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Impact of Foliar Spray of PGR Nutrient Consortium on Growth, Photosynthesis and Yield of Horsegram (*Macrotyloma uniflorum* Lam) under Rainfed Condition

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ABSTRACT

Background: Horsegram is considered as poor man's crop and it has high nutritive value for human being. Apart from its photo and thermo sensitive in nature, formation of tendrils with excessive vegetative growth is major constraints and reason for poor yield. The tendrils act as sink and utilize photo-assimilates for its continuous growth. Hence, a strategy is required for reduction of vegetative growth and tendril formation for yield improvement. The current study is aimed to enhancement of yield in horsegram through physiological approach.

Methods: An experiment was conducted to study the impact of plant growth regulators and nutrients *viz.,* brassinolide (1 ppm), CCC (250 ppm), nutrient consortium (K_2SO_4 (0.5%) + MAP (0.5%) + $FeSO_4$ (0.5%) + boric acid (0.3%) and TNAU Horsegram Wonder (1%) on growth, physiological traits and yield of horsegram *(Macrotyloma uniflorum)* variety Paiyur 2 under rainfed condition during 2018-2020. Plant growth regulators and nutrient consortium were used as foliar spray at flowering stage (50 days after sowing) under field condition.

Result: TNAU Horsegram Wonder showed supremacy to enhance photosynthetic rate, SPAD value, soluble protein and yield compared to other treatments. Early flowering and reduced number of tendrils were observed in CCC nutrient consortium and TNAU Horsegram Wonder treatments. Foliar spray of 1% TNAU Horsegram Wonder recorded highest SPAD value of 22.8 which is on par with BL nutrient consortium. Highest photosynthetic rate of 16.94 µmol m^{-2} s^{-1} and lowest number of tendrils (2.6) were registered by 1% TNAU Horsegram Wonder. Foliar application of TNAU Horsegram Wonder at flowering stage registered highest grain yield of 1090 kg ha^{-1} and increased yield of 23% over control with BC ratio of 2.24.

Key words: Horsegram, Photosynthetic rate, Tendrils, TNAU horsegram wonder, Yield.

INTRODUCTION

Horsegram known as poor men's crop and a crop of poor resources is widely grown in India in almost 200-700 mm rainfall regions. Hence, called drought hardy, typically adapted to a wide range of soils. Horsegram is considered as a miracle super food widely consumed as a staple diet in the southern parts of India. It has rich protein (44%) and plenty of minerals too - iron (87.5%), phosphorus (44.4%) and calcium (28.7%). Horsegram is not as popular as blackgram, greengram and redgram, but still it is one of the most nutritious legumes. It has natural qualities that work as fat burners and can reduce the LDL cholesterol and increase the HDL cholesterol (Sathiskumar *et al.,* 2013).

The US National Academy of Sciences has identified that horsegram as a promising food source for the future and to meet food and nutritional requirements in malnourished populations. Hence, it is known to be very beneficial for human health, as much as it is useful for animal health too. Among over dozen of pulses crop grown in India, it ranks third in area covering 17.02 lakh hectares with an annual production of 7.19 lakh tonnes. The national average productivity of horse gram is 494 kg ha^{-1} (Suthar *et al.,* 2017). However, its photo and thermo-sensitive nature, it does not permit its horizontal expansion in non-traditional and remote regions and considered as a major production constraint.

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Its excessive vegetative growth, results in the formation of tendrils is another major constraint which cause poor pod set, lower yield and harvest index. The tendrils act as a sink and affect the better source-sink relationship. Greater proportion of photo-assimilates is diverted for production and maintenance of vegetative plant parts (tendril in horsegram) rather than translocation to reproductive parts leads to more plant height (Secondo and Reddy, 2018). Based on the above statement, it is clearly indicated that diversion of photosynthates to tendril which is vegetative part of horsegram can reduce the economic yield. Reduce the formation of number of tendrils in horsegram can achieve

the optimum vegetative growth. It enhances better translocation of photosynthates in to developing pods in which plant growth regulators and nutrients play an excellent tool for productivity enhancement.

