoA) was conducted using five populations identified by STRUCTURE. The overall variation explained by the PCoA was 64% with dimensions 1, 2 and 3 explaining 26, 21and 19%, respectively. PCoA separated the bean genotypes into their corresponding centers of domestication (Andean/ Mesoamerican) along the first axis (Figure 4). Exceptions were Andean Cluster 4 genotypes in the second quadrant (four in number) and one genotype of the Andean Control cluster (quadrant III), which showed mixed cluster membership with the Mesoamerican Cluster. The mixed membership of Andean Cluster 1 (K4) was consistent between the STRUCTURE and neighbor-joining analysis results. However, the mixed clustering of Andean Control Cluster (K1) with the Mesoamerican groups was exhibited only in the PCoA and neighbor-joining tree.

Microsatellite diversity of Ethiopian common bean landrace accessions with respect to collections sites

Allelic patterns/diversity

A total of 149 alleles were identified, giving an average of 8.8 alleles per locus for the 17 microsatellites evaluated, of which 12 were genomic markers and 5 were genic (gene-based) (Supplementary Table 2). The range in allele number was 4 to 15, with the marker BM143 having the highest number of alleles, followed by GATS91, GATS54 and BM140, with 14, 13, and 13 alleles, respectively. All these markers were

Table 1. Pairwise population matrix of Nei unbiased genetic distance (below diagonal); Pair-wise N_m values (above diagonal); and F-values of the five cluster groups identified at Structure preset K=5.

Parameter	Andean Cluster1 K4	Andean Cluster2 K5	Andean Control K1	MA Cluster1 K2	MA Control K3	F-values
Andean Cluster1 K4	**0.000**	**1.8**	**1.5**	**1.5**	**0.8**	F_{st}=0.186
Andean Cluster2 K5	0.327	**0.000**	**1.4**	**1.1**	**0.7**	F_{is}=0.385
Andean Control K1	0.525	0.435	**0.000**	0.99	0.7	F_{it}=0.500
MA Cluster1 K2	0.182	0.259	0.384	**0.000**	0.75	
MA Control K3	0.488	0.451	0.517	0.321	**0.000**	

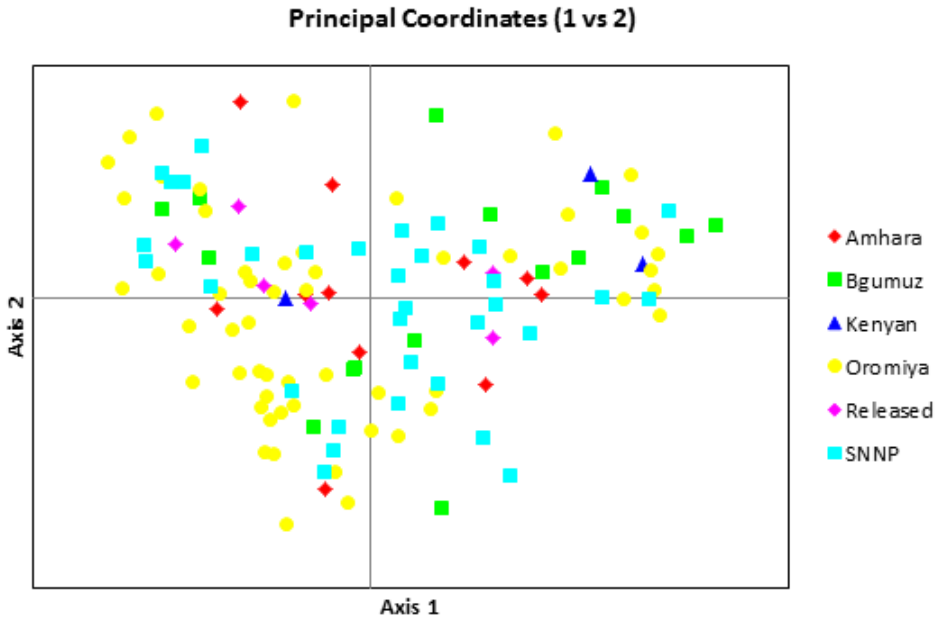


Figure 6. PCoA graph of the 125 common bean accessions from 6 populations.

genomic. The highest number of alleles found for a gene-based microsatellite was for BMd53 with 9 alleles, followed by BMd36 and BMd42 having 6 alleles each. The mean number of alleles for genomic microsatellites was 1.5 times more than that of genic microsatellites. The observed heterozygosity on average was 0.51 across all the 17 markers evaluated. The markers with the highest levels of observed heterozygosity were GATS91 (0.68) and BM143 (0.67), whereas the genic marker PV-CCTT001 had the lowest value, 0.01. With respect to the values recorded for expected heterozygosity (H_e), the SSR markers had an average of 0.564, with the highest being the genomic SSR, GATS91 (0.817), and the lowest for the genic SSR marker, PV-CCTT001 (0.011).

On the other hand, the allelic patterns across the studied populations are presented in Supplementary Figure 2. The figure also depicts the number of alleles, number of effective alleles, Shannon's diversity index, number of private alleles, and number of less common alleles in bars of different colors. The line above the bars indicates pattern of variation in expected heterozygosity among the different groups of accessions. The 'Amhara' and Southern Nations, Nationalities, and People (SNNP) had the highest expected heterozygosity. Nonetheless, the overall variation observed in accessions from different populations (collection sites) vis-à-vis the expected heterozygosity values was moderate. The calculated values for each of the aforementioned allelic measures are given in Table 5. The table corroborates the patterns depicted by Supplementary Figure 2. According to Table 5, accessions from Oromiya and SNNP had the highest average number of alleles (6.9and 6.4, respectively) (Table 5). On the other hand, accessions from 'Amhara' and the released varieties' group had the highest number of alleles with frequencies ≥5% (measurement taken to alleviate the sampling error associated with the sampling of race or distinct alleles, that is, with frequencies ≤ 5%), (N_a Freq. ≥5%), whereas accessions from 'Amhara' and

Table 5. Observed/effective number of alleles, genetic diversity, PIC, total number of alleles, average and expected heterozygosity and Shannon index of the 17 SSR markers used in the study.

Locus	Sample Size	n_a*	n_e*	I*	Average heterozygosity	F_{st}	Genetic Diversity	PIC	H_e
BM205	258	8	2.69	1.4	0.5428	0.25	0.618	0.591	0.590
AG-1	224	4	1.66	0.76	0.3716	0.33	0.409	0.376	0.438
GATS91	234	14	6.57	2.19	0.684	0.28	0.845	0.831	0.817
GATS54	254	13	3.06	1.52	0.5277	0.27	0.665	0.627	0.633
BMd42	242	6	2.89	1.38	0.567	0.28	0.638	0.608	0.607
PV-CCTT001	250	4	1.07	0.08	0.0126	0.11	0.025	0.025	0.011
BMd53	258	9	3.03	1.40	0.6134	0.13	0.664	0.605	0.659
BM156	250	11	2.98	1.40	0.56	0.284	0.655	0.595	0.602
BM187	216	11	2.86	1.35	0.651	0.421	0.648	0.584	0.574
BMd18	216	5	1.69	0.8	0.35	0.423	0.420	0.384	0.381
BMd36	220	6	3.06	1.30	0.53	0.34	0.669	0.621	0.610
BM151	218	8	3.44	1.43	0.56	0.336	0.705	0.656	0.596
BM140	232	13	4.1	1.72	0.64	0.31	0.752	0.717	0.677
BM141	242	7	2.56	1.18	0.48	0.31	0.603	0.536	0.589
BM143	242	15	4.17	1.91	0.67	0.223	0.757	0.736	0.765
BM165	226	6	3.51	1.39	0.54	0.366	0.717	0.671	0.559
BM139	244	9	1.88	1.08	0.41	0.251	0.457	0.437	0.479
Mean	**237**	**8.8**	**3.01**	**1.31**	**0.51**	**0.289**	**0.603**	**0.565**	**0.564**
St. Dev		**3.53**	**1.245**	**0.471**	**0.16**				**0.042**

SNNP had the highest number of effective alleles (N_e) (Table 5). From the perspective of this study, the 'Amhara' and SNNP regions may be the most important population of accessions owing to the higher number of alleles with frequencies ≥5% (excluding rare alleles) and number of effective alleles. Similar to the observations regarding the number of effective alleles (N_e), 'Amhara' and SNNP had the highest genetic diversity measures (Shannon's index=I) (Table 5). This may further strengthen the argument made above regarding the two populations, namely that the 'Amhara' and SNNP regions contain the highest level of bean diversity. Furthermore, accessions from 'Oromiya' and SNNP had the highest numbers of private alleles (0.82 and 0.59, respectively), and fewer common alleles with frequencies less than 50% (1.77and 1.60, respectively). This may imply that, upon further determination of what functional characters, if any, these private/less common alleles encode for or which genome region they mark, it may be possible to harness the potential of accessions in the population in future common bean breeding/improvement and genetic conservation endeavors in Ethiopia. Finally, the highest values for both expected and unbiased expected heterozygosity were recorded for accessions from 'Amhara', SNNP, and the released varieties' group.

Analysis of Molecular Variance (AMOVA) in the ecological/geographic population groups

Results of AMOVA are presented in Table 6 and Figure 7. Figure 7 shows that 58% of the total variation was attributed to genetic diversity prevalent within individuals from different populations, whereas 40% was due to variation among individuals within the same population. In contrast, a smaller portion (2%) of the total variation differentiated populations. In comparison, when the cluster groups identified at STRUCTURE preset K=5 (discussed below) were considered, AMOVA showed that 50% of allelic diversity was attributed to individuals within each of the groups ($P<0.001$); 31% among individuals in the total population; and the rest 19% was attributed to the diversity among populations. Moreover, highly-significant genetic differentiation among subpopulations (0.186, $P<0.01$) was observed.

In view of the F-statistics values (Table 7), the extent of genetic differentiation among the six populations in terms of allele frequencies measured was small (F_{st}=0.015*). Furthermore, the pair-wise Nm values among the six populations studied indicate that the highest values for putative gene flow were recorded for the following pairs of populations: BenishangulGumuz and SNNP (N_m=63); BenishangulGumuz and Kenya (N_m=55); and Oromiya and SNNP (N_m=31) (Supplementary Table 4).

