

# EFFECT OF PINK PIGMENTED FACULTATIVE METHYLOTROPH AND PLANT GROWTH REGULATORS ON GROWTH, CHLOROPHYLL, TRANSPIRATION, SOD ACTIVITY AND YIELD OF TOMATO UNDER DROUGHT

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## KEYWORDS

PPFM  
Brassinolide  
SOD  
Transpiration

## Received on :

31.12.2020

## Accepted on :

05.08.2021

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## ABSTRACT

An experiment was carried out to find out the effect of Pink Pigmented Facultative Methyloph and Plant Growth Regulators on alleviating the drought effects in tomato. Pot culture experiment was conducted in tomato variety PKM 1 and foliar spray of PGRs like brassinolide (1 ppm), salicylic acid (100 ppm), benzyl amino purine (100 ppm), gibberellic acid (10 ppm) and PPFM (1%), PPFM (2%), and PPFM (3%) were carried out under drought condition. Among the PGRs and different concentrations of PPFM used, PPFM (2%) was found to superior in improving root length (25.90 cm), transpiration rate (32.12 mmol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup>) and SOD activity (358.45 Units mg / protein) under drought. The higher plant height of 78.66 cm was registered by GA3 followed by 2% PPFM (75.33 cm). Foliar spray of BAP (100 ppm) maintained higher total chlorophyll content of 2.31 mg g<sup>-1</sup> followed by 2% PPFM (2.15) and brassinolide (1.82) under drought. Among the treatments, foliar spray of PPFM (2%) registered highest fruit yield of 477.9 g plant<sup>-1</sup>. Hence, PPFM (2%) is used to mitigate the drought effect efficiently and improving the drought tolerance capacity of tomato crop.

## INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill.) is one of the most popular and widely grown vegetable crops in the world. The basic idea of this research paper is to study the impact of PPFM (Pink Pigmented Facultative Methyloph) and plant growth regulators on drought effect alleviation in tomato.

It is estimated that 40% to 60% of total crop yield losses in the world are due to drought stress (Shao *et al.*, 2008).

Mollasadeghi *et al.* (2011) reported that drought is one of the most important factors for yield reduction in the majority of the cultivated areas of the world's agriculture lands. Besides affecting plant growth and productivity, drought also causes secondary stresses like oxidative stress, which in turn leads to denaturation of functional and structural proteins (Wang *et al.*, 2003). The major task in the upcoming years will be growing crops with less water availability, especially in areas which have inadequate water recourses. Hence, there is a need to cope up with this problem by adopting strategies which result in more efficient use of water (Nasrullah *et al.*, 2011). Many strategies such as foliar application of nutrients (Raza *et al.*, 2012) and growth regulators (Ahmadi *et al.*, 2015) have been developed to alleviate the drought effect, is very essential. Green (1992) found that methylobacterium species are a group of bacteria known as pink-pigmented facultative methylophs, or PPFMs. The bacterial inoculants that provide cross protection against both biotic and abiotic stress would be highly preferable in environmentally sustainable

agricultural systems (Van Loon *et al.*, 1998). Hayat *et al.* (2010) reported that the adverse effects of drought stress were effectively mitigated by the exogenous application of PGRs and also improves growth and yield of crop plants.

The effect of drought can be recovered to a certain extent with the applications of plant growth regulators (PGRs) that play an important role in several physiological and molecular processes of plants. The beneficial effect of gibberellins in maize (Tuna *et al.*, 2008), cytokinins in *vigna radiata* (Chakrabarti and Mukherji, 2002), salicylic acid in tomato (Hayat *et al.*, 2008), brassinosteroid in tomato (Vardhini and Rao, 2001) and PPFM in tomato (Sivakumar *et al.*, 2017) were documented under drought condition. Hence, PGRs used as potential tools to increase defense mechanisms against any stress conditions. Different approaches were adopted to alleviate the effect of drought and one among those is the exogenous application of plant growth regulators and PGPR (Plant Growth Promoting Rhizobacteria). The present study was intended to find out the suitable PGR / PGPR with optimum concentration to alleviate the drought effect in tomato through physiological interventions. Hence, the study was undertaken with the objective to study the impact of PPFM and different plant growth regulators on growth, chlorophyll, transpiration and yield of tomato under drought condition.

