

EFFECT OF GROWTH REGULATING SUBSTANCES ON THE CHLOROPHYLL, NITRATE REDUCTASE, LEGHAEMOGLOBIN CONTENT AND YIELD IN GROUNDNUT (*ARACHIS HYPOGAEA*)

PUSHP SHARMA* AND VIRENDER SARDANA

Department of Plant Breeding and Genetics,
Punjab Agricultural University, Ludhiana - 141 004
E-mail: pushp20@yahoo.com

KEY WORDS

Arachis hypogaea,
Nitrate reductase,
Leghaemoglobin,
Chlorophyll,
Growth regulating
substances

Received on :
21.11.2011

Accepted on :
02.01.2012

*Corresponding
author

ABSTRACT

Regulation of the plant metabolism by exogenous growth regulating substances offers new possibilities in circumventing environmental limitations, relaxing genetic restraints, improving the quality and aiding production. The effect of foliar application of growth regulating substances *i.e.* indole acetic acid (IAA)@5 and 7.5ppm followed by a second spray of Ethrel@25ppm, sequential spray of Mepiquat chloride @125ppm and Mepiquat chloride@125ppm+Ethrel@25ppm was studied in groundnut (*Arachis hypogaea L*) cultivars SG99 and M13 under field conditions. Chlorophyll content increased with the foliar application of IAA @5ppm+Ethrel 25ppm in both the cultivars. Chla, Chlb and total chlorophyll enhanced with the Mepiquat chloride @125ppm + Ethrel 25ppm in SG99. Nitrate reductase activity did not vary much in SG99 with foliar applications; however in cultivar M13, it increased in leaves with IAA@ 7.5ppm+ Ethrel 25ppm. Both the concentrations of IAA increased the nitrate reductase activity in the nodules of SG99. Leghaemoglobin content increased with IAA@ 5ppm+ Ethrel 25ppmin both the cultivars, whereas Mepiquat chloride @125ppm + Ethrel 25ppm was effective only in M13 as compared to control. Number of mature/developed pods, immature pods and gynophores increased with the foliar sprays. Sequential application of IAA@ 7.5ppm+ Ethrel 25ppm increased the pod yield of SG99 and M13 to the tune of 10 and 8 per cent respectively over control.

INTRODUCTION

Groundnut or peanut (*Arachis hypogaea*) is an important crop world wide distributed across the vast area in tropical, subtropical and temperate zones. It is considered one of the most important legume and oilseed crops, which is valued not only for edible oil and protein for human beings but also as fodder for livestock. Peanut accounts for approximately 50% of oilseed production in India and like China, half of the peanuts produced are used for oil production. Groundnut has indeterminate growth habit, hence growth and development of reproductive and vegetative organs overlap. This causes low fruiting efficiency due to interorgan competition for photo-assimilates and other metabolites. Consequently there is improper partitioning of assimilates to the developing pods and seeds. Most prominent constraint in the low yield is extended duration of flowering and variable pods sizes. Malik *et al.* (1995) and Parmar *et al.* (1989) have demonstrated the translocation of photosynthates within groundnut plant is not random but has a definite pattern, and this pattern is changed during different phases of plant growth. Different growth regulators are shown to influence different crop physiology parameters *e.g.* altering plant archetype, promote photosynthesis, alter assimilate partitioning, stimulate uptake of mineral ions, enhance nitrogen metabolism, promote flowering, uniform pod formation, increase mobilization of assimilates to defined sinks, improve seed quality, induce synchrony in flowering and delay senescence

of leaves. The response of groundnut varieties to different growth regulators, aliphatic alcohols, phenolic compounds etc. varies and for details reference be made to the studies of Parmar *et al.*, (1989, 2003), Sharma and Malik (1994), Verma *et al.*, (2008, 2009). Malik (1995) in his presidential address has detailed plant growth regulators: software for plant development and crop productivity. Further Verma *et al.* (2008, 2009) has investigated the role of some PGR's on crop productivity. Several studies earlier too have demonstrated the effect of growth regulators in altering several physiological traits and hence yield (Malik *et al.*, 1990 and 1995). Menon and Srivastava (1984) have emphasized the importance of PGR's in source and sink relationship leading to enhanced translocation of photoassimilates. Wang *et al.* (1995) and later Parmar *et al.* (2003) have demonstrated in their field studies that application of mepiquat chloride decreased partitioning of photo assimilates to the main stem branches but increased the mobilization of assimilates into the reproductive sinks. Sharma and Malik (1994) used three PGR's to investigate chemical regulation of carbon acquisition in groundnut.

