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Trends of antinutritional compounds in groundnut pod at development stages during drought conditions

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Abstract
This study was investigated for the anti-nutritional compounds in groundnut kernels of two varieties at various stages of pod development in moisture deficit condition. The pods were collected at difference stages at 90 DAS, 105 DAS and 120 DAS of GJG-22 and TG-37A. Irrigation was withdrawn for moisture deficit condition and these days sampling was determined for phytic acid, oxalic acid and trypsin inhibitors. The GJG-22 and TG-37A groundnut verities shown significant differences in anti-nutritional compound *viz,* oxalic acid, phytic acid a trypsin inhibitor in seed and it was found to be increased in control condition as compared to drought condition. In drought condition verities TG-37A was shown decreased in content of phytic acid and oxalic acid. While in case of GJG-22, it was found to be decreased in trypsin inhibitors in all stages of pod development. One surprising effect was observed at 90-DAS in control condition as compared to drought condition for oxalic acids content. Overall anti-nutritional compounds were found to be decreased at 105-DAS to 120-DAS. The quality of groundnut seed kernel is based on anti-nutritional factors. It is fact proven notes that seeds with lower values in oxalate and trypsin inhibitors are being designated as biochemical markers for moisture deficit conditions.

Keywords: Anti-nutritional, drought stress, *Arachis hypogaea*, phytic acid, oxalic acid, trypsin inhibitors

Introduction
Groundnut (*Arachis hypogaea* L.) is one of the world's most important edible oil seed crops. India, China, Nigeria, Senegal, Sudan, Burma, and the United States are the world's top groundnut producers. These countries account for 69% of the area and 70% of the output, with a total area of 18.9 million hectares and a production of 17.8 million tonnes. The 'King' of nuts is groundnut. It is one of our country's most important food and income crops. Groundnut oil has a light yellow colour and a sweet in taste and flavor. This oil is made up of around 20% saturated and 80% unsaturated fatty acids. Groundnuts are well known oils for economy of Saurashtra farmers as well as having higher prices then soybean and cotton seed oils. Raw and roasted nuts are used for edible purpose.

Groundnut is a good cash crop for home markets as well as international trade in a number of developing and developed countries. Kernels are frequently in processed meals such as sweets and powdered dry goods. Cattle feed and organic manure are made from groundnut haulms and oil cake Groundnut crop is suffered them abiotic and biotic stress, out of them abiotic stress can be managed through agronomy practices there for tolerance/resistance traits that provide protection against losses caused by biotic and abiotic stresses are important target traits. Leguminous crops' nodule production is also slowed by moisture stress (Reddi and Reddy 1995) [8]. Drought stress appears to limit groundnut uptake of nitrogen, phosphorus, and potassium (Kulkarni *et al.* 1998) [7].

But one other facts that it has some antinutritional factors like pulse crops because this crops also comes under legumes crops too. Anti-nutritional factors are present in different food substances in varying amounts, depending on the kind of food, mode of its propagation, chemicals used in growing the crop as well as those chemicals used in storage and preservation of the food substances. As everyone knows those each crops has anti-nutritional factors for their survival in nature against abiotic and biotic stress to plants but it is harmful to humans or sometimes making unavailable for certain nutrient in our body and animal too.

Some of the proven facts for animal system for anti-nutrients are given here. The toxicity of oxalate developed from cases of severe or deadly human poisoning after consuming significant quantities of the leaves of certain plant. Phytic acid (Inositol hexaphosphoric acid) forms insoluble compounds with important minerals in meals, making them inaccessible for absorption into the bloodstream (Bingham, 1978) [3].

In rats, phytic acid and its hydrolysis products are linked to a reduction in calcification (Robert and Yudkin, 1999) [9]. When trypsin inhibitors are present in the diet, it decreased the protein digestibility, resulting in slower animal growth (Bolhuis, 2003) [4].

