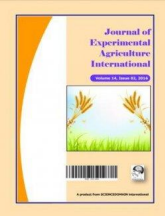

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AMMI Determination of Stability for Oil and Protein Content in Soybean (*Glycine max* L. Merrill) Seed in Zambia

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The aim of the study was determine the yield and stability of oil and protein content of different genotypes in different locations in Zambia. The specific objectives were to characterize the test soybean genotypes for oil and protein content across selected environments and to understand the environments in Zambia with respect to Soybean quality stability.
Study Design: A Randomised Complete Block Design with four (4) replications at each location was used to carry out the experiment. Each plot had 4 rows of 6 m long each.
Place and Duration of Study: A multi- environment trial was carried out in the 2013/2014 agricultural season in five locations (Golden Valley Agricultural Research Trust (GART), Kabwe, Msekera, Misamfu and Masumba Research stations) spread in the three (3) agro -ecological regions of Zambia.
Methodology: As this study focused on seed variables, protein and oil, seed was collected at harvest and was dried at a moisture content of 13.5%. The field trial had four replications, however for the current study; only two replications were used for analysis due to the inhibiting cost of

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determining the oil and protein content. Samples of 35 g were collected and analyzed for chemical composition. Protein and oil concentration was determined by Department of Crop Sciences at University of Illinois using a Perten DA7200 Diode Array Near Infrared Reflectance (NIR) analyzer with built in calibration.

Results: Msekera had the highest location mean (18.98%) with regards to oil content among the five locations and GART had the lowest location mean (16.38%). The genotypes were equally significantly different for oil content with Lukanga having the highest across location with an average oil content of 19.47% and TGX 1830-20E as the lowest oil containing genotype with 16.73%. As regards to the Protein content, GART had the highest location mean (38.23%) across all five locations and Misamfu had the lowest location mean (33.47%) Significant differences among genotypes were evident when all fifteen genotypes were considered across the five locations with TGX 1830–20E having the highest genotype mean (37.57%) across locations and Lukanga having the lowest mean (33.1%) for protein content across locations. The genotype G11 (TGX 1989-60F) exhibited the best stability with regards to oil content and the most unstable was G15 (Lukanga).

Conclusion: The study was able to establish the performance of the genotypes across the locations and understand the locations with respect to oil and protein content.

Keywords: AMMI; genotype by environment; oil; protein; Zambia.

1. INTRODUCTION

Soybean (*Glycine max* (L) Merrill) is an important legume with multifarious uses and its cost effectiveness is ensured through biological nitrogen fixation and rotation with exhaustive crops since it replenishes and maintains soil fertility [1]. The crop has a wide adaptation being cultivated in tropical, subtropical, and temperate climates. Soybean seeds are composed of approximately 20% oil, 40% protein, 30% carbohydrate, 9% crude fiber, and 5% ash [2]. It is known to have the highest protein content among all food crops and is second only to groundnut in terms of oil content among food legumes [3].

Soybean since its introduction in Zambia remained for a long time being grown mostly by commercial farmers. However, due to its industrial properties and nutritional benefits, the crop has gained popularity and is now grown by both small and large scale farmers. Soybean is well adapted to regions II and III of Zambia and grows well wherever maize grows [4]. The main driver for soybean production has been increased demand in edible oils consumption and the growth of the Poultry industry in the country and the region [5]. Hence the importance of the nutritional quality cannot be overemphasized in soybean production. The current study was therefore done to; (1) characterize the test soybean genotypes for oil and protein content across selected environments in Zambia and to (2) understand the environments in Zambia with respect to soybean protein and oil stability.

Quality of plant extracts like oil though mostly studied in crops has been done in other plants as seen among forest trees studies by Bilir and Avci [6] and Avci and Bilir [7]. Among the important findings in the study was the strong effect of the environment on the oil content of the populations as compared to the genotypic effect [7]. This, therefore, necessitates a careful understanding of the genotype responses in different environments to have good results in line with the nutritional breeding objectives. Henceforth, during selection of soybean for a particular seed breeding program or food application, it is important to know the major factors affecting soybean quality such as the protein and oil contents [8]. Several researchers have also reported that the oil and protein content of soybean seed apart from the genotypic effect varies with environmental conditions encountered during the growing period, particularly temperature [9,10] and rain [11].

Other than the effect of the genes and the environment, genotype by environment interactions are known to have effects on biochemical and physical characteristics of soybean seed [12] and these have been reported to affect soybean protein and oil content [13]. Genotype by environment interactions represents differential responses of genotypes and renders mean performance less useful as genotypes' relative ranking or degree of magnitudes, vary across the environments (Allard and Bradshaw, 1964 [14].

Various methods have been proposed for analysis of adaptability and stability of genotypes

tested in multiple environments. Additive Main effects and Multiplicative Interaction (AMMI) is one of the methods that have been used in studies of G x E interaction in soybean [15]. AMMI is a useful tool in the analysis of G X E data because of its superiority to other methods in splitting the G from the GE [16]. This property of AMMI has been reported to be able to exploit broad and specific adaptations hence being able to practically help in recommendations aimed at increasing yield [17].

Henceforth, the study was done using AMMI to determine the yield and stability of oil and protein content of different genotypes in different locations in Zambia.

2. MATERIAL AND METHODS

Zambia is located on the African subcontinent between latitude 8-18° S and longitudes 22-33° E and covering an area of 752,620 km^2, which is 2.5% of the African continent [4]. It is a country with three agro-ecological zones which are characterized by differences in climatic conditions most important of which is the amount of rainfall received annually [4]. The other climatic parameters which are notable in these agro-ecological regions are temperature, soil characteristics and the vegetation type.