The intent of the present study was to evaluate the chlorophyll pigments, enzymes related to symbiotic nitrogen fixation, pod categorization contributing to the yield as affected by different growth regulators.

MATERIALS AND METHODS

Field experiments were conducted at Punjab Agricultural

University, Ludhiana, India (30°54'N, 75°56E, 472 m above mean sea level) during summer seasons of 2006 and 2007 to study the effect of growth regulating substances on the productivity of groundnut. Four treatments, seven rows each of 5 metre row length were sown at spacing of 30x15cm. Two cultivars SG-99 (bunch type) and M-13(semi-spreading) were sown on 11th July, 2006 and 9th June, 2007. The recommended package of practices (except treatments) was followed to raise the crop. The first spray was given at 40 days after sowing (DAS) followed by a sequential application at 50DAS in the following combinations:

Treatments

T₁: IAA@ 5ppm+ Ethrel 25ppm

T₂: IAA@ 7.5ppm+ Ethrel 25ppm

T₃: Mepiquat chloride @125ppm+ Mepiquat chloride I 25ppm

T₄: Mepiquat chloride @125ppm + Ethrel 25ppm

T₅: Water spray

T₆: Control (no spray)

Observations

Photosynthetic pigments: Chlorophyll content was estimated at two times of the day i.e. 9A.M and 2 P.M by the method of Hiscox and Israelstam (1979) at 60 days. Further chlorophyll a (chl.a), chlorophyll b (chl. b), total chlorophyll and chlorophyll a/chlorophyll b (chla/chlb) were calculated as per the formulae in the method.

Nitrate reductase and leghaemoglobin content: Nitrate reductase activity was assayed by the method of Jaworski (1971) from the leaves and nodules at 60 days after sowing. The leghaemoglobin content was determined from freshly excised nodules by the method of Wilson and Reisenauer (1963).

Pod yield: Number of mature/ fully developed pods and immature pods were counted from five randomly selected plants per replicate from each treatment and, were averaged to express the values as percent of the total pods. Pod yield was computed on hectare basis.

RESULTS AND DISCUSSION

Variation in chlorophyll at different times of the day

Chl a, Chl b and total chlorophyll content enhanced with foliar application of IAA@ 5ppm+ Ethrel 25ppm (T₁). Chl b was comparable in Mepiquat chloride @125ppm+Ethrel 25ppm(T₄) and control (no spray) in M-13 at 9A.M. However chl a/chl b ratio increased with all the foliar sprays except with Mepiquat chloride @125ppm+Ethrel 25 ppm (T₄) with respect to control.(Fig 1). In bunch type of cultivar SG-99, Chl a was more with IAA@ 5ppm+ Ethrel 25ppm(T₁) and Mepiquat chloride @125ppm+Ethrel 25ppm(T₄). Chl b content increased with all the foliar applications except for IAA@ 7.5ppm+ Ethrel 25ppm(T₂). Total chlorophyll content enhanced with Mepiquat chloride @125ppm+Ethrel 25ppm(T₃). Chl a/Chl b ratio was more with IAA@ 5ppm+ Ethrel 25ppm(T₁) and was comparable with Mepiquat chloride @125ppm+Ethrel 25ppm(T₄) and no spray treatment.