The potentiality of horsegram has not been exploited in India due to growing in rainfed situation and the use of plant growth regulators and nutrients is meagre. And also the source and sink alteration has not taken up in horsegram by using plant growth regulators and nutrients. Application of plant growth regulators and nutrients change both morphology and physiology of plants.

CCC known as 2 chloro ethyl trimethyl ammonium chloride has been most widely used for reduce plant height and enhance distribution of assimilates to sink (Emam, 2011). Kothule *et al.* (2003) found that the foliar application of CCC 200 ppm reduced the number of days to 50 percent flowering in soybean. Sengupta *et al.* (2011) found that foliar spray of 0.5 ppm brassinolide significantly improved dry matter accumulation in greengram. Foliar application of potassium stimulated flowering in Tommy Atkins mango (Yeshitela *et al.,* 2005) under unfavorable situation.

Brahmachari *et al.* (1996) reported that all the growth substances showed positive result in fruit yield and quality of guava. However, CCC (500 ppm) induced the earliest flowering and recorded highest number of flowers, fruit set and yield. Foliar spray of $FeSO_4$ at 0.5% on 25 days after sowing registered maximum yield in greengram (Saini and Singh, 2017). Boron is an important for pollen germination and pollen tube development and ultimately it boosts flowering and fruit setting. Suganiya *et al.* (2015) reported that the foliar application of boron increased the number of flower buds in brinjal.

TNAU (Tamil Nadu Agricultural University) Horsegram Wonder is a PGR (CCC) and nutrient consortium (K, P, Fe and B) developed by Tamil Nadu Agricultural University which contains essential nutrients and PGR required for horsegram. Hence, the experiment was conducted to study the performance of different plant growth regulator nutrient consortium along with TNAU Horsegram Wonder on yield of horsegram by assessing growth parameters, physiological traits and yield of horsegram under rainfed condition.

MATERIALS AND METHODS

The study was conducted at Regional Research Station, Tamil Nadu Agricultural University, Paiyur, Tamil Nadu during *rabi* season of 2019. The experiment was carried out by using horsegram variety Paiyur 2 seeds, sown directly to the field with the spacing of 30 × 10 cm after the receipt of sufficient rainfall. Brassinolide (BL), CCC with nutrient solutions were prepared separately and combinations along with TNAU Horsegram Wonder applied as foliar spray at 50 days after sowing and the control was maintained with water spray. The experiment was carried out with one variety imposed with seven treatments, replicated thrice and adopted statistics of randomized block design.

Plant growth regulators like brassinolide (1 ppm), CCC (250 ppm), mixed with nutrients like K_2SO_4 (0.5%), boric acid (0.3%), MAP (0.5%), $FeSO_4$ (0.5%) and TNAU Horsegram Wonder (1%) were used as foliar spray at 50 days after sowing. In this study, the growth parameters like plant height, leaf area, days to flowering, number of tendrils, and physiological traits like photosynthetic rate, SPAD value, soluble protein, NR activity and yield of horsegram were estimated under rainfed condition.

Measurement of growth parameters

Plant height was measured from the ground level to the tip of the growing point. Leaf area per plant was measured using a leaf area meter (LICOR, Model LI 3000) and using the formula of dry weight method expressed as cm^2 $plant^{-1}$. Number of tendrils per plant was counted manually and average was taken. Days to flowering was taken at which 50% of the plants in the plot started flowering.

Measurement of photosynthetic rate

Photosynthetic rate measurement was performed by using Portable Photosynthesis System (PPS) (Model LI-6400 of LICOR inc., Lincoln, Nebraska, USA) equipped with a halogen lamp (6400-02B LED) positioned on the cuvette. Third leaf from top was used for the measurements with replicated thrice. Leaf was inserted in a 3 cm^2 leaf chamber and PPFD at 1200 µmol photons m^{-2} s^{-1} and relative humidity (50-55%) were set. The readings were taken between 9 am to 11.30 am and the value is expressed as µmol CO_2 m^{-2} s^{-1}.

