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Relationship of altitude, individual seed weight, and kernel colonization by *Aspergillus flavus* with biochemical parameters of various Ethiopian groundnut (*Arachis hypogaea* L.) accessions

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KEYWORDS

Individual seed weight

Kernel Colonization

Total carbohydrate

Protein

Total free amino acids

ABSTRACT

Groundnut is one of the five extensively grown oil crops of Ethiopia. Groundnut kernels contain 40-50% fat, 20-50% protein, and 10-20% carbohydrate and are rich in vitamin E, niacin, riboflavin, thiamine, falacin, calcium, phosphorus, magnesium, zinc, iron, and potassium. This study aimed to determine individual seed weight, kernel colonization by *Aspergillus flavus*, and biochemical parameters in groundnut seeds collected from different areas of Ethiopia. Groundnut germplasm in the form of seeds was collected from the Ethiopian Biodiversity Institute (EBI), further information about each accession location and altitude was also collected from the institute. Parameters such as individual seed weight (ISW), kernel colonization by *A. flavus*, and biochemical parameters (i.e. total carbohydrate, protein, and total free amino acids) were measured in the groundnut seeds. A negative relationship between ISW and altitude ($p<0.01$) was observed in this study which indicates that an increase in altitude would result in a decrease in seed weight. A positive relationship between altitude with total carbohydrate ($p<0.01$) and with total free amino acids ($p<0.05$) was also observed. Low-ISW accessions exhibited significantly higher total carbohydrates while high-ISW accessions exhibited significantly higher total free amino acids in their seeds. Groundnut seeds of EBI accessions with 0% kernel colonization contained less total carbohydrate, and the accessions with 100% kernel colonization contained the highest total carbohydrates. Besides, total carbohydrates correlated positively with kernel colonization ($p<0.01$). So, it is postulated that high total carbohydrate makes groundnut seeds susceptible to *A. flavus* colonization.

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1 Introduction

Groundnut (*Arachis hypogaea L.*) is one among the five extensively grown oil crops of Ethiopia (Wijnands et al. 2009). The crop is relatively new to Ethiopia and it was introduced from Eritrea to Hararghe in the early 1920s by the Italian explorers (Ahmed et al. 2016). The major groundnut producer region in Ethiopia is the Oromiya region (41,089 ha), followed by Benishangul-Gumuz (14,759 ha) and Amhara (3,161 ha) regional states (Ahmed et al. 2016). Under rain-fed conditions, it is generally grown and is utilized for extracting cooking oil, and also for confectionary in Ethiopia (Kudama 2013). Besides, this crop helps small-scale producers in getting significant revenue and also helps Ethiopia in getting foreign money earnings through export (Geleta et al. 2007).

Groundnut which is additionally referred to as peanut, earthnut, monkey nut, and goobers is an annual legume. It is one of the world's most important oilseed crops, ranking the 13th most important food crop and the 4th most important oilseed crop in the world is cultivated in more than 100 countries on six continents (Guchi 2015). Groundnut kernels contain 40-50% fat, 20-50% protein, and 10-20% carbohydrate and are rich in vitamin E, niacin, riboflavin, thiamine, falacin, calcium, phosphorus, magnesium, zinc, iron, and potassium (Guchi 2015).

Groundnut plays a crucial role in the livelihoods of the poor farmers and the rural economy of many developing countries (Daniel 2009). Poor agricultural exercise and post-harvest treatments of groundnut can lead to an infection by mold fungus *Aspergillus flavus* and *A. parasiticus* releasing the poisonous substance called aflatoxins (Waliyar et al. 2006). The size of groundnut seeds is one important criterion that determines the market value of groundnut. In addition, the size of the seed is one characteristic that is important in selecting parent plants before a hybridization experiment is performed to get improved varieties with heterosis. Therefore, the study deals with an investigation of individual seed weight, kernel colonization by *A.flavus*, and biochemical parameters in groundnut seeds collected from different areas of Ethiopia. The results of the study are expected to form a foundation for further studies in the future on plant breeding/improvement programs.

2 Materials and Methods

2.1 Sample Collection

Seeds of 50 groundnut accessions were collected from the Ethiopian Biodiversity Institute (EBI), Addis Ababa. The accessions collected from EBI are landraces. Further information about each accession namely, locality of collection and altitude were also collected from EBI. These accessions belong to various places out of 50 accessions 23 accessions belong to Babile woreda of Misrak Harerge zone in the Oromiya region, 17 accessions belong to Gursum woreda of Misrak Harerge zone in the Oromiya region, 9 accessions belong to Benishangul Gumuz region, Metekel Zone (7 from Guba woreda, 1 from Bulen woreda and 1 from Assosa woreda) and 1 accession belong to Benishangul Gumuz region, Kamash Zone, Blo Jiganifado woreda. Based on the sampling area a total of 50 groundnut accessions from EBI were used for this study.

2.2 Individual Seed Weight Determination

Individual seed weight (g) was calculated from 100 seed weight (g) as per the Jeyaramraja and Woldesenbet (2014) procedure.

2.3. Kernel Colonization by *Aspergillus flavus*

Kernel colonization was analyzed as per the method described by Arunyanark et al. (2009). Ten seeds from each accession were used for measuring *A. flavus* colonization. Seeds were surface-sterilized for 3 min in a 10% aqueous solution of Clorox (0.525% NaOCl), rinsed three times with sterile water, and placed on a moistened pre-sterilized paper towel in a sterile plastic box. After incubating for 7 days at 25°C, the number of *A. flavus* infected seeds having a greenish mold on the seed coat, was counted and the results were expressed in percent (%).