Region I comprise the valley areas of the country and lie between 300 and 900 m above sea level. The annual rainfall received in this area is low, not exceeding 800 mm with relatively high mean temperatures of 38°C received in October. Region 2 is the most agricultural active region receiving between 800 mm to 1000 mm of annual rainfall. The elevation of this region is between 900 and 1300 meters above sea level. The mean daily temperatures during the growing season range between 23-25°C. Most of the national soybean production in Zambia is done in region II. The last region is region III at an elevation ranging between 1100-1700 meters above sea level and receives above 1000 mm of rainfall per year. The average monthly temperature in the growing season is 16°C. This region has a soil acidity set back in agricultural production. Table 1 shows the soil characteristics of the three agro-ecological regions of Zambia and their limitations to crop production.

2.1 Experimental Sites

The multi-environment trials were carried out in the 2013/2014 agricultural season at five locations found in the three agro ecological regions and the locations are described in Table 2.

Table 1. Soils in the agro-ecological regions and their limitations to crop production

Region	General description of soils	Limitations
Region I	Loamy and clay with course to fine tops	Slightly acidic to alkaline. Minor fertility limitations
	Reddish course sandy soils	Low pH, available water and nutrient capacity reserve
	Poorly drained sandy soils	Severe wetness, acidic and low fertility
	Shallow and gravel soils in rolling to hilly areas	Not suitable for cultivation
Region II	Moderately leached clayey to loamy soils	Low nutrient and water holding capacity
	Slightly leached soils	Slight to moderate acidity. Heavy textured soils
	Course sandy loams in large dambos	Imperfectly to poorly drained. Limitations due to wetness
	Sandy soils on Kalahari sand	Medium to strong acidity, course textured topsoil, low water holding capacity and nutrient capacity.
Region III	Red brown clayey loamy soils	Very strong acidity and highly leached
	Shallow and gravel soils	Limited depth
	Clayey soil, red in color	Fewer limitation but moderately leached
	Poorly to very poorly drained floodplain soils	Variable texture and acidity
	Course sandy soils in pan dambos on Kalahari sand	Very strong acidity

Source: Compiled from Bunyolo. A. Chirwa. B and Muchinda M. Agro ecological and Climatic conditions in Muliokela. S (ed), 1997: Zambia Technology handbook, Ministry of Agriculture Food and Fisheries, Lusaka

Table 2. Experiment sites description

Location name	Coordinates	Agroecological region	Altitude (M)	Assigned Code
Kabwe research station	14.39 S, 28.49 E	II	1176	E1
Golden valley agriculture research trust (GART)	14. 50 S, 28.10 E	II	1139	E2
Misekera research Station	13.38 S, 32.34 E	II	1032	E3
Masumba	13.22 S, 31.93 E	I	546	E4
Misamfu research station	10.17^{o}S, 31.22° E	III	1536	E5

Table 3. Soil analysis results for the four (4) trial locations

Location	pH	N	Organic matter	P	K	Na	Ca	Mg	Cu	Fe	Mn	Zn	S	Sand	Clay	Silt	Class
			%	mg/kg	cmol/kg		cmol/kg		mg/kg		mg/kg		mg/kg	%	%	%	
Kabwe	5.52	0.063	0.56	15.21	0.17	0.05	1.83	0.57	0.14	6.44	6.43	0.58	14.79	80	6	14	Loamy sand
GART	5.95	0.07	1.92	7.56	0.66	0.08	6.50	2.47	3.24	3.38	6.26	0.92	17.75	64	16	20	Sandy loam
Msekera	5.63	0.08	2.40	12.27	0.90	0.10	10.00	2.25	0.64	9.46	8.03	0.74	13.81	70	10	20	Sandy loam
Masumba	5.52	0.07	3.52	1.99	0.43	0.06	6.83	1.51	0.97	6.92	9.61	0.55	12.82	64	12	24	Sandy loam
Misamfu	4.62	0.22	1.68	11.58	0.16	0.06	0.82	0.36	0.05	10.2	3.34	0.3	23.18	82	8	10	Loamy sand

Composite soil samples were collected at the 5 locations to a depth of 30 cm and soil analysis was done at the University of Zambia soil science laboratories. The soil analysis results are indicated in Table 3 and show that the locations had relatively similar soil texture of sandy loam in three locations namely Masumba, Msekera and Golden Valley Research Trust (GART) while two locations Kabwe and Misamfu had loamy sands. The pH range for the locations was between 4.62 and 5.95. The locations varied on NPK and the trace elements.

Climatic conditions namely rainfall and temperature were recorded and aggregated by month. The data for four locations; Masumba, Kabwe, Misamfu and Msekera was obtained from the Zambia Meteorology Department, while the data for Golden Valley Agriculture Research Trust was obtained from the research station. The recorded data is tabulated in Table 4. The highest amount of rainfall was received at Misamfu (1, 348.4 mm) followed by Msekera (1097.7 mm). The other locations received 642.8 mm (Masumba), 601.2 mm (GART) and 583.3 mm (Kabwe). The mean temperatures for the locations were 32.88°C (Masumba), 29.5°C (Msekera), 23.12°C (kabwe), 24.24°C (GART) and 21.66°C (Misamfu).

2.2 Experimental Design

The experimental material consisted of 15 genotypes of soybean (Table 5). There were twelve promiscuous lines obtained from the International Institute of Tropical Agriculture, two lines from Zambia Agricultural Research Institute (ZARI) and one from SeedCo.