SPAD value

Chlorophyll content can be assessed indirectly through the mini instrument SPAD meter. SPAD readings were recorded by using chlorophyll meter (SPAD 502) designed by the soil plant analytical development (SPAD) section, Minolta, Japan. The Minolta SPAD-502 measures chlorophyll content as ratio of transmittance of light at wavelength of 650 nm and 940 nm. Five readings were taken from each replication and the average value was computed using the method described by Monje and Bugbee (1992).

Soluble protein

Soluble protein content of leaf was estimated as per the method of Lowry *et al.* (1951). 250 mg of leaf sample was weighed and macerated with 10 ml of phosphate buffer solution. The content was centrifuged at 3000 rpm for 10 minutes and the supernatant was collected and made up to 25 ml. 1 ml of the supernatant was pipette out to a test tube and 5 ml of alkaline copper tartarate reagent and 0.5 ml of folin reagent were added. The colour intensity was measured at 660 nm in spectrophotometer and the amount of soluble protein present in the sample was calculated by using bovine serum albumin as standard and expressed as mg g^{-1} fresh weight.

Nitrate reductase activity

Leaf samples were kept in 10 ml of assay medium containing substrate (1% KNO_3) for 1 hour (Nicholas *et al.,* 1976). After 1 hour, amount of NO_2 produced by the enzyme was

quantified by adding sulphanilamide and naphthyl ethylene diamine dihydrochloride (NEDD). The absorbance was measured at 540 nm. The quantity of NO_2 produced by the enzyme was calculated using standard curve and expressed as µg NO_2 g^{-1} hr^{-1}.

Yield

The total weight of grains harvested from each plot of replications was measured and average yield per plot was worked out and expressed in kg per plot. The plot yield recorded after harvest, the estimated yield was calculated and expressed in terms of kilogram per hectare. The data on various parameters were analyzed statistically as per the procedure suggested by Gomez and Gomez (1984).

RESULTS AND DISCUSSION

Plant height is an important parameter that determines the growth and development of a plant. Indeterminate increase in plant height is an undesirable issue that utilizes more assimilates for increased plant height and production of more leaves. Hence, optimum plant height is desirable for higher productivity especially in indeterminate crops. In the present study, highest plant height of 39.8 cm was recorded in foliar spray of brassinolide nutrient consortium (BL + MAP + K_2SO_4 + boric acid + $FeSO_4$) while lowest (32.7 cm) was registered in foliar spray of CCC nutrient consortium (CCC + K_2SO_4 + boric acid) (Table 1). The increased plant height by BL nutrient consortium might be due to BL act as growth promoting hormone and the nutrients supports the growth especially by potassium and iron. The decreased plant height by CCC nutrient consortium might be due to CCC act as growth retardant leads to reduced plant height. However, the decreased plant height by the application of PGR nutrient consortium is not covert the indeterminate to determinate. The PGR nutrient consortium is effective for 25 to 30 days which is coincide with the pod formation and pod development stages. During this time, maximum photosynthates are utilized by the reproductive parts rather than vegetative parts. The reduction of vegetative growth at critical stage like flowering and pod development stages are important for the enhancement of yield and harvest index of the crop.

Rademacher (2000) reported that CCC is a plant growth retardant regulating the plant height physiologically mainly through reducing cell elongation and cell division. Plant growth retardants reduce unwanted shoot elongation through inhibition of the formation of growth active gibberellins (Mansuroglu *et al.,* 2009). Present study corroborated with earlier findings. However, moderate plant height of 35.6 cm was observed in TNAU Horsegram Wonder. This might be due to the application of CCC act as growth retardant along with potassium, iron and boron which are act as growth nutrients and enzyme activators. Lodeta *et al.* (2010) reported that the application of CCC reduced the plant height in Poinsettia.