2.4 Analysis of Biochemical Parameters

2.4.1 Estimation of Total Carbohydrates

Total carbohydrates in the seeds of groundnut accessions were measured by following the anthrone method described by Sadasivam and Manickam (2008). 100mg of powdered groundnut seeds were measured and transferred into a boiling tube. Hydrolysis was done by keeping it in a boiling water bath for 3 hours with 5 ml of 2.5 N HCl and then, it was cooled to room temperature. It was then, neutralized with solid sodium carbonate until the effervescence ceased. The volume was made up to 100 ml and then, centrifuged. The supernatant was collected and, 0.5 and 1 ml aliquots were taken for analysis. Standards were prepared by taking 0, 0.2, 0.4, 0.6, 0.8, and 1 ml of the working standard. Here "0" serves as blank. The volume was made up to 1 ml in all the tubes including the sample tubes by adding distilled water. All the tubes were kept on ice and then, 4 ml of ice-cold anthrone reagent was added. All the tubes were heated for 8 minutes in a boiling water bath. Then, the tubes were cooled rapidly and read the green to dark color at 630 nm using a UV-VIS spectrophotometer (RS-295). A standard graph was drawn by plotting the concentration of the standard on the X-axis versus absorbance on the Y-axis. From the graph, the amount of carbohydrate present in the sample tube was calculated.

2.4.1.1 Standard

100mg of glucose was dissolved in 100 ml distilled water in a conical flask. 10ml of this stock standard was taken and diluted to 100 ml of distilled water in another volumetric flask for a working standard solution. A standard curve was drawn using absorbance versus concentration. Total carbohydrate present in the sample was expressed as mg carbohydrate in 100 mg sample (in other words, percent equivalent of glucose).

2.4.2 Estimation of Protein

Soluble proteins in the seeds of groundnut accessions were measured by following Lowry's method described by Sadasivam and Manickam (2008). For this, 500mg of groundnut seeds were peeled and ground with a grinder. To this homogenate, 5ml of buffer was added. This was centrifuged and the supernatant was used for protein estimation. 0, 0.2, 0.4, 0.6, 0.8, and 1ml of the working standard were pipetted out into a series of test tubes. 0.1 ml and 0.2ml of the sample extracts were pipetted out in two other test tubes. The volume was made up to 1ml in all the test tubes. A tube with 1ml of water serves as the blank. 5ml of the alkaline copper solution was added to each tube including the blank, mixed well, and allowed to stand for 10min. Then, 0.5ml of Folin-Ciocalteau reagent was added, rapidly mixed well, and incubated at room temperature in the dark for 30min. The blue color was developed. Readings were taken at 660nm using a UV-VIS spectrophotometer (RS-295). A standard graph was drawn and the amount of protein in the sample was calculated.

2.4.2.1 Standard

50mg of bovine serum albumin was dissolved in 50ml distilled water in a conical flask. 10ml of this stock standard was taken and diluted to 50ml of distilled water in another volumetric flask for a working standard solution. A standard curve was drawn using absorbance versus concentration. The amount of protein was expressed as mg protein/100 mg sample (in other words, percent equivalent of BSA).

2.4.3 Estimation of Total Free Amino Acids

Total free amino acids in the seeds of groundnut accessions were measured by following the method described by Sadasivam and Manickam (2008). For this, 500mg of the powdered groundnut seeds were weighed and ground with a grinder. To this homogenate, 5ml of hot 80% ethanol was added. This was centrifuged and the supernatant was used for amino acids estimation. To 0.1ml of extract, 1ml of ninhydrin solution was added. The volume was made up to 2ml with distilled water. The tube was heated in a boiling water bath for 20min. Then, 5ml of the diluent was added and the contents were mixed. After 15min, the intensity of the purple color was read against a reagent blank in a colorimeter at 570nm using a UV-VIS spectrophotometer (RS-295). The reagent blank was prepared as above by taking 0.1ml of 80% ethanol instead of the extract.

2.4.3.1 Standard

50 mg leucine was dissolved in 50 ml of distilled water in a volumetric flask. 10 ml of this stock standard was taken and diluted to 100 ml in another volumetric flask for a working standard solution. A standard curve was drawn using absorbance versus concentration. The concentration of the total free amino acids in the sample was found out and expressed as the percent equivalent of leucine (in other words, mg amino acids/100 mg sample).

2.5 Statistical Analysis

The data were subjected to statistical analysis using WASP (WEB AGRI STAT PACKAGE Version 2.0), developed by Ashok Kumar Jangam and Pranjali Thali, ICAR Research Complex for Goa, India. Critical difference (CD) values were computed at 0.05 and 0.01 levels to find out whether statistically significant differences existed among the treatments in terms of studied parameters. Correlation coefficient (r) values were computed to find out the relationships among the studied parameters.