In M-13 at 2 P.M Chl a, Chl b and total chlorophyll were

higher than the control with Mepiquat chloride @125ppm+Ethrel 25ppm (T₄) foliar spray. Total chlorophyll also increased with both the IAA concentrations and Mepiquat chloride @125ppm+ Mepiquat chloride I 25 ppm (T₃). However Chl a/Chl b ratio did not improve with foliar applications at 2 P.M. In SG-99, chl.a and total chlorophyll were higher with IAA@ 5 ppm+ Ethrel 25ppm (T₁) however with Mepiquat chloride @125ppm+ Mepiquat chloride I 25ppm (T₃) Chl b enhanced whereas chl a/chl b was comparable in IAA@ 5ppm+ Ethrel 25ppm (T₁) and the control. The chlorophyll content did not vary significantly at two times of the day in M-13 and SG- 99.

Overall with the foliar application of IAA@ 5ppm+ Ethrel 25ppm (T₁) increased Chl a in cultivar M-13 and SG-99. Mepiquat chloride @125ppm+Ethrel 25ppm (T₄) was also effective in enhancing chl.a in the latter cultivar. Chl b in M-13 increased with both the concentrations of IAA whereas IAA@ 5ppm+ Ethrel 25ppm(T₁), Mepiquat chloride @125ppm+ Mepiquat chloride I 25ppm (T₃) and Mepiquat chloride @125ppm+Ethrel 25ppm(T₄) improved this component in SG-99 as depicted in Fig. 2. Total chlorophyll content improved with IAA@ 5ppm+ Ethrel 25ppm(T₁) and Mepiquat chloride@ 125ppm+ Ethrel 25ppm(T₄) only in M-13.

Unarguably, the two cultivars responded differently to various treatments of growth regulating substances. Total chlorophyll content was higher in SG-99, a bunch type cultivar as compared to M-13, a semi-spreading type. Salicylic acid significantly increased chlorophyll a, chlorophyll b recording maximum values at 100mg/L. On the contrary the content of such pigments changed reversely with the higher concentration of SA (Jayalaskhmi et al., 2010). These results are in agreement with those obtained by Gharib (2006) in sweet basil and marjoram plants where SA at 10⁻⁵ M stimulated total chlorophyll synthesis and 10⁻³M had a reverse effect.

Nitrate reductase and leghaemoglobin content

Nitrate reductase increased with the foliar application of IAA@ 7.5ppm+ Ethrel 25ppm in leaves of M-13. However in the nodules of SG-99 the enzyme activity enhanced with both the concentrations of IAA. Significant differences were recorded for nitrate reductase within varieties and foliar sprays in both leaves and nodules (Table1) but the interactions were significant only in the nodules. Nodules of both the cultivars had higher nitrate reductase activity at 60 DAS in comparison to the leaves. Interestingly, the activity was higher in leaves and nodules of cultivar M-13.

Leghaemoglobin increased with IAA@ 5 ppm+ Ethrel 25ppm in SG- 99 whereas increase in M-13 was recorded with higher concentration of IAA and the foliar spray of Mepiquat chloride @125ppm + Ethrel 25ppm. Significant variation in the nitrate reductase and leghaemoglobin content was observed in SG-99, a bunch type of variety by Sharma et al. (2011). SA (10⁻⁵M) was found to be beneficial in enhancing the NR activity when applied under ambient conditions and also helps partially in overcoming negative effect of heat stress (Hayat et al., 2009).

