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

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

ABSTRACT
Regulation of the plant metabolism by exogenous growth regulating substances offers new possibilities in 
circumventing environmental limitations, relaxing genetic restrains, 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 archetypae, 
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 
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:

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Mepiquat chloride @125ppm+ Ethrel 25ppm</th>
<th>Mepiquat chloride @125ppm+ Mepiquat chloride 1.25ppm</th>
<th>Mepiquat chloride @125ppm</th>
<th>Ethrel 25ppm</th>
<th>Water spray</th>
<th>Control (no spray)</th>
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</thead>
<tbody>
<tr>
<td>T&lt;sub&gt;1&lt;/sub&gt;</td>
<td>IAA@ 5ppm + Ethrel 25ppm</td>
<td>Mepiquat chloride @125ppm + Mepiquat chloride 1.25ppm</td>
<td>Mepiquat chloride @125ppm</td>
<td>Ethrel 25ppm</td>
<td>Water spray</td>
<td>Control (no spray)</td>
</tr>
<tr>
<td>T&lt;sub&gt;2&lt;/sub&gt;</td>
<td>IAA@ 7.5ppm + Ethrel 25ppm</td>
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<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>T&lt;sub&gt;3&lt;/sub&gt;</td>
<td>Mepiquat chloride @125ppm + Mepiquat chloride 1.25ppm</td>
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</tr>
<tr>
<td>T&lt;sub&gt;4&lt;/sub&gt;</td>
<td>Mepiquat chloride @125ppm</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T&lt;sub&gt;5&lt;/sub&gt;</td>
<td>Ethrel 25ppm</td>
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</tr>
<tr>
<td>T&lt;sub&gt;6&lt;/sub&gt;</td>
<td>Water spray</td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T&lt;sub&gt;7&lt;/sub&gt;</td>
<td>Control (no spray)</td>
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</table>

**Observations**

**Photosynthetic pigments:** Chlorophyll content was estimated at two times of the day i.e. 9 A.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 (chl/a/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 (T<sub>1</sub>), Chl b was comparable in Mepiquat chloride @125ppm + Ethrel 25ppm (T<sub>2</sub>) 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 25ppm (T<sub>1</sub>) with respect to control.(Fig 1). In bunch type of cultivar SG-99, Chl a was more with IAA@ 5ppm + Ethrel 25ppm (T<sub>1</sub>) and Mepiquat chloride @125ppm + Ethrel 25ppm (T<sub>2</sub>) than with water spray and control. The activity was higher in leaves and nodules of both the cultivars where SA at 10<sup>-5</sup>M 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@ 5ppm + 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<sup>-5</sup>M) 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 higher than the control with Mepiquat chloride @125ppm+Ethrel 25ppm (T<sub>1</sub>) foliar spray. Total chlorophyll also increased with both the IAA concentrations and Mepiquat chloride @125ppm + Mepiquat chloride l 25 ppm (T<sub>1</sub>). 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 (T<sub>1</sub>) however with Mepiquat chloride @125ppm + Mepiquat chloride l 25ppm (T<sub>2</sub>) Chl b enhanced whereas chl a/chl b was comparable in IAA@ 5ppm + Ethrel 25ppm (T<sub>1</sub>) 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 (T<sub>1</sub>) increased Chl a in cultivar M-13 and SG-99. Mepiquat chloride @125ppm + Ethrel 25ppm (T<sub>2</sub>) 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 (T<sub>3</sub>), Mepiquat chloride @125ppm + Mepiquat chloride l 25ppm (T<sub>4</sub>) and Mepiquat chloride @125ppm + Ethrel 25ppm (T<sub>5</sub>) improved this component in SG-99 as depicted in Fig. 2. Total chlorophyll content improved with IAA@ 5ppm + Ethrel 25ppm (T<sub>1</sub>) and Mepiquat chloride@ 125ppm + Ethrel 25ppm(T<sub>5</sub>) 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 marjanorplants where SA at 10<sup>-5</sup>M stimulated total chlorophyll synthesis and 10<sup>-5</sup>M had a reverse effect.
Table 1: Effect of foliar sprays on the nitrate reductase activity and leghaemoglobin content in cultivar M-13 and SG-99 at 60 DAS

