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Protein Prophyl Analysis in *Arachis hypogaea* Seeds Grown under Different Combination of Fly Ash, Soil and IAA, GA

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Abstract

Fly ash is a coal combustion residue of thermal power plants having solid waste all over the world. Practical value of fly ash in agriculture and related application as an eco- friendly and economic fertilizer or soil amendments can be established after repeated field experiments for each type of soil to confirm its quality and safety. Fly-ash has great potentiality in agriculture due to its efficacy in modification of soil health and crop performance. The high concentration of elements (K, Na, Zn, Ca, Mg and Fe) in fly-ash increases the yield of many agricultural crops. Fly-ash has great potentiality in agriculture due to its efficacy in modification of soil health and crop performance. Fly ash can play important role in increasing content of protein by the uses of HPLC in *Arachis hypogaea*.

Ground nut is an important source of protein and is a reasonable source of essential amino acid too(young 1973). Ground nut seeds containing 50%oil and 50% protein. Ground nut requires slightly acidic soil pH of 6.0 – 6.5. In this experiment analyze the increases of protein level in ground nut seeds by determination of total N. Liming has been found to be beneficial in increasing crop yield of Ground nut in the acid soil. NPK enhance nodulation and protein synthesis in the plant. Application of NPK gives better yield of ground nut. Nitrogen and phosphorus can increase the number of flowers and phosphate can increase the flowering period. Auxine synthesized a new mRNA for producing new enzyme protein. IAA and GA together regulate biochemical processes by promotion of various enzymes. The use of fly ash, NPK and IAA and GA has helped in increases of amino acid content.

Key words: - Protein, Seeds, Fly-ash

Introduction:-

Fly ash has similar Physic-chemical properties with soil. It can mix homogeneously and can improve agronomic properties of soil (chang *et. al*, 1977). Fly ash is treasure

of trace elements. It makes the trace element readily available to the crop when mixed with soil (Dreiher, *et. al*, 1975 and plank *et. al*, 1974). Arachis hypogaea (Ground nut) is a small branched herb, which grows erect or trails on the ground and bears small yellow flowers. The cylindrical reticulated pods or nuts (1"-2") usually contain two seeds within the outer shell. Seeds are rich in oil and protein. ground nut can adapt to variety of soil and climatic conditions. Use of fly ash as a liming agent in mono and dicotyledonous plants for better crop yields (Ahmad, et.al 1986; Sarangi, et.al, 1998; Millar, et.al,1997; Rai et.al,2003; singh,et. al, 2003). Auxin increased respiration rates is suggestive of parallel relationship of growth and respiratory activity. Auxins also increased water uptake and amino acid uptake by cells (Tyronely, et. al, 1979). Auxin has been found to increases RNA synthesis in tissue of higher plants (Webster, et. al,1962). Use of fly ash to ameliorate soil acidity for maximum uptake of trace elements from fly ash which acted as a reserve of trase element when mixed in soil. Fly ash helps to retain water in the soil. It helped CO2 evolution. The plant hormone Indole acetic acid and Gibberellic acid helped protein and oil synthesis and also increased respiration rate.

Material and method:

In the present experiment, 120 pots containing 40 different combinations of soil, fly ash, NPK, IAA and GA has been used in triplicate. The plant growth parameters viz seed germination (%), number of leaves, leaf area (cm^2), plant root length (cm) and plant shoot length (cm) has been taken in to the consideration. After estimation of the plant growth parameters the five combinations A, B, C, D & E of pots has selected for further study.

The combinations patterns are as following:

Combination A= Plain soil (fig 1)

Combination B = Plain soil +NPK (600:400:200) mg/pot (fig 2)

Combination C = Soil 90% + Fly ash 10% + NPK (600:400:200) mg/pot (fig3)

Combination D = Soil 80% + Fly ash 20% + NPK (600:400:200) mg/pot (fig 4)

Combination E = Soil 70% + Fly ash 30% + NPK (600:400:200) mg/pot + $1.42x10^{-5}$m

IAA + $6.42x10^{-5}$ m GA (fig 5).