Cluster analysis and Principal Coordinate Analysis (PCoA)

Cluster analysis with respect to populations (collection sites) was performed on the allelic frequency data using

Table 6. Important allelic values recorded in the landrace and control genotypes in six population groups.

Parameters	Populations					
	Amhara	**Bgumuz**	**Kenyan**	**Oromiya**	**Released**	**SNNP**
Na	5.059	4.647	2.529	**6.882**	3.824	**6.353**
Na Freq. ≥ 5%	**3.824**	3.765	2.529	3.294	**3.824**	3.647
Ne	**3.262**	2.791	2.195	2.612	2.815	**2.970**
I	**1.239**	1.114	0.757	1.179	1.065	**1.236**
No. Private Alleles	0.353	0.176	0.000	**0.824**	0.412	**0.588**
No. Less common Alleles (≤50%)	1.294	1.412	0.588	**1.765**	0.765	**1.588**
He	**0.619**	0.571	0.457	0.565	**0.574**	**0.597**
uHe	**0.652**	0.593	0.578	0.571	**0.627**	**0.607**

N_a (number of alleles), N_a Freq >= 5% (number of alleles with frequencies greater than or equal to 5%, N_e (number of effective alleles), I (Shannon's index), number of private alleles, number of less common alleles (with frequencies less than or equal to 25% and 50%, and He (expected heterozygosity). Populations refer to geographical administrative regions from which accessions had been collected.

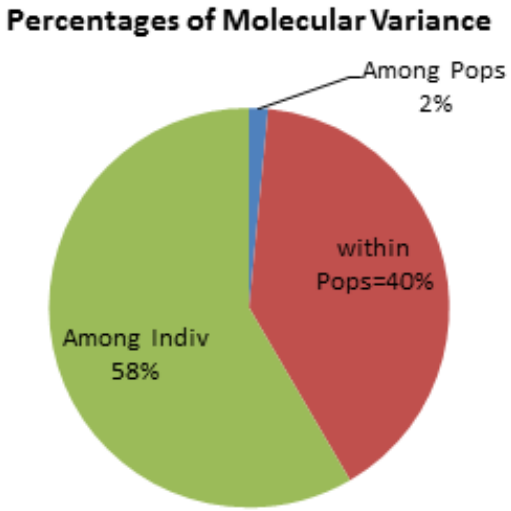


Figure 7. AMoVA variation pie chart for 125 common bean accessions from six populations in Ethiopia.

Table 7. Values of sum of squares; mean squares; and F-values among populations; among individuals in a population; and among individuals in all the populations.

Source	df	SS	MS	Est. Var.	%	F-Statistics	Value	P (random ≥ data)
Among Pops	5	53.742	10.75	0.085	2	F_{st}	0.015	0.020
Within pops	119	924.562	7.77	2.247	40	Fi_s	0.407	0.010
Among Indiv	125	409.500	3.28	3.276	58	F_{it}	0.416	0.010
Total	249	1387.804		5.608	100	N_m	16.282	

the Neighbor-joining method as implemented in the Darwin 5 and PowerMarker V3.25 software programs. Figure 8 shows the dendrogram clustering pattern for individual accessions in different populations (collection sites). As can be seen from the dendrogram, five different groups were identified. Furthermore, accessions from

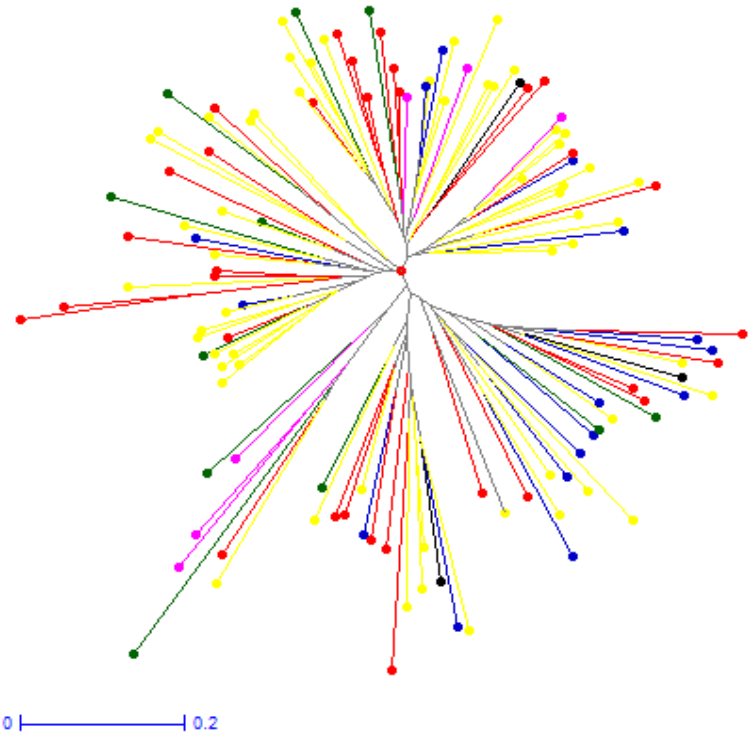


Figure 2. Neighbor-joining dendrogram of the 125 common bean accessions constructed by Darwin V5 software program. Green: Amhara; Blue: Benishangul Gumuz; Yellow: Oromiya; Red: Southern region; Purple: Released varieties; Orange: Kenya.

different populations (collection sites) clustered together. On the other hand, Supplementary Figure 3 shows the results of the cluster analysis done based on Nei's average unbiased genetic distance (Nei, 1983) among the accessions studied. Based on these results, four groups of populations were identified among the common bean landrace accessions from six different populations. Group 1 belonged to accessions from the Amhara region/population; the second group comprised accessions from the southern Ethiopia and Oromiya regions/populations. Another neighbor-joining dendrogram was constructed based on the shared-allele frequency genetic distances measured (Supplementary Figure 4). In comparison, this dendrogram identified five groups (compared to the four groups identified in the Nei's genetic distance NJ dendrogram), with Oromiya and Southern regions in the farthest end and Benishangul-Gumuz and Amhara, being group 3 and 4. Finally, yet importantly, the shared-allele frequency NJ dendrogram, similarly with the Nei's NJ dendrogram, clustered accessions from Kenya and the released varieties' group.

On the other hand, the first three axes of the PCoA accounted together for 65 % of the total variation, with 27, 21 and 17% explained by PC axis 1, 2, and 3, respectively. Results of the PCoA are displayed in Figure 6. It can be seen from this figure that accessions from different collection sites often clustered together.

DISCUSSION

The hierarchical classification scheme into subpopulations comprised of Andean and Mesoamerican genotypes obtained here was in agreement with that reported for common bean germplasm in various studies (Singh et al., 1991a; Gepts, 1998; Diaz and Blair, 2006; Blair et al., 2007, 2010a, 2011; Okii et al., 2014b). Moreover, the moderate to mostly large differentiation among subpopulations (F_{st} values) were higher than was reported in other studies (Asfaw et al., 2009; Okii et al., 2014b). On the other hand, the higher differentiation recorded in the present study among Mesoamerican subpopulations compared to their Andean counterparts was in contrast with the results of Asfaw et al. (2009) and Okii et al. (2014b). The separation of bean accessions into the two gene pools was also evidenced in the NJ and PCoA analyses. Five cluster groups were identified, which supports the findings reported in previous studies (Kwak and Gepts, 2009; Burle et al., 2011). Furthermore, the presence of moderate admixture level agrees with previous reports (Asfaw et al., 2009; Blair et al., 2010b; Okii et al., 2014b). Similarly, the concurrence of

STRUCTURE results with that of PCoA and NJ analyses was in agreement with that reported by Asfaw et al. (2009) and Okii et al. (2014b).

Five subpopulations with moderate admixture level and some switching of membership were observed in the present study. In line with this, Okii et al. (2014b) noted that the high level presence of admixture is indicative of the considerable mixing of common bean germplasm in planting and consumption and in hybridization in breeding. The considerable presence of admixture and switching of membership in some instances was supported by the PCoA and NJ analyses. These agree with some previous reports (Asfaw et al., 2009; Blair et al., 2010b; Burle et al., 2011; Okii et al., 2014b). In addition, the PCoA and NJ analyses in terms of geographic/ecological sampling of accessions indicated that accessions from different collection sites clustered together, which implied there was significant exchange of planting materials among farmers in different growing regions in the country. Moreover, the analysis of hybrid/ non-hybrid accessions indicated the Mesoamerican genotypes had higher instances of hybridity than the Andean counterparts. This observation supported previous results (Asfaw et al., 2009; Kwak and Gepts, 2009). On the other hand, from a population differentiation viewpoint, Andean genotypes were more differentiated than those from the Mesoamerican gene pool, which concurs with the findings reported previously for East African common bean germplasm (Asfaw et al., 2009; Okii et al., 2014b).

Accessions with Andean origin had higher allelic parameter values (Na, Ne, PIC, etc.) than Mesoamerican accessions. This contrasted with results from other related studies (Asfaw et al., 2009; Burle et al., 2011; Okii et al., 2014 a,b). Such differences may be attributed to differences in genetic samples and respective sampling methods employed. On the other hand, the hybridity values recorded in our study were much higher than those reported previously (Blair et al., 2010b; Okii et al., 2014a). This might be explained by the fact that most of the accessions (>90%) were acquired from the National Gene Bank, which, in turn, had collected these accessions from subsistence farmers with a culture of keeping mixed seeds for consumption and subsequent planting seasons. Moreover, the higher allelic values of genomic than genic markers were comparable to those reported in some previous studies (Asfaw et al., 2009; Blair et al., 2010b; Okii et al., 2014b). The high genetic diversity of the Ethiopian common bean landraces was also evident when considering their ecological or geographical distribution. Such presence of high diversity in terms of both gene pools and the existence of ecologically- or geographically differentiation populations can have potential applications prospective common bean breeding programs in Ethiopia.