<table>
<thead>
<tr>
<th>No.</th>
<th>Treatments</th>
<th>Nodules Nitrate reductase (μmole/h/g F.W.)</th>
<th>Leaves Leghaemoglobin content (mg /g nodule wt.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>SG-99 M-13 Mean</td>
<td>SG-99 M-13 Mean</td>
</tr>
<tr>
<td>T1</td>
<td>IAA@5ppm + Ethrel 25ppm</td>
<td>0.471 0.518 0.494 1.68 1.22 1.44 1.74 1.39 1.66</td>
<td></td>
</tr>
<tr>
<td>T2</td>
<td><a href="mailto:IAA@7.5ppm">IAA@7.5ppm</a> + Ethrel 25ppm</td>
<td>0.460 0.612 0.536 1.36 1.05 1.2 1.38 1.68 1.52</td>
<td></td>
</tr>
<tr>
<td>T3</td>
<td>Mep.chloride@125ppm + Mep.chloride 125ppm</td>
<td>0.440 0.565 0.503 1.22 1.56 1.39 1.39 1.35 1.37</td>
<td></td>
</tr>
<tr>
<td>T4</td>
<td>Mep.chloride @125ppm + Ethrel 25ppm</td>
<td>0.408 0.469 0.438 1.04 1.70 1.36 0.899 1.82 1.36</td>
<td></td>
</tr>
<tr>
<td>T5</td>
<td>Water spray</td>
<td>0.549 0.581 0.568 1.74 2.52 2.13 1.52 1.73 1.62</td>
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</tr>
<tr>
<td>T6</td>
<td>Control (No spray)</td>
<td>0.487 0.533 0.510 1.29 2.63 1.96 1.40 1.59 1.49</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>0.469 0.546 1.38 1.78 1.38 1.62</td>
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</tbody>
</table>
| C.D@5% | Var (V)=0.033  Sprays Var (V)=0.102 Sprays Var(V)=NS | Sprays Var(V)=NS Sprays Var(V)=NS Sprays Var(V)=NS Sprays |}

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

<table>
<thead>
<tr>
<th>No.</th>
<th>Treatments</th>
<th>M-13 Total % of pods per plant (kg/ha)</th>
<th>SG-99 Total % of pods per plant (kg/ha)</th>
<th>Mature Yield</th>
<th>Immature Yield</th>
<th>Mature %</th>
<th>Immature %</th>
<th>Mature %</th>
<th>Immature %</th>
<th>Mature %</th>
<th>Immature %</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>IAA@5ppm + Ethrel 25ppm</td>
<td>45.4 57.4 42.5 1956 61.8 72.8 27.2 2532</td>
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<td></td>
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<tr>
<td>T2</td>
<td><a href="mailto:IAA@7.5ppm">IAA@7.5ppm</a> + Ethrel 25ppm</td>
<td>39.8 63.3 36.6 2028 59.4 74.1 25.9 2722</td>
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<tr>
<td>T3</td>
<td>Mep.chloride@125ppm + Mep. chloride 125ppm</td>
<td>36.1 63.9 36.0 2111 65.2 75.5 24.5 2621</td>
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<tr>
<td>T4</td>
<td>Mep.chloride @125ppm + Ethrel 25ppm</td>
<td>37.8 60.9 38.1 1972 57.2 72.2 27.8 2635</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>T5</td>
<td>Water spray</td>
<td>44.2 58.1 41.8 2046 56.2 74.7 25.3 2741</td>
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<tr>
<td>T6</td>
<td>Control (No spray)</td>
<td>40.4 56.2 40.8 1870 58.0 72.4 27.6 2458</td>
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<tr>
<td></td>
<td>C.D@5%</td>
<td>1.2 1.44 0.786 170.8 1.3 1.19 0.431 94.9</td>
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</table>

Figure 1: Variation in chlorophyll content at different times of the day in M-13 and SG-99
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 alcohals 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 Ashraf, 2006) and recently in groundnut (Jayalakshmi et al., 2010).

REFERENCES


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