Estimation of protein

At first total nitrogen in seed is determined in all combination

Determination of total N:

Requirement:

Kjeldahl digestion and distillation set.

Reagent:

1) Conc. H2SO4
2) NaOH solution
3) CuSO4
4) Phenolphtalein indicator solution

Procedure:
5 gm of sample from each combination was taken in the Kjeldahl flask, 1 gm CuSO4 was added. Heated the mixture the for about two hours. Continued till the color of the mixture becomes more or less transparent. Cooled the mixture and diluted with distilled water. Transfer the mixture to distillation unit. The solution was neutralized with Conc. NaOH solution. A few glass beads were added. 25 ml of 0.1N H2SO4 solution was taken in a conical flask. The mixture was heated and collected in the receiver. The heating was stopped and the flask was removed. The distilled with 0.1N NaOH solution was titrated and observed and calculated the total nitrogen. Nitrogen percentage is multiplied by a conversion factor, I.e. 6.4. it gives the protein percentage of protein (Kjeldalhls 1984).

Preparation of protein hydrolysate (Block *et.al.* 1961):
10 gm of seeds obtained from different combinations to get protein hydrolysate 8 N H2SO4 is employed at atmospheric pressure, heated at 110◦ C the advantage of H2SO4 lies in the ease with the excess of acid can be removed by Ba(OH)2. the filtarate to subjected to HPLC.

High Performance Liquid Chromatography of Protein (Aliet.al., 1997)
Seeds of ground nut (*Arachis hypogaea*) obtained from different combination after pot experiment (A to E) were dried to remove excess of moisture in the oven, at 105◦ C for 10 hrs. Now the seeds are treated with 8 N NH2SO4 at 110◦ C for 15 hrs. Ba (OH)2 solution was added to precipitate SO4-2 as BaSO4. Then the hydrolysate is filtered.

This filtrate contains amino acids. For separation of amino acids, hydrolysate was analyzed by HPLC.

HPLC condition:

[1]	Instrument	: Varine Modal 4000
[2]	Column	: Micro pac Si 1
[3]	Packing	: ISRP, 5 micron
[4]	Pressure	: 1000 Psi
[5]	Flow rate	: 1.5 ml/min
[6]	Porous material	: Silica(Zorbax, 5µm, spherical 300m2/gm)
[7]	Columna material	: Zipax 30 lim (1m2/gm
[8]	Solution	: 5% Ethenol in methylene chloride
[9]	Temperature	: 50◦ C
[10]	Detector	: 254 nm UV, 0.08 Auts.

Result and discussion:
In the ground nut showed 40.60% protein in the seeds (Kachot, *et. al*. 2002). In the present data showed the percentage of protein in A to E combination A – 10.60%, B – 11.25%, C – 12.50%, D – 13.20% and E – 14.25% respectively (fig1-6). In the pot experiment plain soil could provide 10.60 gm protein/100 gm of seeds, In B combination the value enhanced to 11.25 gm protein /100 gm of seeds, this is due to

the contribution of NPK, which causes plant growth parameter to increases and therefore seed quality was improved (Arnon,1958; Betlts *et.al.*, 1966; Evas *et. al.*,1966 and Beevers, 1972). The E combination showed the highest value of protein 14.25 gm/100 gm seeds, this improvement in percentage of protein is due to combination of NPK, fly ash and IAA and GA (Rainhold *et. al*.1956). The concentration of amino acid from the hydrolysate of protein from protein Glycine max seeds obtained from 40% fly ash and 60% soil in very much higher as compared to that of seeds obtained from plain soil (Goyal, *et. al*, 2002). Fly ash regulated the soil pH up to 7, where by the trace element uptake is maximum (Change, *et. al*., 1979 and Hill, *et. al*, 1979).IAA and GA contribute amino acid to synthesis, which further combined to form protein (Nooden, *et. al*.1963).