The presence of higher levels of gene flow within each gene pool than that found between gene pools observed in our study agrees with the result of Asfaw et al. (2009). This may be explained, in part, due to the lack of flowering synchronization, which could reduce inter-gene pool gene flow. A larger proportion of the accessions (i.e., 58%) were introgressions, which contradicts the report of Asfaw et al. (2009) about the lower level of introgression with Ethiopian and Kenyan bean landraces/cultivars. This, in turn, negates the assumption of the aforementioned authors implying that the genetic divergence in Ethiopian bean germplasm could be mainly due to the original differences in introduced germplasm from the primary centers of origin. Rather, the presence of a higher number of introgressions may be partially explained by the fact that the accessions were gene bank collections from farmers' fields often characterized by a higher level of mixtures. The common practice of subsistence farmers in the country who cultivate for consumption and save segregant genotypes, resulting from any natural hybridization, as planting materials for subsequent generations, could result in such type of introgressions (Blair et al., 2010b; Worthington et al. 2012). A final noteworthy remark may be the fact that inter-gene pool introgressions are often endowed with useful combination of traits, including enhanced adaptation to environmental stresses, higher resistance to diseases and pests, and higher nutritional quality; hence, the introgressions identified in this study are of considerable importance in future bean breeding and conservation endeavors in Ethiopia. These merits of hybrids were evidenced in Islam et al. (2005) and Blair et al. (2010b), who reported that introgressions had higher mineral compositions than their respective non-hybrid parents. Consequently, it may be essential to tap into the useful genetic diversity found in such types of inter-gene pool introgressions, to be harnessed in further common bean breeding, improvement, and genetic conservation programs of beans in Ethiopia.

Conclusion

This study formulates new insights about the pattern and extent of genetic diversity and population structure of common bean landrace germplasm in Ethiopia. The results in the context of both the two gene pools of origin and ecological/geographic populations shed light on the presence of adequate genetic diversity organized into the Andean and Mesoamerican gene pools, and distributed across various ecological/geographic populations. This in turn should be strengthened by identifying the cluster groups identified by STRUCTURE via integrating molecular marker evaluations with phenotypic data.

Conflicts of interest

The authors have not declared any conflict of interest.

ACKNOWLEDGMENTS

We are grateful to the financial and material support provided by the Rural Capacity Building Project (RCBP)-Ministry of Agriculture, Ethiopia; the African Biosciences Challenge Fund (ABCF) at the BecA-ILRI hub, Nairobi, Kenya; and Addis Ababa University. Moreover, we are thankful to the Ethiopian Biodiversity Institute, Ethiopia, and the National Common Bean Research Project based at Melkassa Agricultural Research Center, Ethiopia for availing the plant materials for our study.

REFERENCES

Allen DJ, Edje OT (1990). Common bean in Africa Farming System. In: 1990 Progress in Improvement of Common Bean in Eastern and Southern Africa; Smithson JB. Ed. Bean Research, CIAT Africa Workshop Series. 5(20):32.

Asfaw A, Blair MW, Almekinders C (2009). Genetic diversity and population structure of common bean (*Phaseolus vulgaris* L.) landraces from the East African highlands. Theor. Appl. Genet. 120:1-12.

Blair MW, Díaz LM, Buendía HF, Duque MC (2010a). Genetic diversity, seed size associations and population structure of a core collection of common beans (*Phaseolus vulgaris* L.). Theor. Appl. Genet. 119:955-73

Blair MW, Díaz LM, Gill-Langarica HR, Rosales-Serna R, Mayek-Perez N, Acosta-Gallegos JA (2011). Genetic relatedness of Mexican common bean cultivars as revealed by microsatellite markers. Crop Sci. 51: 1–13.

Blair MW, Giraldo MC, Buendia HF, Tovar E, Duque MC, Beebe SE (2007). Microsatellite marker diversity in common bean (*Phaseolus vulgaris* L.). Theor. Appl. Genet. 113:100-109.

Blair MW, Gonzalez LF, Kimani PM, Butare L (2010b). Genetic diversity, inter-gene pool introgression and nutritional quality of common beans (*Phaseolus vulgaris* L.) from Central Africa. Theor. Appl. Genet. 121:237-248.

Broughton WJ, Hernandez G, Blair MW, Beebe SE, Gepts P, Vanderleyden J (2003). Beans (*Phaseolus spp*.) model food legumes. Plant Soil 252:55-128.

Burle L, Fonseca JR, Peloso M, Melo L, Temple SR, Gepts P (2011). Integrating phenotypic evaluations with a molecular diversity assessment of a Brazilian collection of common bean landraces. Crop Sci. 51:2668-2680.

Diaz LM, Blair MW (2006). Race structure within the Mesoamerican gene pool of common bean (*Phaseolus vulgaris* L.) as determined by microsatellite markers. Theor. App. Genet. 114:143-154.

Doyle JJ, Doyle JL (1987). A rapid DNA isolation procedure from small quantities of fresh leaf tissue. Phytochem. Bull. 19:11-15.

Ehrich D (2006). AFLPDAT: a collection of R functions for convenient handling of AFLP data. Mol. Ecol. Notes 6:603-604.

Evanno G, Regnaut S, Goudet J (2005). Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Mol. Ecol. 14:2611-2620.

Freytag GF, Debouck DG (2002). Taxonomy, Distribution, and Ecology of the Genus *Phaseolus* (Leguminosae-Papilionoideae) in North America, Mexico and Central America. Bot. Res. Inst. Of Texas, Brit. Press, Ft. Worth, TX.

Gepts P (1988). A Middle American and an Andean Common Bean Gene Pool. In. Genetic resources of *Phaseolus* beans; Gepts, P. Ed. Kluwer, Dordrecht, The Netherlands. Pp. 375-390.

Gepts P (1998). Origin and evolution of common bean: Past events and recent trends. HortScience 33:1124-1130.

Gepts P, Aragão FJL, Barros E, Blair MW, Broughton RBW, Galasso I, Hernández G, Kami J, Lariguet P, McClean P, Melotto M, Miklas PN, Pauls P, Pedrosa-Harand A, Porch T, Sánchez F, Sparvoli F, Yu K (2008). Genomics of *Phaseolus* Beans, a Major Source of Dietary Protein and Micronutrients in the Tropics. In. Genomics of Tropical Crop Plants; Moore PH, Ming R. Eds. Springer Science+Business Media, New York, USA. 1: 132-162.

Gepts P, Bliss FA (1986). Phaseolin variability among wild and cultivated common beans (*Phaseolus vulgaris*) from Colombia. Econ. Bot. 40:469-478.

Habtu A (1990). Research Highlights on Haricot Bean in Ethiopia: An Assessment of the Progress, Priorities, and Strategies. In. Proceedings of National Workshop; 1-3 October, 1990, Addis Ababa, Ethiopia. pp. 50-53.

Hornakova O, Zavodna M, Zakova M, Kraic J, Debre F (2003). Diversity of common bean landraces collected in the Western and Eastern Carpatien. Czech. J. Genet. Plant Breed. 39(3):73-83.

Imru A (1985). Bean production in Ethiopia. In. Regional Workshop on Potential for Field Beans in West Asia and North Africa. Allepo, Syria, 10-13 October 1983, pp. 15-38.

Islam FM, Basford KE, Jara C, Redden RJ, Beebe S (2005). Seed compositional and disease resistance differences among gene pools in cultivated common bean. Genet. Res. Crop. Evol. 49:285-293.

Koenig R, Gepts P (1989). Allozyme diversity in wild *Phaseolus vulgaris*: further evidence for two major centers of diversity. Theor. Appl. Genet. 78:809-817.

Kwak M, Gepts P (2009). Structure of genetic diversity in the two major gene pools of common bean (*Phaseolus vulgaris* L., Fabaceae). Theor. Appl. Genet. 118:979-992.

Kwak M, Toro O, Debouck D, Gepts P (2012). Multiple origins of the determinate growth habit in domesticated common bean (*Phaseolus vulgaris* L.). Ann. Bot. 110:1573-1580.

Liu KJ, Muse SV (2005). PowerMarker: an integrated analysis environment for genetic marker analysis. Bioinformatics 21:2128-2129.

Martin GB, Adams MW (1987). Landraces of *Phaseolus vulgaris* (Fabaceae) in Northern Malawi: Generation and maintenance of variability. Econ. Bot. 41:204-215.

Okii D, Tukamuhabwa P, Kami J, Namayanja A, Paparu P, Ugen M, Gepts P (2014b). The genetic diversity and population structure of common bean (*Phaseolus vulgaris* L.) germplasm in Uganda. Afr. J. Biotechnol. 29:2935-2949.

Okii D, Tukamuhabwa P, Namayanja A, Mukabaranga J, Paparu P, Gepts P (2014a). Morphological diversity of tropical common bean germplasm. Afr. Crop Sci. J. 22:59-67.

Pallottini L, Garcia E, Kami J, Barcaccia G, Gepts P (2004). The genetic anatomy of a patented yellow bean. Crop Sci. 44:968-977.

Peakall R, Smouse PE (2012). GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research—an update. Bioinformatics 28:2537-2539.

Pedrosa-Harand A, Porch T, Gepts P (2008). Standard nomenclature for common bean chromosomes and linkage groups. Bean Improvement Cooperative, East Lansing, MI, USA. https://naldc.nal.usda.gov/download/IND44058845/PDF.

Pritchard JK, Stephens M, Donnelly P (2000). Inference of population structure using multilocus genotype data. Genetics 155:945-959.

Rosenberg NA, Pritchard JK, Weber JL, Cann HM, Kidd KK, Zhivotovsky LA, Feldman MW (2002). Genetic structure of human populations. Science 298:2381-2385.

Rousset F (2008). Genepop'007: a complete re-implementation of the genepop software for Windows and Linux. Mol. Ecol. Resour. 8:103-106.

Singh SP, Gepts P, Debouck DG (1991a). Races of common bean (*Phaseolus vulgaris* L., Fabaceae). Econ. Bot. 45:379-396.

Singh SP, Gutierrez JA, Molina A, Urea C Gepts P (1991b). Genetic diversity in cultivated common bean: II. Marker-based analysis of morphological and agronomic traits. Crop Sci. 31:23-29.

Singh SP, Nodari R, Gepts P (1991c). Genetic diversity in cultivated common bean. I. Allozymes. Crop Sci. 31: 19–23.

Sperling L (2001). The effect of the civil war on Rwanda's bean seed systems and unusual bean diversity. Biodivers. Conserv. 10:989-1009.