Therefore it is imperative to study on ant nutritional factors of groundnut crop. Our hypothesis also based on that whether any varietal differences can possible or not? or In moisture deficit or drought condition what will happen to the crops quality. In physiology of plants indicated that tissue often responds to drought stress by leaking solutes as a result of membrane breakdown. Similarly in moisture stress condition many compounds showed different pattern in accumulation. Therefore study was also design specifically for groundnut pods harvest at 90, 105 and 120 DAS for seeing anti-nutritional quality attributes. Since long many literatures cited for other crops at harvest still there is no information available for specific crop growth stages for anti-nutritional components.

Materials and Methods

Experimental conditions: The experiment was laid out in the Food Testing Laboratory, atRabi-Summer 2020-21. Trial was done in root blocks of Department of Biochemistry, College of Agriculture, Junagadh Agricultural University in Biotech Farm. Two variety of Groundnut [*Arachis hypogaea* (L.)] seeds *viz.*, GJG-22(V_1) and TG-37A (V_2) were collected from Main Oilseed Research Station and same was used for grown in blocks. The pods were harvested at S_1-90-DAS, S_2-105-DAS and S_3-120-DAS. For moisture deficit condition. Irrigation was withhold for 15 days before each stage of sampling (i.e. 90, 105, and 120 day after sowing) and crops also irrigated just after sampling to maintain 15 days withhold condition.

Analytic method for phytic acid: Phytic acid was extracted from deshelled kernels of groundnut Pods and analysis and calculation was done according to Megazyme Assay Kits (K-pyt 05/07).

Analytic method for oxalic acid: The method was followed for oxalic acids as described by Day and Underwood (1986) [5]. Impregnate1 gram of sample with 75 mL of 3mol/LH_2SO_4 in an Erlenmeyer flask. The mixture was stirred with a magnetic stirrer for 1 hour and filtered. Collect filtrate (25 mL) and heat to 80–90 °C, then keep at 70 °C.Use0.05 mol/L $KMnO_4$ to continuously titrate in hotaliquots until the end point is pale pink for 15 seconds. The calculation method of oxalate content is to take 1mL of 0.05 mol/L $KMnO_4$ equivalent to 2.2 mg of oxalate.

Analytic method of trypsin inhibitors: Trypsin inhibitor activity was determined by the standard procedure of Hammerstrand *et al.* (1981) [6]. From the five test tubes removed, add 2 ml aliquots of the diluted sample to the four test tubes. Prepare the fifth tube of trypsin standard by adding 2mL of distilled water. Add 2 mL of trypsin solution (prepared by dissolving 0.004 g of trypsin in 200mL of 0.001 N HCl) to three of the four test tubes containing sample extracts, and then keep in a water bath. Incubate at 37°C for 10 minutes. Five milliliters of benzoyl dl arginine p-nitro aniline hydrochloride (BAPNA) (prepared by dissolving 0.08 grams of benzoyl arginine hydrochloride p-nitro aniline in 2 milliliters of dimethylsulfoxide, and use 50 Dilute the Mm Tris buffer pH 8.2 to 200 ml, in which the calcium chloride content is 20 mM, heated to 37 mM C) and quickly add it to each tube. The contents were immediately stirred on a vortex mixer and the tube was placed in a 37°C water bath. After exactly 10 minutes, quickly add 1 mL of 30% ethanol to terminate the reaction. The fourth tube containing the sample extract (sample blank) was prepared according to the same procedure, except that the trypsin solution was added after the reaction was terminated by adding 30% glacial acetic acid. Relative to the blank sample, measure the absorbance of each solution at 410 nm. The values obtained from each of the two sample extracts were subtracted from the trypsin standard. Taking the average of these values, the trypsin content is determined as follows:

$$\text{TIU} = \frac{\text{Differential absorbance} \times \text{dilution factor}}{\text{sample weight}}$$

