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Genotype × Environment Interaction Analysis of Seeds Polyphenols and Antioxidant Activity in Groundnut (Arachis hypogaea L.) Grown in the Sudano-sahelian Zone of Cameroon

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Abstract

The objective of this study is to analyze the effects of genotype × environment interaction on seeds polyphenol content and antioxidant activity of exotic groundnut (Arachis hypogaea) varieties grown in the Sudano-Sahelian zone of Cameroon. The field study was conducted in the localities of Gazawa, Bocklé and Dang during 2019 growing season using a randomized complete block design with three replications. Combined analysis of variance was performed using GEST 98 software. The analysis of variance showed significant difference ($p<0.05$) within the fifteen genotypes for total phenol content and antioxidant activity. The highest total polyphenol contents were noted for 58-619 (1.72 EAG/100g) and R4-A (1.64 EAG/100g) lines while varieties 58-619, EC21164 and R4-A showed largest antioxidant activity (79.96; 76.97 and 76.18 mg TE/g DM). Combined analysis of variance attested that polyphenol content and antioxidant activity were influenced only by genotype effects accounting respectively for 91.96% and 96.05% of the total variation of both traits.

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The effects of genotype × environment interaction (7.14 and 3.82% of the total sum of square) and environmental effects (0.86 and 0.13%) were not significant in our study area. For these traits, varietal improvement should be given due considerations.

Keywords: Arachis hypogaea; Genotype × environment interaction; polyphenols; antioxidant activity; Sudano-Sahelian zone; Cameroon.

1. Introduction

Groundnut (Arachis hypogaea L.) is an oilseed and protein crop, native of South America but grown throughout the tropical and warm temperate regions of the globe between latitudes 40°N to 40°S [1;2]. Peanut global production was estimated at 47.09 million tons with a total area of about 27.94 million hectares [3]. Africa represented proximally 25% of the world production [4]. In Cameroon, groundnut is grown on nearly 395 000 ha with an annual production of about 580 000 t of kernels and Northern Cameroon accounts for more than 57% of the national production [5]. This oleaginous species is used for the extraction of oil, for the preparation of meals, for the realization of cakes and and many industrial products. Nutritionally, groundnuts contain about 47% lipids, 38.6% proteins and 9 to 12% carbohydrates [6]. About 60% of the total groundnut production is crushed for edible oil and industrial products, while 30% is consumed in food uses [7]. The nutritional qualities of kernels play a role in the fight against malnutrition in children [8]. Epidemiological studies revealed that peanut consumption reduces the risk of cardiovascular disease [9; 10]. These health benefits have been attributed in part to the presence of antioxidant compounds in plants [11]. Indeed, natural antioxidants are the subject of much research and new breath towards the exploitation of secondary metabolites generally and polyphenols particularly both in health and in front of pernicious diseases as well as in the food industry [11]. Groundnut contains resveratrol, a polyphenol antioxidant which has been found to provide protective function against cancers, heart disease, and degenerative nerve disease [12]. It is therefore necessary to promote the use of natural antioxidants at the expense of synthetic antioxidants in order to fight against free radicals harmful to the body causing damage in biological molecules. In Cameroon, groundnut production is estimated at 536.187 tons for an area of 337.496 hectares [13]. Groundnut cultivation in North Cameroon, represents 56% of the cultivated area, 59% of national production [5]. However, considered as a cash crop, it suffers yield reductions due to biotic constraints, the main ones being arthropods and parasitic diseases [14]. In addition, drought is a major factor hindering agricultural production worldwide [15]. The lack of improved seeds in sufficient quality and quantity is also another constraint to production [16]. Climate change is the major factor that causes yield fluctuations [17]. Thus, the groundnut crop is faced with the major challenge of adapting to climate change and must have productions with increased resilience to environmental factors [18]. Given these existential variations within environments and cultivated peanut genotypes, it would be ideal to conduct a multi-local study, diversifying the genotypes in this peanut production area [19]. The objective of this study is to analyse the effects of genotype × environment interaction on polyphenol content and antioxidant activity of exotic groundnut (Arachis hypogaea) varieties grown in the Sudano-sahelian zone of Cameroon.