Worthington M, Soleri D, Aragón-Cuevas F, Gepts P (2012). Genetic composition and spatial distribution of farmer-managed bean plantings: an example from a village in Oaxaca, Mexico. Crop Sci. 52:1721-1735.

Wortmann CS, Kirkby RA, Eledu CA, Allen DJ (1998). Atlas of common

bean (*Phaseolus vulgaris* L.) production in Africa. CIAT Pan-African Bean Research Alliance, Vol. 133.
Yeh FC, Yang R, Boyle T (1999). POPGENE VERSION 1.31-Microsoft Window-based Freeware for Population Genetic Analysis. University of Alberta Edmonton, AB Canada T6G 2H1.
Yu K, Park S, Poysa V, Gepts P (2000). Integration of simple sequence repeat (SSR) markers into a molecular linkage map of common bean (*Phaseolus vulgaris* L.). J. Hered. 91:429-434.
Fisseha Z (2015). Genetic diversity and population structure of common bean (*Phaseolus vulgaris*, L.) germplasm from Ethiopia. PhD Thesis. Addis Ababa University, Addis Ababa, Ethiopia.

Supplementary Text 1: Genomic DNA Extraction.

For the molecular diversity assessment, total genomic DNA for each accession was isolated from a bulked leaf tissue sample of five randomly selected, one-week-old plants per accession using cetyltriethylammonium bromide (CTAB) method (Doyle and Doyle, 1990) with some minor modifications, as described in the following sections.

About 200 mg of fresh leaf tissue samples/leaf were placed in a 2 ml autoclaved and labeled Eppendorf tubes, covered by paraffin paper with a small slot at one side for air circulation, and freeze-dried for two days at-80^{0}C. Subsequently, a drop of polyvinyl polypyrrolidone (PVPP) was added to the Eppendorf tubes. Then, 500 µl of 1× CTAB was added to each tube to break open cells and soluble cellular contents. Next, the contents in each tube were mixed using a Vortex, and kept in a gently-shaking water bath for 1 hour at 65°C. After the samples were taken out of the water bath, they were centrifuged at 14,000 rpm for 30 min, using an Eppendorf centrifuge (5417R). Afterwards, the supernatant suspension was transferred into new Eppendorf tubes, and 250 µl of potassium acetate was added. A total of 400 µl of ice-cold isopropanol was added to the supernatant solution harvested, after centrifuging the samples at 14,000 rpm for 30 min. At this point, the samples were left at -20°C overnight. The following day, the samples were removed from the -20°C freezer; centrifuged at 14,000 rpm for 30 min at -4°C. The supernatant was then poured off and the pellet dried. In order to remove the remaining isopropanol drops, the tubes were placed upside down on a paper towel. The pellets were air-dried for 30 min at room temperature.

Subsequently, 200 µl of TE and 3 µl of RNAse were added to each tube, which were then left in a water bath at 37°C. Following this, chlorophyll and some denatured proteins were removed by dissolving in a 200 µl mixture of phenol, chloroform, and isoamyl alcohol at a ratio of 25:24:1, which was mixed with manual inversions from 5 to 10 times. Next, the samples were incubated at room temperature for 10 min. Subsequently, a fixed volume of supernatant (180 µl) was harvested from each tube into new sets of 1.5 ml Eppendorf tubes. Three hundred µl of ice-cold 100% ethanol plus 15 µl of sodium acetate (at pH 5.2) was added to each. Following incubation at -80°C for 5 min, centrifugation was performed at 14,000 rpm for 30 min; the supernatant was poured off and the inside of each tube was washed with 200 µl 70% ethanol, and another centrifuging was applied at 14,000 rpm for 30 min at -4°C. Following this, DNA pellets were air-dried for an hour, and re-suspended with 30 µl of low salt buffer. DNA quality and quantity were measured by gel electrophoresis (using 1% agarose gel for 1 hour using λ-DNA as a size marker) (Figure 11).

Supplementary Text 2.Pictorial display of the Zymoplant seed DNA extraction kit.

BIONEER
innovation . Value . Discovery

AccuPower® Gold Multplex PCR PreMix

Bioneer Corporation
8-11, Munpyeong-ro
Daedeok-gu, Daejeon 306-220
South Korea
Tel. (Korea) 1588-9788
(International) + 82-42-930-8777
Fax. + 82-42-930-8600
E-mail: sales@bioneer.com

Bioneer, Inc.
1000 Atlantic Avenue
Alameda, CA 94501 USA
Toll free: + 1-877-264-4300
Fax: + 1-510-865-0350
E-mail: orderusa@bioneerusa.com

Bioneer Trade(Shanghai) Co., Ltd
403 Room, Building 88, no 887
Zuchongzhi Road, Zhangjiang High Tech Park
PuDong New District, Shanghai 201203 China
Tel. + 86-21-5080-0969
Fax. + 86-21-5080-1620
E-mail: salesch@bioneer.com

Seoul Office
2nd fl. Sansu B/D, 496, Gangnamdaero
30-gil, Seocho-gu, Seoul 137-130
South Korea
Tel. + 82-2-598-1064
Fax. + 82-2-598-1096

Order
Domestic only: 1588-9788
E-mail: order@bioneer.co.kr
URL: www.bioneer.com

6. Maintain the reaction mixture at 4°C after amplification. The sample can be stored at -20°C until use.
7. Load 5 μl of the reaction mixture directly on agarose gel without adding a loading dye to analyze the PCR products.

X. Reaction Example

1. Reaction mixture

Component	Volume	Concentration
Template	1 μl	100 ng/μl
Primers	2 μl	1 pmole/μl each
D.W	17 μl	
Total	20 μl	

2. PCR cycling condition

Step	Temperature	Time	No. of Cycles
Pre-denaturation	95°C	5 min	1
Denaturation	95°C	30 sec	30
Annealing	57°C/65°C	30 sec	
Extension	72°C	1 min	
Final-Extension	72°C	5 min	1

Figure 1, Figure 2. (a)
Figure 2. (b)

XI. Experimental Data

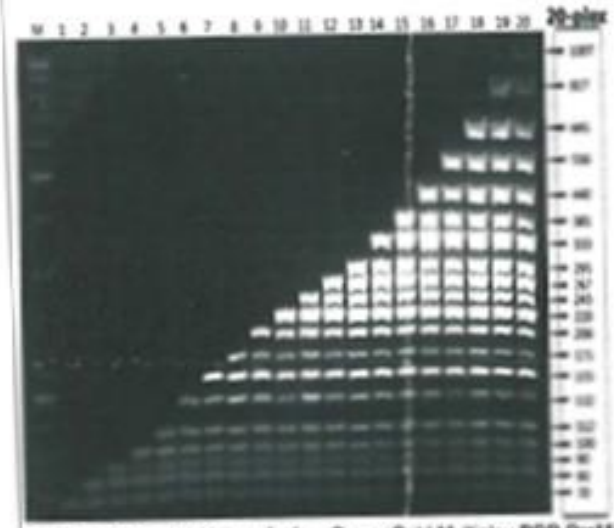
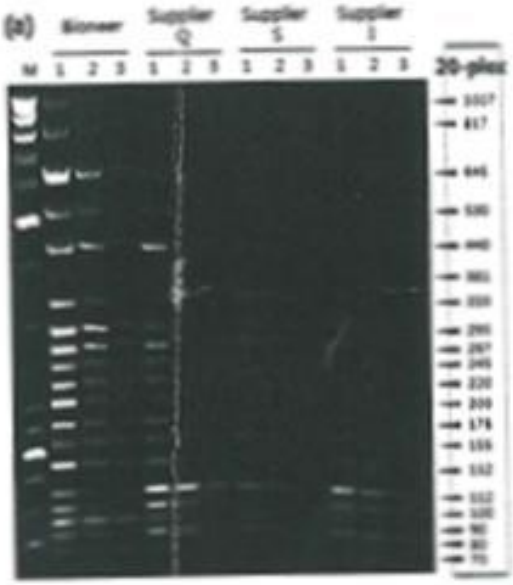


Figure 1. High specificity of *AccuPower* Gold Multiplex PCR PreMix. Each line from left to right represents the progressive number of primer sets up to 20 included in *AccuPower* Gold Multiplex PCR PreMix.



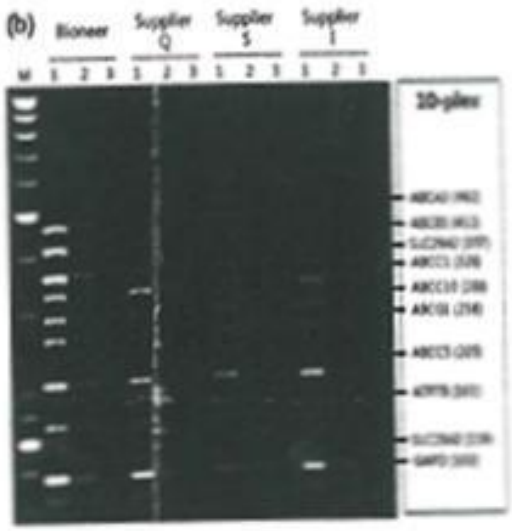


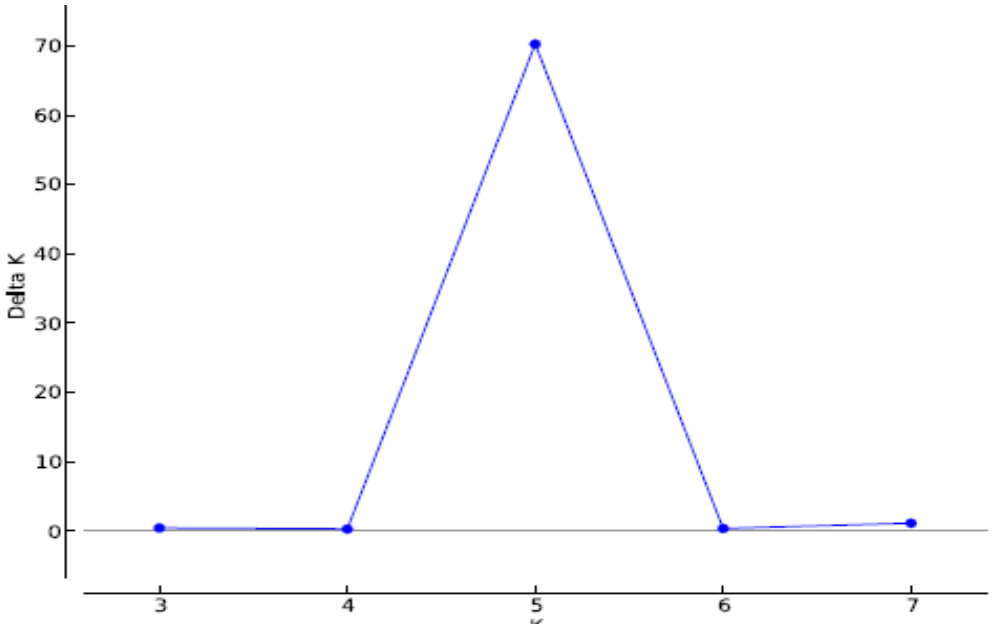
Figure 2. Comparison of amplification quality between *AccuPower* Gold Multiplex PCR PreMix and other supplier's Multiplex PCR kits.