Pod yield

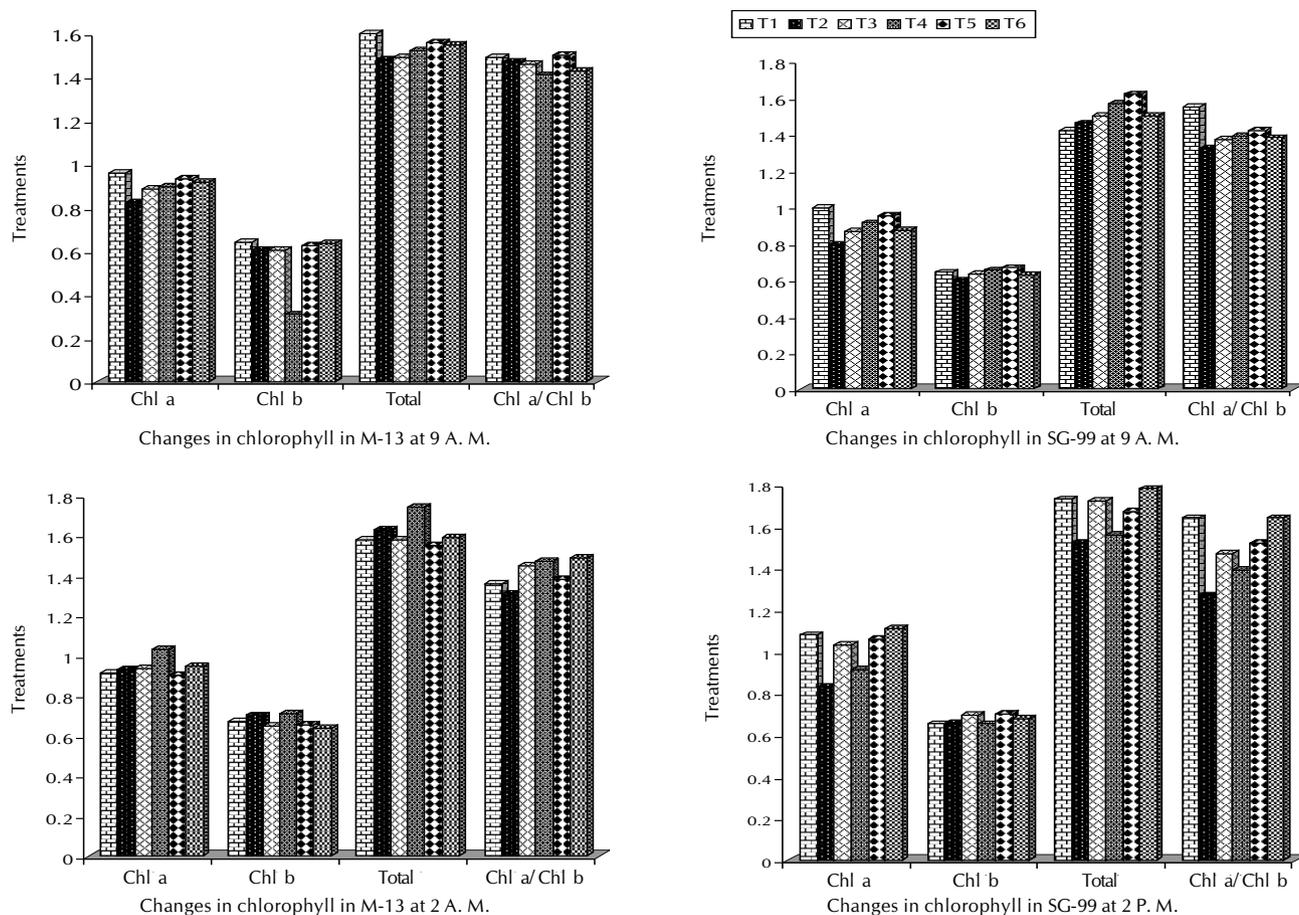
Yield has improved significantly in both the cultivars of groundnut with the foliar sprays of IAA @ 7.5ppm+ Ethrel 25ppm, Mepiquat chloride @125ppm+ mepiquat chloride

Table 1: Effect of foliar sprays on the nitrate reductase activity and leghaemoglobin content in cultivar M-13 and SG-99 at 60 DAS