2. Materials and methods

2.1. Study locations

The field experiments were conducted in 2019 growing season in three selected sites representing the varying agro-ecologies of the major groundnut growing areas: Gazawa (10°3'N, 15°21'E) in the Far North region, Bocklé (9°21'N, 13°31'E)in the North region and Dang (7°13'N, 13°34'E) in the Adamawa region (Table 1).

Table 1: Experimental sites, sowing date and mean environmental variables.

Location	Region	Planting Date	Environmental variables				
			Altitude (m)	Rainfalls (mm)	TP (°C)	RH (%)	Soil type
Gazawa	Far North	10th July	369	844	28	70	Sandy clay
Bocklé	North	28th June	486	945	29	65	Clay loam
Dang	Adamawa	10th June	1289	1539	22	80	Silt clay

TP: temperature, RH: relative humidity

2.2. Plant materials

Fifteen groundnut pure lines obtained from the Institute for Research on Crops of Semi-Arid Tropical Zones (ICRISAT, Niger) were included in the study. These exotic genotypes were selected for the Sudano-Sahelian conditions viz., C5 (ICG817), RCM439 (ICG2378), JH24 (ICG3365), U12-7-1 (ICG1988), EC21164 (ICG3294), U4-4-32 (ICG3786), 58-619 (ICG9098), C29 (ICG824), AP80-43 (ICG10087), C75 (ICG839), R4-A (ICG3750), EC106965 (ICG3621), US17 (ICG3312), 55-437 (ICG1471), SP2B (ICG330).

2.3. Experimental setup

In each location, the experimental design was a randomized complete block design with three replications. The experimental unit is a plot of one meter long and 0.5 meter wide. The trial occupied an area of 54 m^2 with an adjusted density of 90 000 plants ha^{-1}. Two seeds of each variety were sown at an intra-row spacing of 40 cm and thinned to one plant per hill, 20 days after sowing (DAS). Normal farmer's cultural practices were followed with no application of inorganic fertilizers and chemicals throughout the plantings. At maturity, harvesting was done on ten randomly selected plants, when 70-80% of pods were ready for picking. Kernels were later dried in an oven at 60°C for about 12h. Dried groundnut whole seeds were ground in Moulinex model SeB PREP'LINE 850 for biochemical analysis.

2.4. Determination of total polyphenol content

Total phenols content (TPC) flours were determined following the protocol applied by [20] using the Folin Ciocalteu reagent. 0.5 g of sample was homogenate and extracted with 10 mL ethanol 70°, agitated for 30 min and filtered through Whatmann N°3 paper. 200 µl of the extract was mixed with 1 ml of Folin Ciocalteu

reagent, diluted 10 times in distilled water. After 4 min, 800 µl of sodium carbonate 20% (Na_2CO_3) were added. The mixture was incubated at 45°C for 20min and cooled to room temperature. The absorbance is measured at 760 nm using a UV spectrophotometer. The calibration curve is performed with gallic acid at different concentrations (0-250µg/ml). The results are thus expressed in mg equivalent gallic acid per 100 g dry weight (mg GAE/100g) and all measurements are repeated 3 times.

2.5. Determination of antioxidant activity

The antioxidant activity (AA) of flour was estimated by the reduction of 2.2- diphenyl-1-picrylhydrazyl radical (DPPH) according to the method described by [21]. 100 µl of the extract, was mixed with 1ml of DPPH solution followed by homogenization. After 15 min in the dark, the absorbance is read at 517 nm. The percentage of reduction of DPPH (AA %) calculated as:

$$AA\ (\%) = [(Abs\ C - Abs\ E) / Abs\ C] \times 100\ AA \qquad (1)$$

Abs C: Absorbance of the control;

Abs E: Absorbance of sample.