Supplementary Table 1. ID number and names of collection site for the germplasm used in the study.

No.	Accession ID	Region/ Collection Area	No.	Accession ID	Region/ Collection Area	No.	Accession ID	Region/ Collection Area
1	211315	E. Hararghe	43	235692	Bench Maji	85	208703	Wellega
2	211317	E. Hararghe	44	235697	Bale &Arsi	86	211266	Gojam
3	211318	E. Hararghe	45	201066	Jimma	87	211267	Gojam
4	211349	Metekel	46	201293	W. Hararghe	88	211277	South Omo
5	241736	Sidama	47	201294	W. Hararghe	89	211279	South Omo
6	241756	Bench Maji	48	207933	Assosa	90	211290	Bench Maji
7	241757	Bench Maji	49	MWITEMA	Kenyan	91	211291	Bench Maji
8	244805	Sidama	50	E7	Kenyan	92	211299	W. Hararghe
9	211286	South Omo	51	WANJIRU	Kenyan	93	211300	W. Hararghe
10	211294	North Omo	52	211298	W. Hararghe	94	211305	W. Hararghe
11	211293	North Omo	53	211294	North Omo	95	211319	E. Hararghe
12	211301	W. Hararghe	54	211304	W. Hararghe	96	211320	E. Hararghe
13	211331	Somali	55	240190	Jimma	97	211322	E. Haraghe
14	211340	Wellega	56	211347	Metekel	98	211323	E. Haraghe
15	211341	Wellega	57	211349	Metekel	100	211327	W. Haraghe
16	211345	Metekel	58	211361	Metekel	101	211329	W. Haraghe
17	208647	Somali	59	211362	Metekel	102	211332	E. Haraghe
18	208705	Wellega	60	211379	Bale & Arsi	103	211295	E. Haraghe
19	211269	Gojam	61	211382	Shewa & Wello	104	211337	Wellega
20	211271	Wellega	62	211483	Bench Maji	105	211338	Wellega
21	211389	Shewa & Wello	63	219234	E. Hararghe	106	211339	Wellega
22	211394	South Omo	64	219235	E. Hararghe	107	211342	Wellega
23	211551	Shewa & Wello	65	AWASH 1	MA Control	108	211344	Metekel
24	211552	North Omo	66	211386	Shewa & Wello	109	211350	Metekel
25	MELKADIMA	Andean Control	67	211481	Bench Maji	110	211377	Bale &Arsi
26	CHERCHER	Standard Variety	68	241807	Gojam	111	211388	Wellega
27	GOBERASHA	Standard Variety	69	241737	Sidama	112	211546	North Omo
28	NASER	Standard Variety	70	241738	North Omo	113	211349	Metekel
29	237993	North Omo	71	241739	North Omo	114	212860	South Omo
30	240173	Jimma and Illubabor	72	241748	Sidama	115	216819	E. Hararghe
31	240512	Metekel	73	216819	E. Haraghe	116	216820	E. Hararghe
32	241730	Bale &Arsi	74	211278	South Omo	117	211337	Wellega
33	207934	Assosa	75	211292	Bench Maji	118	240522	Metekel
34	207938	Assosa	76	237078	Bale & Arsi	119	241733	Sidama
35	207949	Jimma and Illubabor	77	211348	Metekel	120	241750	North Omo
36	208638	W. Hararghe	78	211356	Metekel	121	241752	Bench Maji
37	212861	Bale &Arsi	79	211378	Bale & Arsi	122	241753	Bench Maji
38	212978	North Omo	80	211387	Shewa & Wello	123	241755	Bench Maji
39	213046	Bench Maji	81	208646	Somali	124	241814	Gojam
40	215719	Shewa & Wello	82	208695	Wellega	125	MEXICAN-142	Standard Variety
41	219233	West Hararghe	83	208698	Wellega			
42	230779	Bale &Arsi	84	208702	Wellega			

Supplementary Table 2. List of microsatellite (SSR) markers with forward/reverse nucleotide sequence, dye color, repeat motif, chromosomal location, and annealing temperature.

No.	SSR marker	Nucleotide Sequence	Dye Color	Repeat motif	Chromo-somal location	Annealing temperature
1	BM139-F BM139-R	TTAGCAATACCGCCATGAGAG ACTGTAGCTCAAACAGGGCAC	NED	(CT)25	2	55 °C
2	BM140-F BM140-R	TGCACAACACACATTTAGTGAC CCTACCAAGATTGATTTATGGG	PET	(GA)30	4	55°C
3	BM-141-F BM-141-R	TGAGGAGGAACAATGGTGGC CTCACAAACCACAACGCACC	VIC	(GA)29	9	55-58°C
4	BM143-F BM143-R	GGGAAATGAACAGAGGAAA ATGTTGGGAACTTTTAGTGTG	6- FAM	(GA)35	2	55-58°C
5	BM151-F BM151-R	CACAACAAGAAAAGACCTCCT TTATGTATTAGACCACATTACTTCC	NED	(TC)14	8	55°C
6	BM156-F BM156-R	CTTGTTCCACCTCCCATCATAGC TGCTTGCATCTCAGCCAGAATC	NED	(CT)32	10	55-58°C
7	BM165-F BM165-R	TCAAATCCCACACATGATCG TTCTTTCATTCATATTATTCCGTTCA	VIC	(TA)3(CA)9	8	52°C
8	BM172-F BM172-R	CTGTAGCTCAAACAGGGCACT GCAATACCGCCATGAGAGAT	6- FAM	(GA)23	2	50°C
9	BM183-F BM183-R	CTCAAATCTATTCACTGGTCAGC TCTTACAGCCTTGCAGACATC	NED	(TC)14	7	52°C
10	BM187-F BM187-R	TTTCTCCAACTCACTCCTTTCC TGTGTTTGTGTTCCGAATTATGA	PET	(CT)10 (CT)14	6	50-52°C
11	BM188-F BM188-R	TCGCCTTGAAACTTCTTGTATC CCCTTCCAGTTAAATCAGTCG	VIC	(CA)18 (TA)7	9	55°C
12	BM205-F BM205-R	CTAGACCAGGCAAAGCAAGC TGAGCTGGGATTTCATTTCTG	6-FAM	(GT)11	7	50°C
13	AG1-F AG1-R	CATGCAGAGGAAGCAGAGTG GAGCGTCGTCGTTTCGAT	NED	GA)8GGTA (GA)5GGGG	3	50°C
14	GATS54-F GATS54-R	GAACCTGCAAAGCAAAGAGC TCACTCTCCAACCAGATCGAA	PET	ACG (GA)5AACAGAGTC	10	56°C
15	GATS91-F GATS91-R	GAGTGCGGAAGCGAGTAGAG TCCGTGTTCCTCTGTCTGTG	VIC	(GA)8(AG)4 (GA)17	2	58°C
16	BMd53-F BMd53-R	TGCTGACCAAGGAAATTCAG GGAGGAGGCTTAAGCACAAA	6-FAM	(GTA)5	5	50°C
17	BMd36-F BMd36-R	CATAACATCGAAGCCTCACAGT ACGTGCGTACGAATACTCAGTC	NED	(TA)8	3	50°C
18	BMd42-F BMd42-R	TCATAGAAGATTTGTGGAAGCA TGAGACACGTACGAGGCTGTAT	PET	(AT)5	10	55°C
19	BMd1-F BMd1-R	CAAATCGCAACACCTCACACAA GTCGGAGCCATCATCTGTTT	VIC	(AT)9	3	54°C
20	BMd16-F BMd16-R	ATGACACCACTGGCCATACA GCACTGCGACATGAGAGAAA	6-FAM	(CATG)4	4	55°C
21	BMd18-F BMd18-R	AAAGTTGGACGCACTGTGATT TCGTGAGGTAGGAGTTTGGTG	NED	(TGAA)3	2	50-53°C
22	PV- AG001-F PV- AG001-R	CAATCCTCTCTCTCTCATTTCCAATC GACCTTGAAGTCGGTGTCGTTT	PET	(GA)1	11	50°C
23	PV- AT001-F PV- AT001-R	GGGAGGGTAGGGAAGCAGTG GCGAACCACGTTCATGAATGA	VIC	(TA)22	11	53°C
24	PV- CTOO1-F PV- CT001-R	CCAACCACATTCTTCCCTACGTC CGCAGGCAGTTATCTTTAGGAGTG	6- FAM	(CTT)3	4	56°C



Supplementary Figure 1. c Results of the Evano et al. (2005) test for ΔK between different sub-groupings of 123 common bean accessions/cultivars and two control genotypes based on analysis of allelic diversity at 17 microsatellite loci.

Supplementary Table 3. Membership coefficients and posterior probability values for K values from 1-5.