No.	Treatments	Nitrate reductase ($\mu\text{mole/h/g F.W.}$)			Leghaemoglobin content (mg /g nodule wt.)					
		Leaves			Nodules					
		SG-99	M-13	Mean	SG-99	M-13	Mean	SG-99	M-13	Mean
T1	IAA@5ppm + Ethrel 25ppm	0.471	0.518	0.494	1.68	1.22	1.44	1.74	1.39	1.66
T2	IAA@7.5ppm + Ethrel 25ppm	0.460	0.612	0.536	1.36	1.05	1.2	1.38	1.68	1.52
T3	Mep.chloride@125ppm + Mep.chloride125ppm	0.440	0.565	0.503	1.22	1.56	1.39	1.39	1.35	1.37
T4	Mep.chloride @125ppm + Ethrel 25ppm	0.408	0.469	0.438	1.04	1.70	1.36	0.899	1.82	1.36
T5	Water spray	0.549	0.581	0.568	1.74	2.52	2.13	1.52	1.73	1.62
T6	Control (No spray)	0.487	0.533	0.510	1.29	2.63	1.96	1.40	1.59	1.49
Mean		0.469	0.546		1.38	1.78		1.38	1.62	
C.D @5%		Var (V)=0.033 Sprays (s)=0.057 VxS= NS			Var (V)=0.102 Sprays (s)=0.120 VxS= 0.170			Var(V)=NS Sprays (s)=NS VxS= 0.348		

Table 2: Effect of foliar sprays on the yield attributes and yields in cultivar M-13 and SG-99 at maturity

No.	Treatments	Total pods/plant	M-13		Yield (kg/ha)	SG-99		Yield (kg/ha)	
			% of pods per plant			% of pods per plant			
			Mature	Immature		Mature	Immature		
T1	IAA@5ppm + Ethrel 25ppm	45.4	57.4	42.5	1956	61.8	72.8	2532	
T2	IAA@7.5ppm + Ethrel 25ppm	39.8	63.3	36.6	2028	59.4	74.1	2722	
T3	Mep.chloride@125ppm + Mep.chloride125ppm	36.1	63.9	36.0	2111	65.2	75.5	2621	
T4	Mep.chloride @125ppm + Ethrel 25ppm	37.8	60.9	38.1	1972	57.2	72.2	2635	
T5	Water spray	44.2	58.1	41.8	2046	56.2	74.7	2741	
T6	Control (No spray)	40.4	56.2	40.8	1870	58.0	72.4	2458	
C.D@5%		1.2	1.44	0.786	170.8	1.3	1.19	0.431	94.9