## MATERIALS AND METHODS

The experiment was carried out in the pot culture under glass

house condition during 2016-17. Red sandy soil mixture was prepared (red soil : vermicompost : sand - 3:2:1) and used for pot culture experiment. Drought was imposed at first day after transplanting onwards by maintaining soil moisture at 50 per cent field capacity. Crop was supplied with fertilizers and other cultivation operations as per recommended package of practices of Tamil Nadu Agricultural University, Coimbatore. The experiment was laid out in completely randomized block design with three replications. The treatments like absolute control (100% FC), Control (50% FC), PPFM (1%), PPFM (2%), PPFM (3%), brassinolide (BL - 1 ppm), salicylic acid (SA - 100 ppm), benzyl amino purine (BAP - 100 ppm) and GA<sub>3</sub> (10 ppm) were used for this experiment. PPFM and PGRs treatments were given as foliar spray at 25 and 45 days after transplanting.

Plant height and root length were measured by using meter scale and expressed as cm. Leaf area per plant was measured using a Leaf Area Meter (LICOR, Model LI 3000) and expressed as cm<sup>2</sup> plant<sup>-1</sup>.

Transpiration 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. Totally, three measurements were taken in the same leaf. Leaves were inserted in a 3 cm<sup>2</sup> leaf chamber and PPFD at 1200 μmol photons m<sup>-2</sup> s<sup>-1</sup> and relative humidity (50-55%) were set. The readings were taken between 9 am to 11.30 and the value was expressed in mmol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup> (Long *et al.*, 1996). Chlorophyll 'a', chlorophyll 'b' and total chlorophyll contents were estimated by using 80% acetone by adopting the procedure of Arnon (1949) and the content was expressed as mg g<sup>-1</sup> of fresh weight.

Chlorophyll stability index was determined by adopting the method of Murthy and Majumdar (1962) as follows. Two samples of 250 mg of leaf sample were weighed and made into 8 to 10 leaf bits separately and transferred to two test tubes. 20 ml of distilled water and 20 ml hot water (54-56°C) was added to the control and treatment tubes respectively. The treatment tubes were kept in a hot water bath for 30 minutes. After, the leaf bits were taken out from the test tube and macerate with 10 ml of 80 per cent acetone. The content was centrifuged at 3000 rpm for 10 minutes. The supernatant was collected and made up to 25 ml by using 80 per cent acetone and the OD was measured at 652 nm in the spectrophotometer .

The CSI was calculated by the following formula and expressed as percentage.

$$CSI = \frac{\text{Total chlorophyll content in control} - \text{Total chlorophyll content in treatment}}{\text{Total chlorophyll content in control}} \times 100$$

The total chlorophyll content was estimated by using the method given by Arnon (1949).

Superoxide dismutase (SOD) activity was estimated by adopting the protocol of Beau-Champ and Fridovich (1971). 500 mg of leaf sample was weighed and macerated with 10 ml HEPES-KOH buffer containing 0.1 mM EDTA.

The content was centrifuged at 15000 ppm for 15 min and the supernatant was collected and made up to 50 ml. All the operations were carried out at 4°C One ml of enzyme extract

was pipette out and mixed with 3 ml of reaction mixture (50 mM HEPES-KOH, 0.1 mM EDTA, 50 mM Na<sub>2</sub>CO<sub>3</sub>, 12 mM methionine, NBT 75 M, and 1 M riboflavin). One unit SOD activity was defined as the amount of enzyme required to result a 50 per cent inhibition of the rate of NBT reduction at 560 nm and the results were expressed as units mg / protein. Number of fruits harvested from each plant / replication at each picking was counted and total number of fruits per plant was calculated. The total weight of fruits harvested from each plant of all picking was added and average yield per plant was worked out and expressed in gram per plant.