2.6. Statistical and genetic analysis

In each location, variability among genotypes was determined by simple analysis of variance (ANOVA) using Statgraphics plus version 5.0 software. Significant means were separated using Least Significant Difference at 5% level of probability (LDS). Combined analysis of variance was performed using the pooled analysis procedure to partition the total variance into component due to genotype (G), environment (E) and G × E interaction effects. The combined analysis of variance across locations was done using by 5-model with genotypes being considered as fixed effects and replications within environments being random mode in order to evaluate the effect of difference between genotypes, across locations and also to determine whether their interaction was significant [22]. Analysis of genotype x environment interaction was performed using GEST 98 software [23].

3. Results and discussion

3.1. Variability of total polyphenol content.

Analysis of variance shows that there is a significant difference ($p<0.05$) (Table 2). The results show that varieties 58-619 and R4-A have high levels of total polyphenols (1.72±0.02 mg EAG/100g and 1.64±0.01 mg EAG/100g) compared to variety SP2B which has a polyphenol content of 1.17±0.01 mg EAG/100g. These results would be due to the insufficiency of mineral elements such as nitrogen and sulphate as well as the effect of a hydric shock and the drought at the end of the season in these two agro ecological zones. Our results are different from those obtained by [24]. They report that, the cultivar Chounfakhi has a high polyphenol content (2.1 mg EAG/DM). On the other hand, low values (0.91 and 0.92 mg EAG/100g) of polyphenols in peanut were obtained by [25; 26].

Table 2: Variability and heritability for seeds polyphenols (mg EAG/ 100g DM) of fifteen Arachis hypogaea lines grown in three locations of Northern Cameroon.

Genotypes	Gazawa	Bocklé	Dang	**Varietal means**
C5	1.51±0.02^{h}	1.41±0.00^{e}	1.36±0.01^{ef}	**1.42±0.08**
RCM439	1.54±0.02^{i}	1.51±0.02^{g}	1.54±0.03^{i}	**1.53±0.02**
JH24	1.30±0.01^{c}	1.47±0.00^{f}	1.50±0.02^{h}	**1.42±0.11**
U12-7-1	1.41±0.02^{f}	1.37±0.01^{d}	1.44±0.01^{g}	**1.41±0.05**
EC21164	1.62±0.02^{k}	1.57±0.02^{h}	1.49±0.02^{h}	**1.56±0.09**
U4-4-32	1.35±0.01^{d}	1.33±0.00^{c}	1.22±0.01^{b}	**1.30±0.07**
58-619	1.74±0.02^{l}	1.73±0.02^{j}	1.71±0.03^{k}	**1.72±0.02**
C29	1.46±0.02^{g}	1.38±0.01^{de}	1.30±0.02^{d}	**1.38±0.07**
AP80-43	1.27±0.01^{b}	1.33±0.02^{c}	1.18±0.016^{a}	**1.26±0.06**
C75	1.56±0.03^{j}	1.57±0.01^{h}	1.51±0.01^{hi}	**1.55±0.04**
R4-A	1.63±0.01^{k}	1.67±0.01^{i}	1.,62±0.02^{j}	**1.64±0.04**
EC106965	1.29±0.01^{bc}	1.36±0.04^{cd}	1.39±0,01^{f}	**1.34±0.06**
US17	1.44±0.02^{fg}	1.39±0.01^{de}	1.34±0.02^{e}	**1.39±0.03**
55-437	1.18±0.01^{a}	1.26±0.03^{b}	1.25±0.03^{c}	**1.23±0.05**
SP2B	1.20±0.01^{a}	1.16±0.02^{a}	1,17±0.02^{a}	**1.18±0.02**
Means	**1.43±0.41**	**1.43±0,57**	**1.40±0.38**	**1.42**
σ_i^2	**0.0033**	**0.0121**	**0.0184**	
σ_I^2	**0.1681**	**0.3249**	**0.1444**	
h^2	**0.98**	**0.96**	**0.87**	