**Figure 1: Variation in chlorophyll content at different times of the day in M-13 and SG-99**

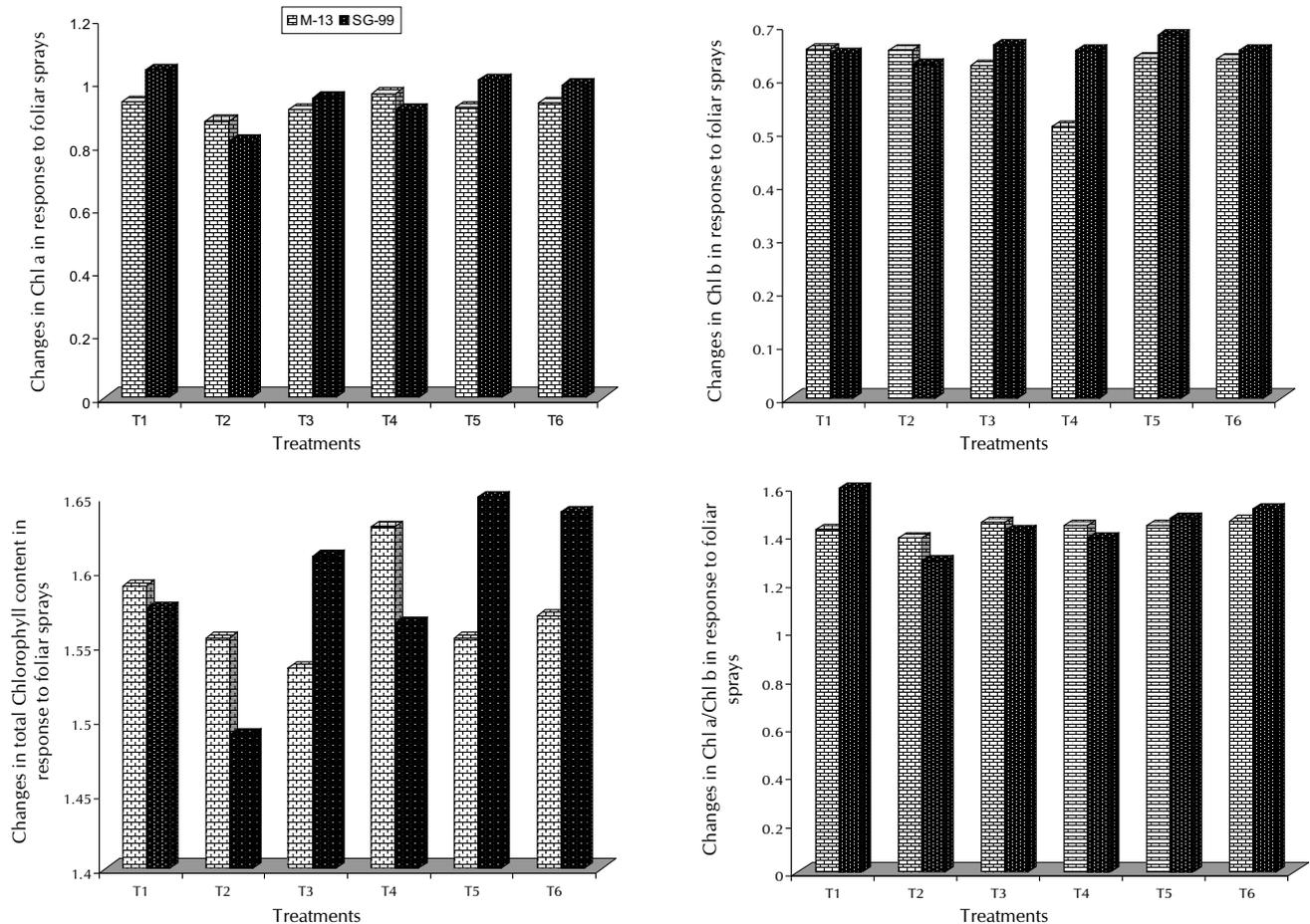


Figure 2: Influence of foliar applications on the chlorophyll content of two cultivars of groundnut

and Mepiquat chloride @125ppm + Ethrel 25ppm (Table 2). This could be attributed to higher percentage of mature pods, lesser number of immature pods, increase in nitrate reductase, leghaemoglobin and chlorophyll content. Foliar spray of IAA @ 7.5ppm + Ethrel 25ppm improved pod yield to 8.4 % in M-13 and 10.7 in SG-99. Sequential spray of Mepiquat chloride @125ppm enhanced yield to the tune of 12.8% in M-13 and 6.6 % in SG-99. These foliar sprays of growth regulating substances have altered the source –sink relationship by diverting the assimilates to the desirable sinks i.e. more number of filled or mature pods. Verma *et al.* (2008 and 2009) have reported the foliar application of aliphatic alcohols had stimulated the mobilization of photosynthates to the kernels. Foliar feeding with salicylic acid has increased yield and yield components as reported in maize (Shehata *et al.*, 2001; Abdel-Wahed *et al.* 2006), wheat (Shakirova *et al.* 2003; Iqbal and Ashtaf, (2006) and recently in groundnut (Jayalakshmi *et al.*, 2010).

REFERENCES

- Abdel-Wahed, M. S. A., Amin, A. A. and EL-Rashad, S. M. 2006. Physiological effect of some bioregulators on the vegetative growth, yield and chemical constituents of yellow maize plants. *J. Agric. Sci.* **2(2)**:149-155.
- Gharib, F. A. 2006. Effect of salicylic acid on growth metabolic activities and oil content of basil and marjoram. *Int. J. Agric. Biol.* **4**:

485-492.

