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Study the impact of plant growth regulators on tendril, physiological traits and flowering in *kharif* horse gram

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Abstract
Horse gram is an important pulse crop grown as rabi crop in Tamil Nadu and not grown during kharif season due to absence of flowering. An attempt was made to induce flowering in horse gram at kharif season by using plant growth regulators (PGRs). Field experiment was conducted to study the impact of PGRs *viz.*, salicylic acid (SA-100 ppm), chlormequat chloride (CCC-250 ppm), mepiquat chloride (MC-250 ppm), tri iodo benzoic acid (TIBA-200 ppm) and nitrobenzene (0.2%) on growth, physiological traits and flowering in horse gram during kharif season by foliar spray at 25 days after sowing. Among the PGRs, TIBA showed its supremacy on growth traits and CCC proved its dominance on physiological traits. Foliar spray of TIBA registered lowest plant height (37.9 cm) and number of tendrils (2.3) compared to other treatments. Among the PGRs, salicylic acid recorded highest NR activity (128.43 µg NO_2 $g^{-1}h^{-1}$) followed by TIBA. Higher Chlorophyll Content Index (CCI) value (25.1) and photosynthetic rate (14.96 µmol cm^{-2} s^{-1}) were recorded by CCC. However, flowering did not take place in any treatments. Among the plant growth regulators used, TIBA registered its positive action on flowering by reducing number of tendrils, however not enough.

Keywords: TIBA, CCC, tendril, CCI, NR activity, horse gram

Introduction
Horse gram known as poor men's crop is widely grown in India in almost 200-700 mm rainfall situations at a temperature range of 20-35°C. Hence, called drought hardy, typically adapted to a wide range of soils. Among over dozens 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) [19]. However, its photo and thermo-sensitive nature does not permit its horizontal expansion in non-traditional and remote regions considered as a major constraint in horse gram production.

The main season for sowing horse gram is rabi and it is sown during second week of October in Tamil Nadu. However, it is not grown as kharif crop in Tamil Nadu. Kharif sown crop does not flower and mainly used as fodder. Formation of a greater number of tendrils may be the one of the main reasons for refusal flowering during kharif season.

Induction of flowering in horse gram during kharif season is an immense task and if achieved it is highly valuable for higher production. Use of plant growth regulators is one of the possible ways for the induction of flowering. Abdelkadir *et al.* (2010) [1] registered that the application TIBA yielded more flowers per plant in *Jatropha curcas* with heavier than the control. CCC induced the earliest flowering and highest number of flowers, fruit set, retention and yield (Brahmachari *et al.*, 1996) [3]. Pacheco *et al.* (2013) [14] found that the foliar application of 1 mM salicylic acid registered higher number of inflorescences. Vinothini *et al.* (2018) [21] registered that foliar application of CCC and mepiquat chloride increased the number of flowers. Nitrobenzene is a new generation plant energizer and yield booster through increases nutrient use efficiency and induces profuse flowering (Mithila *et al.*, 2012) [10].

Excessive vegetative growth, results in the formation of tendrils is major constraint and it acts as a sink and affects source-sink relationship. Greater proportion of photo-assimilates is diverted for production and growth of tendril rather than translocation to reproductive parts leads to more plant height (Secondo and Reddy, 2018) [16]. This statement clearly indicated that the diversion of photosynthates to tendril which is vegetative part of horse gram can arrest the flowering. Based on this background, an attempt was undertaken for the induction of flowering in horse gram under kharif season by using plant growth regulators. This is a first attempt was made to induce flowering in horse gram under kharif season by using plant growth regulators through physiological approach.

Materials and Methods

The experiment was conducted at field number C2, Regional Research Station, Tamil Nadu Agricultural University, Paiyur during 2019 to 2021. Horse gram variety Paiyur 2 seeds were sown directly to the field with the spacing of 30 x 10 cm after the receipt of sufficient rainfall. Plant growth regulators includes salicylic acid (SA - 100 ppm), chlomequat chloride (CCC-250 ppm), mepiquat chloride (250 ppm), tri iodo benzoic acid (TIBA-200 ppm) and nitro benzene (0.2%) were applied as foliar spray at 25 days after sowing along with control was maintained with water spray and absolute control maintained without any spray. The experiment was carried out in randomized block design with one variety imposed with seven treatments in three replications.