3 Results and Discussions

3.1 Classification of EBI Accessions Based on Altitude of Cultivation

The accessions collected from EBI were classified based on the altitude of cultivation to study the relationship between each studied parameter. To classify the accessions based on altitude, the "Quantiles Classification" method was used in essence. All the accessions were first ranked from lowest to highest altitudes. After ranking, the first 13 accessions (from 554 to 1480 meters) are regarded as "low-altitude" accessions, and the last 13 accessions (from 1699 to 1898 meters) are regarded as "moderate-altitude" accessions. These are presented in Table 1. Of the 13 "low-altitude" accessions, 9 accessions belong to the Benishangul Gumuz region and 4 accessions belong to the Oromiya region. All the "moderate-altitude" accessions belong to the Oromiya region, the Misrak Harerge zone.

3.2 Analysis of Individual Seed Weight and Kernel Colonization in EBI Accessions

Since EBI provided only 40 seeds for each accession, individual seed weight (ISW) was calculated from 40 seeds weight (g). Data on ISW and kernel colonization by *A. flavus* in EBI accessions belonging to the Oromiya region, Misrak Harerge zone, and Benishangul Gumuz region are presented in Tables 2 and 3, respectively.

Table 1 Classification of groundnut accessions collected from EBI based on altitude

Low-altitude accessions					
S.N.	Accession no.	Altitude (meter)	Region	Zone	Woreda
1	23523	554	Benishangul Gumuz	Metekel	Guba
2	23522	559			
3	23524	601			
4	23521	614			
5	23529	808			
6	23525	837			
7	23528	850			
8	29488	1227		Kamash	Blo Jiganifado
9	29572	1288	Oromiya	MisrakHarerge	Babile
10	29573	1350			
11	29574	1350			
12	19755	1459			
13	23531	1480	Benishangul Gumuz	Metekel	Bulen
Moderate-altitude accessions					
S.N.	Accession no.	Altitude (meter)	Region	Zone	Woreda
1	19752	1699	Oromiya	MisrakHarerge	Babile
2	19747	1700			
3	19748	1709			
4	19749	1709			
5	19750	1744			
6	19766	1753			Gursum
7	19767	1753			
8	19764	1762			
9	19765	1765			
10	19763	1805			
11	19762	1839			
12	19760	1898			
13	19761	1898			

The accession no. 29572 which belongs to the Oromiya region, Misrak Harerge Zone, Babile woreda, had very low ISW (0.417 g). The accession no. 23531 and 23521 which belong to the Benishangul Gumuz region, Metekel Zone, Bulen, and Guba woreda respectively registered very high ISW (0.788 g). Groundnut seed size was found to be highly correlated with seed weight and both traits contributed significantly to yield gains (Chiow and Wynne 1983). High ISW is due to the larger seed size. According to Kabbia et al. (2017) development of groundnut genotypes with a large seed size and seed weight and improved seed, quality attracts consumers' immediate attention. Nautiyal (2002) noted that seed size is another important characteristic that also determines both quality and value. In confectionary type groundnut, there are some important trade attributes. In developing new varieties with quality characteristics for confectionary products seed size is one of the important traits (Amarasinghe et al. 2016).

Table 2 Individual seed weight and Kernel colonization by *A. flavus* in the groundnut accessions from Oromiya region, Misrak Harerge zone

Region, Zone	Woreda	Accession no.	Individual seed weight (g)	Kernel colonization (%)
Oromiya region, Misrak Harerge Zone	Babile	19739	0.519	20
		19740	0.591	10
		19741	0.580	30
		19742	0.525	100
		19743	0.585	90
		19745	0.621	100
		19746	0.580	20
		19747	0.666	30
		19748	0.663	10
		19749	0.584	20
		19750	0.660	0
		19751	0.704	10
		19752	0.555	20
		19753	0.589	20
		19754	0.623	0
		19755	0.626	60
		19756	0.562	10
		19757	0.599	40
		19758	0.516	10
		19759	0.591	20
		29572	0.417	10
		29573	0.569	10
		29574	0.522	10
	Gursum	19760	0.553	0
		19761	0.528	10
		19762	0.578	10
		19763	0.550	0
		19764	0.609	10
		19765	0.505	50
		19766	0.571	50
		19767	0.612	30
		19768	0.606	70
		19769	0.472	10
		19770	0.570	50
		19771	0.522	70
		19772	0.478	30
		19773	0.540	10
		19774	0.531	10
		19775	0.506	0
		19776	0.563	10

Table 3 Individual seed weight and Kernel colonization by *A. flavus* in the groundnut accessions from Benishangul Gumuz region

Region, Zone	Woreda	Accession no.	Individual seed weight (g)	Kernel colonization (%)
Benishangul Gumuz region, Metekel Zone	Guba	23521	0.788	0
		23522	0.586	30
		23523	0.594	0
		23524	0.738	0
		23525	0.776	0
		23528	0.644	0
		23529	0.697	0
	Bulen	23531	0.788	0
	Assosa	23532	0.457	10
Benishangul Gumuz region, Kamash Zone	Blo Jiganifado	29488	0.531	0

Table 4 Classification of groundnut accessions collected from EBI based on individual seed weight

