Influence of nutrient levels and plant growth regulators on relative chlorophyll content (SPAD value), days to 50% flowering and nodulation of soybean (Glycine max)

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Abstract
A field experiment was conducted to study the influence of nutrient levels and foliar application of plant growth regulators on relative chlorophyll content (SPAD value), days to 50% flowering and nodulation of soybean [Glycine max (L.) Merrill] during kharif 2017. The experiment was laid out using randomized complete block design (factorial concept) with 14 treatments including control and replicated thrice. The treatments consisted of two nutrient levels 125% RDF and 100% RDF, six plant growth regulator (PGR) dosages: salicylic acid @ 50 and 100 ppm, ethrel @ 100 and 200 ppm, chlormequat chloride (CCC) @ 250 and 500 ppm; independent control: RPP, without PGR spray and RPP + KNO₃ @ 1%. PGRs were sprayed at 25 and 40 DAS. Results shows that application of 125% RDF + chlormequat chloride @ 500 ppm at 25 and 40 days after sowing (DAS) as foliar spray recorded significantly higher relative chlorophyll content (SPAD value) compared to other treatment combinations and control, followed by 125% RDF + ethrel @ 200 ppm. Numerically application of ethrel (200 ppm) initiated early flowering (42.9 days for 50% flowering) in soybean. 125% RDF treatment statistically observed higher number of nodules and nodule dry weight on per plant basis.

Keywords: Chlormequat chloride, ethrel, SPAD value, nodule, flowering, soybean

Introduction
The soybean [Glycine max (L.) Merril] is also called as “Miracle crop” is a source of protein for human beings, animal feeds and many prepackaged meals. It is excellent in its nutritive value with enhanced protein (40-42%) and oil (20%) content and is also rich in vitamins, minerals, salts and other essential amino acids (Dass et al., 2018) [2]. In India it is grown over an area of 11.25 million hectare with production of 11.73 million tonnes and productivity of 1042 kg ha⁻¹ (Anon., 2018) [1]. Soybean is characterized by prolific flower production with an extremely low proportion of pod set. The extent of flower shedding is said to be 60-92% in soybean (Nahar and Ikeda, 2002) [4], which causes low yield. Plant growth regulators are well known to improve the source-sink connection and encourage the translocation of photosynthates thereby helping in effective flower formation, fruit, and seed development and ultimately increase the yield of crops. Some of the growth regulators like salicylic acid, ethrel and chlormequat chloride (cycoel) may play a greater role to increase the growth and yield attributing characters of soybean. Salicylic acid is an endogenous growth regulator of phenol nature, which participates in regulation of physiological processes in plant, stomata closure, ion uptake, inhibition of ethylene biosynthesis, transpiration and stress tolerance (Khan et al., 2003) [3]. And also its application increase carbon dioxide (CO₂) and assimilation and photosynthetic rate, thus increasing dry matter. Ethylene released from ethrel (2-Chloro ethyl phosphonic acid) could possibly be utilized for promoting pod growth and early pod development in chickpea and tomato are related to higher ethylene levels, thus decreasing flower and pod shedding and thereby reducing abscission and improving better pod set. Ethrel induced increase in cell division, resulting in increased fruit size and yield have been reported in tomato fruits. Chlormequat chloride (CCC) or Cycoel is a plant growth regulator and known as antagonist of the plant hormone gibberellin. It acts by inhibiting gibberellin biosynthesis, reducing intermodal growth to give stouter stems, enhanced root growth.
causing early fruit set and increasing seed set in plants. Shinde (2015) [7] also observed that the application of PGRs viz., Progibb (20, 40 and 60 ppm), CCC (500 and 1000 ppm) and TIBA (100 and 200 ppm) hastened the days to flower initiation and fifty per cent flowering in soybean.

Considering the importance of above stated points a field experiment was conducted with an objective to know the influence of nutrient levels and plant growth regulators on relative chlorophyll content (SPAD value), days to 50% flowering and nodulation of soybean [Glycine max (L.) Merrill].

