

## The Performance of Five Selected Pepper Accessions in Comparison to Two Local Varieties

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Received 14th November 2016      Accepted: 19th April 2017

### Abstract

In Ghana three species of pepper (*Capsicum annum*, *C. chinense* and *C. frutescens*) are commonly found among which *C. annum* is the most widely cultivated in farms and gardens. This study sought to compare the growth and yield of 5 pepper accessions against two local cultivars. Seedlings of the accessions were grown under field conditions in a randomised complete block design with 3 replications. Data on shoot height, canopy spread, days to 50% flowering and yield parameters were recorded. Molecular analysis was also undertaken on four of the pepper accessions. Accession CH8 showed earlier flowering and fruit ripening than the others and could be important for early market. Yield of fruits per plant was significantly ( $P = 0.015$ ) highest in accession A11A followed by accession A12A. The two accessions also showed higher number of fruits per plant than the checks. A dendrogram of the molecular analysis confirmed the morphological similarity of accessions CH8 and BAG14002 which were both round and small in size and the variation of accession SBL26 which had large and wrinkled fruits.

**Keywords:** *Capsicum*, pepper, accession, fruit shape, molecular analysis

### La Performance De Cinq Accessions De Poivre Sélectionnées Par Rapport À Deux Variétés Locales

#### Résumé

Au Ghana, on trouve couramment trois espèces de poivre (*Capsicum annum*, *C. chinense* et *C. frutescens*) dont *C. annum* est le poivre le plus largement cultivé dans les fermes et dans les jardins. Cette étude a cherché à comparer la croissance et le rendement de 5 accessions de poivre sélectionnées contre deux cultivars locaux. Les semis des accessions ont été cultivés dans des conditions de champ dans un ensemble de blocs aléatoirement complet avec 3 répétitions. Les données sur la hauteur des pousses, la propagation des plantes, les jours à 50% des paramètres de floraison et de rendement ont été enregistrés. Une analyse moléculaire a également été réalisée sur quatre des accessions de poivrons. L'accession CH8 a montré une floraison précoce et une maturation des fruits que les autres et pourrait être importante pour le marché précoce. Le rendement des fruits par plante était

considérablement ( $P = 0,015$ ) le plus élevé dans l'accession A11A et a été suivi par l'accession A12A. Les deux accessions ont également montré un nombre plus élevé de fruits par plante que les contrôles. Un dendrogramme généré par l'analyse moléculaire a confirmé la similarité morphologique des accessions CH8 et BAG14002 qui étaient rondes et de petite taille et la variation de l'accession SBL26 qui avait des fruits grands et ridés.

**Mots-clés:** *Capsicum*, poivre, accession, forme des fruits, analyse moléculaire

### Introduction

It is estimated that there are more than 30 species of pepper (USDA, 2011) and these originated from South America (Eshbaugh, 1993, Olmstead *et al.*, 2008). Five of these which are domesticated are *Capsicum annuum*, *C. frutescens*, *C. chinense*, *C. baccatum* and *C. purpurium* (Andrews, 1984). *C. annuum* has been found to interbreed with *C. frutescens* and *C. chinense* and forms a complex leading to some referring to all as *C. annuum* (El Tahir, 2004). *Capsicum annuum* L. ( $2n=24$ ) belongs to the solanaceae family and it is a vegetable crop grown worldwide for spice, ornamental and source of vitamins A, C and K (Deepa *et al.*, 2007). In Ghana three species of pepper are mostly cultivated (*Capsicum annuum*, *C. chinense* and *C. frutescens*). *C. annuum* is the most commonly cultivated and can be found in farms and gardens. *C. frutescens* (birds' eye pepper) is mostly seen in farms as voluntary crop and mostly propagated by birds although there are now attempts to domesticate them and grow for export. *C. frutescens* are therefore found in the wild and are known to be very pungent and hot. *C. chinense* comes in a wide variety of shapes and sizes and can be very hot and pungent (Deepa *et al.*, 2007). The different uses of pepper have led to breeding of varieties that suit different consumer needs in terms of flavour, colour and shape (El Tahir, 2004).