No.	Accession	Member Coefficient				Posterior Probability Values			
1	211269	0.09	0.132	0.082	0.696	(0.000,0.301)	(0.000,0.389)	(0.000,0.263)	(0.393,0.976)
2	241807	0.06	0.032	0.043	0.866	(0.000,0.206)	(0.000,0.106)	(0.000,0.145)	(0.666,0.997)
3	211266	0.853	0.017	0.066	0.064	(0.648,0.996)	(0.000,0.052)	(0.000,0.216)	(0.000,0.210)
4	211267	0.316	0.014	0.628	0.042	(0.069,0.553)	(0.000,0.046)	(0.406,0.838)	(0.000,0.145)
5	241814	0.012	0.964	0.011	0.013	(0.902,1.000)	(0.902,1.000)	(0.000,0.034)	(0.000,0.039)
6	211389	0.242	0.425	0.114	0.218	(0.000,0.606)	(0.004,0.833)	(0.000,0.331)	(0.000,0.605)
7	211551	0.565	0.301	0.107	0.027	(0.270,0.859)	(0.033,0.552)	(0.000,0.325)	(0.000,0.086)
8	215719	0.765	0.022	0.049	0.164	(0.545,0.968)	(0.000,0.068)	(0.000,0.166)	(0.001,0.341)
9	211382	0.017	0.016	0.4	0.568	(0.000,0.052)	(0.000,0.050)	(0.191,0.601)	(0.370,0.774)
10	211386	0.19	0.02	0.024	0.766	(0.000,0.422)	(0.000,0.061)	(0.000,0.080)	(0.528,0.985)
11	211387	0.252	0.016	0.498	0.234	(0.000,0.562)	(0.000,0.051)	(0.237,0.742)	(0.040,0.452)
12	ANDEAN Ctrl	0.012	0.964	0.008	0.015	(0.000,0.038)	(0.901,1.000)	(0.000,0.027)	(0.000,0.050)
13	207934	0.015	0.341	0.027	0.617	(0.000,0.047)	(0.174,0.519)	(0.000,0.089)	(0.430,0.796)
14	207938	0.035	0.053	0.745	0.167	(0.000,0.116)	(0.000,0.177)	(0.540,0.935)	(0.002,0.361)
15	207933	0.544	0.291	0.018	0.146	(0.237,0.814)	(0.001,0.603)	(0.000,0.057)	(0.000,0.387)
16	211349	0.876	0.025	0.07	0.029	(0.655,0.999)	(0.000,0.075)	(0.000,0.238)	(0.000,0.088)
17	211345	0.881	0.022	0.022	0.075	(0.673,0.999)	(0.000,0.066)	(0.000,0.070)	(0.000,0.249)
18	240512	0.879	0.057	0.035	0.029	(0.717,0.996)	(0.000,0.167)	(0.000,0.117)	(0.000,0.095)
19	211347	0.017	0.015	0.019	0.949	(0.000,0.056)	(0.000,0.046)	(0.000,0.061)	0.860,1.000)
20	211349	0.148	0.044	0.668	0.14	(0.000,0.384)	(0.000,0.150)	(0.444,0.894)	(0.000,0.374)
21	211361	0.063	0.038	0.826	0.073	(0.000,0.217)	(0.000,0.124)	(0.608,0.994)	0.000,0.245)
22	211362	0.044	0.032	0.014	0.91	(0.000,0.141)	(0.000,0.100)	(0.000,0.046)	(0.781,0.998)
23	211348	0.442	0.016	0.018	0.524	(0.057,0.736)	(0.000,0.050)	(0.000,0.055)	(0.237,0.881)
24	211356	0.063	0.012	0.046	0.879	(0.000,0.208)	(0.000,0.039)	(0.000,0.156)	(0.689,0.998)
25	211344	0.829	0.021	0.116	0.035	(0.624,0.991)	(0.000,0.067)	(0.000,0.288)	0.000,0.111)

Table 3. Contd.

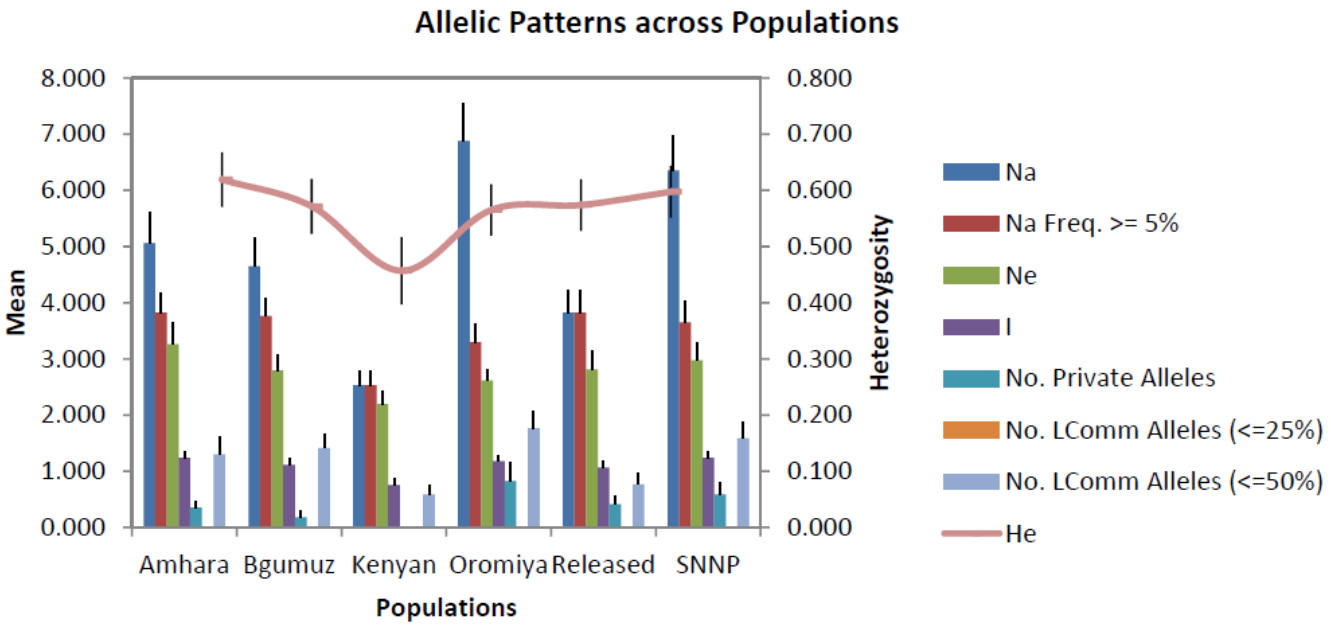
26	211350	0.448	0.074	0.135	0.343	(0.009,0.855)	(0.000,0.264)	(0.000,0.319)	(0.000,0.794)
27	211349	0.13	0.016	0.421	0.434	(0.000,0.406)	(0.000,0.050)	(0.164,0.646)	(0.234,0.634)
28	240522	0.214	0.028	0.736	0.022	(0.000,0.467)	(0.000,0.092)	(0.486,0.972)	(0.000,0.068)
29	241756	0.69	0.026	0.256	0.028	(0.406,0.967)	(0.000,0.084)	(0.001,0.524)	(0.000,0.089)
30	241757	0.697	0.041	0.194	0.069	(0.324,0.988)	(0.000,0.137)	(0.000,0.509)	(0.000,0.232)
31	213046	0.098	0.017	0.805	0.079	(0.000,0.333)	(0.000,0.054)	(0.531,0.996)	(0.000,0.266)
32	235692	0.027	0.069	0.871	0.033	(0.000,0.086)	(0.000,0.178)	(0.733,0.986)	(0.000,0.109)
33	211483	0.021	0.072	0.809	0.099	(0.000,0.067)	(0.000,0.216)	(0.636,0.965)	(0.000,0.273)
34	211481	0.023	0.018	0.012	0.947	(0.000,0.075)	(0.000,0.058)	(0.000,0.038)	(0.853,1.000)
35	211292	0.028	0.022	0.015	0.934	(0.000,0.093)	(0.000,0.073)	(0.000,0.047)	(0.822,1.000)
36	211290	0.814	0.014	0.148	0.024	(0.603,0.990)	(0.000,0.044)	(0.000,0.348	(0.000,0.074)
37	211291	0.876	0.013	0.061	0.05	(0.684,0.998)	(0.000,0.042)	(0.000,0.206)	(0.000,0.166)
38	241752	0.33	0.304	0.13	0.237	(0.000,0.802)	(0.000,0.739)	(0.000,0.372)	(0.000,0.751)
39	241753	0.923	0.032	0.012	0.032	(0.796,0.999)	(0.000,0.109)	(0.000,0.038)	(0.000,0.105)
40	241755	0.955	0.014	0.011	0.02	(0.876,1.000)	(0.000,0.046)	(0.000,0.033)	(0.000,0.063)
41	211294	0.044	0.189	0.346	0.422	(0.000,0.144)	(0.000,0.446)	(0.108,0.577	(0.130,0.719)
42	211293	0.698	0.098	0.047	0.157	(0.413,0.966)	(0.000,0.336)	(0.000,0.160)	(0.000,0.410)
43	211552	0.498	0.095	0.154	0.253	(0.223,0.751)	(0.000,0.302)	(0.000,0.392)	(0.000,0.550)
44	237993	0.655	0.148	0.129	0.068	(0.313,0.942)	(0.000,0.356)	(0.000,0.399)	(0.000,0.222)
45	212978	: 0.797	0.139	0.028	0.036	(0.514,0.995)	(0.000,0.409)	(0.000,0.093)	(0.000,0.119)
46	211294	0.027	0.018	0.026	0.929	0.000,0.090)	(0.000,0.057)	(0.000,0.086)	(0.810,0.999)
47	241738	0.013	0.011	0.742	0.234	(0.000,0.040)	(0.000,0.034)	(0.582,0.885)	(0.095,0.391)
48	241739	0.182	0.014	0.292	0.513	(0.000,0.458)	(0.000,0.044)	(0.002,0.554)	(0.299,0.733)
49	211546	0.885	0.011	0.043	0.061	(0.720,0.996)	(0.000,0.033)	(0.000,0.148)	(0.000,0.175)
50	241750	: 0.760	0.022	0.019	0.2	(0.506,0.988)	(0.000,0.069)	(0.000,0.061)	(0.000,0.449)
51	241736	0.815	0.021	0.136	0.028	(0.574,0.994)	(0.000,0.065)	(0.000,0.366)	(0.000,0.091)
52	244805	0.082	0.03	0.609	0.279	(0.000,0.283)	(0.000,0.099)	(0.196,0.957)	(0.000,0.617)
53	241737	0.016	0.026	0.014	0.943	(0.000,0.052)	(0.000,0.087)	(0.000,0.044)	(0.847,1.000)
54	241748	0.036	0.049	0.762	0.153	(0.000,0.119)	(0.000,0.131)	(0.601,0.909)	(0.012,0.308)
55	241733	0.106	0.045	0.835	0.014	(0.000,0.306)	(0.000,0.146)	(0.641,0.988)	(0.000,0.043)
56	211286	0.014	0.959	0.013	0.014	(0.000,0.044)	(0.890,1.000)	(0.000,0.042)	(0.000,0.044)
57	211394	0.165	0.208	0.43	0.197	(0.000,0.414)	(0.000,0.523)	(0.225,0.636)	(0.000,0.490)
58	211278	0.084	0.012	0.032	0.872	(0.000,0.281)	(0.000,0.038)	(0.000,0.106)	(0.657,0.999)
59	211277	0.124	0.011	0.842	0.023	(0.000,0.386)	(0.000,0.035)	(0.580,0.998)	(0.000,0.074)
60	211279	0.065	0.011	0.908	0.016	(0.000,0.223)	(0.000,0.035)	(0.734,0.999)	(0.000,0.050)
61	212860	0.923	0.032	0.025	0.02	(0.802,0.999)	(0.000,0.103)	(0.000,0.082)	(0.000,0.063)
62	MWITEMA	0.107	0.159	0.565	0.169	(0.000,0.349)	(0.000,0.416)	(0.296,0.811)	(0.000,0.439)
63	E7	0.026	0.253	0.014	0.707	(0.000,0.083)	(0.003,0.501)	(0.000,0.046)	(0.456,0.962)
64	WANJIRU	0.362	0.194	0.269	0.176	(0.009,0.701)	(0.000,0.500)	(0.000,0.598)	(0.000,0.508)
65	MA Ctrl	0.01	0.011	0.968	0.01	(0.000,0.032)	(0.000,0.036)	(0.914,1.000)	(0.000,0.031)
66	241730	0.104	0.149	0.691	0.055	(0.000,0.310)	(0.000,0.352)	(0.487,0.887)	(0.000,0.192)
67	212861	0.014	0.021	0.956	0.01	(0.000,0.042)	(0.000,0.068)	(0.882,1.000)	(0.000,0.031)
68	230779	0.271	0.677	0.021	0.031	(0.026,0.489)	(0.468,0.895)	(0.000,0.068)	(0.000,0.101)
69	235697	0.204	0.189	0.262	0.346	(0.000,0.521)	(0.001,0.399)	(0.000,0.547)	(0.006,0.692)
70	211379	0.053	0.014	0.018	0.916	(0.000,0.180)	(0.000,0.042)	(0.000,0.056)	(0.770,0.999)
71	237078	0.596	0.014	0.121	0.269	(0.337,0.828)	(0.000,0.043)	(0.000,0.368)	(0.093,0.468)
72	211378	0.067	0.016	0.064	0.854	(0.000,0.227)	(0.000,0.050)	(0.000,0.210)	(0.646,0.997)
73	211377	0.581	0.061	0.286	0.073	(0.259,0.880)	(0.000,0.191)	(0.029,0.558)	(0.000,0.234)
74	216819	0.211	0.183	0.396	0.211	(0.000,0.483)	(0.000,0.416)	(0.194,0.599)	(0.024,0.420)
75	211319	0.79	0.009	0.181	0.019	(0.574,0.984)	(0.000,0.027)	(0.000,0.397)	(0.000,0.063)
76	211320	0.877	0.059	0.049	0.015	(0.706,0.996)	(0.000,0.173)	(0.000,0.168)	(0.000,0.047)
77	211322	0.15	0.018	0.808	0.025	(0.000,0.340)	(0.000,0.059)	(0.624,0.969)	(0.000,0.082)