Materials and Methods
A field study was conducted at University of Agricultural Sciences, Dharwad, Karnataka on medium deep black soil during kharif 2017. The experiment was laid out using randomized complete block design (factorial concept) with nutrient levels (N₁): 100% RDF (40:80:25 N:P:K kg ha⁻¹) and (N₂): 125% RDF (50:100:31.2 N:P:K kg ha⁻¹); Plant growth regulators (G₁): Salicylic acid @ 50 ppm, (G₂): Salicylic acid @ 100 ppm, (G₃): Ethrel @ 100 ppm, (G₄): Ethrel @ 200 ppm, (G₅): Chlormequat chloride @ 250 ppm and (G₆): Chlormequat chloride @ 500 ppm; Controls (C₁): Independent control RPP-without plant growth regulator spray and (C₂): RPP + KNO₃ @ 1% foliar spray. The experiment consisted of twelve treatment combinations (Table 1) and replicated thrice. The N, P₂O₅ and K₂O were applied as per the treatments to each plot in the form of Urea, SSP and MOP at the time of sowing along with gypsum at the rate of 100 kg ha⁻¹. Plant growth regulators were sprayed in different concentration as per the treatment at 25 and 40 DAS on 1st Aug 2017 and 16th Sept 2017, respectively. During the crop growth period, a total rainfall of 582.8 mm was received which was optimum for good growth and higher yield. The soil of the experimental site was clay with pH of 7.02 and electrical conductivity of 0.29 ds m⁻¹. The soil was medium in organic carbon (0.51%) and low in available nitrogen (258.5 kg ha⁻¹) and medium in available P (32.5 kg ha⁻¹) and available K (285.5 kg ha⁻¹). The land was ploughed by tractor once after the harvest of previous crop followed by harrowing twice. At the time of sowing, the land was prepared to a fine seed bed, FYM was added at the rate of 5 tonnes per hectare and the plots were laid out as per the plan of layout of the experiment. Gross plot size and net plot size were 4.0 × 3.6 m and 3.8 × 3.0 m, respectively. Soybean variety DSh-21 seeds were treated using 1250 g each Rhizobium and PSB per hectare seeds. Two seeds per hill were dibbled 5 cm deep in furrows at a spacing of 30 × 10 cm on 06th July 2017. The seed rate used was 62.5 kg ha⁻¹. Two protective irritations were given to the crop at 2nd and 4th week of August 2017 as rainfall received was less. Relative chlorophyll content (SPAD value) was measured using SPAD meter in randomly selected five fully opened leaves from the top of plants and value was averaged. The number of days taken for 50% flower production was recorded by counting the days from sowing to the date at which the plants produced 50% of flowers in each treatment. The number of effective root nodules was counted at 50 DAS in randomly selected five plants. The plants were carefully removed from the soil without damaging the roots, and roots were dipped gently in a bucket containing tap water to remove the adhering soil and then nodules were counted. After counting the number of nodules per plant, nodules were oven dried at 70 °C for two days and dry weight was recorded and expressed as g per plant.

Table 1: Influence of nutrient levels and plant growth regulators on relative chlorophyll content (SPAD value), days to 50% flowering and nodulation of soybean

| Treatments | Nutrient levels | Days to 50% flowering | Number of nodules plant⁻¹ | Dry weight of nodules plant⁻¹ (g) |
|------------|----------------|-----------------------|---------------------------|----------------------------------|
|            | 30 DAS | 60 DAS | | |
| N₁ | 32.00 | 41.89 | 43.81 | 31.49 | 0.52 |
| N₂ | 33.40 | 42.80 | 43.50 | 33.08 | 0.55 |
| S. Em. ± | 0.22 | 0.17 | 0.50 | 0.43 | 0.01 |
| C. D. at 5% | 0.66 | 0.49 | NS | 1.32 | 0.02 |
|            |  |  |  |  |  |
| G₁ | 31.52 | 41.53 | 44.18 | 31.72 | 0.51 |
| G₂ | 32.05 | 41.96 | 44.04 | 32.00 | 0.52 |
| G₃ | 32.10 | 41.81 | 43.44 | 32.11 | 0.52 |
| G₄ | 33.84 | 42.98 | 42.90 | 32.47 | 0.54 |
| G₅ | 32.20 | 41.38 | 44.00 | 32.37 | 0.54 |
| G₆ | 34.48 | 44.42 | 43.42 | 33.06 | 0.55 |
| S. Em. ± | 0.39 | 0.29 | 0.86 | 0.75 | 0.01 |
| C. D. at 5% | 1.14 | 0.85 | NS | NS | NS |
|            |  |  |  |  |  |
| N₁ | 30.87 | 41.27 | 44.33 | 30.88 | 0.49 |
| N₂ | 31.30 | 41.72 | 44.07 | 31.04 | 0.51 |
| N₃ | 31.83 | 41.42 | 43.86 | 31.36 | 0.51 |
| N₄ | 32.80 | 42.86 | 43.08 | 31.68 | 0.52 |
| N₅ | 31.40 | 41.08 | 44.08 | 31.72 | 0.53 |
| N₆ | 33.77 | 43.02 | 43.51 | 32.28 | 0.54 |
| N₇ | 32.17 | 41.78 | 44.03 | 32.55 | 0.53 |
| N₈ | 32.80 | 42.21 | 44.01 | 32.95 | 0.54 |
| N₉ | 32.37 | 42.19 | 43.01 | 32.86 | 0.54 |
| N₁₀ | 34.88 | 43.11 | 42.72 | 33.25 | 0.56 |
| N₁₁ | 32.99 | 41.68 | 43.91 | 33.01 | 0.54 |
| N₁₂ | 35.19 | 45.83 | 43.34 | 33.84 | 0.57 |
| S. Em. ± | 0.55 | 0.41 | 1.22 | 1.06 | 0.02 |
| C. D. at 5% | NS | 1.21 | NS | NS | NS |
|------------|----|------|----|----|----|
| Control    |    |      |    |    |    |
| C₁         | 29.73 | 39.94 | 46.10 | 30.76 | 0.50 |
| C₂         | 30.78 | 40.35 | 45.31 | 31.77 | 0.52 |
| S. Em. ±   | 0.54 | 0.47 | 1.20 | 1.07 | 0.02 |
| C. D. at 5% | 1.56 | 1.37 | NS | NS | NS |