## RESULTS AND DISCUSSION

Plant height is an important trait for growth and increased plant height would allow greater biomass production and yield potential in crops (Zhang *et al.*, 2004). In the present study, significant reduction of plant height was observed up to 34.80 per cent under drought condition (Table 1).

Hussain *et al.* (2008) reported the reduced plant height, leaf area and crop growth under drought in sunflower. Among the PGRs and PPFM used, the maximum plant height of 78.66 cm was recorded in gibberellic acid treatment and found significantly superior over PPFM (2%) (75.33 cm) and brassinolide (75.20 cm). However, the minimum plant height was recorded in control (53.82 cm) compared to absolute control (82.30 cm) (Table 1). The increment of plant height by

**Table 1: Effect of PPFM and PGRs on growth parameters of tomato under drought**

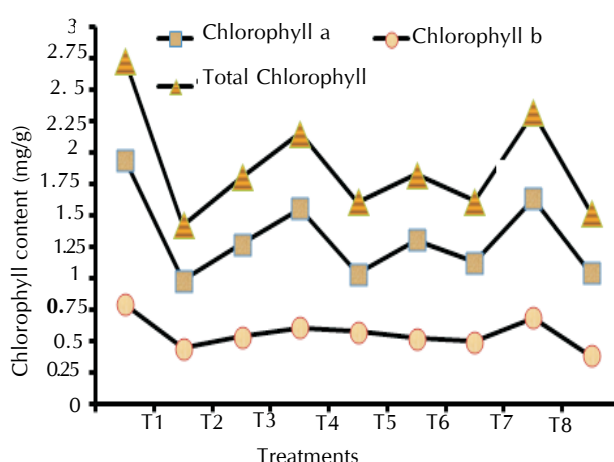
Treatments	Plant height (cm)	Root length (cm)	Leaf area (cm <sup>2</sup> )
Absolute control (100% FC)	82.3	27.9	413.7
Control (50% FC)	53.82	13.5	263.9
PPFM (1%)	70.32	22.6	292.9
PPFM (2%)	75.33	25.9	320.7
PPFM (3%)	69.62	22.1	286.6
Brassinolide (1 ppm)	75.2	25.2	304.5
Salicylic acid (100 ppm)	68.9	22.93	312.1
BAP (100 ppm)	70.81	19.5	283.8
GA <sup>3</sup> (10 ppm)	78.66	18.1	269.9
SEd	1.4	0.48	6.42
CD (P=0.05)	2.96	1.02	13.49

**Table 2: Effect of PPFM and PGRs on CSI and Transpiration rate of tomato under drought**

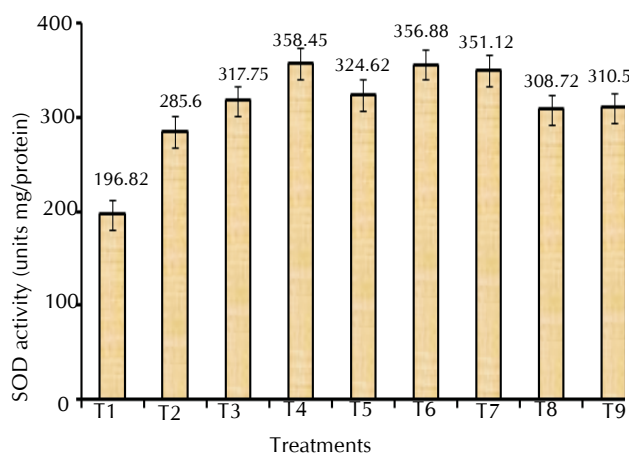
Treatments	Chlorophyll Stability Index (%)	Transpiration Rate (mmol H <sub>2</sub> O m <sup>-2</sup> s <sup>-1</sup> )
Absolute control (100% FC)	82.33	36.03
Control (50% FC)	53.66	24.17
PPFM (1%)	70.32	28.89
PPFM (2%)	75.33	32.12
PPFM (3%)	69.62	28.63
Brassinolide (1 ppm)	78.66	30.86
Salicylic acid (100 ppm)	76.9	31.32
BAP (100 ppm)	76.2	30.02
GA <sup>3</sup> (10 ppm)	70.81	30.29
SEd	1.4	0.72
CD (P=0.05)	2.96	1.51