Table 4. Monthly meteorological data of sites used in the study during the 2013/2014 rainy season

Location		Month				
		Dec	Jan	Feb	Mar	Apr
Masumba	Rainfall (mm)	106.9	246.3	214.1	75.5	0
	Mean Temp (°C)	35.6	31.8	31.8	33	32.2
Msekera	Rainfall (mm)	143.1	306.5	407.8	216.8	23.5
	Mean Temp (°C)	31.6	28.5	28.5	30.1	28.8
Kabwe	Rainfall (mm)	191.7	204.2	97	88.4	2
	Mean Temp (°C)	24.9	23.5	22.95	22.95	21.3
GART	Rainfall (mm)	307.6	69.2	99.4	65.1	60.2
	Mean Temp (°C)	25.2	25.1	24.4	24.1	22.4
Misamfu	Rainfall (mm)	315.9	234.4	464.1	256.3	77.7
	Mean Temp (°C)	21.9	21.75	21.5	21.95	21.2

Table 5. List of genotypes used in the trial and their assigned codes

Genotype	Genotype assigned code	Source
TGX 1740-2F	G 1	IITA
TGX 1830-20E	G 2	IITA
TGX 1835-10E	G 3	IITA
TGX 1887-65F	G 4	IITA
TGX 1904-6F	G 5	IITA
TGX 1987-11F	G 6	IITA
TGX 1987-23F	G 7	IITA
TGX 1988-9F	G 8	IITA
TGX 1988-18F	G 9	IITA
TGX 1988-22F	G 10	IITA
TGX 1989-60F	G 11	IITA
TGX 1990-129F	G 12	IITA
Magoye	G 13	ZARI
Safari	G 14	SeedCo
Lukanga	G 15	ZARI

The IITA lines were obtained from a pool recommended for Zambian trials under the USAID-funded feed the future project

2.3 Data Collection and Statistical Analysis

As this study focused on seed variables, protein and oil, seed was collected at harvest and was dried at a moisture content of 13.5%. The field trial had four replications, however for the current study; only two replications were used for analysis due to the inhibiting cost of determining the oil and protein content. Samples of 35 g were collected and analyzed for chemical composition. Protein and oil concentration was determined by the Department of Crop Sciences at University of Illinois using a Perten DA7200 Diode Array Near Infrared Reflectance (NIR) analyzer with built in calibration.

GENSTAT Statistical package version 16 was used for the analysis of variance (ANOVA) for each of the measured and derived parameters as well as for the Additive Main Effect Multiplicative Interaction (AMMI) Model [18].

3. RESULTS

3.1 Analysis of Variance

Combined analysis of variance for soybean oil content revealed highly significant differences (P≤ 0.01) among locations and among genotypes (Table 6). The results also showed significant genotype by location interactions for oil content. The combined analysis of variance with regards to protein content showed highly significant differences (P≤ 0.001) among Locations and Genotypes. Significant Genotype by Location interactions were also observed (Table 6).

Further analysis of the results with respect to oil content (Table 7) showed Msekera to have had the highest location mean (18.98%) among the five locations and GART had the lowest location mean (16.38%). Similarly, the genotypes were significantly different for oil content with Lukanga(a check) having the highest across location with an average oil content of (19.47%) and TGX 1830-20E as the lowest oil containing genotype with 16.73%.The check entries had a higher overall mean oil content (18.92%) than the IITA genotypes (17.87%). Results also revealed that all the three checks used in the study had oil content above the grand mean for all the genotypes (18.03%) with Safari and Magoye ranking second and sixth respectively among all the genotypes tested at all five locations. It was also observed that genotypes changed in both, oil content magnitudes, and ranking. The non-consistent performance of the genotypes manifested interactions with environments. For instance, the best genotype at Masumba (TGX 1988-9F) was fourth at Misamfu, tenth at Kabwe, thirteenth at GART and eleventh at Msekera.

With regards to protein content, GART had the highest location mean (38.23%) across all five locations and Misamfu had the lowest location mean (33.47%) (Table 8). Similarly, significant differences among genotypes were evident when all fifteen genotypes were considered across the five locations with TGX 1830–20E a promiscuous (self nodulating) genotype having the highest genotype mean (37.57%) across locations and Lukanga (non- promiscuous) having the lowest genotype mean (33.1%) for protein content across locations. The results in Table 8 further showed that there was high interaction between genotypes and locations as the genotypes performance and ranking varied from one location to another. For instance, the best genotype at Kabwe, TGX 1987-23F was third at GART and Msekera while being fourth and thirteenth at Masumba and Misamfu, respectively. Similarly, genotype TGX 1830-20E which was the second best performing genotype at Kabwe, fluctuated to fifth and first ranks at GART and Msekera, respectively. On the other hand Safari showed similar fluctuations though generally having lower protein content.

Table 6. Combined analysis of variance across five locations for soybean oil and protein content

		(%)	
Source of variation	**d.f.**	**m.s (Oil)**	**m.s.(Protein)**
Location	4	32.35**	107.30**
Reps (L)	5	0.87	2.07
Genotype	14	5.08**	15.94**
Location*Genotype	56	1.10**	3.48*
Residual	65	0.31	2.11
Total	144		