σ_i^2: Intravarietal variance; σ_I^2: Intervarietal variance; h^2: broad-sense heritability; Means followed by the same letters are not significantly different at 5% level of probability

3.2. Variability of antioxidant activity

Analysis of variance of antioxidant activity shows a significant difference ($p<0.05$) (table 3). Variety 58-619 has the best total antioxidant capacity of about 80±0.60; 79.33±0.70 and 80.56±0.64 mg TE/g DM for Gazawa, Bocklé and Dang locations. While, SP2B variety contains low antioxidant activity (54.26±0.44 mg TE/g DM). The remarkable antioxidant properties of variety 58-619 would be attributed to Vitamin E in the oil or caffeic acid, p-coumaric acid, ferulic acid, flavonoids and resverastrol in the seeds C. The work conducted by [27] on sixty peanut varieties belonging to Spanish and Virginia group show that there is a significant difference between the antioxidant activity values ($p<0.05$). They record an antioxidant activity in the Spanish group in the range of 6.8 to 15.2 µMTE/g and in the Virginia group 12.5 µMTE/g and 16.5 µMTE/g. In comparison to our results, these antioxidant activities are different from those of the exotic varieties studied. Our results are however, different from those of [28]. They note in seven varieties of Vigna unguiculata grown in the Sudano-sahelian zone of Cameroon antioxidant activities ranging from 70.98 to 266.93 mg TE/g DM with an average of 136.41 mg TE/g DM. These results indicate that groundnut has the potential to be used in the nutraceutical industry. Based on this, it is suggested that peanut consumption could potentially provide some health benefits [29].

Table 3: Variability and heritability for antioxidant activity of fifteen Arachis hypogaea lines grown in three locations of Northern Cameroon.

Genotypes	Gazawa	Bocklé	Dang	**Varietal means**
C5	76.66±1.20^{i}	73.16±1.15^{h}	68.43±1.55^{d}	**72.75±3.97**
RCM439	69.56±0.66^{g}	71.83±0.83^{g}	70.66±1.80^{e}	**70.68±1.77**
JH24	64.40±1.10^{e}	66.16±0.58^{f}	67.7±0.97^{d}	**66.08±2.86**
U12-7-1	60.36±1.40^{d}	62.33±2,36^{e}	64.6±0.34^{c}	**62.42±1.37**
EC21164	78.76±0.47^{j}	77.76±1,72^{i}	72.03±2.70^{ef}	**76.18±3.30**
U4-4-32	60.4±1.35^{d}	61.03±1.63^{d}	58.2±0.43^{b}	**59.87±1.80**
58-619	80.0±0.66^{j}	79.33±0.70^{j}	80.56±0.64^{i}	**79.96±0.65**
C29	73.93±0.50^{h}	71.0±0.33^{g}	72.91±2.55^{fg}	**72.61±1.46**
AP80-43	57.51±1.34^{c}	59.16±0.32^{c}	58.56±1.37^{b}	**58.41±1.34**
C75	76.63±0.65^{i}	70.96±1.41^{g}	74.33±1.22^{g}	**73.97±2.76**
R4-A	77.03±1.25^{i}	76.73±2.51^{i}	77.16±0.77^{h}	**76.97±0.83**
EC106965	66.93±2.32^{f}	65.23±0.73^{f}	65.2±1.01^{c}	**65.78±0.69**
US17	61.0±2,.52^{d}	59.6±1.34^{c}	60.5±1.72^{c}	**60.36±1.52**
55-437	55.13±0.58^{b}	55.16±0.51^{a}	57.43±3.85^{b}	**55.91±2.64**
SP2B	53.67±1.94^{a}	56.33±0.64^{b}	52.86±0.50^{a}	**54.26±3.44**
Environmental means	**67.46±11.94**	**67.05±7.96**	**66.74±8.05**	**67.08**
σi^2	**2.58**	**1.39**	**2.99**	
σI^2	**142.66**	**63.38**	**64.82**	
h^2	**0.98**	**0.97**	**0.96**	