Leaf area determines the light interception capacity of the crop which decides the dry matter production. The maximum leaf area of 408.4 cm^2 $plant^{-1}$ was recorded by BL nutrient consortium followed by TNAU Horsegram Wonder (358.40) and minimum was recorded in CCC nutrient consortium (318.8) compared to control (326.10) (Table 1). The declined leaf area by the application of CCC nutrient consortium might be due to reduced plant height leads to reduced number of leaves. However, the increment of leaf area by the application of TNAU Horsegram Wonder over control might be due to the action of additional nutrients like iron and phosphorus. Kumar *et al.* (2019) found that the application cycocel reduced the plant height and leaf area in *Nerium odorum*. Present study corroborated with earlier findings.

Under rainfed condition, days to flowering was negatively correlated with grain yield. Delayed flowering under rainfed is a strong indication of susceptibility to any stress (Hanamaratti and Salimath, 2012). Induction of earlier flowering is an important physiological trait which directly correlated with the grain yield especially under rainfed condition. In the present study, lowest number of days taken to flowering (43.6) was recorded in foliar spray of CCC nutrient consortium followed by TNAU Horsegram Wonder (44.3) and highest number in control (47.7) and also BL combination (Table 1). Application of CCC nutrient consortium induces earlier flowering by 4.1 days followed by TNAU Horsegram Wonder by 3.4 days compared to control. Foliar application of cycocel induced early emergence of inflorescences approximately 10 days earlier in chrysanthemum was reported by Qureshi *et al.* (2018). The present study corroborated with earlier finding.

Table 1: Impact of PGR nutrient consortium on growth traits and SPAD value in horsegram.

Treatments	Plant height (cm)	Leaf area (cm^2 $Plant^{-1}$)	Days to flowering	SPAD value
Control	33.9	326.1	47.7	20.2
BL + K_2SO_4 + boric acid	36.8	348.7	46.7	20.7
CCC + K_2SO_4 + boric acid	32.7	318.8	43.6	21.4
MAP + K_2SO_4 + boric acid	33.2	332.4	47.0	20.9
MAP + K_2SO_4 + $FeSO_4$ + boric acid	35.5	363.3	47.3	21.8
BL + MAP + K_2SO_4 + boric acid + $FeSO_4$	39.8	408.4	47.7	22.2
TNAU Horsegram Wonder	35.6	358.4	44.3	22.8
SEd	0.68	8.16	0.85	0.42
CD (P=0.05)	1.42	17.46	1.82	0.93

Kumar *et al*. (2019) reported that the early flowering due to the application of CCC might be due to build up of sufficient food reserves at initial stages. These reserve foods utilized for the reproductive growth with a restriction in vegetative growth. Murali and Gowda (1988) registered that the CCC treated plants showed earlier flowering due to its anti - gibberellin action. A reduction in the level of endogenous gibberellins might be a prerequisite for floral induction.

Chlorophyll content is a key factor affecting the performance of plant photosynthesis through efficient light absorption (Taiz and Zeiger, 2006). In the present study, SPAD value taken indirectly measures the relative chlorophyll content and indicator for greenness of the plant. Foliar spray of 1% TNAU Horsegram Wonder registered higher SPAD value of 22.8 which is on par with BL nutrient consortium (22.2) while lower recorded in control (20.2).

An increment of 12.9% SPAD value was observed by the application of TNAU Horsegram Wonder compared to control (Table 1). The positive effect of TNAU Horsegram Wonder on SPAD value might be due to its contents especially CCC and iron. Bhagure and Tamble (2013) reported that CCC has the ability to arresting the chlorophyll degradation in okra. Maximum chlorophyll content was obtained by foliar application of 500 ppm CCC in soybean was recorde by Devi *et al*. (2011). The positive effect on SPAD value was enhanced by the addition of potassium and iron might be due to enzyme activator of chlorophyll synthesis and protect the chloroplast through osmolytic water balance. Adhikari *et al*. (2019) registered that the foliar application of 2.5% potassium sulphate increased the chlorophyll content in soybean. Foliar application of 0.5% $FeSO_4$ at flowering and peg formation stages significantly increased the chlorophyll content over rest of the treatments (Yadav *et al.,* 2019) in groundnut. The supremacy effect of iron on increment of SPAD value is clearly registered in the present study (Table 1).