The worldwide use of pepper has led to its export to various countries and Ghana is one of the countries that exports pepper to Europe

and North America. The production and marketing of pepper leads to agribusiness chain that comes with employment and wealth opportunities. Information on the yield potential of different varieties of chilli (a cultivar of *C. annuum*) and scotch bonnet (a cultivar of *C. chinense*) peppers are lacking. This study was therefore undertaken to collect, characterize and select high yielding chilli and scotch bonnet pepper varieties for cultivation by farmers to boost the non-traditional export of the country. Consequently, missions were organized to many parts of the country and collected 120 accessions of chilli and scotch bonnet pepper for conservation, characterization and utilization. After characterization of the accessions, 5 promising ones were selected for further study. In this study, the five most promising accessions were compared to two local varieties in order to ascertain their competitive growth and yield potentials.

### Materials and Methods

#### Field evaluation:

Various collecting missions were undertaken on chilli and scotch bonnet pepper and collected germplasm were grown on the field for characterisation and evaluation. Consequently, five elite accessions were selected and used for this study. Seeds of the selected accessions (A9A, A11A, A12A, CH8 and SBL26) and the local varieties (BAG14001A and BAG14002A) were sown in separate boxes. Randomised Complete block design was used for this field experiment with 7 treatments and 3 replications.

Data on the following plant characteristics were collected: shoot height, canopy diameter, days to 50% flowering, fruiting and ripening and yield.

Data on the parameters were analysed using the analysis of variance (ANOVA) test. Least significant difference (LSD) at 5% was used to separate treatment means. All statistical analyses were performed using the Genstat 14th edition.

#### Molecular analysis:

Genomic DNA was isolated from both the leaf and the fruit pulp of the four pepper accessions (SBL26, CH8, BAG14002A, A11A). Approximately 0.2g of the samples were weighed and crushed with liquid nitrogen for isolation of genomic DNA. A modified Sweet Potato DNA extraction protocol of De la Porta (Egnin *et al.*, 1998) was used to extract genomic DNA from the samples. The spectrophotometer (UV-1800-240V) was used to estimate the quantity and quality of DNA at 260 nm ( $OD_{260}$ ) and 280 nm ( $OD_{280}$ ), respectively. The DNA was resolved on 0.8% agarose gel in 1x TAE buffer stained with ethidium bromide. The DNA in the gel was visualized with an ultra violet trans-illuminator in an alpha imager.

Genomic DNA was diluted to an approximate concentration of  $50\text{ ng}\mu\text{l}^{-1}$ , prior to being used for PCR. The PCR Cocktail volume of  $10\ \mu\text{l}$  was used and the composition (final concentration) of cocktail was  $50\ \text{ng}/\mu\text{l}$  DNA,  $5.0\ \mu\text{l}$  of Thermoscientific 2x dreamtaq green mastermix (2x dreamTaq buffer, 0.4 mM each of dATP, dCTP, dGTP and dTTP and 4 mM  $\text{MgCl}_2$ ),  $10\ \mu\text{M}$  random primer, and nuclease free sterile water to top up to the  $10\ \mu\text{l}$  volume and spined down. The Thermoscientific PCR kit was used for the preparation of cocktail and 14 decamere random primers used are in Table 1. The PCR conditions used for the amplification of DNA were as follows: an

Table 1: List of Random Primers used

Random primer label	Sequence
11	GGGTTTAGGG
14	GTCGCTCAGA
15	CGAAGCTACC
17	GAATGGGAGG
20	AATCGGGCTG
22	GAAACGGGTG
23	GTGATCGCAG
24	CAATCGCCGT
27	GTGAGGCGTC
29	GAACGGACTC
31	TGGACCGGTG
2	CCTGGGCTTG
4	CCTGGGCTGG
85	GTGCTCGTGC
368	ACTTGTGCGG

initial denaturation at  $94^\circ\text{C}$  for four min, 35 cycles of denaturation at  $94^\circ\text{C}$  for 30 sec, annealing at  $45^\circ\text{C}$  for one minute, extension at  $72^\circ\text{C}$  for one minute, and final extension for 10 mins at  $72^\circ\text{C}$  holding at  $4^\circ\text{C}$  was included. PCR product was loaded on 1.5% agarose in 1x TBE buffer and ethidium bromide stain (0.003% vol/vol) and electrophoresis was conducted in a multi Sub Maxi Gel Electrophoresis system at 120 volts for 45 minutes. Following electrophoresis, gel was visualised by means of a MiniBis Gelcapture system.