Low-individual seed weight accessions					
S.N.	Accession no.	Individual seed weight (g)	Region	Zone	Woreda
1	29572	0.417	Oromiya	MisrakHarerge	Babile
2	23532	0.457	Benishangul Gumuz	Metekel	Assosa
3	19769	0.472	Oromiya	MisrakHarerge	Gursum
4	19772	0.478			
5	19765	0.505			
6	19775	0.506			
7	19758	0.516			Babile
8	19739	0.519			
9	29574	0.522			
10	19771	0.522			Gursum
11	19742	0.525			Babile
12	19761	0.528			Gursum
13	19774	0.531			
High-individual seed weight accessions					
S. N.	Accession no.	Individual seed weight (g)	Region	Zone	Woreda
1	19745	0.621	Oromiya	MisrakHarerge	Babile
2	19754	0.623			
3	19755	0.626			
4	23528	0.644	Benishangul Gumuz	Metekel	Guba
5	19750	0.66	Oromiya	MisrakHarerge	Babile
6	19748	0.663			
7	19747	0.666			
8	23529	0.697	Benishangul Gumuz	Metekel	Guba
9	19751	0.704	Oromiya	MisrakHarerge	Babile
10	23524	0.738	Benishangul Gumuz	Metekel	Guba
11	23525	0.776			
12	23521	0.788			
13	23531	0.788			Bulen

To study the influence of ISW on other studied parameters, the accessions collected from EBI were classified based on ISW by the "Quantiles Classification" method. All the accessions were first ranked from lowest to highest individual seed weight. After ranking, the first 13 accessions (from 0.417 to 0.531g) are regarded as "low-ISW" accessions, and the last 13 accessions (from 0.621 to 0.788g) are regarded as "high-ISW" accessions. These are presented in Table 4. Of the 13 "low-ISW" accessions, 12 accessions belong to the Oromiya region, Misrak Harerge zone while among the 13 "high-ISW" accessions, 7 accessions belong to the Oromiya region, Misrak Harerge zone while the remaining 6 accessions belong to Benishangul Gumuz region, Metekel Zone.

Kernel colonization (%) values ranged from 0 to 100 (Tables 2 and 3). To study the effect of kernel colonization on other studied parameters, the groundnut accessions were classified based on kernel colonization. All the accessions were first ranked from lowest to highest kernel colonization values and then, a few representative accessions are picked up from each kernel colonization value randomly (Figure 1). It is interesting to note that out of the 10 accessions collected from the Benishangul Gumuz region, 8 accessions showed 0 % kernel colonization (Table 3). On the other hand, out of the 40 accessions collected from the Oromiya region, Misrak Harerge zone, only 5 accessions showed 0 % kernel colonization (Table 2).

3.3 Relationship among Altitude, Individual Seed Weight and Kernel Colonization in theEBI Accessions

Table 5 shows statistically significant ($p<0.01$) negative relationship between ISW and altitude ($r = -0.415$). This indicates that as altitude increases, the seed weight decreases. Thus, groundnut cultivation in low-altitude areas is preferable. Altitude has a negative relationship with temperature. In the studies conducted by Haile and Keith (2017) the optimum temperature for growing groundnut is between 25-30° C. Temperature above 35°C is detrimental to groundnut production. Under lower temperatures the germination is delayed; the delay in germination exposes the seeds to soil pathogen attack for a longer period. Below 17°C, crop growth almost ceases. Cooler temperatures, especially at night, will also delay harvesting. Groundnut should not be grown in areas more than 1,500 meters above sea level as the temperature is likely to be low for groundnut.

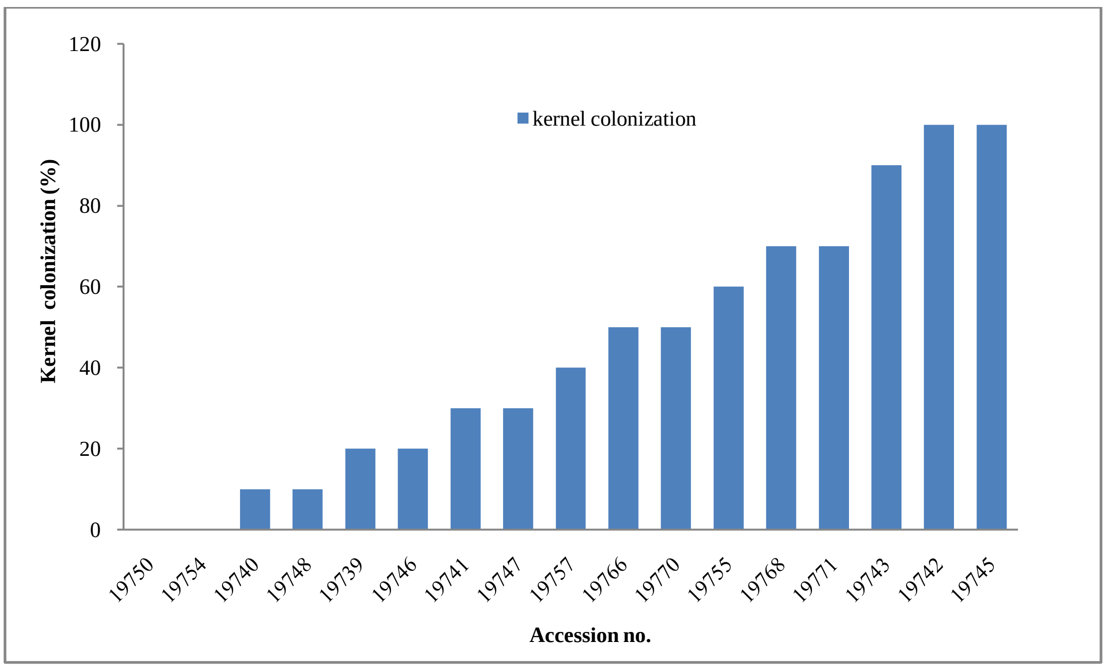

Figure 1 Few representative EBI groundnut accessions with varying levels of kernel colonization values