In the plain soil seeds contained less protein, which is attributed to poor plant growth parameter due to soil acidity and Al ion toxicity (Millar,*et. al*. 1997; Fargeria, *et.al*,1988 and Margina, *et. al*,2003). When the NPK added to soil, seeds showed increase in numberand quality of seeds. Due to NPK protein contain was increased because of synthesis of protein (Beevers, *et. al*. 1969 and Wolosiuk, *et.al*, 1977).When fly ash is added to the soil, apart from NPK and pH regulation, the trace element increased (Zn, Mo) and thus contribute to increase in protein content synthesis (Kapur, *et. al*. 1977; Thornely *et. al*.1979 and Sharma, *et. al*, 1989). The addition of IAA and GA contribute to rise in protein content by inducing enzymes activities in plant cell (Nitsan, *et. al*, 1966 and Gaylor, *et. al*. 1968). Activity of the enzyme catechol 1,2 dioxygenase was induced during growth on IAA suggesting that catechol is an intermediate of the IAA catabolic pathway.(Leaveau and Lindow, 2005) the root is most sensitive to fluctuation in IAA and its response to increasing amount of exogenous IAA extends from elongation of the primary root.

Liu, *et. al*.(1997) reported that the accumulation of andogenous IAA are in proportion to the callus growth in soybean hypocotyls explants. GA stimulate enzymes production (α – amylase) in geminating cereal grains for mobilization of seed resevers (Davis, 1995, Raven, *et. al*, 1992 and Salisbury and Ross, 1992).GA stimulate the transcription of genes of the hydrolytic enzyme in the seed's aleurone layer. GA stimulate the transcription of amylase mRNA. Amylase break down starch to glucose which diffuses to the embryo and is used for the early stages of plant growth IAA stimulated the H+ pumps in the cell wall which activates pH dependent enzyme that break bond, between cellulose micro fibril as short term effect in long term effect and involve the transcription of DNA into mRNA and its subsequent translation into protein responsible for growth.

IAA and GA have been implicated in the regulated of inducible enzyme. Recent evidence points to hormonal regulation of the specific mRNA required for regulation of a given enzyme. Wonkers in Australia have shown that apart from the glucose role in cane invertase regulation, there is a hormone hormone sensitive mechanism governing the synthesis of invertase of mRNA.

Auxine increased respiration has also been correlated with increased water uptake and with increased amino acid uptake by cell (Reinhold and Powell, 1956). Nooden and Thiman (1963) presented evidence that protein synthesis is among the requirement for cell elongation. It has further been show that GA stimulated

elongation of lentil epicotyl cell is accompanied by increased synthesis of DNA (Nitasan and Long, 1966). Roychaoudhary and Sen (1964) reported that auxin stimulate the release as well as the synthesis of RNA. Michaelis, (1951) has described at length the role of RNA in protein synthesis, growth and morphogenesis. Numerous reports have appeared relative to induced changes in enzyme behavior. The control mechanism of an enzyme, synthesis appears to reside at the formation of specific mRNA for that enzyme. In recent years ground nut protein has been resuming technical importance. It can be readily prepared from groundnut cake of low oil content (1.0% - 1.5%).

Determination of Amino acids

HPLC of protein hydrolysate (obtained by acid hydrolysis) gives amino acids. Tryptophan is destroyed during acid hydrolysis. Alkaline hydrolysis gives consistent results for tryptophan (Savage et. al., 1994).

The use of fly ash, NPK and IAA and GA has helped in increase of amino acid, content such as Methionine and Lysine and two limiting amino acid (Abdul Rahman, 1982). These have been found to enhance the concentration from A to E combination. Ground nut a reasonable source of essential amino acid (Young, 1973).