**, ** Significantly different at p≤0.05 and p≤ 0.001 levels respectively, d.f. = degree of freedom, m.s. = Mean Square*

Table 7. Genotype mean oil content (%) within and across locations

Genotype	Kabwe	GART	MsekeraOi%	Masumba	Misamfu	Overall genotype means
TGX 1740-2F	18.44	17.03	19.31	17.79	19.19	18.35
TGX 1830-20E	15.75	15.28	17.04	16.61	18.96	16.73
TGX 1835-10E	17.59	15.17	17.8	17.21	18.48	17.25
TGX 1887-65F	17.38	16.09	18.71	17.43	19.09	17.74
TGX 1904-6F	18.23	16.21	19.64	18.18	18.18	18.09
TGX 1987-11F	18.27	16.25	19.68	18.13	19.64	18.39
TGX 1987-23F	15.54	15.05	17.65	17.49	19.7	17.09
TGX 1988-9F	18.2	15.2	18.46	19.58	19.21	18.13
TGX 1988-18F	18.21	16.58	18.8	18.73	18.36	18.13
TGX 1988-22F	19.24	17.12	19.87	18.75	18.19	18.63
TGX 1989-60F	18.21	16.57	19.27	18.29	19.01	18.27
TGX 1990-129F	18.12	15.67	18.07	17.59	18.67	17.62
Magoye (check)	18.65	16	19.53	17.97	19.5	18.33
Safari (check)	19.03	18.24	19.79	18.98	18.71	18.95
Lukanga (check)	19.6	19.3	21.09	19.3	18.09	19.47
Location means	**18.03**	**16.38**	**18.98**	**18.13**	**18.86**	**18.08**
Min	15.54	15.05	17.04	16.61	18.09	16.73
Max	19.6	19.3	21.09	19.58	19.7	19.47
Mean of IITA entries	17.77	16.02	18.69	17.98	18.89	17.87
Mean of checks	19.09	17.85	20.14	18.75	18.77	18.92
LSD 5%	0.5481	0.7673	0.4277	0.8306	1.451	
CV	2	3.1	1.5	3	4.9	

Table 8. Genotype mean protein content (%) within and across locations

Genotype	Kabwe	GART	Msekera	Masumba	Misamfu	Overall genotype means
TGX 1740-2F	35.36	38.23	34.07	37.19	33.11	35.59
TGX 1830-20E	38.87	39.02	37.02	39.15	33.8	37.57
TGX 1835-10E	35.39	37.77	33.52	36.27	29.12	34.41
TGX 1887-65F	37.86	39.03	36.05	38.1	35.39	37.28
TGX 1904-6F	35.9	38.62	32.44	36.05	34.17	35.44
TGX 1987-11F	36.78	39.34	32.67	38.25	31.95	35.8
TGX 1987-23F	39.29	39.23	36.56	37.9	31.91	36.98
TGX 1988-9F	38.31	40.12	36.34	35.43	35.1	37.06
TGX 1988-18F	35.63	37.68	34.39	36.42	33.32	35.49
TGX1988-22F	36.61	38.33	35.95	39.16	35.02	37.01
TGX 1989-60F	36.39	39.03	34.22	37.05	32.87	35.91
TGX 1990-129F	37.64	38.88	36.84	37.15	34.59	37.02
Magoye (check)	33.89	37.66	33.98	36.06	33.73	35.06
Safari (check)	34.66	36.88	34.6	35.22	32.38	34.75
Lukanga (check)	32.56	33.55	29.61	34.1	35.65	33.1
Location means	**36.34**	**38.23**	**34.55**	**36.9**	**33.47**	**35.90**
Min	32.56	33.55	29.61	34.1	29.12	33.1
Max	39.29	40.12	37.02	39.16	35.65	37.57
Mean of IITA genotypes	**37.00**	**38.77**	**35.01**	**37.34**	**33.36**	**36.30**
Mean of checks	**33.70**	***36.03***	***32.73***	**35.13**	**33.92**	**34.30**
LSD 5%	1.0504	0.4375	1.359	1.756	4.702	
CV	1.9	0.8	2.6	3.1	9	

3.2 Stability Analysis for Oil and Protein Content in Soybean

3.2.1 AMMI model and pattern analysis for soybean oil content

The AMMI analysis of variance for soybean oil content of the fifteen genotypes tested in five environments showed highly significant differences (P<0.001) among genotype main effects, environment main effects and genotype x environment interaction (Table 9). The model revealed that the differences between the environments accounted for 49.41% of the variation while the genotypes and the G x E interaction accounted for 27.15% and 23.44% of the variation respectively. The IPCA1 and IPCA 2 parameters were significant at P> 0.001 and P> 0.05 respectively. The IPCA 1 accounted for 65.88% of the GE interaction sum of squares while IPCA 2 accounted for 18.88% of the variability. The two IPCA parameters explain 84.76% of the G x E sum of squares while the remaining 15.24% would be residual.

The AMMI 1 biplot for soybean oil content Fig. 1 showed that the variability due to environments was higher than that due to genotypes as the points for environments were more scattered in the biplot than the points for genotypes. One check G15 (Lukanga) and one environment E5 (Misamfu) were dispersed away from the axes of the biplot showing high instability. The IITA genotypes G11 (TGX 1989-60F), G1 (TGX 1740-2F), G12 (TGX 1990-129F) and the check G13 (Magoye) with IPCA1 scores close to zero and oil content close to the mean showed stability and general adaptability with negligible interaction. Genotype G11 (TGX 1989-60F) was the closest to the centre of the biplot exhibiting general adaptability and the best stability.

According to the AMMI 1 biplot in Fig. 1, the ideal genotypes that were stable with high oil content above the mean were G13 (Magoye) a check and the IITA genotypes G6 (TGX 1987-11F) and G8 (TGX 1988-9F)in quadrant II. The environments were spread around the biplot with the high soybean oil yielding environments in quadrant II and III and the lower soybean oil yielding environments in quadrants IV. The high oil potential environments falling on the right hand side of the midpoint of the main effect axis were E4 (Masumba) in quadrant II and E3 (Msekera) in quadrant III while environment E2 (GART) was a low oil yielding environment as it lay in quadrant IV.

A further analysis to determine the top four (4) performing genotypes with regards to oil content across the locations was done and the results are shown in Table 10. The results showed two checks (Lukanga and Safari) and two IITA genotypes (TGX 1988-18F and TGX 1988-9F) were among the first four performing genotypes at four of the five locations tested. The check G15 (Lukanga) was the best performing genotype at three of the five environments used in the study namely E1 (Kabwe), E2 (GART) and E3 (Msekera) while G8 (TGX1988-9F) and G7 (TGX 1987-23F) were the best performing genotypes at locations E4 (Masumba) and E5 (Misamfu) respectively.