#### Data Analysis

The Random primers were subjected to primer test and primers that were generating

Table 2. Jaccard, similarity matrix generated based on analysis with 11 Random Primers

Unit	SBL26	CH8	BAG	AIIA
SBL 26	1.00			
CH8	0.32	1.00		
BAG14002	0.36	0.55	1.00	
AIIA	0.26	0.28	0.41	1.00

reproducible amplified bands were used for analysis. All the primers tested were used as they produced reproducible bands. Gels were scored and data entered as present or absent alleles. Bands were sized by means of an axygen 100 bp ladder. Genetic distances and clusters analysis were conducted using GENSTAT 14th edition statistical package. Similarity matrices and dendograms were generated for all the three analysis conducted.

**Results**

*Field Evaluation*

There was no significant difference between the five selected pepper accessions and the local cultivars ( $P = 0.471$ ) in the number of days to 50% flowering. However, accession CH8 showed the earliest number of days to 50% flowering and fruit ripening with a range from 111 in CH8 to 119 days in SBL26 and the two local varieties (Fig. 1)

Figure 2 shows that the total number of fruits harvested was highest in accession A11A followed by accession A12A and then accession CH8. The number of fruits of accessions A11A and A12A were significantly ( $P = 0.023$ ) higher than one of the local accessions (BAG14002A).

The number of fruits per plant was similarly higher in accessions A11A and A12A than the other accessions. They were not however significantly ( $P = 0.087$ ) different from the