Table 5 Linear relationship of altitude with individual seed weight and kernel colonization, using data obtained from EBI groundnut accessions

Y	Correlation coefficient (r)	a	b	n
Individual seed weight (g)	-0.415**	0.7254	-0.00009	48
Kernel colonization (%)	0.254*	-0.0607	0.0002	48

**p<0.01; *p<0.1; Regression formula Y = a + b X, where X is altitude, and a and b are regression constants; n is the number of observations

A positive relationship was found between kernel colonization and altitude (r = 0.254), which is weakly significant at p<0.1. This indicates that with an increase in altitude, there may be an increase in kernel colonization by *A. flavus*.

3.4 Analysis of Biochemical Parameters in EBI Groundnut Accessions

Biochemical parameters content namely total carbohydrates, total free amino acids, and protein, were measured in EBI groundnut accessions of different categories *viz*. (a) "low-altitude" accessions vs. "moderate-altitude" accessions, (b) "low-ISW" accessions vs. "high-ISW" accessions, (c) few representative accessions picked up from each kernel colonization value.

To study the effect of altitude, ISW, and kernel colonization on the biochemical parameter, the biochemical parameter were measured only in the above categories of groundnut accessions. The standard graphs used for the calculation of total carbohydrate, protein, and total free amino acid in the groundnut samples are presented in Figures 2, 3, and 4. Data on total carbohydrate, protein, and total free amino acids in various categories of EBI groundnut accessions are shown in Tables 6, 7, and 8.