Plant height was measured by using meter scale and expressed as cm. Number of tendrils per plant was counted manually and average was taken. These growth parameters were measured at 60 days after sowing. Photosynthetic rate measurement was performed by using Portable Photosynthesis System (PPS) (Model LC*pro*-SD., ADC BioScientific Ltd., Hoddesdon, UK) 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 3 cm^2 leaf chamber and PPFD at 1500 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}.

Chlorophyll content can be assessed indirectly through the mini-instrument chlorophyll content meter (CCM-200 plus, Opti-Sciences, Inc., Hudson, NH, USA). It measures chlorophyll content as ratio of optical absorbance in two different wavebands 653 nm (Chlorophyll) and 931 nm (Near Infra-Red) providing Chlorophyll Content Index (CCI) value. Five readings were taken from each replication and the average value was computed using the method described by Monje and Bugbee (1992) [12]. NR activity was estimated through the leaf samples were kept in 10 ml of assay medium containing substrate (1% KNO_3) for 1 hour (Nicholas *et al.*, 1976) [13]. 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}. Number of flower buds formed per plant was counted manually and average of five plants was taken. The data on various parameters were analyzed statistically as per the procedure suggested by Gomez and Gomez (1984) [7].

Results and Discussion

Plant height is an important parameter that determines the growth and development of a plant. However, continuous increment of plant height is not favorable for yield attributes. Highest plant height of 55.6 cm was recorded by absolute control which is on par with control (54.7 cm) while lowest was recorded by the application of TIBA (37.9 cm). Foliar spray of TIBA decreased the plant height up to 31.8% followed by CCC (23%) when compared to absolute control (Table 1). Reduction of plant height by TIBA might be due to its anti-auxin task and CCC due to its anti-gibberellin activity. In the present study, TIBA and CCC reduced the plant height and divert the photosynthates for the induction of flowering rather than utilize for vegetative growth.

Buzzello *et al.* (2013) [4] reported that the smaller plant height was achieved by the application of TIBA. Plant height reduced by cycocel due to inhibitory effect on cell division and expansion and reduces the utilization of photosynthates for growth (Singh *et al.*, 2018) [18].

Tendrils are contact-sensitive, filamentous organs that permit climbing plants together to their taller neighbours. Horse gram 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 flowering of the plant except support, however it utilizes photosynthates ultimately inhibit flowering. Hence, decrease the number of tendrils is a pre requisite for the induction of flowering.

In the present study, highest number of tendrils (6.7) per plant was recorded in control while lowest number of tendrils (2.3) in foliar spray of TIBA followed by CCC (3.0) (Table 1). It clearly indicated that TIBA reduced the number of tendrils might be due to arresting the apical dominance and CCC reducing the shoot elongation through anti-gibberellin activity. Mansuroglu *et al.* (2009) [9] reported that the most plant growth retardants inhibit the growth by arresting the active gibberellins synthesis and reduce unwanted shoot elongation. CCC inhibits cell elongation can decrease the growth of stem, leaves, and runners was concluded by Sharma *et al.* (1998) [17] in potato.

In the present study, CCI value taken which indirectly measures the relative chlorophyll content and indicator for greenness of the plant. Foliar spray of CCC registered higher CCI value of 25.1 which is on par with salicylic acid (24.3) followed by mepiquat chloride (24.0) while lower recorded in absolute control (22.3). An increment of 12.6% CCI value was observed by the application of CCC compared to absolute control (Table 1). Bhagure and Tamble (2013) [2] 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 recorded by Devi *et al.* (2011) [5]. Salicylic acid is an important plant hormone involved in chlorophyll synthesis might be the reason for enhancement of CCI value. Farahbakhsh and Saiid (2011) [6] found that foliar application of 200 ppm salicylic acid increased the chlorophyll content by 38.7% in maize. Salicylic acid improving the chlorophyll content in plant tissues by synthesis and increase the rate of de-epoxidation (Moharekar *et al.*, 2003) [11].