The AMMI analysis of variance for soybean protein content of the 15 genotypes tested in five environments (Table 11) showed that soybean protein content was significantly affected by environments, genotypes (P> 0.001) and genotype x environment interaction (P> 0.05). The results further showed that 50.67% of the treatment Sum of Squares (SS) was attributable to environmental effects, 26.35% to genotypic

Table 9. ANOVA for the AMMI analysis of soybean oil content across five locations/environments

Source	Df	SS	MS	F	F_prob	Explained %
Treatments	74	261.84	3.538	11.51	0.00000**	
Genotypes	14	71.08	5.077	16.52	0.00000**	27.15
Environments	4	129.38	32.345	37.25	0.00000**	49.41
Block	5	4.34	0.868	2.82	0.02276*	
Interactions	56	61.38	1.096	3.57	0.00000**	23.44
IPCA1	17	40.44	2.379	7.74	0.00000**	65.88
IPCA2	15	11.59	0.773	2.51	0.00544*	18.88
Residuals	24	9.35	0.39	1.27	0.22331	
Error	65	19.98	0.307			
Total	149	286.16	1.921			

**, **: Significant at p=0.05 and p=0.001 level*

effects and 23%% to G x E interaction sum of squares effects The AMMI model demonstrated the presence of G x E interactions, and this was partitioned among the first and second IPCA (Interaction Principal Component Axes). The first principal component (IPCA1) was highly significant ($p < 0.001$) while the second principal component (IPCA 2) was not significant. The IPCA 1 and the IPCA 2 of the AMMI analysis accounted for 62.17% and 18.94% of the variability for soybean protein content respectively. These two IPCA parameters combined captured 81.11% of the G x E sum of squares.

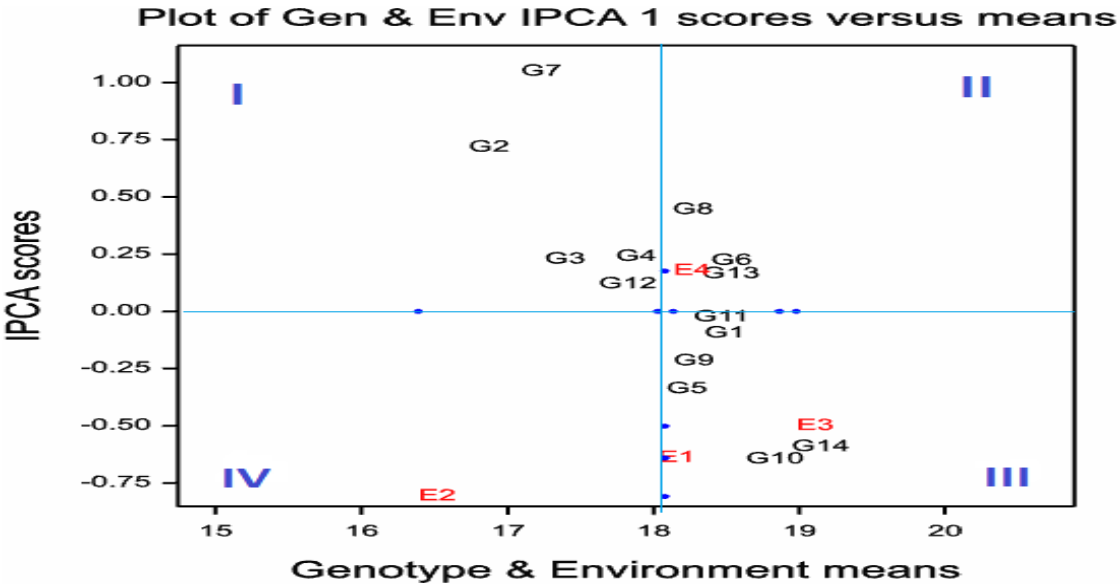

Fig. 1. AMMI 1 biplot of genotypes and environment IPCA 1 scores versus the soybean oil means of fifteen genotypes and five environments

E= Environment, G= Genotype; E1= Kabwe, E2= GART, E3= Msekera, E4= Masumba, E5= Misamfu

Table 10. The AMMI model's first four genotype selections for mean soybean oil content across five environments

Code	Environment	Environment mean	IPCA 1 Score	1	2	3	4
E1	Kabwe	18.03	-0.6408	G15	G10	G14	G8
E2	GART	16.38	-0.8088	G15	G14	G10	G1
E3	Msekera	18.98	-0.5013	G15	G14	G10	G1
E4	Masumba	18.13	0.1762	G8	G15	G10	G14
E5	Misamfu	18.86	1.7747	G7	G6	G8	G13

Table 11. ANOVA for the AMMI analysis of soybean protein content across five environments

Source	Df	SS	MS	F	F. prob	Explained %
Treatments	74	847.1	11.45	5.43	0.00000**	
Genotypes	14	223.2	15.94	7.55	0.00000**	26.35
Environments	4	429.2	107.3	51.93	0.00000**	50.67
Block	5	10.3	2.07	0.98	0.43722^{NS}	
Interactions	56	194.8	3.48	1.65	0.02631*	23.00
IPCA 1	17	121.1	7.13	3.38	0.0002**	62.17
IPCA 2	15	36.9	2.46	1.17	0.31949^{NS}	18.94
Residuals	24	36.7	1.53	0.72	0.80863	
Error	65	137.2	2.11	*	*	
Total	149	994.6	6.68	*	*	