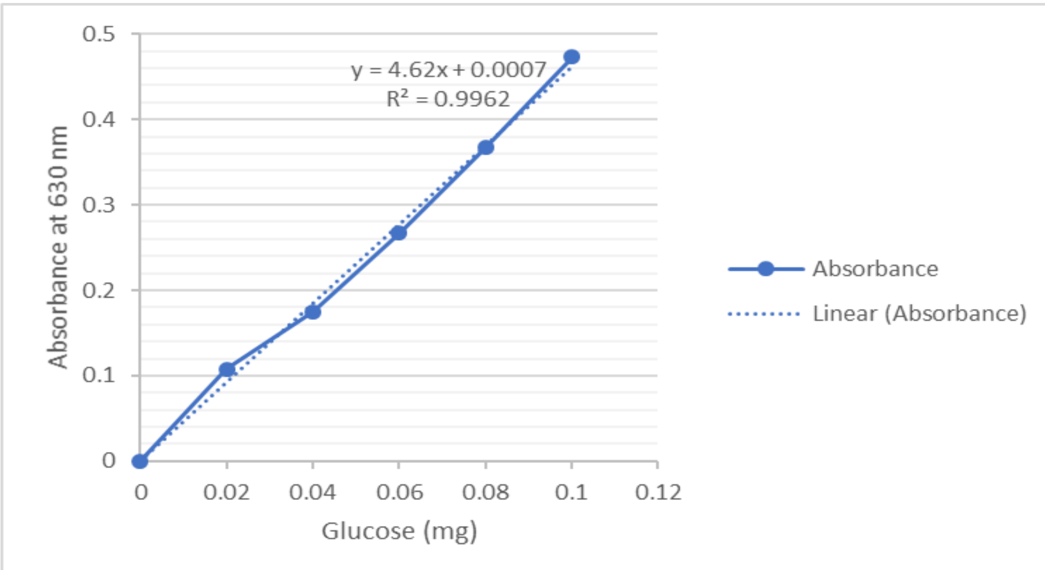

Figure 2 Standard graph used for total carbohydrate estimation

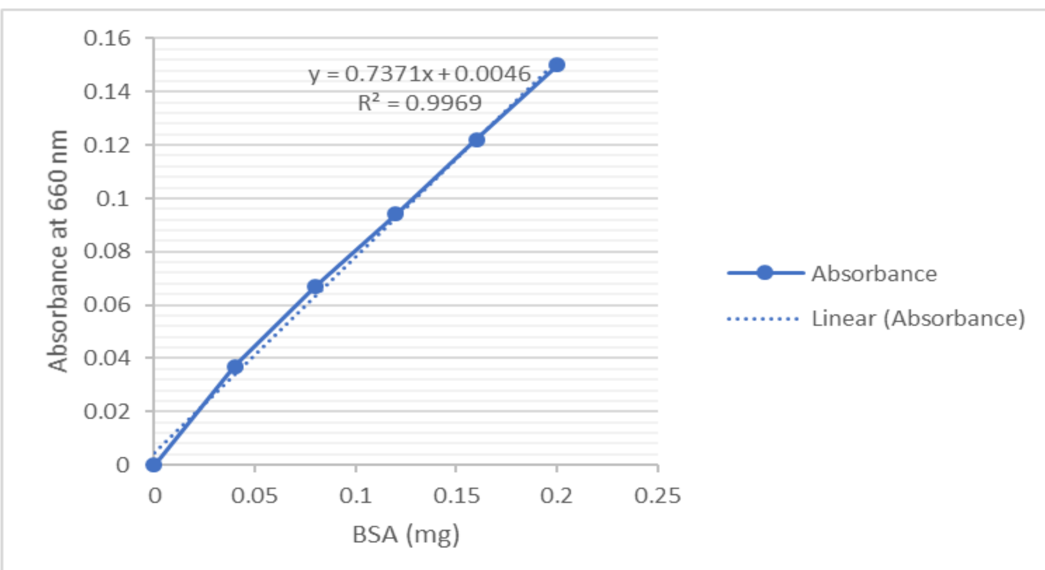

Figure 3 Standard graph used for protein estimation

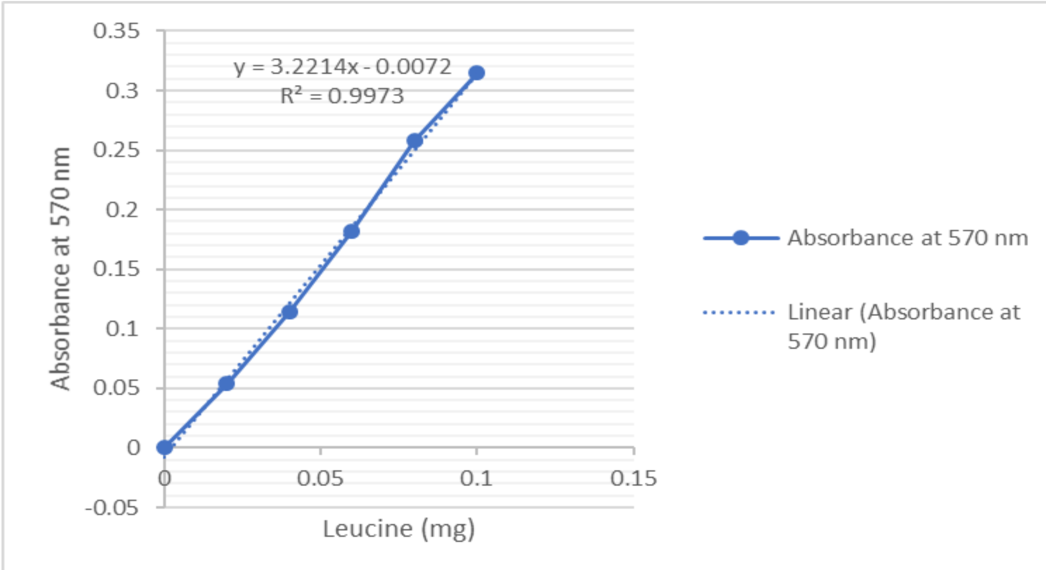

Figure 4 Standard graph used for the estimation of total free amino acids

Total carbohydrate was found to be the highest in the accession 19745 (21.9 %) which belongs to the Oromiya region, Misrak Harerge Zone, Babile woreda, and the lowest in the accession 23521(7.02 %) that belongs to Benishangul Gumuz region, Metekel Zone, Guba woreda. Groundnut seeds are reported to contain 9.5 to 19.0% total carbohydrates as both soluble and insoluble carbohydrates (Abdel 1982; Woodroof 1983). Carbohydrate content ranging from 6.0 to 24.9% was reported by Duke (1981) in groundnut. Adedeji and Onyema (2019) noticed a total carbohydrate content of 8.2 % in groundnut kernels.

In the two accessions from the Oromiya region, Misrak Harerge Zone, one accession 19768 which belongs to Gursum woreda contained the highest protein (39.5 %), while the other accession 19758 which belongs to Babile woreda contained the lowest protein (16.8 %). Yaw et al. (2008) while studying the chemical composition of groundnut landraces of Ghana observed that the crude protein of the seeds ranged from 18.92 to 30.53%. In the two accessions from the Oromiya region, Misrak Harerge Zone, one accession 19750 belonging to Babile woreda possessed the highest total free amino acids (6.9 %), while the other accession 19771 belonging to Gursum woreda had the lowest total free amino acids (0.37 %).

Table 6 shows the biochemical parameters analyzed in low *Vs*. moderate altitude groundnut accessions, collected from EBI. Moderate-altitude accessions exhibited significantly ($p<0.01$) higher total carbohydrate (13.26 %) in their seeds than the low-altitude accessions (10.20 %). Bresson et al. (2009) provided evidence for an altitudinal increase in photosynthetic capacity. Their study showed that the maximum assimilation rate (A_{max}) increases significantly with increasing elevation. Hence, it can be understood that an increase in carbohydrates at high altitudes may be due to an increase in photosynthetic rate.

However, in the case of protein, no significant differences were found to exist between low-altitude accessions and moderate-altitude accessions. According to Suranto et al. (2015), no significant relationship between the contents of protein and the environmental conditions, such as the altitudes, soil pH, temperature, relative humidity, or even soil moisture. On the contrary, Berry and Bjorkman (1980) reported that the plants grown under the low temperature of high altitudes contain a greater amount of proteins including rubisco.

Moderate-altitude accessions exhibited significantly ($p<0.05$) higher total free amino acids (3.22 %) in their seeds than the low-altitude accessions (1.72 %). Nautiyal (2002) inferred that the increase in the amount of amino acids at higher altitudes could be an adaptational metabolic modification of temperate plant species to high altitudes. Melesse et al. (2012) reported that amino acid concentrations in *Moringa stenopetala* leaves at mid-elevation were higher than those at low elevation, except for aspartic acid, phenylalanine, and serine.