$N_{1} = 100\%$ RDF (40:80:25 N:P:O₂:K₂O kg ha$^{-1}$) | $G_{1} =$ Ethrel @ 100 ppm | $C_{1} =$ RPP, without plant growth regulator
$N_{2} = 125\%$ RDF(50:100:31.25 N:P:O₂:K₂O kg ha$^{-1}$) | $G_{1} =$ Ethrel @ 200 ppm | $C_{2} =$ RPP + KNO₃ @ 1% (Foliar spray)
$G_{1} =$ Salicylic acid @ 50 ppm | $G_{2} =$ Chlormequat chloride @ 250 ppm | RDF = Recommended dose of fertilizer
$G_{2} =$ Salicylic acid @ 100 ppm | $G_{3} =$ Chlormequat chloride @ 500 ppm | RPP = Recommended package of practice
Note: Spray at 25 and 40 DAS | NS = Non - significant

### Results and Discussion

#### Relative chlorophyll content (SPAD value)

The data on relative chlorophyll content (SPAD value) as influenced by nutrient levels and plant growth regulators are presented in Table 1. At 30 and 60 DAS, among the various plant growth regulators significantly higher chlorophyll content was recorded with application of 125% RDF (33.40 and 42.80, respectively) over 100% RDF (32.00 and 41.89, respectively). With respect to different plant growth regulators significantly higher chlorophyll content was recorded with application of chlormequat chloride @ 500 ppm (34.48 and 44.42, respectively). The combined application of ethrel (200 ppm) and chlormequat chloride @ 500 ppm treatment combination significantly recorded higher SPAD reading (45.83) at 60 DAS followed by 100% RDF + ethrel @ 200 ppm (43.11) over control [C₁] (39.94). Whereas, interaction effect at 30 DAS was found to be non-significant. Control treatments (C₁ and C₂) were on par to each with respect to SPAD value at 30 (29.73 and 30.78, respectively) and 60 DAS (39.94 and 40.35, respectively). Higher SPAD values may be due to the increase in greenness and chlorophyll content. Similarly Singh and Sarkar (1976) and Raksha (2010) reported higher SPAD value due application of cycoceil in soybean.

#### Days to 50% flowering

With respect to different levels of nutrient, plant growth regulators and interaction between the treatments were found to be non-significant with respect to days to 50% flowering. However, numerically application of ethrel (200 ppm) initiated early flowering (42.9 days to 50% flowering) in soybean and statistically higher number of nodules and nodule dry weight on per plant basis were observed with 125% RDF treatment.

#### Nodulation

The data on number of nodules plant$^{-1}$ as influenced by nutrient levels and plant growth regulators are presented in Table 1. Application of 125% RDF recorded significantly higher number nodules plant$^{-1}$ (33.08) and dry weight nodules plant$^{-1}$ (0.55 g) at 50 DAS compared to 100% RDF (31.49 and 0.52 g, respectively). There was no significant difference observed with plant growth regulator treatments and interaction effect of nutrient levels and plant growth regulators application on number of nodules and nodule dry weight at 50 DAS. Number of nodules plant$^{-1}$ recorded in control treatments C₁ (RPP, without plant growth regulators) and C₂ (RPP + KNO₃ @ 1%) were on par to each other. The number of nodules per plant and dry weight of nodules per plant (g) indicate the N fixing ability. Nodule number of soybean was significantly influenced by nutrient levels. So, application of 125% RDF recorded higher nodules number per plant (33.08) and dry weight of nodules (0.55 g). These results are in line with the findings of Son et al. (2006) and Nastasija et al. (2008).

### Conclusion

The results confirm that relative chlorophyll content (SPAD value) was significantly increased with the combined application 125% RDF + chlormequat chloride @ 500 ppm at 25 and 40 DAS as foliar spray as compared to other treatment combinations and control. Numerically application of ethrel (200 ppm) initiated early flowering (42.9 days to 50% flowering) in soybean and statistically higher number of nodules and nodule dry weight on per plant basis were observed with 125% RDF treatment.

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