Table 3. Contd.

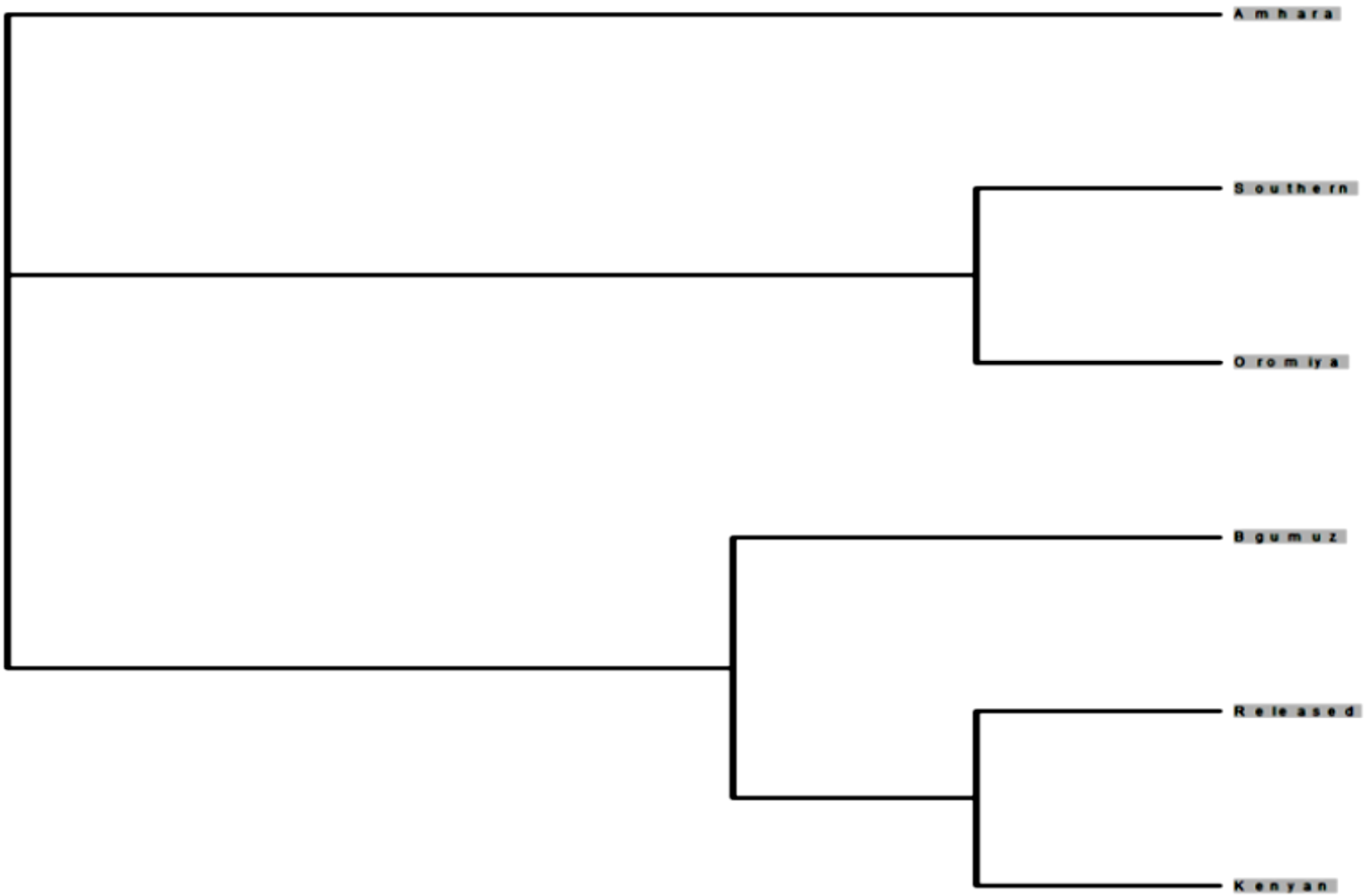
78	211323	0.342	0.054	0.584	0.02	(0.134,0.565)	(0.000,0.159)	(0.368,0.791)	(0.000,0.061)
79	211332	0.805	0.013	0.169	0.013	(0.594,0.980)	(0.000,0.042)	(0.002,0.372)	(0.000,0.041)
80	211295	0.49	0.048	0.44	0.021	(0.218,0.760)	(0.000,0.161)	(0.188,0.700)	(0.000,0.069)
81	211315	0.937	0.024	0.02	0.019	(0.828,1.000)	(0.000,0.079)	(0.000,0.061)	(0.000,0.058)
82	211317	0.032	0.354	0.568	0.046	(0.000,0.104)	(0.188,0.526)	(0.386,0.740)	(0.000,0.160)
83	211318	0.536	0.206	0.203	0.056	(0.206,0.880)	(0.000,0.447)	(0.000,0.441)	(0.000,0.196)
84	211331	0.373	0.145	0.041	0.441	(0.001,0.756)	(0.000,0.500)	(0.000,0.136)	(0.008,0.900)
85	208647	0.116	0.118	0.013	0.753	(0.000,0.333)	(0.000,0.346)	(0.000,0.039)	(0.528,0.976)
86	219234	0.016	0.016	0.909	0.059	(0.000,0.053)	(0.000,0.052)	(0.771,0.999)	(0.000,0.182)
87	219235	0.06	0.028	0.647	0.265	(0.000,0.204)	(0.000,0.086)	(0.323,0.947)	(0.000,0.584)
88	208646	0.102	0.23	0.644	0.024	(0.000,0.303)	(0.093,0.385)	(0.440,0.826)	(0.000,0.078)
89	216819	0.566	0.017	0.397	0.02	(0.340,0.787)	(0.000,0.055)	(0.182,0.618)	(0.000,0.062)
90	216820	0.703	0.028	0.245	0.023	(0.456,0.938)	(0.000,0.093)	(0.025,0.477)	(0.000,0.071)
91	240173	0.219	0.053	0.643	0.085	(0.000,0.486)	(0.000,0.188)	(0.399,0.922)	(0.000,0.273)
92	207949	0.923	0.021	0.035	0.021	(0.793,0.999)	(0.000,0.069)	(0.000,0.117)	(0.000,0.064)
93	201066	0.021	0.062	0.9	0.016	(0.000,0.069)	(0.000,0.164)	(0.778,0.995)	(0.000,0.053)
94	240190	0.057	0.017	0.423	0.504	(0.000,0.183)	0.000,0.052)	(0.228,0.620)	(0.311,0.699)
95	211340	0.62	0.231	0.064	0.085	(0.303,0.913)	(0.000,0.479)	(0.000,0.220)	(0.000,0.274)
96	211341	0.274	0.517	0.141	0.068	(0.000,0.806)	(0.029,0.900)	(0.000,0.407)	(0.000,0.215)
97	208705	0.373	0.229	0.122	0.277	(0.000,0.733)	(0.001,0.494)	(0.000,0.345)	(0.000,0.747)
98	211271	0.077	0.035	0.357	0.532	(0.000,0.250)	(0.000,0.116)	(0.176,0.543)	(0.333,0.727)
99	208695	0.282	0.012	0.684	0.022	0.000,0.591)	(0.000,0.036)	(0.380,0.977)	(0.000,0.073)
100	208698	0.607	0.01	0.324	0.06	(0.256,0.966)	(0.000,0.031)	(0.000,0.675)	(0.000,0.209)
101	208702	0.766	0.021	0.195	0.018	(0.545,0.973)	(0.000,0.069)	(0.000,0.415)	(0.000,0.056)
102	208703	0.496	0.185	0.054	0.265	(0.060,0.812)	(0.001,0.389)	(0.000,0.185)	(0.000,0.632)
105	211337	0.019	0.024	0.281	0.677	(0.000,0.061)	(0.000,0.076)	(0.067,0.492)	(0.462,0.892)
106	211338	0.378	0.026	0.567	0.03	(0.082,0.661)	(0.000,0.086)	(0.296,0.837)	(0.000,0.093)
107	211339	0.493	0.012	0.481	0.014	(0.279,0.711)	(0.000,0.037)	(0.265,0.694)	(0.000,0.044)
108	211342	0.93	0.013	0.036	0.021	(0.809,1.000)	(0.000,0.039)	(0.000,0.121)	(0.000,0.065)
109	211388	0.915	0.038	0.028	0.019	(0.788,0.998)	(0.000,0.118)	(0.000,0.093)	(0.000,0.059)
110	211337	0.383	0.086	0.482	0.05	(0.082,0.654)	(0.000,0.207)	(0.254,0.706)	(0.000,0.175)
111	211298	0.775	0.014	0.196	0.015	(0.548,0.975)	(0.000,0.044)	(0.003,0.418)	(0.000,0.047)
112	211299	0.787	0.171	0.021	0.02	(0.615,0.945)	(0.025,0.329)	(0.000,0.071)	(0.000,0.066)
113	211300	0.962	0.009	0.017	0.012	(0.895,1.000)	(0.000,0.028)	0.000,0.053)	(0.000,0.038)
114	211305	0.727	0.011	0.248	0.015	(0.506,0.943)	(0.000,0.033)	(0.033,0.465)	(0.000,0.047)
115	211325	0.931	0.02	0.016	0.032	(0.831,0.999)	(0.000,0.066)	(0.000,0.051)	(0.000,0.106)
116	211301	0.245	0.03	0.058	0.667	(0.000,0.688)	(0.000,0.099)	(0.000,0.202)	(0.235,0.985)
117	208638	0.018	0.012	0.96	0.01	(0.000,0.057)	(0.000,0.037)	(0.892,1.000)	(0.000,0.030)
118	219233	0.074	0.025	0.883	0.018	(0.000,0.213)	(0.000,0.085)	(0.732,0.995)	(0.000,0.057)
119	201293	0.875	0.019	0.083	0.023	(0.661,0.998)	(0.000,0.060)	(0.000,0.282)	(0.000,0.075)
120	201294	0.012	0.945	0.016	0.026	(0.000,0.037)	(0.854,1.000)	(0.000,0.052)	(0.000,0.087)
121	211304	0.027	0.018	0.026	0.929	(0.000,0.087)	(0.000,0.058)	(0.000,0.087)	(0.810,0.999)
122	CHERCHER	0.039	0.366	0.58	0.015	(0.000,0.128)	(0.188,0.547)	(0.388,0.766)	(0.000,0.046)
123	GOBERASHA	0.014	0.925	0.025	0.036	(0.000,0.045)	(0.805,0.999)	(0.000,0.083)	(0.000,0.125)
124	NASER	0.242	0.272	0.469	0.018	(0.008,0.473)	(0.099,0.459)	(0.272,0.658)	(0.000,0.057)
125	Mexico-142	0.418	0.237	0.311	0.035	(0.185,0.648)	(0.053,0.428)	(0.128,0.501)	(0.000,0.112)