Biochemical parameters analyzed in low *Vs*. high-ISW groundnut accessions, collected from EBI are shown in Table 7. Low-ISW accessions exhibited significantly ($p<0.05$) higher total carbohydrate (14.51%) in their seeds than the high ISW accessions (11.22 %). Pattee et al. (1981) reported that carbohydrate concentration was generally highest for the smallest seed size. In addition, immature seeds have higher sugar content than mature seeds (Manda et al. 2004). These observations are in agreement with the results of the present study.

Table 6 Biochemical parameters in low- Vs. moderate-altitude groundnut accessions, collected from EBI

Low-altitude accessions				
S.N.	Accession no.	Carbohydrate (mg/100g)	Protein (mg/100g)	Amino acid (mg/100g)
1	23523	11.61	25.61	1.59
2	23522	11.00	26.82	1.49
3	23524	8.64	24.91	2.04
4	23521	7.02	31.76	1.64
5	23529	10.64	25.45	1.90
6	23525	9.94	26.33	2.29
7	23528	8.32	22.53	2.27
8	29488	11.13	22.17	0.76
9	29572	13.56	28.95	0.47
10	29573	7.75	29.12	2.28
11	29574	12.08	29.98	0.82
12	19755	10.05	23.89	3.78
13	23531	10.84	22.47	1.03
Mean		10.20	26.15	1.72
Moderate-altitude accessions				
S. N.	Accession no.	Carbohydrate (mg/100g)	Protein (mg/100g)	Amino acid (mg/100g)
1	19752	11.24	20.98	3.33
2	19747	10.78	26.02	3.64
3	19748	12.90	21.78	5.85
4	19749	10.88	23.56	4.19
5	19750	12.18	23.15	6.89
6	19766	17.41	29.90	1.28
7	19767	12.91	25.90	1.17
8	19764	15.99	27.59	4.56
9	19765	11.89	23.57	1.37
10	19763	11.53	24.06	1.28
11	19762	15.34	28.46	3.12
12	19760	17.49	25.76	2.42
13	19761	11.78	20.38	2.75
Mean		13.26	24.70	3.22
Statistical significance				
Between "low-altitude" & "moderate-altitude" accessions				
CD (0.01)		2.38	NS	NS
CD (0.05)		1.76	NS	1.16
CV (%)		18.5	11.73	57.57

Values are means of triplicates

Table 7 Biochemical parameters in low *Vs*. high-individual seed weight groundnut accessions, collected from EBI

Low-individual seed weight accessions				
S. N.	Accession no.	Carbohydrate (mg/100g)	Protein (mg/100g)	Amino acid (mg/100g)
1	29572	13.55	28.95	0.47
2	23532	13.30	21.44	0.51
3	19769	14.60	32.52	1.43
4	19772	13.53	26.73	0.61
5	19765	11.89	23.57	1.37
6	19775	18.96	27.22	2.26
7	19758	14.45	16.79	3.70
8	19739	15.47	26.86	3.76
9	29574	12.08	29.98	0.82
10	19771	14.83	23.95	0.37
11	19742	21.05	23.69	4.11
12	19761	11.78	20.38	2.75
13	19774	13.12	31.31	1.75
Mean		14.51	25.64	1.84
High-individual seed weight accessions				
S. N.	Accession no.	Carbohydrate (mg/100g)	Protein (mg/100g)	Amino acid (mg/100g)
1	19745	21.89	21.49	4.20
2	19754	8.44	29.20	1.34
3	19755	10.05	23.89	3.78
4	23528	8.24	22.53	2.27
5	19750	12.12	23.15	6.89
6	19748	12.92	21.78	5.85
7	19747	10.77	26.02	3.64
8	23529	10.56	25.45	1.90
9	19751	14.52	20.19	4.14
10	23524	8.60	24.91	2.04
11	23525	9.90	26.33	2.29
12	23521	7.01	31.76	1.64
13	23531	10.84	22.47	1.03
Mean		11.22	24.55	3.16
Statistical significance				
Between "low-ISW" & "high-ISW" accessions				
CD (0.01)		NS	NS	NS
CD (0.05)		2.67	NS	1.284
CV (%)		25.71	15.769	63.55

Values are means of triplicates

No significant differences existed between low ISW accessions and high ISW accessions, in terms of protein. On the contrary, Prathiba and Reddy (1994) reported that the protein content of small seed varieties of groundnut was high compared to bold seeded varieties. However, it has to be noted that Prathiba and Reddy (1994) worked with only seven groundnut varieties from ICRISAT (International Crops Research Institute for the Semi-Arid Tropics) to arrive at their conclusion while the present investigation dealt with 26 EBI accessions (Table 7).

High ISW accessions exhibited significantly ($p<0.05$) higher total free amino acids (3.16 %) in their seeds than the low ISW accessions (1.84 %). These results are contradictory to the findings of Prathiba and Reddy (1994) who reported that amino acid composition has no relationship with seed size in groundnut. Again here, it has to be noted that Prathiba and Reddy (1994) worked with only seven groundnut varieties while the present study examined 26 groundnut accessions.