Tendrils are contact-sensitive, filamentous organs that permit climbing plants together to their taller neighbors. Horsegram leaves are compound with each leaf carrying one or more pairs of leaflets along the leaf axis. The leaf is further specialized organ formed at the terminal position of the leaf is a tendril, rather than a leaflet. Formation of tendrils indicates that the vegetative growth of the plant is continues. Even though tendril is a vegetative part, it is not useful for photosynthesis and yield of the plant except support rather than it utilizes photosynthates ultimately reduced the yield. Hence, decrease the number of tendrils is a pre requisite for higher productivity. In the present study, highest number of tendrils (5.6) per plant was recorded in control while lowest number (2.6) in foliar spray of TNAU Horsegram Wonder followed by CCC nutrient consortium (3.7) (Fig 1). It clearly indicated that CCC reduced the number of tendrils might be due to arresting the apical dominance and reducing the shoot elongation through anti-gibberellin activity.

Mansuroglu *et al*. (2009) reported that most plant growth retardants inhibit the formation of growth active gibberellins and can thus be used to reduce unwanted shoot elongation. Present study corroborated with earlier findings. CCC inhibits cell elongation can decrease the growth of stems, leaves, and runners was concluded by Sharma *et al*. (1998) in potato.

Photosynthetic rate is an important physiological trait which directly indicates photosynthetic efficiency and productivity of crop plants. Highest photosynthetic rate of 16.94 µmol m^{-2} s^{-1} was registered by foliar spray of 1% TNAU Horsegram Wonder followed by BL nutrient consortium (15.96) which is on par with CCC + K_2SO_4 + boric acid treatment (15.76). The positive effect of TNAU Horsegram Wonder is might be due to its constituent's *viz.* CCC and Fe, which enhanced the photosynthetic rate of the crop. In the present study, 18.8% increment of photosynthetic rate was observed by 1% TNAU Horsegram Wonder (Table 2). The additive effect of Fe on photosynthetic rate might be due to its catalyst action on the formation of aminolevulinic acid (ALA), the initial step in the chlorophyll synthesis. An increment of 13.4% photosynthetic rate was registered by 100 ppm CCC compared to control in Bt cotton was reported by Patil *et al*. (2014). Phogat *et al*. (2020) reported that combined application of phosphorus along with sulphur showed synergistic effect on seed yield of black gram probably due to the action of balanced nutrition on photosynthesis. Wang *et al*. (2018) reported that the phosphorus application increased the leaf protein, rubisco activity and photosynthesis compared to non application of phosphorus.

The soluble protein content of the leaf, being a measure of rubisco activity is considered as an indirect index for assessing photosynthetic efficiency of crop plants. Myat *et al*. (2014) reported that rubisco enzyme forms more than

Table 2: Impact of PGR nutrient consortium on physiological traits and yield in horsegram.

Treatments	Photosynthetic rate (µmol m^{-2} s^{-1})	Soluble protein (mg g^{-1})	Yield (kg ha^{-1})
Control (Water spray)	14.26	13.6	885
BL + K_2SO_4 + boric acid	14.45	14.0	910
CCC + K_2SO_4 + boric acid	15.76	14.6	975
MAP + K_2SO_4 + boric acid	15.08	14.3	900
MAP + K_2SO_4 + $FeSO_4$ + boric acid	15.49	15.2	980
BL + MAP + K_2SO_4 + boric acid + $FeSO_4$	15.96	15.3	940
TNAU Horsegram Wonder	16.94	15.7	1090
SEd	0.31	0.31	11.41
CD (P=0.05)	0.66	0.63	23.68

50 per cent of the soluble proteins and 25% of nitrogen in leaves. Among the treatments, foliar spray of 1% TNAU Horsegram Wonder registered higher soluble protein content of 15.7 mg g^{-1} which is on par with BL nutrient consortium (15.3 mg g^{-1}) while lower was recorded in control (13.6).