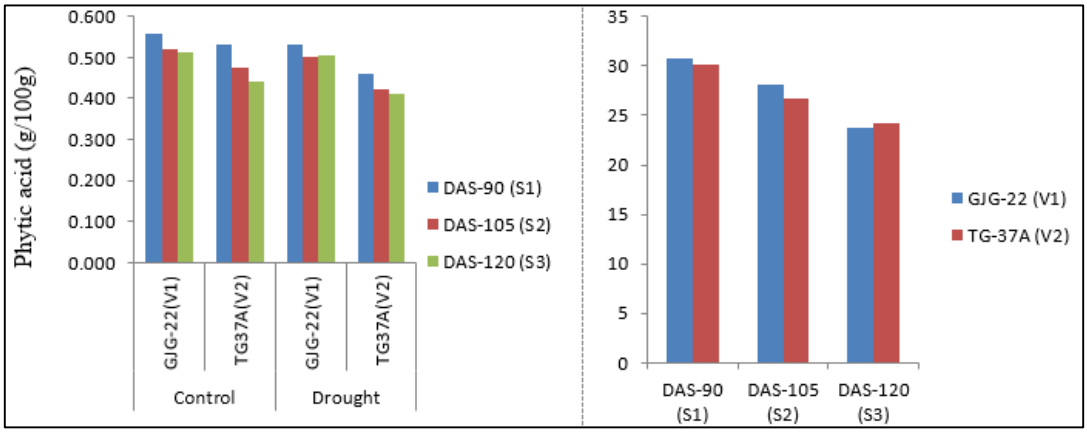
Statistical analysis

All experimental conditions and design of experiment were done according to the Completely Randomized Design (CRD) –Factorial.

Results and Discussion

The results of anti-nutritional compounds in groundnut kernels *Viz.*, phytic acid (Table.1), oxalic acid (Table.2) and trypsin inhibitors (Table.3) were found to be significant in all stages of developments.

The results for changes in phytic acid (g/100g) in response to control and drought stress in groundnut seeds at different stages of pod development is presented in Table 1 and graphically depicted in Fig 1.



S.Em±: 0.008 C.D. @ 5%: 0.023

Fig 1: Interaction effect of drought stress on phytic acid (g/100g) in seed of groundnut verities at pod development stages.

The data showed significant differences for all over stage (S), the general mean for phytic acid at different stage of pod development was significantly highest values at stage (S_1) 90 DAS (0.520 g/100g) followed by 105-DAS (S_2) (0.480 g/100g). 120-DAS stage (S_3) (0.467 g/100g) recorded significantly the lowest phytic acid compared to the other stages.

Interaction effect for treatments and verities (T X V) was found significant (Table 1). Irrespective of treatment and verities, in control condition the general mean for phytic acid was higher T_1V_1 (0.506 g/100g) than the drought condition T_2V_2 (0.472 g/100g) and it was shown to be 6.71%.

Interaction effect for verities and stages (V X S) was found significant (Table 1). In control condition, the lowest phytic acid was observed in TG-37A (0.482 g/100g) treatment while highest was GJG-22 (0.530 g/100g) with the treatment with percent variation of 9.05%. In drought condition the lowest phytic acid was observed in TG-37A (0.431 g/100g) in comparison with GJG-22 (0.513 mg/100g). TG-37A showed 15.98% then the GJG-22.

Interaction effect for treatments and stages (T X S) was found significant (Table 1). In control condition, the highest phytic acid was observed with the T_1S_1 (0.544 g/100g) treatment while lowest was T_1S_3 (0.477 g/100g) with the treatment. Treatments T_1S_1 were statistically at par with T_1S_2 and T_1S_3 treatment. In drought condition the phytic acid was minimum with T_2S_3 (0.458 mg/100g) treatment and maximum with the T_2S_1 (0.497 g/100g) treatments in the drought condition (T_2). Stage wise decreased from S_1 to S_3 stage in control (T_1) and drought condition (T_2). The reduction of phytic acid (g/100g) was lowest in S_3 stage compared to S_1 stage.