Table 8 illustrates biochemical parameters in EBI groundnut accessions with varying levels of kernel colonization. Statistically significant differences were found to exist among the mean values of biochemical parameters namely, total carbohydrate ($p<0.05$), protein ($p<0.01$), and total free amino acids ($p<0.01$) analyzed in EBI accessions with varying levels of kernel colonization. It was observed that seeds of EBI accessions with zero kernel colonization contained less total carbohydrate, and the accessions with 100 % kernel colonization contained the highest total carbohydrate. Hence, it can be inferred that the high carbohydrate content in groundnut kernels makes them susceptible to *A. flavus* colonization. Sugars are found in soil and glucose is used by most fungi as a source of carbon and energy. Several authors have reported that the genera *Fusarium*, *Aspergillus*, and *Penicillium* use glucose in different metabolic processes (Beyer et al. 2004; Daynes et al. 2008; Olsson et al. 1994; Panagiotou et al. 2008). In most fungi, glucose plays a central role in metabolism (Carlile et al. 2001).

Table 8 Biochemical parameters in EBI groundnut accessions with varying levels of kernel colonization

S. N.	Accession no.	Kernel colonization (%)	Carbohydrate (mg/100g)	Mean Carbohydrate	Protein (mg/100g)	Mean Protein	Amino Acid (mg/100g)	Mean Amino acid
1	19750	0	12.12	10.28	23.15	26.18	6.89	4.11
2	19754		8.44		29.20		1.34	
3	19740	10	13.31	13.12	24.88	23.33	4.96	5.41
4	19748		12.92		21.78		5.85	
5	19739	20	15.47	15.01	26.86	24.79	3.76	3.86
6	19746		14.56		22.72		3.97	
7	19741	30	15.32	13.05	23.57	24.79	4.24	3.94
8	19747		10.78		26.02		3.64	
9	19757	40	14.30	14.3	17.09	17.09	3.48	3.48
10	19766	50	17.41	15.39	29.90	29.15	1.28	1.27
11	19770		13.38		28.40		1.27	
12	19755	60	10.05	10.05	23.89	23.89	3.78	3.78
13	19768	70	17.16	15.99	39.49	31.72	1.20	0.78
14	19771		14.83		23.95		0.37	
15	19743	90	11.33	11.33	21.21	21.21	3.96	3.96
16	19742	100	21.05	21.47	23.69	22.59	4.11	4.15
17	19745		21.89		21.49		4.20	
Statistical significance								
Between the mean values of biochemical parameters in accessions with different kernel colonization values								
CD (0.01)				NS		7.414		1.951
CD (0.05)				5.53		5.547		1.464
CV (%)				32.98		18.921		36.63

Values are means of triplicates

Table 9 Relationships among biochemical parameters in EBI groundnut accessions

Relationship between	r	Significant at	n
Altitude (m) vs. total carbohydrate (%)	0.522	1%	43
Altitude (m) vs. total free amino acids (%)	0.316	5%	43
Individual seed weight (g) vs. total carbohydrate (%)	-0.399	1%	45
Kernel colonization (%) vs. total carbohydrate (%)	0.489	1%	45
Protein (%) vs. total free amino acids (%)	-0.413	1%	45

r = is correlation coefficient and n = is number of observations

3.5 Relationships among Biochemical Parameters in EBI Groundnut Accessions

Linear relationships among biochemical parameters in EBI groundnut accessions are shown in Table 9. Altitude had a significantly positive relationship with total carbohydrate ($p<0.01$), and with total free amino acids ($p<0.05$). An increase in altitude increases total carbohydrate and total free amino acids in EBI groundnut accessions. These observations are in line with the observation that moderate-altitude accessions had higher total carbohydrate and total free amino acids than the low-altitude accessions (Table 6).

Total carbohydrate was found to correlate positively with kernel colonization ($p<0.01$). An increase in carbohydrates increases the chances of kernels being colonized by *A. flavus*. That's why, accessions with zero kernel colonization had fewer carbohydrates, and the accessions with 100 % kernel colonization had the highest carbohydrate (Table 8). It has also to be noted that accessions with high ISW showed less total carbohydrate and *vice versa*, as shown in Table 9. This is the reason for the negative relationship ($p<0.01$) between individual seed weight and total carbohydrates (Table 9). On the contrary, a positive correlation between sugar content and seed size ($r=0.56$) was observed by Prathiba and Reddy (1994) who worked with only seven groundnut varieties. Nayak et al. (2020) observed a positive relationship between seed size with crude protein content, but not with any other nutritional traits understudy in their investigation employing 60 groundnut genotypes.

Significant ($p<0.01$) negative relationship has been established between protein and total free amino acids. This observation is supported by the findings of Pfarr et al. (2018) who reported that amino acids such as lysine, methionine, cysteine, tryptophan, and threonine are negatively correlated with soybean seed protein concentration.

Conclusions

A negative relationship between ISW and altitude ($p<0.01$) observed in this study indicated that an increase in altitude would result in a decrease in seed weight. A positive relationship between kernel colonization and altitude ($p<0.1$) observed in this study indicated that an increase in altitude would increase kernel colonization. Since total carbohydrate correlated positively with kernel colonization ($p<0.01$), it is postulated that high total carbohydrate makes groundnut seeds susceptible to *A. flavus* colonization. Altitude had a significantly positive relationship with total carbohydrate ($p<0.01$), and with total free amino acids ($p<0.05$). Total carbohydrate was found to correlate positively with kernel colonization ($p<0.01$). Total carbohydrate was found to correlate positively with kernel colonization ($p<0.01$). A negative relationship ($p<0.01$) between individual seed weight and total carbohydrate was observed. A significant ($p<0.01$) negative relationship has been established between protein and total free amino acids.

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