NS,, **: Non Significant, Significant at p=0.05 and p=0.001 level*

3.2.2 AMMI model and pattern analysis for soybean protein content

The AMMI biplot in Fig. 2 shows that the points for environment are more scattered than the points for genotypes indicating that variability due to environments was higher than that due to genotypes differences. One check genotype (Lukanga) and one environment Misamfu dispersed away from the area of the biplot showing their large variability. Three IITA genotypes namely TGX 1740 2F, TGX 1988-18F, TGX1988-9F, and one Safari as well as one environment E4 (Masumba) were clustered near the center of the biplot indicating an average performance of the genotypes and environment. IITA Genotypes TGX 1740-2F, TGX1988- 18F TGX 1988-9F TGX 1989-60F TGX 1990-129F and Safari with IPCA scores close or equal to zero and protein content close to the mean exhibited stability and general adaptability with negligible interaction. TGX 1740-2F was closest to the centre of the Biplot and was therefore the most stable genotype. The ideal genotypes which were stable and had high protein content were all IITA genotypes namely TGX 1988-9F, TGX 1990-129F, TGX 1989-60F and TGX 1830-20E.Kabwe, GART and Masumba falling on the right hand side of the midpoint of the main effect axis, were favorable environments for soybean protein content among the environments in the study. They were also high protein potential environments as they were found in quadrant II. The lower protein potential environment was Msekera in quadrant I. The Biplot also indicated that GART was the highest yielding environment as it was the furthest to the right of the midpoint.

Analysis of the four best perfoming genotypes in protein content was done and the results are presented in Table 12. The results revealed that among the three checks used in the study, only one (Lukanga) made it to the top four highest protein yielding genotypes across environments. The IITA genotype TGX 1830-20E was well adapted to four of the five environments tested but was best adapted to environment Kabwe and GART. The genotypes TGX 1988-9F, TGX 1987-11F and Lukanga were best adapted to environments Msekera, Masumba, and Misamfu respectively TGX 1987-23F on the other hand performed well across three of the five environments tested namely Kabwe, GART and Msekera.

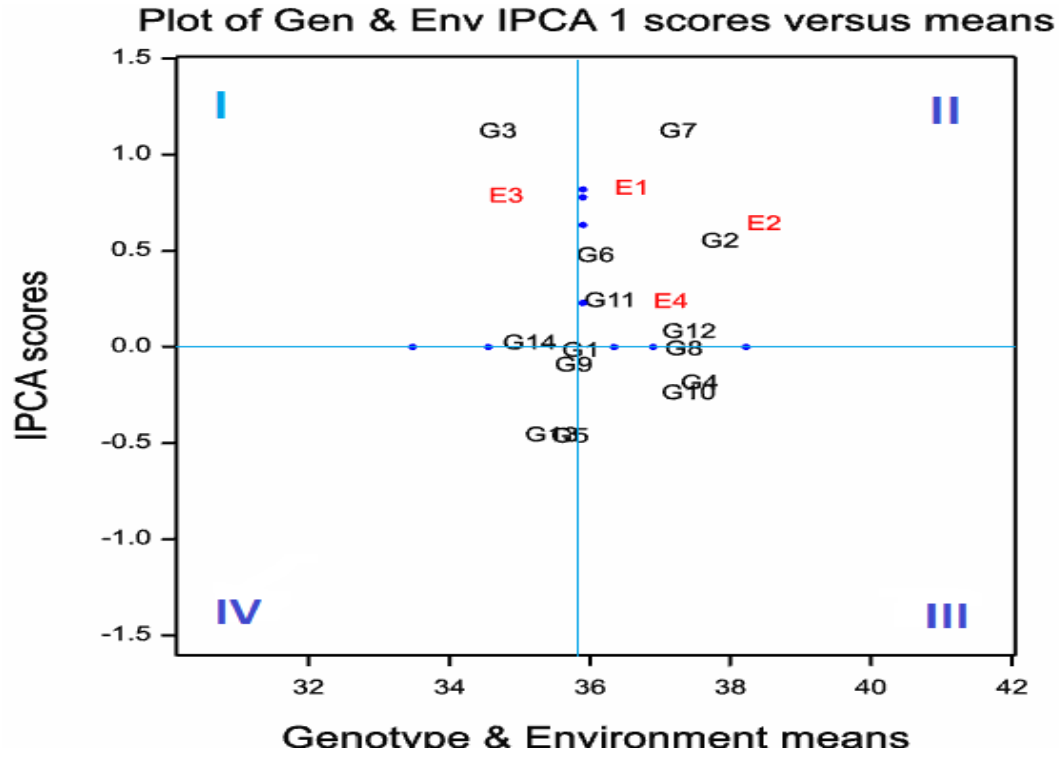

Fig. 2. AMMI 1 Biplot of genotypes and environment IPCA1 scores versus the soybean protein means of fifteen genotypes and five environments

G=Genotype, E= Environment

Table 12. The AMMI model's first four genotype selections for mean soybean protein content across five environments

Code	Envt	Envt. Mean	Score	1	2	3	4
E1	Kabwe	36.34	0.819	G2	G7	G8	G12
E2	GART	38.23	0.6341	G2	G7	G4	G10
E3	Msekera	34.55	0.778	G8	G7	G2	G12
E4	Masumba	36.9	0.229	G6	G2	G10	G4
E5	Misamfu	33.47	-2.4601	G15	G4	G10	G8

3.3 Relationships and Interrelationships among Measured Variables

Simple correlation analysis was conducted to establish the associations among soybean oil content and the various climatic and soil parameters as well as protein content and climatic and soil parameters. The results (Table 13) revealed simple relationships between oil and iron (r = 0.84), oil and rainfall (r = 0.50), oil and copper (r = -0.83) and oil and Zinc (r = -0.49). A stepwise regression analysis to adduce the main causal factors to the fluctuations of oil content in soybean revealed that two factors namely, iron (Fe) and rainfall, being edaphic and climatic factors respectively, were the most important causal factors with b-values of 0.53 and -0.00145 respectively and explaining up to 48% of the variation in oil content (R2 = 47.78). The sensitivity of oil to change in rainfall is implied by the magnitude of the associated b-value (b = - 0.00145). These results also showed that the other factors were not important determinants, in nature, to the changes in oil content.