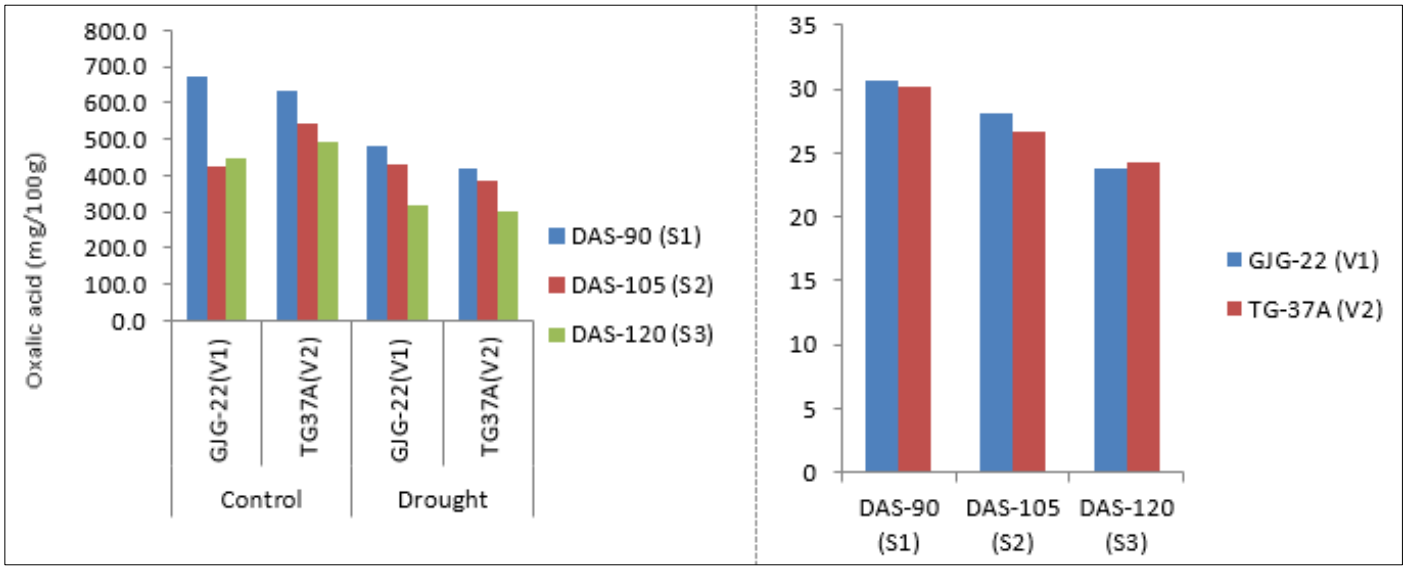
Interaction effect of T X V X S for phytic acid content revealed significant differences (Fig.1). In Control and drought treatment, GJG-22 had higher value at 90 DAS (S_1) in comparison to S_2 and S_3. The minimum content was observed at S_3 stage in both control and drought condition. It showed percent decrease 4.50%, 3.98%, and 1.38% respectively in S_1, S_2 and S_3 in comparison to control and drought condition and TG-37A had higher value at 90 DAS (S_1) in comparison to S_2 and S_3 in control and drought condition. It was also shown decreasing in phytic acid content at S_2 to S_3 stages. It showed percent decrease 15.43%, 12.05%, and 7.80% respectively in S_1, S_2 and S_3 in comparison to control and drought condition.

These results are in agreement with (schlemmer *et al.* 2009) [10] studied phytate in foods and significance for humans: Food sources, intake, processing, bioavailability, protective role and analysis. Beneficial activities of dietary phytate such as its effects on calcification and kidney stone formation and on lowering blood glucose and lipids, antioxidative property and its potential anti cancerogenic activities were reported.

Table 1: Effect of drought stress on phytic acid (g/100g) in seed of groundnut verities at pod development stages.