- Hayat, S., Asim, M., Mohammad, Y., Qazi, F. and Aqil, A. 2009. Growth of Indian mustard (*Brassica juncea*L.) in response to salicylic acid under high temperature stress. *Braz. J. Plant Physiol.* **21(3)**: 187-195.
- Hiscox, J. D. and Israelstam, G. F. 1979. A method for the extraction of chlorophyll from leaf tissue without maceration. *Can. J. Bot.* **57**: 1332-1334.
- Iqbal, M. and Ashraf, M. 2006. Wheat seed priming in relation to the salt tolerance, growth, yield and the level of free salicylic acid and polyamines. *Ann. Bot. Fennici.* **43(4)**: 250-259.
- Jaworski, E. G. 1971. Nitrate reductase in the intact tissue. *Biochem. Biophys. Res. Commun.* **43**: 1274-79.
- Jayalakshmi, P., Suvanalatha, D. P., Prasanna, N. D., Revathi, G. 2010. Morphological and physiological changes of groundnut plants by foliar application with salicylic acid. *The Bioscan.* **5(2)**:193-195.
- Malik, C. P. 1995. Plant growth regulators, software for plant development and crop productivity. Presidential address (Botany Section) *Indian Science Congress Association. pp.* 1-35.
- Malik, C. P., Singh, P., Kaur, S., Malik, S., Parmar, U., Grewal, M. and Bhatia, D. S. 1990. Modification of leaf photosynthesis by foliar application of aliphatic alcohols. *J. of. Agon. and Crop Sci.* **165**: 198-201.
- Malik, C. P., Thind, S. K. and Bhatia, D. S. 1995. Altering plant archetype with plant growth regulators and genetic transformation-Biological software in agrobiotechnology *In Agro's Annual Rev. of Plant Physiology.* **2**:13-64.

- Menon, K. K.G. and Srivastava, H. S. 1984.** Increasing plant productivity through improved photosynthesis. *Proc. Ind. Acad. Sci. (Plant Sci.)*. **93**: 359-378.
- Parmar, U., Malik, C. P., Grewal, M. and Bhatia, D. S. 1989.** Flowering pattern and pod development responses in a spreading type of groundnut (cv.M-13) to a monophenol and aliphatic alcohol mixture. *Proc. Ind. Acad. Sci.* **99**:147-153.
- Parmar, U., Kaur, A. and Singh, P. 2003.** Effect of mepiquat chloride e foliar application on dry matter accumulation and setting percentage in groundnut (*Arachis hypogaea L.*) cv. M-335. *J. Pl. Sci. Res.* **19**: 29-32.
- Shakirova, F. M., Sakhabutdinova, A. R., Bezrukova, M. V., Fathkutdinova, R. A. and Fathkutdinova, D. R. 2003.** Changes in the hormonal status of wheat seedling induced by salicylic acid and salinity. *Plant Sci.* **164**: 317.
- Sharma, P. and Malik, C. P. 1994.** Triacylglycerol synthesis in the developing kernel of groundnut as influenced by aliphatic alcohols. *Phytochemistry*. **36(4)**: 899-902.
- Sharma, P., Sardana, V. and Kandhola, S. S. 2011.** Response of groundnut (*Arachis hypogaeaL.*) to Rhizobium inoculation. *Libyan Agri. Res. Center J. Internation.* **2(3)**:101-104.
- Shehata, S. A. M., Ibrahim, S. I. and Zaghlool, S. A. M. 2001.** Physiological response of flag leaf and ears of maize plant induced by foliar application of kinetin (kin) and acetyl salicylic acid (ASA). *Ann. Agric. Sci. Shams. Uni. Cario.* **46(2)**: 435-449.
- Verma, A., Kaur, B., Malik, C. P., Sinsinwar, Y. K. and Gupta, V. K. 2009.** Recent development in Groundnut: Carbon assimilation, Pathology, Molecular Biology. In Crop Breeding and Biotechnology Ed. Malik, C. P., Wadhvani, C. and Kaur, B. Pointer Publishers, Jaipur, pp:161-191.
- Verma, A., Malik, C. P., Sinsinwar, Y. K. and Gupta, V. K. 2008.** Role of some growth regulators on crop physiology parameters influencing productivity in peanut. *J. Pl. Sci. Res.* **24**: 167-170.
- Wang, Z., Yanping, Y. and Xuenhen, S. 1995.** The effect of DPC (N, N-dimethyl piperidinium chloride) on the $^{14}\text{C}_2$ -assimilation and partitioning of the ^{14}C assimilates within the cotton plants inter planted in a wheat stand. *Photosynthetica.* **31**: 197-202.
- Wilson, D. O. and Reisenauer, A. 1963.** Determination of leghaemoglobin in legume nodule. *Analy. Biochem.* **6**: 27-30.