Methionine and Lysine are two limiting amino acids; these have been found to be present in the 'E' combination in sufficiently higher quantity as compared to the 'A' combination (fig.). The present work is significant due to higher percentage of methionine and Lysine because ground nut are deficient in some of the essential amino acids (notably lysine and methionine). But further application of fly ash (beyond 30%) after treatment resulted in decrease of essential amino acids.

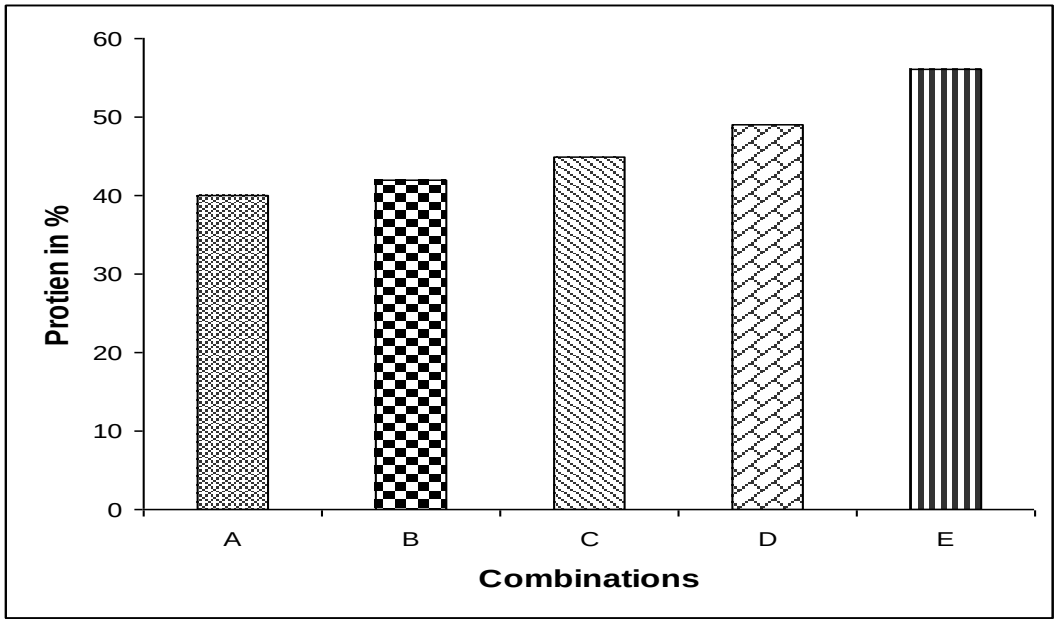

Figure. 1 percentage of protein in combination A- E.

Plain soil gives poor quality of seeds containing less proteins so less amino acid are available after protein hydrolysis (Cowell, *et. al.,* 1945 and Anderson, 1970). In addition of NPK amino acid content shows mild rise due to involvement of N and P in the formation of the amino acids (Beevers, et. al.., 1969; Wolosiuk, et. al., 1977 and Rossignol, 1977). The addition of fly ash rectified the soil acidity to make available the essential trace elements acidity. Because of this soil amylase activity were increased (Sarangi and Mishra, 1996). IAA and GA contributed to rise in enzymatic activities of plant, due to which amino acids contents were increased in different combination.