Supplementary Table 4. Pair-wise number of migrants (Nm) based on Fst values.

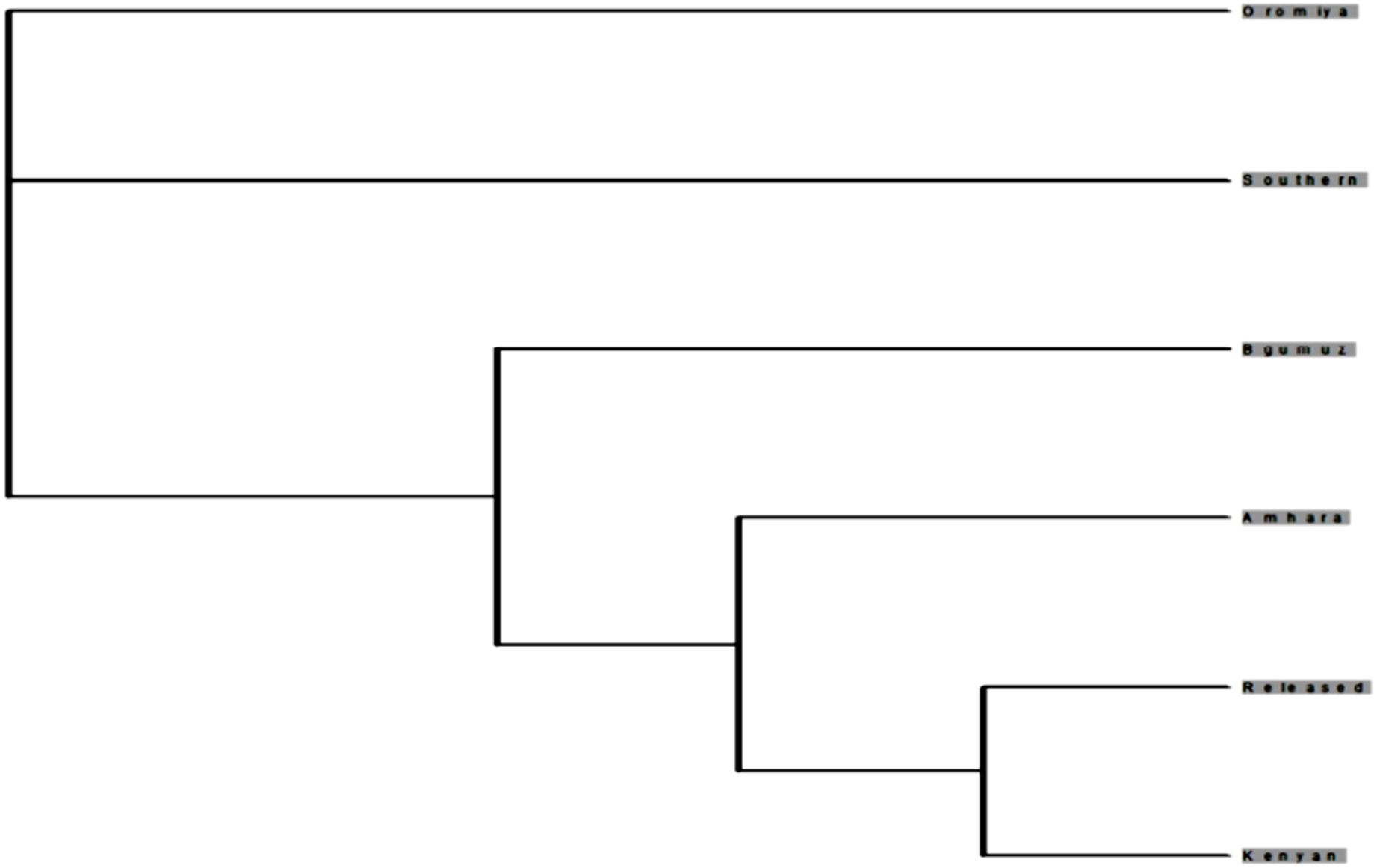
	Amhara	Bgumuz	Kenyan	Oromiya	Released	SNNP
Amhara	0.000					
Bgumuz	27.570	.000				
Kenyan	19.226	**54.601**	0.000			
Oromiya	14.973	14.168	5.207	0.000		
Released	10.537	3.838	2.738	6.193	0.000	
SNNP	0.000	**63.186**	9.461	**30.790**	6.480	0.000



Supplementary Figure 2. Patterns of allelic variation observed in the study populations along with important allelic values. Na (number of alleles), Na Freq>= 5% (number of alleles with frequencies greater than or equal to 5%, Ne (number of effective alleles), I (Shannon'sindex), number of private alleles, number of less common alleles (with frequencies less than or equal to 25% and 50%, and He (expected heterozygosity).



Supplementary Figure 3. Neighbor-joining dendrogram for the six (geographical) populations based on Nei's unbiased genetic distance (Nei,1983). Populations (from top to bottom): 'Amhara'; Southern; 'Oromiya'; 'Bensihangul-Gumuz'; 'standard' or 'released'; Kenyan.



Supplementary Figure 4. Neighbor-joining dendrogram for the six (geographical) populations based on shared-allele genetic distance values measured. Populations (from top to bottom): 'Oromiya'; Southern; 'Bensihangul-Gumuz'; 'Amhara'; 'standard' or 'released'; Kenyan.

African Journal of Biotechnology

Related Journals Published by Academic Journals

- *Biotechnology and Molecular Biology Reviews*
- *African Journal of Microbiology Research*
- *African Journal of Biochemistry Research*
- *African Journal of Environmental Science and Technology*
- *African Journal of Food Science*
- *African Journal of Plant Science*
- *Journal of Bioinformatics and Sequence Analysis*
- *International Journal of Biodiversity and Conservation*

academicJournals