Treatment (T)	Variety (V)	Stages (S)			Mean V X S	Mean T X V
		DAYS-90 (S_1)	DAYS-105 (S_2)	DAYS-120 (S_3)		
Control (T_1)	GJG-22(V_1)	0.557	0.522	0.512	0.530	0.506
	TG37A(V_2)	0.531	0.474	0.442	0.482	
	Mean T X S	0.544	0.498	0.477		
Drought (T_2)	GJG-22(V_1)	0.533	0.502	0.505	0.513	0.472
	TG37A(V_2)	0.460	0.423	0.410	0.431	
	Mean T X S	0.497	0.463	0.458		
	Mean S	0.520	0.480	0.467		
	T	V	T X V	S	T X S	V X S
S.Em. ±	0.003	0.003	0.005	0.004	0.006	0.006
C.D. at 5%	0.009	0.009	0.013	0.012	0.016	0.016
	C.V%= 2.03					

The results for changes in oxalic acid (mg/100g) in response to control and drought stress in groundnut seeds at different stages of pod development is presented in Table 2 and graphically depicted in Fig 2.



S.Em±: 0.008 C.D. @ 5%: 0.023

Fig 2: Interaction effect of drought stress on oxalic acid content (mg/100g) in seed of groundnut verities at pod development stages.

The data showed significant differences for stages (S) the general mean for oxalic acid at different stage of pod development was significantly highest values at stage (S_1) 90 DAS (550.8 mg/100g followed by 105-DAS (S_2) (447.2 mg/100g). 120-DAS stage (S_3) (388.7 mg/100g) recorded significantly the lowest oxalic acid compared to the other stages.

Interaction effect for treatments and verities (T X V) was found significant (Table 2). Irrespective of treatment and verities, in control condition the general mean for oxalic acid was higher T_1V_1 (535.5 mg/100g) than the drought condition T_2V_2 (388.9 mg/100g) and it was shown to be 27.37%.

Interaction effect for verities and stages (V X S) was found significant (Table 2). In control condition, the highest oxalic acid was observed in TG-37A (555.9 mg/100g) treatment while lowest was GJG-22 (515.1 mg/100g) with the treatment with percent variation of 7.33%. In drought condition the lowest oxalic acid was observed in TG-37A (368.9 mg/100g) in comparison with GJG-22 (409 mg/100g). TG-37A showed 15.98% then the GJG-22.

Interaction effect for treatments and stages (T X S) was found significant (Table 2). In control condition, the highest oxalic acidwas observed with the T_1S_1 (652.5 mg/100g) treatment while lowest was T_1S_3 (469.3 mg/100g) with the treatment. Treatments T_1S_1 were statistically at par with T_1S_2 and T_1S_3 treatment. In drought condition the oxalic acid (%) was minimum with T_2S_3 (308.2 mg/100g) treatment and maximum with the T_2S_1 (449.0 mg/100g) treatments in the drought condition (T_2). Stage wise decreased from S_1 to S_3 stage in control (T_1) and drought condition (T_2). The reduction of oxalic acid (%) was lowest in S_3 stage compared to S_1 stage.