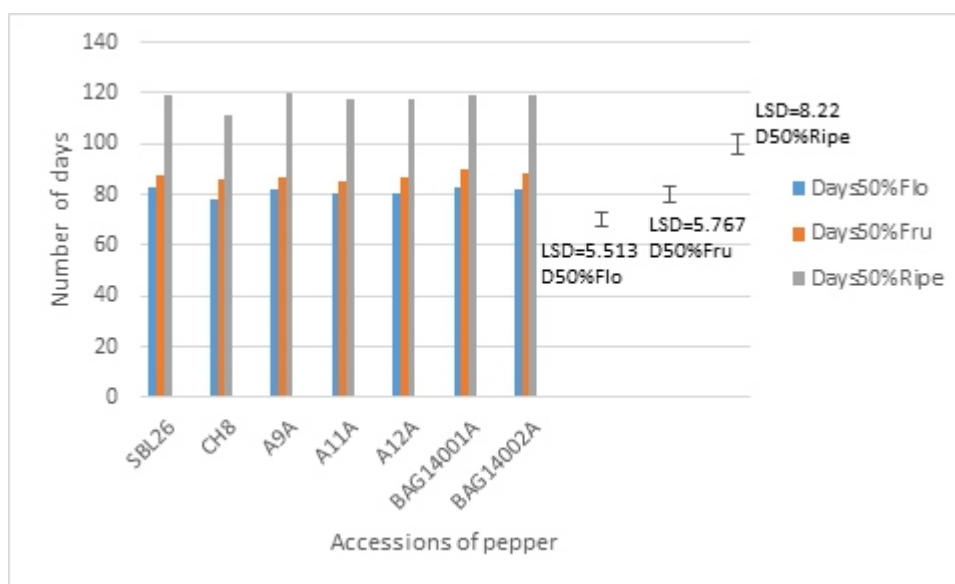


Fig. 1 Number of days to 50% flowering stage of pepper accessions

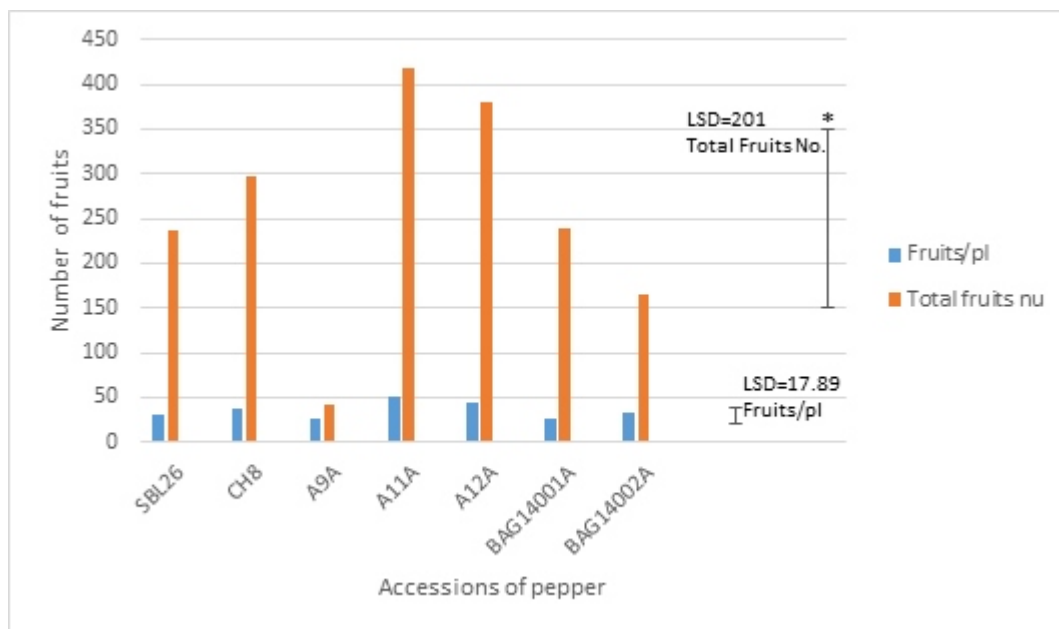


Fig. 2 Total number of harvested fruits and fruits per plant of pepper accessions.