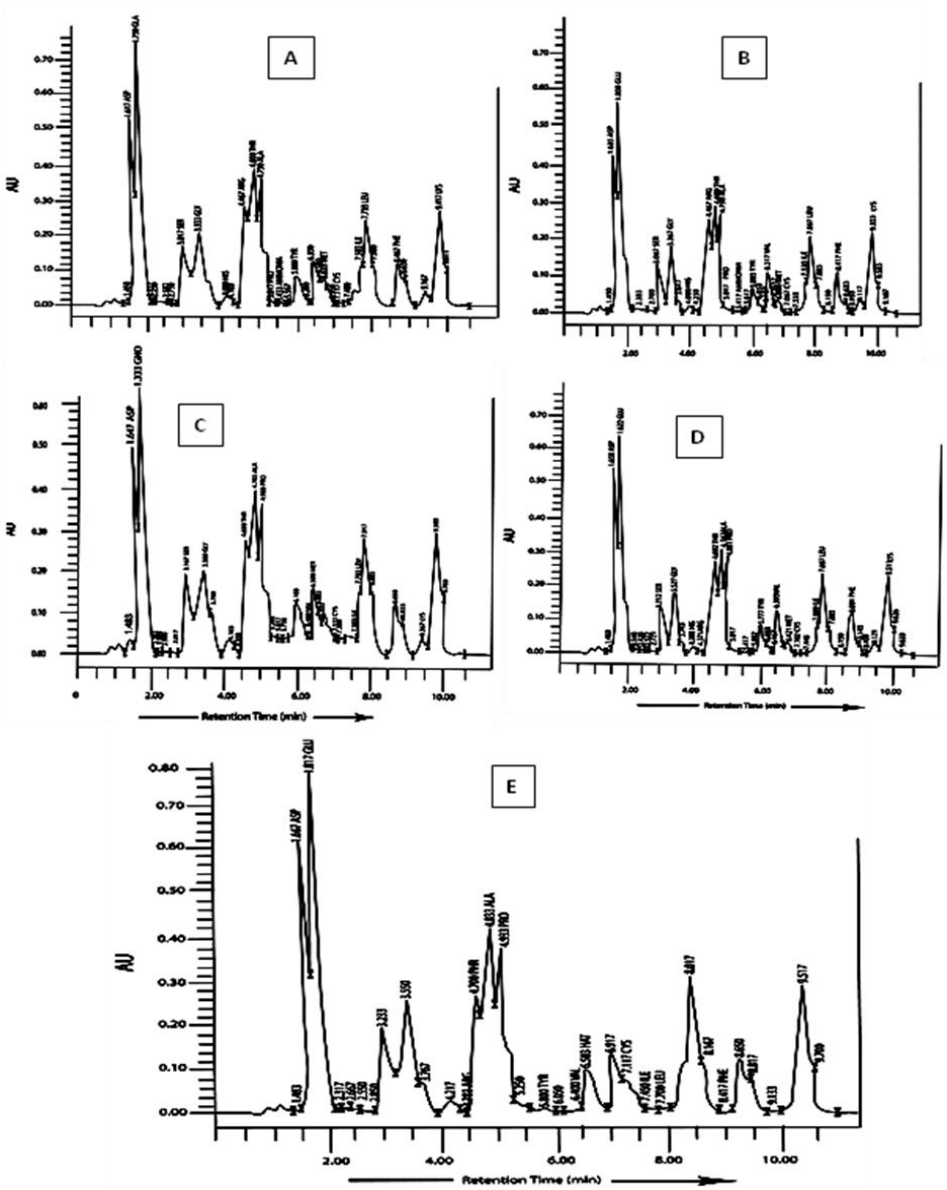

Figure 2. Shows Amino acid of protein hydrolysate from seeds of ground nut from combination A, B,C,D and E.

Table 1. Amino acid of protein hydrolysate from seeds of ground nut from combination A.

S.N.	Retention time (min)	Names	Area(uV* Sec.	Amount	Unit
1.	1.633	ASP	2234948	2700.08	PM
2.	1.300	GLU	968853	3644.16	PM
3.	3.067	SER	2185393	27008.8	PM
4.	3.367	GLY	134530	21674.30	PM
5.	4.00	HIS	549973	19585.53	PM
6.	4.467	ARG	546015	18825.97	PM
7.	4.600	THR	315201	16275	PM
8.	4.75	ALA	1286556	14008.04	PM
9.	5.017	PRO	4459112	12208.87	PM
10.	5.883	TRY	2285435	10897.45	PM
11.	6.317	VAL	155690	10171.44	PM
12.	6.600	MET	320550	9419.04	PM
13.	7.067	CYS	2029319	7547.00	PM
14.	7.533	ILE	3152011	6305.50	PM
15.	7.66	LUE	12865544	5700	PM
16.	8.417	THE	4459112	4648.14	PM
17.	9.33	LYS	2285435	2910.13	PM