INSTRUCTION TO AUTHORS

The Bioscan

An International Quarterly Journal of Life Science

THE JOURNAL

The Bioscan is an international quarterly journal of life sciences with international editorial board. The journal is online and details can be seen (downloaded from the site. www.thebioscan.in). For any query e-mail at m_psinha@yahoo.com & dr.mp.sinha@gmail.com can be used.

AIM & SCOPE

The journal aims to publish original peerly reviewed/ refereed research papers/reviews on all aspects of life sciences.

SUBMISSION OF MANUSCRIPT

Only original research papers are considered for publication. The authors may be asked to declare that the manuscript has not been submitted to any other journal for consideration at the same time. Two hard copies of manuscript and one soft copy, complete in all respects should be submitted. The soft copy can also be sent by e-mail as an attachment file for quick processing of the paper.

FORMAT OF MANUSCRIPT

All manuscripts must be written in English and should be typed double-spaced with wide margins on all sides of good quality A4 paper.

First page of the paper should be headed with the title page, (in capital, font size 16), the names of the authors (in capitals, font size 12) and full address of the institution where the work was carried out including e-mail address. A short running title should be given at the end of the title page and 3-5 key words or phrases for indexing.

The main portion of the paper should be divided into Abstract, Introduction, Materials and Methods, Results, Discussion (or result and discussion together), Acknowledgements (if any) References and legends.

Abstract should be limited to 200 words and convey the main points of the paper-outline, results and conclusion or the significance of the results.

Introduction should give the reasons for doing the work. Detailed review of the literature is not necessary. The introduction should preferably conclude with a final paragraph stating concisely and clearly the aims and objectives of your investigation.

Materials and Methods should include a brief technical description of the methodology adopted while a detailed description is required if the methods are new.

Results should contain observations on experiment done illustrated by tables and figures. Use well known statistical tests in preference to obscure ones.

Discussion must not recapitulate results but should relate the author's experiments to other work on the subject and give their conclusions.

All tables and figures must be cited sequentially in the text. Figures should be abbreviated to Fig., except in the beginning of a sentence when the word Figure should be written out in full.

The figures should be drawn on a good quality tracing/ white paper with black ink with the legends provided on a separate sheet. Photographs should be black and white on a glossy sheet with sufficient contrast.

References should be kept to a minimum and listed in alphabetical order. Personal communication and unpublished data should not be included in the reference list. Unpublished papers accepted for publication may be included in the list by designating the journal followed by "in press" in parentheses in the reference list. The list of reference at the end of the text should be in the following format.

1. **Witkamp, M. and Olson, J. S. 1963.** Breakdown of confined and non-confined Oak Litter. *Oikos*. **14**:138-147.
2. **Odum, E.P. 1971.** *Fundamentals of Ecology*. W. B. Sauder Co. Publ. Philadelphia.p.28.
3. **Macfadyen, A.1963.** The contribution of microfauna to total soil metabolism. In:*Soil organism*, J. Doeksen and J. Van Der Drift (Eds). North Holland Publ. Comp., pp 3-16.

References in the text should be quoted by the **author's name and year** in parenthesis and presented in year order. When there are more than two authors the reference should be quoted as: first author followed by *et al.*, throughout the text. Where more than one paper with the same senior author has appeared in on year the references should

Cont. P. 22