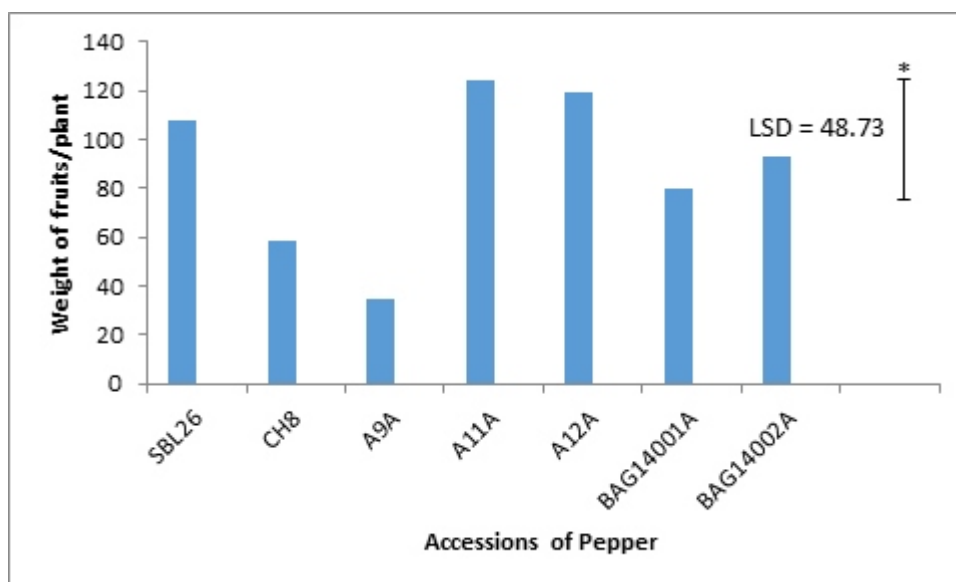


Fig. 3 Weight of fruits per plant of Pepper accessions

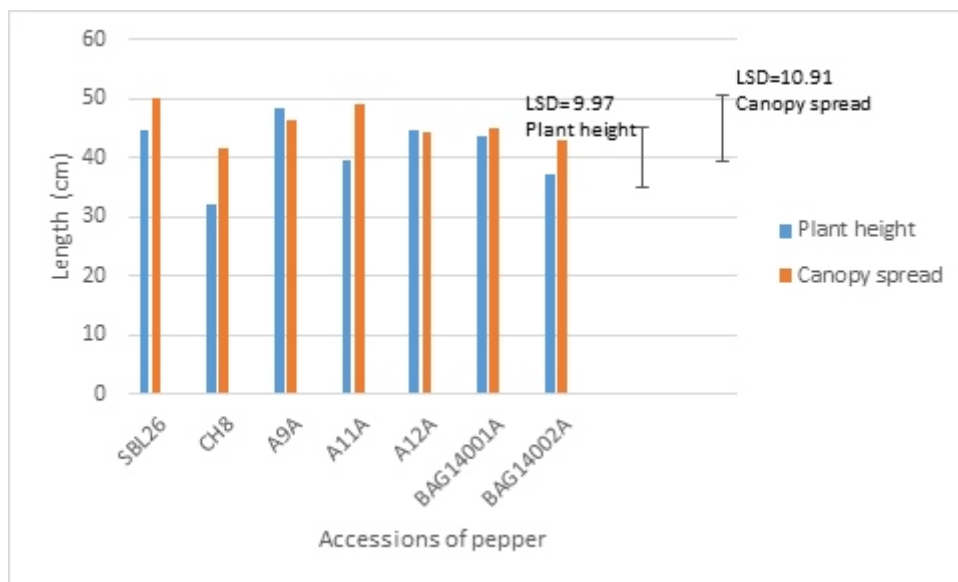


Fig. 4 Plant height and canopy spread of pepper plants

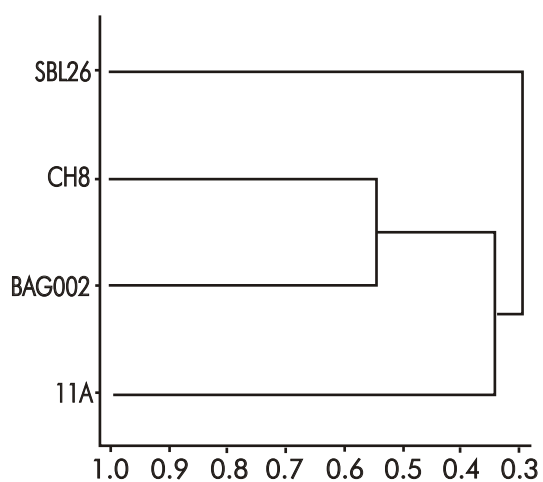


Figure 5. Dendrogram of relationship of 4 pepper genotypes

rest of the selected accessions and the two local varieties (Controls).

Figure 3 shows that the yield of the plants in the various accessions in terms of weight of fruits in grammes was highest in A12A (119.7) followed by accession SBL26 (107.4) and lowest in accession A9A (58.8). There were significant differences between the means of the accessions ( $P=0.015$ ).

Plant height was highest in accession A9A followed by Accession SBL26. Accession CH8 had the lowest plant height (32 cm). The height of accession A9A was significantly ( $P=0.05$ ) higher than accession CH8 and BAG14002A, a local variety. Canopy spread was widest in SBL26 (49.9 cm) followed by accession A11A (49.1 cm). These were not significantly different from the two local varieties (Fig. 4).

The dendrogram generated (Fig. 5) linked the three accessions and one local line on a scale of 1 to 0.3. Two major clusters were generated where

SBL26 was separated from the remaining three which were clustered together. The local variety BAG14002A was closely related to the accession CH8. The accession A11A was in a separate cluster and SLB26 and A11A were the most distantly related.

### Discussion

*Capsicum annum*, *C. frutescens* and *C. chinense* originated from South America and introduced to West Africa by the Europeans. *C. chinense* was introduced to West Africa at a later period (El Tahir, 2004). The accessions of pepper in Ghana probably developed over a period of time and also through cross-pollination. Accession CH8 differed from the other accessions in that it showed earlier number of days to 50% flowering and fruit ripening. Such traits show the variety could be in early market and as well be useful when selecting varieties for early maturity to avoid drought in the face of climate change.