σ_i^2: Intravarietal variance; σ_I^2: Intervarietal variance; h^2: broad-sense heritability; Means followed by the same letters are not significantly different at 5% level of probability

3.3. Genotype × environment interaction effects

A combined analysis of variance according to the [22] model for total polyphenol content and seed antioxidant activity of the 15 varieties tested across three environments is presented in Tables 4 and 5. The main effect differences among genotypes and the G × E interaction were highly significant for both traits ($p<0.01$). In contrast, the main effect differences among environments were not significant at 5% level of probability ($p>0.05$). Of the total variance for total polyphenol, genotype main effect accounted for 91.96%, whereas genotype and G × E interaction effects accounted for 0.89% and 7.14% of the total variation, respectively. For seed antioxidant, the sum of square values showed that the genotype main effect captured 82.91% of the total variation, while G × E interaction and environment effects captured only 1.65% and 0.78% of the total sum of square. These results showed that seed polyphenols and antioxidant capacity were significantly affected by genotypic effect followed by G × E interaction. According to [30], this is G>GEI>E with a non-significant environment effect. The highly significant genotypic effect and its high variance component could be attributed to the large difference among the tested varieties for seed total polyphenol content and antioxidant activity. Changes in environments do not affect significantly these biochemical traits suggesting poor differences among the test locations for environmental factors. The amount of variance contributed by G × E interaction was larger

than that contributed by environments indicating a marked G × E interaction effect and leading to the presence of substantial differences in genotypic responses across the test locations. Thus, a large difference could be noted in genotypic performances and their rank orders across environments. This result is consistent to that of [31] for seed oil content of peanut across the state of Texas. It is evident that selection and recommendation of new varieties basing on these criteria depend mainly on genotypic values but would be difficult due to G × E interaction effect that minimizes the utility of genotypes by confounding their performance [31]. Thus, it is very important to study in depth the genotypic performances, adaptation patterns and stability of these varieties in multiple environments.

Table 4: Combined analysis of variance for seed total polyphenols of fifteen groundnut varieties grown in three locations.

Sources	DF	SS	% SS	MS	F
Genotypes	14	1.03	91.96	0.074	24.66**
Environments	2	0.01	0.89	0.005	1.66 ^{ns}
GEI	28	0.08	7.14	0.003	1.00**
Residual	14	0.04		0.003	
Total	44	1.12			

DF: Degree of freedom; GEI: genotype by environment interaction; SS: Sum of Squares; % SS: percentage of the sum of square; MS: Mean Square; F: Fisher's test; **: significant at 1% level of probability; ns: not significant at 5% level of probability.

Table 5: Combined analysis of variance for seed antioxidant activity of fifteen groundnut varieties grown in three locations.

Sources	DF	SS	% SS	MS	F
Genotypes	14	2867.32	96.05	204.81	77.29**
Environments	2	3.89	0.13	1.94	0.74 ^{ns}
GEI	28	114.17	3.82	4.08	1.53**
Residual	14	37.08		2.65	
Total	44	2985.38			

DF: Degree of freedom; GEI: genotype by environment interaction; SS: Sum of Squares; % SS: percentage of the sum of square; MS: Mean Square; F: Fisher's test; **: significant at 1% level of probability; ns: not significant at 5% level of probability.

4. Conclusion

The objective of this study was to evaluate the effects of genotype × environment interaction on total polyphenol content and antioxidant activity of 15 exotic varieties of Arachis hypogaea grown in three locations

of the sudano-sahelian zone of Cameroon. The results showed a highly significant variability among the different varieties for these biochemical parameters. Exotic varieties such as: 58-619, R4-A and EC21164 are varieties with high polyphenol content and antioxidant activity and could be used in the nutraceutical industry. Total polyphenol content and antioxidant activity of the seeds are more influenced by the genotype main effect and the genotype × environment interaction effect while the environment effect is not significant. However, both mean performance and stability should be considered simultaneously to exploit the useful effects of G × E interaction and to make the selection of favourable genotypes more precise.

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