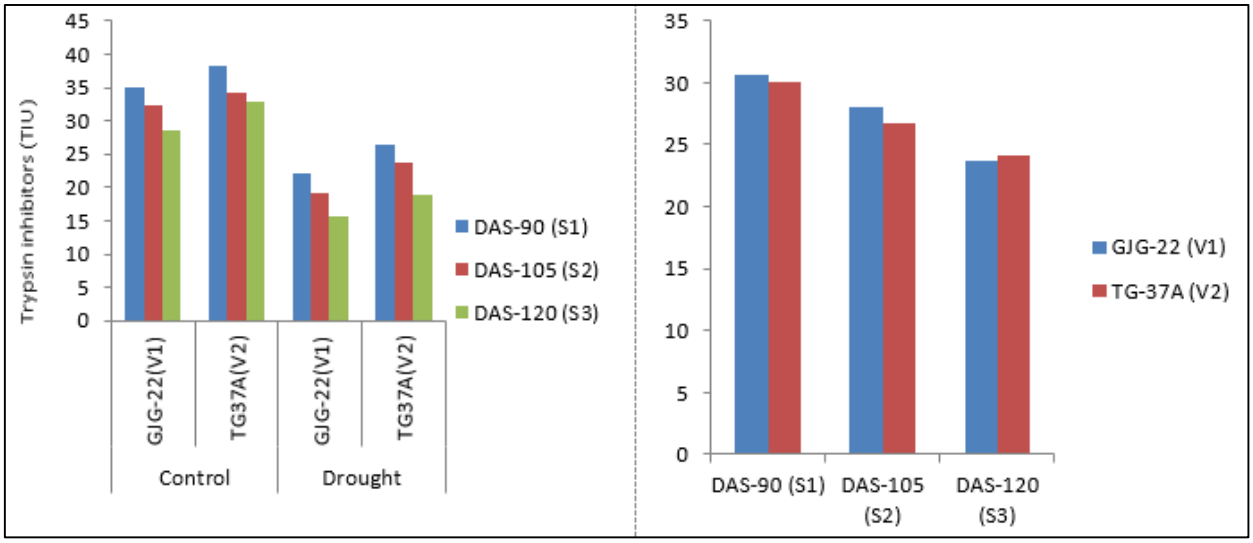
Interaction effect of T X V X S for oxalic acid content revealed significant differences (Fig. 2). In Control and drought treatment, GJG-22 had higher value at 90 DAS (S_1) in comparison to S_2 and S_3. The minimum content was observed at S_3 stage in both control and drought condition. It showed percent decrease 40.14%, 1.38%, and 41.86% respectively in S_1, S_2 and S_3 in comparison to control and drought condition and TG-37A had higher value at 90 DAS (S_1) in comparison to S_2 and S_3 in control and drought condition. It was also shown decreasing in oxalic acid content at S_2 to S_3 stages. It showed percent decrease 51.24%, 40.34%, and 39.30% respectively in S_1, S_2 and S_3 in comparison to control and drought condition.

These results are agreement in with (Adegbaju*et al.* 2019) [1] studied anti-nutrient content of *Celosia argentea* at three stages of maturity i.e. Pre-flowering (PRF), flowering (FLW) and post-flowering (PST) to establish the best time of harvest for optimal nutritional benefits. They found that alkaloid and saponin contents were highest at the PRF stage while oxalate content was dependent on growth stages.

Table 2: Effect of drought stress on oxalic acid (mg/100g) in seed of groundnut verities at pod development stages.

Treatment (T)	Variety (V)	Stages (S)			Mean V X S	Mean T X V
		DAYS-90 (S_1)	DAYS-105 (S_2)	DAYS-120 (S_3)		
Control (T_1)	GJG-22(V_1)	671.3	426.7	447.3	515.1	535.5
	TG37A(V_2)	633.7	542.7	491.3	555.9	
	Mean T X S	652.5	484.7	469.3		
Drought (T_2)	GJG-22(V_1)	479.0	432.7	315.3	409.0	388.9
	TG37A(V_2)	419.0	386.7	301.0	368.9	
	Mean T X S	449.0	409.7	308.2		
	Mean S	550.8	447.2	388.7		
	T	V	T X V	S	T X S	V X S
S.Em. ±	3.073	3.073	4.346	3.764	5.323	5.323
C.D. at 5%	8.972	8.972	12.689	10.989	15.541	15.541
	C.V%= 1.72					

The results for changes in trypsin inhibitors (TIU) in response to control and drought stress in groundnut seeds at different stages of pod development is presented in Table 3 and graphically depicted in Fig 3.



S.Em±: 0.029 C.D. @ 5%: 0.084

Fig 3: Interaction effect of drought stress on trypsin inhibitors (TIU) in seed of groundnut verities at pod development stages.

The data showed significant differences for stages (S) the general mean for trypsin inhibitors at different stage of pod development was significantly highest values at stage (S_1) 90 DAS (30.40 TIU) followed by 105-DAS (S_2) (27.40 TIU). 120-DAS stage (S_3) (23.97 TIU) recorded significantly the lowest trypsin inhibitors compared to the other stages.

Interaction effect for treatments and verities (T X V) was found significant (Table 3). Irrespective of treatment and verities, in control condition the general mean for trypsin inhibitors was highest T_1V_1 (33.53 TIU) than the drought condition T_2V_2 (2.98 TIU) and it was shown to be 37.43%.