Table 2. Amino acid of protein hydrolysate from seeds of ground nut from combination B

S. N.	Retention time (min)	Names	Area(uV* Sec.	Amount	Unit
1.	1.667	ASP	228627	167.61	PM
2	1.817	GLU	190662	151.63	PM
3.	4.383	ALG	840424	697.21	PM
4.	4.700	PHR	201864	343.55	PM
5.	4.833	ALA	1096342	4211.95	PM
6.	4.933	PRO	102824	145.25	PM
7.	5.800	TYR	446286	155.14	PM
8.	6.400	VAL	26964	1211142	PM
9.	6.583	HIS	242276	7390.22	PM
10.	7.117	CYS	1762482	1562.60	PM
11.	7.450	ILE	461218	141.98	PM
12.	7.70	LEU	2241491	8617.47	PM
13.	8.417	PHE	2840418	5275.80	PM

Table 3. Amino acid of protein hydrolysate from seeds of ground nut from combination C

S. N.	Retention time (min)	Names	Area(uV* Sec.	Amount	Unit
1.	1.647	ASP	5910154	7669.45	PM
2	1.33	GLU	2627529	4190.35	PM
3.	3.167	SER	401272	287.12	PM
4.	2.500	GLY	171916	293.76	PM
5.	4.650	THR	670750	535.72	PM
6.	4.786	ALA	83057	68.90	PM
7.	4.933	PRO	1044041	1156.84	PM
8.	6.500	MET	130638	171.07	PM
9.	7.33	CYS	2838958	2878.98	PM
10.	7.783	ILE	2934990	6888.46	PM
11.	9.367	LEU	1608755	2601.59	PM

Table 4. Amino acid of protein hydrolysate from seeds of ground nut from combination D

S.No.	Retention time (min)	Names	Area(uV* Sec.	Amount	Unit
1.	1.658	ASP	698741	269.87	PM
2	1.622	GLU	365902	185.25	PM
3.	3.212	SER	301357	773.42	PM
4.	3.527	GLY	2926161	423.81	PM
5.	4.208	HIS	261827	411.23	PM
6.	4.371	ARG	451247	160.25	PM
7.	4.692	THR	20318	190.21	PM
8.	4.662	ALA	606698	1191.48	PM
9.	4.881	PRO	21037	7501.53	PM
10.	5.777	TRY	563959	1781.29	PM
11.	6.393	VAL	207489	156.07	PM
12.	6.421	MET	440892	8822.79	PM
13.	7.102	CYS	314272	5503.13	PM
14.	7.689	ILE	532576	289.83	PM
15.	7.667	LEU	451247	2162.41	PM
16.	8.001	PHE	41683	3162.86	PM
17.	9.511	LYS	3014272	2621.52	PM

Table 5. Amino acid of protein hydrolysate from seeds of ground nut from combination E.

S.N.	Retention time (min)	Names	Sl96342.No Area(uV* Sec.	Amount	Unit
1.	1.667	ASP	228627	167.61	PM
2	1.817	GLU	190662	151.63	PM
3.	4.383	ALG	840424	697.21	PM
4.	4.700	PHR	201864	343.55	PM
5.	4.833	ALA	10	4211.95	PM
6.	4.933	PRO	102824	145.25	PM
7.	5.800	TYR	446286	155.14	PM
8.	6.400	VAL	26964	1211142	PM
9.	6.583	HIS	242276	7390.22	PM
10.	7.117	CYS	1762482	1562.60	PM
11.	7.450	ILE	461218	141.98	PM
12.	7.70	LEU	2241491	8617.47	PM
13.	8.417	PHE	2840418	5275.80	PM

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