The resulting regression equation for oil was;
Oil = 15.4 + 0.531 Fe – 0.00145 Rainfall
R2 adj = 47.78%

Simple correlation analysis of protein content with climatic and edaphic parameters (Table 14) showed simple relationships between protein and pH (r = 0.81), protein and calcium (r = 0.77), protein and Zinc (r = 0.69), protein and magnesium (r = 0.50), protein and manganese (r =0.46), protein and iron (r = -0.97), Protein and rainfall (r = -0.93), protein and nitrogen (r = -0.75), protein and phosphorus (r = -0.47) and protein and sulphur (r= -0.45).

Stepwise regression analysis was done in order to determine the main causal factors to the fluctuations in protein content and results revealed that three edaphic factors namely, Phosphorus (P), Sulphur (S) and iron (Fe) were the most important causal factors with b-values of -0.055, -0.082 and -0.609 respectively and explaining up to 48% of the variation in oil content (R2 = 48.48). The sensitivity of protein to change in phosphorus is implied by the magnitude of the associated b- values (b = -0.055). These results also showed that the other factors were not important determinants to the observed variation in protein content.

The resulting regression equation for protein was;
Protein = 42.2 - 0.0548 P – 0.0825 S – 0.609 Fe
R2 adj = 47.8%

4. DISCUSSION

4.1 Oil and Protein Content Variations of Soybean Genotypes

The mean oil and protein content for the soybean genotypes tested in this study ranged from 16.73% to 19.47% and 33.09% to 37.57% respectively across locations. The contents are in agreement with the ranges reported for oil and protein in soybean by Ramana and Satyanarayana [19] and Arslanoglu et al. [20]. The differences of the oil and protein content among the genotypes in the current study could be as a result of specific and inherent genotypic expression. These assertations are supported by the findings of Rodrigues et al. [9]; Brumm and Hurburgh, [21] who reported that oil content is affected by the inherent genotypic expression of genotypes.

The protein content differences apart from the inherent genotypic differences of the genotypes could be attributed to differences in the efficiency on the ability to fix atmospheric nitrogen. The present study had promiscuous varieties (IITA varieties) and non promiscuous varieties (Checks) which require inoculation with suitable rhizobia strains in order to fix nitrogen from the air. Symbiotic nitrogen fixation is the main source of nitrogen in legumes such as soybean and is regarded as the main factor for seed protein content [22]. Maphosa, [23] in his study on effect of inoculation of soybean on nutritional quality

parameters confirmed that seed of promiscuous varieties contained significantly higher crude protein as compared to non-promiscuous varieties. This could be among the major reasons why a non promiscuous genotype Lukanga was the lowest in terms of protein among the genotypes.

The other very important aspect with regards to the oil and protein content of the genotypes is with regard to the relationship of the two traits. Studies have shown that there is an inverse relationship between the oil and the protein content in soybean [24]. This could be the reason as to why among the genotypes, Lukanga which had the highest mean oil content (19.47%) had the lowest Protein content (33.1 %) while TGX 1830-20E which had the lowest oil content of 16.73% had the highest protein content of 37.57%.

4.2 Location Variations for Oil and Protein Content

The study showed location differences for both oil and protein content. The fact that the locations were different with regards to amount of rainfall, temperature and indeed soil types implied that the effect of these edaphic and climatic conditions could have affected the physiological processes in the synthesis of oil and protein in soybean. The current study results further showed that the key environmental factors important for changes in oil content were iron (Fe) and rainfall, explaining 48% of the variation in the oil content while the key environmental factors important for changes in protein content were iron (Fe), Sulphur (S) and Phosphorus (P) explaining 48% of the variation in the protein content.

The low rainfall areas GART and Kabwe had relatively low percentages of oil as compared to the high rainfall areas. This is supported by Yamagata et al. [25] who stated that oil and carbohydrate synthesis by the seed, is primarily dependent on concurrent carbon fixation during the seed filling stage which is in turn affected by adequate moisture availability. A reduction in assimilate supply due to water stress during seed fill could therefore directly impact on the synthesis of oil and residual components.

The current study revealed that the soils at Misamfu had the highest iron (10.23 mg/kg) while soils at GART had the lowest iron (3.38 mg/kg). Additionally, the second highest oil content (18.86%) and lowest protein content (33.47%) among the locations tested were realized from Misamfu while the lowest oil content (16.38%) and the highest protein (38.23%) were from GART. Kobraee and Shamsi [26] substantiates the present study results as they reported that availability of iron increased oil contents, however, excess amounts of iron in the soil reduced protein content of soybean. Iron is an important element for synthesis of chlorophyll, metabolism and is also used in many plant enzyme systems. Further, the reduced protein content of genotypes at GART could have been as a result of the known inverse relationship between protein and oil [27,28] as genotypes at this site yielded the highest oil content.

4.3 Stability of Oil Content and Protein Content across Varying Environments

The AMMI biplots identified stable and genotypes with specific adaptation. Accordingly, the genotypes which are characterized by means greater than the grand mean and with IPCA score nearly zero are considered as generally adaptable to all environments. However, genotypes with high mean performance and with large value of IPCA score are considered as having specific adaptability to the environments. The study further identified stable genotypes with high oil or protein content (ideal genotypes). Pacheco et al. [29] stated that for cultivar recommendation purposes, stable genotypes should also have desirable characteristics. This agrees with Ebehart and Russel [30] who recommended that breeders aim at developing varieties that are not only stable but also have above average performance in other traits. This means the 'ideal' genotypes can be selected for breeding for high oil and protein content respectively in all the five environments. In other words, these genotypes can be recommended for wider adaptation and for production of high oil/ protein content in soybean.