The highest number of fruits was recorded in accession A11A followed by accession A12A. These pepper accessions surpassed the local cultivars, BAG14001A and BAG14002A in yield (Fig.2). Accessions A11A in particular showed higher yield per plant than the checks, BAG14001A, BAG14002A and significantly ( $P = 0.015$ ) higher yield than one of them (BAG14001A). Accessions A11A and A12A showed the highest values of most of the yield parameters studied namely total number of fruits produced, number of fruits per plant and weight of fruits. These accessions with high yields have the potential for use by plant breeders, seed producers and growers to improve upon the crop and increase productivity. The high canopy spread of these accessions, especially accession A11A would lead to increased yield as more branches and leaves would increase sunlight interception leading to more photosynthetic carbon assimilation.

Plant height was highest in accession A9A and was significantly ( $P = 0.054$ ) higher than accessions BAG14002A and CH8. Accession A9A is the bird's eye chilli pepper which grows in the wild and have to compete with other weeds for sunlight. It has therefore evolved to be higher than the cultivated varieties. The wild pepper accession has evolved in the wild where the species needed little or no pesticide application. Such accessions produce safer and healthier fruits.

Canopy spread was widest in SBL26 followed by A11A. Wide branching increases the interception of light by the leaves and result in increased photosynthesis and therefore higher yield (Figures 3 and 4). Canopy spread correlated positively with yield.

The 3 morphologically similar accessions (CH8, A11A and BAG14002A) and the SBL26 which were subjected to molecular differentiation were all hot aromatic peppers and were therefore cultivars of *Capsicum chinense*. Accessions CH8, A11A and BAG14002A were small in size and were hot spicy peppers and shared common characteristics of shape, fruit width and colour. Accession SBL26 also of *C. chinense* had large and wrinkled pepper fruit. The size (fruit length and fruit width) and the wrinkled fruits of SBL26 made it different and quite distant from the other 3 aromatic peppers. This agrees with a previous study by Deepa *et al.* (2007) who noted that *C. chinense* has a large variation in the colour and size of fruits. Fruit diameter and fruit length featured highly in distinguishing between the fruits of the accessions and were prominent characters used to differentiate 11 landraces in a study in Tunisia (Lahbib *et al.*, 2013). The differentiation of the dendrogram from the molecular analysis clusters of accessions therefore confirmed the morphological characterisation. Accessions CH8 and BAG14002A looked similar in terms of shape and size and

it is therefore not surprising that CH8 is closely related to BAG14002A on the dendrogram. Accession A11A had longer fruits than the other two accessions. The molecular analysis also showed the phenotypic variation of accession SBL26 and A11A from the other two accessions. These variations could be useful in genetic diversity studies for the improvement of the crop.

This study showed that out of the selected 5 pepper accessions, two accessions (A11A and A12A) gave higher yields than the 2 local varieties. These therefore have the potential for use by farmers to increase productivity and maximise profit. These could as well be used by plant breeders to improve upon the yield of the pepper varieties. The study also showed the potential of accession A9A which is a wild pepper variety. Wild crop relatives could harbour genes which could be tolerant to drought and resistant to some pests and diseases. These attributes of the accession could be harnessed for crop improvement. The dendrogram generated from the molecular analysis confirmed the similarity in the morphological characterization of accessions CH8 and BAG14002A and morphological variation of accession SBL26 which had wrinkled and larger fruit size and these could be used to generate diversity for pepper improvement.

#### Acknowledgements

This study is part of the CSIR-PGRRI/Export Development and Agricultural Investment Fund (EDAIF) project on Pepper which was started in 2006 to select Chilli pepper and Scotch bonnet pepper to boost the non-traditional export. The funds provided by EDAIF for this study is gratefully acknowledged. The Authors wish to express sincere thanks to Mr. Abednedgo Owusu Mensah for his assistance in field data collection.

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