An increment of 15.4% soluble protein was observed in the present study by the application of TNAU Horsegram Wonder (Table 2). Bhagure and Tamble (2013) reported that the CCC has the ability to promoting the synthesis of soluble protein and enzyme. The additive effect of potassium with CCC on soluble protein attributed to increases rubisco carboxylation activity in chloroplast (Zahoor *et al.,* 2017). Potassium facilitates chloroplast integrity and the efficiency of light absorption, rubisco diffusion and, as a consequence, carbon assimilation (Tranknera *et al.,* 2018). The impact of CCC on soluble protein was higher when combined with iron and phosphorus.

Nandan *et al.,* (2018) found significantly higher protein content with the treatment where RDF followed by foliar application of 0.05% Fe at pre flowing and pod formation stages in chickpea cultivars. Singh and Reddy (2016) also reported that Rubisco activity, RUBP regeneration, and maximum quantum yield related to the photochemical system were decreased under phosphorus deficient condition. The present study corroborated with the earlier findings. Hence, both iron and phosphorus were showed positive effect on soluble protein along with CCC (Table 2).

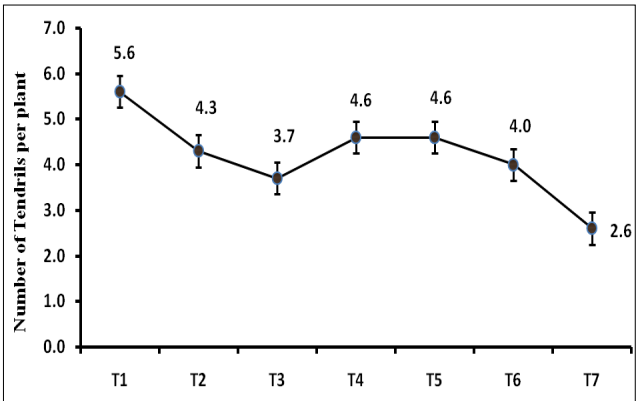
The first step in nitrogen assimilation pathway is the reduction of NO_3 to NO_2 catalyzed by the enzyme nitrate reductase, which is a rate-limiting step that regulates the conversion of inorganic nitrogen to organic form in plants (Beevers and Hageman, 1969). Nitrate reductase is the key enzyme involved in basic step of protein synthesis in which it converts NO_3 in to NO_2. Hence, assessment of NR activity is an excellent tool to assess productivity of crop plants. In the present study, greater nitrate reductase activity of 130.8 µg NO_2 g^{-1} hr^{-1} was observed in foliar spray of TNAU Horsegram Wonder (Fig 2) followed by BL nutrient consortium (125.0) and lower in control (106.3). Hemalatha (2002) reported that the application of CCC stimulated nitrate reductase activity effectively at 5×10^{-5} M concentration in rice seedlings.

NR activity decrease was much more in iron deficient leaves than root evident suggesting that iron deficiency affected more the NR activity in the leaves was reported by Borlotti *et al.* (2012) in cucumber. Dar *et al.* (2017) reported that adequate supply of phosphorus improved the NR activity in *Phaseolus vulgaris*. The present study corroborated with earlier findings in which TNAU Horsegram Wonder has CCC, P and Fe are stimulated the NR activity (Fig 2).

Significant difference was observed between the treatments with respects to grain yield. Among the PGR nutrient combinations, TNAU Horsegram Wonder recorded the maximum grain yield of 1090 kg ha^{-1} followed by nutrient consortium (980) which is on par with CCC + K_2SO_4 + boric acid (975) compared to control (Table 2). The yield increment by TNAU Horsegram Wonder might be due to the reduced plant height, increased SPAD value, soluble protein content, NR activity and photosynthetic rate which are directly contribute to the photosynthesis and ultimately yield.