In Table 10 and Table 12, the AMMI model successfully summarizes the patterns and relationships of genotypes and environments by showing the best four performing genotypes at each location for soybean oil and protein content respectively. This is an indication of the AMMI model's ability to analyse the GEI and identification of superior genotypes. From these results, it is evident the AMMI model can also be used in the selection of the most suitable environments for production and/ or evaluation of specific genotypes.

Table 13. Correlations among soybean oil content and environmental parameters

	Oil%	R/fall	Temp	pH	N	Org. matter	P	K	Na	Ca	Mg	Cu	Fe	Mn	Zn	S
Oil%	1.000															
Rainfall	0.495	1.000														
Temp	0.383	-0.231	1.000													
pH	-0.368	-0.763	0.388	1.000												
N	0.131	0.843	-0.493	-0.921	1.000											
Org. Matter	0.161	0.019	0.859	0.142	-0.110	1.000										
P	0.274	0.313	-0.666	-0.229	0.181	-0.864	1.000									
K	0.060	-0.015	0.515	0.641	-0.444	0.455	-0.188	1.000								
Na	0.139	0.264	0.316	0.419	-0.185	0.325	0.009	0.950	1.000							
Ca	0.164	-0.143	0.757	0.667	-0.560	0.643	-0.383	0.949	0.827	1.000						
Mg	-0.272	-0.283	0.482	0.794	-0.559	0.473	-0.384	0.932	0.813	0.895	1.000					
Cu	-0.829	-0.487	0.048	0.690	-0.395	0.209	-0.451	0.495	0.370	0.413	0.761	1.000				
Fe	0.839	0.866	0.032	-0.760	0.644	0.077	0.318	-0.130	0.080	-0.128	-0.456	-0.819	1.000			
Mn	0.241	-0.544	0.924	0.647	-0.783	0.641	-0.542	0.501	0.243	0.737	0.527	0.154	-0.241	1.000		
Zn	-0.485	-0.526	0.178	0.920	-0.704	0.069	-0.122	0.757	0.627	0.669	0.890	0.822	-0.709	0.382	1.000	
S	-0.295	0.613	-0.771	-0.698	0.884	-0.357	0.257	-0.436	-0.191	-0.641	-0.431	-0.046	0.258	-0.943	-0.410	1

Table 14. Correlations among soybean protein content and environmental parameters

	Protein %	Rain fall	Temp	pH	N	Org. matter	P	K	Na	Ca	Mg	Cu	Fe	Mn	Zn	S
Protein %	1.000															
Rain fall	-0.928	1.000														
Temp	0.205	-0.231	1.000													
pH	0.810	-0.763	0.388	1.000												
N	-0.748	0.843	-0.493	-0.921	1.000											
Org. Matter	0.115	0.019	0.859	0.142	-0.110	1.000										
P	-0.473	0.313	-0.666	-0.229	0.181	-0.864	1.000									
K	0.183	-0.015	0.515	0.641	-0.444	0.455	-0.188	1.000								
Na	-0.077	0.264	0.316	0.419	-0.185	0.325	0.009	0.950	1.000							
Cu	0.255	-0.143	0.757	0.667	-0.560	0.643	-0.383	0.949	0.827	1.000						
Mg	0.500	-0.283	0.482	0.794	-0.559	0.473	-0.384	0.932	0.813	0.895	1.000					
Ca	0.770	-0.487	0.048	0.690	-0.395	0.209	-0.451	0.495	0.370	0.413	0.761	1.000				
Fe	-0.969	0.866	0.032	-0.760	0.644	0.077	0.318	-0.130	0.080	-0.128	-0.456	-0.819	1.000			
Mn	0.460	-0.544	0.924	0.647	-0.783	0.641	-0.542	0.501	0.243	0.737	0.527	0.154	-0.241	1.000		
Zn	0.686	-0.526	0.178	0.920	-0.704	0.069	-0.122	0.757	0.627	0.669	0.890	0.822	-0.709	0.382	1.000	
S	-0.446	0.613	-0.771	-0.698	0.884	-0.357	0.257	-0.436	-0.191	-0.641	-0.431	-0.046	0.258	-0.943	-0.410	1.000

5. CONCLUSION

This study revealed that genotypes were different for oil content and protein content. The plausible reason for these differences could be inherent genotypic differences of the genotypes. The differences were however not consistent due to significant environmental influence on oil and protein content of soybean manifested through significant GE interactions. There was inconsistency in the ranking of the genotypes within individual locations for oil content and protein content which made it difficult to identify superior genotypes.

Msekera was the most suitable environment for production of soybean genotypes with high oil content while GART was the most suitable environment for production of soybean genotypes with high protein content as these environments were the largest contributors to phenotypic stability of the named traits respectively. The two environments can also be recommended for use in the improvement of the two traits.

The genotypes Lukanga, Safari, TGX 1988-22F and TGX 1740-2F were best suited for Msekera with regard to oil content while genotypes TGX 1830-20E, TGX 1987-23F, TGX 1887-65F and TGX 1988-22F were best suited for protein content at GART. These genotypes can therefore be said to be best adapted to these environments and could be deployed to these areas as they would fully exploit their potential in the named environments.

The study further revealed that genotypes TGX 1989-60F and TGX 1740-2F were the most stable for soybean oil and protein content respectively and were therefore adaptable to a wide range of growing areas and may be suitable as a parental line in crosses to improve soybean for oil stability or for commercial exploitation.

Clearly, every genotype has a set of environments that are best suitable for them, with respect to particular characteristic(s), but the implication of specific adaptation presents challenges in crop variety development and deployment as such, the concept of stability should be employed to enhance crop productivity in the wake of unpredictability of climate.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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