Photosynthetic rate is an important physiological trait which directly indicates photosynthetic efficiency of crop plants. Highest photosynthetic rate of 14.96 µmol m^{-2} s^{-1} was registered by foliar spray of CCC followed by salicylic acid (14.55) and mepiquat chloride (14.52) and lowest was recorded by control (Fig. 1). In the present study, 20.6% increment of photosynthetic rate was observed by CCC. The positive effect of CCC on photosynthetic rate might be due to its increment action on chlorophyll, soluble protein and also enhancement of carboxylation reaction by the enzyme rubisco. Patil *et al.* (2014) [15] found that an increment of 13.4% photosynthetic rate by 100 ppm CCC compared to control in Bt cotton.

Nitrate reductase enzyme reduces NO_3 to NO_2 is first step in the nitrogen assimilation pathway in plants. Nitrate reductase

is the key enzyme involved in basic step of protein synthesis which decides the growth and development of the plants. In the present study, highest NR activity of 128.43 µg NO_2 g^{-1} hr^{-1} was observed in foliar spray of salicylic acid (Table 1) and lowest in absolute control (107.03). The positive effect of SA on nitrate reductase activity might be due to its active role in activation of the inactive NR protein and prevention of enzyme degradation by proteolysis. Hayat *et al.* (2014) [8] reported that foliar spray of 10^{-5} M SA increased the number of nodules, nitrogenase activity and enhanced the activities of the NR enzyme involved in nitrogen assimilation. Salicylic acid protects NR activity under any stress condition and maintained NR level in the plant (Umebese *et al.*, 2009) [20] was reported by many researchers. The present study is also corroborated with earlier findings. In the case of flowering, no flower buds formed in any treatments. Number of flower buds counted was zero in all the treatments and transformed value was presented in the Table 1. Hence, there was no difference observed among the treatments in number of flower buds formed.

Table 1: Impact of PGRs on growth parameters and physiological traits in horse gram during kharif season.

Treatments	Plant height (cm)	No. of tendrils plant^{-1}	CCI	NR activity (µg NO_2 g^{-1} h^{-1})	Number of flowers per plant*
Absolute control	55.6	6.3	22.3	107.03	0.432
Control (water spray)	54.7	6.7	22.5	107.80	0.432
SA (100 ppm)	50.4	5.3	24.3	128.43	0.432
CCC (250 ppm)	42.8	3.0	25.1	120.13	0.432
MC (250 ppm)	43.8	4.0	24.0	121.00	0.432
TIBA (200 ppm)	37.9	2.3	23.1	122.27	0.432
Nitro benzene (0.2%)	49.0	5.3	23.5	118.23	0.432
SEd	1.52	0.15	0.40	4.787	0.0092
CD (P = 0.05)	3.16	0.31	0.82	9.695	0.0217

* Transformed data

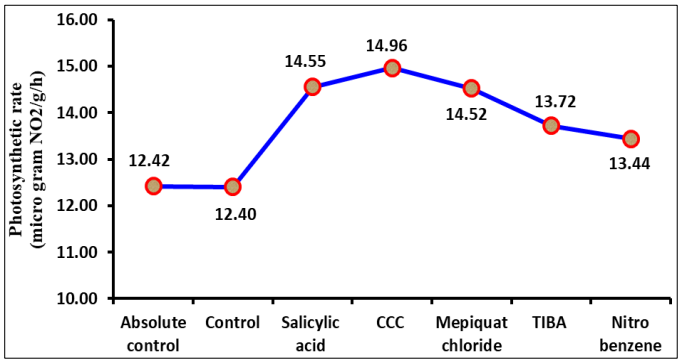

Fig 1: Impact of PGRs on photosynthetic rate in horse gram during *kharif* season.

Conclusion

Horse gram does not flower in kharif season and produce a greater number of tendrils compared to *rabi*. In the present study, TIBA (200 ppm), CCC (250 ppm) and salicylic acid (100 ppm) showed some positive initiation to induce flowering by reduced the number of tendrils, enhancement of chlorophyll in terms of CCI and NR activity respectively. However, it is not enough to produce flowers even in the favorable physiological traits achieved by PGRs. Further study is required on gene profiling under kharif season to achieve the target.

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