Interaction effect for verities and stages (V X S) was found significant (Table 3). In control condition, the lowest trypsin inhibitors was observed in GJG-22 (31.95 TIU) treatment while highest was TG-37A (35.11 TIU) with the treatment with percent variation of 9.00%. In drought condition the lowest trypsin inhibitors was observed in TG-37A (18.90 TIU) in comparison with GJG-22 (23.04 TIU). TG-37A showed 17.96% then the GJG-22.

Interaction effect for treatments and stages (T X S) was found significant (Table 3). In control condition, the highest trypsin inhibitors was observed with the T_1S_1 (36.6 TIU) treatment while lowest was T_1S_3 (30.67 TIU) with the treatment. Treatments T_1S_1 were statistically at par with T_1S_2 and T_1S_3 treatment. In drought condition the trypsin inhibitors (%) was minimum with T_2S_3 (17.26 TIU) treatment and maximum with the T_2S_1 (24.18 TIU) treatments in the drought condition (T_2). Stage wise decreased from S_1 to S_3 stage in control (T_1) and drought condition (T_2). The reduction of trypsin inhibitors (%) was lowest in S_3 stage compared to S_1 stage.

Interaction effect of T X V X S for trypsin inhibitors revealed significant differences (Fig. 3). In Control and drought treatment, GJG-22 had higher value at 90 DAS (S_1) in comparison to S_2 and S_3. The minimum content was observed at S_3 stage in both control and drought condition. It showed percent decrease 37.21%, 40.56%, and 45.46% respectively in S_1, S_2 and S_3 in comparison to control and drought condition and TG-37A had higher value at 90 DAS (S_1) in comparison to S_2 and S_3 in control and drought condition. It was also shown decreasing in trypsin inhibitors at S_2 to S_3 stages. It showed percent decrease 30.99%, 30.63%, and 42.26% respectively in S_1, S_2 and S_3 in comparison to control and drought condition.

These results are agreement in with (Ahmed *et al.* 1988) [2] studied the characterization of trypsin inhibitor in florunner peanut seeds and they showed that high levels of aspartic acid, cysteine, and serine and low levels of histidine and tyrosine in trypsin inhibitor. They found low molecular weight of the inhibitor i.e 8.3 KDa. The existence of various forms of this inhibitor was possible.

Table 3: Effect of drought stress on trypsin inhibitors (TIU) in seed of groundnut verities at pod development stages.

Treatment (T)	Variety (V)	Stages (S)			Mean V X S	Mean T X V
		DAYS-90 (S_1)	DAYS-105 (S_2)	DAYS-120 (S_3)		
Control (T_1)	GJG-22(V_1)	35.01	32.32	28.53	31.95	33.53
	TG37A(V_2)	38.23	34.28	32.82	35.11	
	Mean T X S	36.6	33.3	30.675		
Drought (T_2)	GJG-22(V_1)	21.98	19.21	15.56	23.04	20.98
	TG37A(V_2)	26.38	23.78	18.95	18.92	
	Mean T X S	24.18	21.50	17.26		
	Mean S	30.40	27.40	23.97		
	T	V	T X V	S	T X S	V X S
S.Em. ±	0.012	0.012	0.017	0.014	0.02	0.02
C.D. at 5%	0.034	0.034	0.048	0.042	0.059	0.059
	C.V%= 1.16					

Conclusion

It can be concluded that the GJG-22 and TG-37A groundnut verities is anti-nutritional compound *viz*, oxalic acid, phytic acid and trypsin inhibitors in seed was increased with higher concentration of control condition as compared to drought condition. In drought condition verities TG-37A was decreased *viz.*, phytic acid, oxalic acid, moisture content and verities GJG-22 was decreased in total protein, trypsin inhibitors and relative water content. Identified developing stages can be used to get maximum or minimum content of specific nutritional compound. The increases oxalic acid in 90-DAS in control condition as compared to drought condition. Anti-nutritional compound *viz*, phytic acid and trypsin inhibitor it was also shown decreasing content at 105-DAS to 120-DAS. The expression of all gene was higher and lowest in seed of groundnut genotype GJG-22 and TG-37A as compared to drought condition.

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