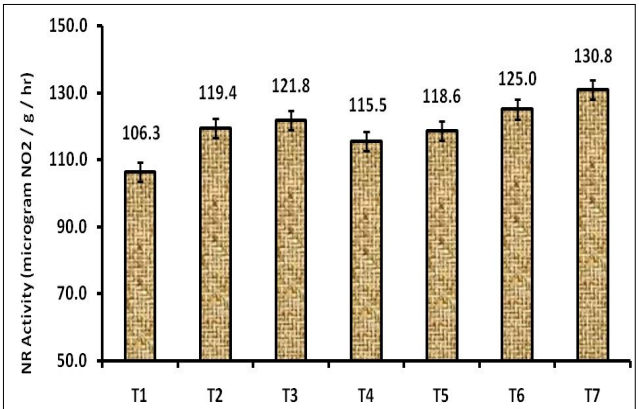
The positive effect of CCC on grain yield might be due to reduced plant height which improves the partitioning efficiency and sink activity. The spectacular impact of CCC on yield improvement in horsegram might be due to reduction tendril numbers. Anosheh *et al.* (2016) registered the positive role of CCC on yield components such as greater fertile tillers, spike number, fertile spikelets, grain number and grain yield. Foliar application of boron showed significant improvement in physiological and biochemical parameters especially photosynthesis and yield of all the genotypes of blackgram (Pegu *et al.,* 2013).

The present investigation coincided with these earlier studies. Potassium is known to enhance the source-sink



T_1 - Control, T_2 - BL + K_2SO_4 + boric acid, T_3 - CCC + K_2SO_4 + boric acid, T_4 - MAP + K_2SO_4 + boric acid, T_5 - MAP + K_2SO_4 + $FeSO_4$ + boric acid, T_6 - BL + MAP + K_2SO_4 + boric acid + $FeSO_4$, T_7 - TNAU Horsegram Wonder.

Fig 1: Impact of PGR nutrient consortium on number of tendrils in horsegram.



T_1 - Control, T_2 - BL + K_2SO_4 + boric acid, T_3 - CCC + K_2SO_4 + boric acid, T_4 - MAP + K_2SO_4 + boric acid, T_5 - MAP + K_2SO_4 + $FeSO_4$ + boric acid, T_6 - BL + MAP + K_2SO_4 + boric acid + $FeSO_4$, T_7 - TNAU Horsegram Wonder.

Fig 2: Impact of PGR nutrient consortium on nitrate reductase activity in horsegram.

relationship and stimulate the translocation of photo-assimilates thereby helping in effective flower formation ultimately enhance productivity of the crops. Boron can improve flowering ability via pollen germination and pollen tube growth and can also enhance the effective partitioning of assimilates from source to sink. Iron and phosphorus supported the photosynthesis through chlorophyll and protein synthesis.

Foliar spray of plant growth regulators and nutrients might have exploited favorably for continuous translocation of photosynthates to pods ultimately yield increment in indeterminate crops like pulses. Apart from this, reduction of tendril numbers may also be attributed for yield increment in horsegram. The yield improvement in horsegram by TNAU Horsegram Wonder might be due to the CCC with combination of nutrients resulted in decreased tendril numbers and better translocation of photosynthates to pods.

CONCLUSION

Our results showed that PGR (CCC) nutrient consortium (P, K, Fe and B) called as TNAU Horsegram Wonder developed by Tamil Nadu Agricultural University has a greater role on yield improvement in horsegram. It had a positive role on chlorophyll (SPAD value), soluble protein, photosynthetic rate and nitrate reductase activity. Its supremacy action showed on reduction of plant height and number of tendrils, and early flowering improved the grain yield under rainfed condition.

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