**Table 3: Effect of PPFM and PGRs on yield parameters of tomato under drought**

Treatments	Yield parameters	
	Fruit number plant <sup>-1</sup>	Fruit yield (g plant <sup>-1</sup> )
Absolute control 100% FC)	21	674.1
Control (50% FC)	13	386.3
PPFM (1%)	16	412.4
PPFM (2%)	19	477.9
PPFM (3%)	16	428.8
Brassinolide (1 ppm)	18	464.5
Salicylic acid (100 ppm)	17	445.6
BAP (100 ppm)	16	439.2
GA <sup>3</sup> (10 ppm)	16	425.6
SEd	0.36	11.03
CD (P = 0.05)	0.74	23.18



T1 - Absolute control (100% FC); T2 - Control (50% FC); T3 - PPFM (1%); T4 - PPFM (2%); T5 - PPFM (3%); T6 - Brassinolide (1 ppm); T7 - Salicylic acid (100 ppm); T8 - BAP (100 ppm) T9 - GA<sup>3</sup> (10 ppm)

**Figure 1. Effect of PPFM and PGRs on chlorophyll contents of tomato under drought**

T1 - Absolute control (100% FC); T2 - Control (50% FC); T3 - PPFM (1%); T4 - PPFM (2%); T5 - PPFM (3%); T6 - Brassinolide (1 ppm); T7 - Salicylic acid (100 ppm); T8 - BAP (100 ppm) T9 - GA<sup>3</sup> (10 ppm)

**Figure 2. Effect of PPFM and PGRs on superoxide dismutase activity of tomato under drought**

GA<sup>3</sup> might be due to involvement in cell elongation leads to internode elongation. Increased plant height by PPFM might

be due to increment of cytokinin. This finding is corroborated with earlier study in soybean by Mauney and Gerik (1994).

Root length plays a major role in the absorption of water and nutrients necessary for normal plant growth. PPFM (2%) marked the highest root length of 25.90 cm, followed by brassinolide (25.20 cm) and salicylic acid (22.93 cm) while compared to control (13.50 cm). The reduction of root growth under stress condition is a result of the inhibition of cell cycle progression and reduction in root apical meristem size (West *et al.*, 2004).

In the present study, the application of PPFM and PGRs invariably increased the root length under drought which ultimately increasing the water uptake. Root length was increased up to 36.29 per cent by PPFM (2%) compared to control followed by brassinolide (34.52%) (Table 1).

Increment of root length by PPFM might be due to its excretion of auxins and cytokinin. Madhaiyan *et al.* (2004) found that PPFMs excrete auxins and cytokinins that increase root growth and play critical roles in a plant's response to water stress. Trotsenko *et al.* (2001) reported that the PPFMs exude osmo-protectants like sugars and alcohols on the surface of host plants and this matrix may help to protect the plants from desiccation. These findings support the present study.

The enhancement of root length by BL under drought might be due its triggering effect on ethylene. Clouse (2011) reported that the application of brassinosteroids increase the root growth under drought might be due to stimulate ethylene biosynthesis in roots. The role of brassinosteroids has been implicates abiotic and biotic tolerance in plants as presented by Choudhary *et al.* (2012).

Leaf area development aids in the effective interpretation of light leading to high dry matter production (Shibles and Weber, 1966). The highest leaf area of 320.70 cm<sup>2</sup> plant<sup>-1</sup> was recorded by PPFM (2%) followed by salicylic acid (312.10 cm<sup>2</sup> plant<sup>-1</sup>) and brassinolide (304.50 cm<sup>2</sup> plant<sup>-1</sup>) compared to control (263.9). The decline in leaf area under drought might be due to the loss of cell turgor leads to reduced cell enlargement, transport of assimilates from the leaves to the developing sink which later caused senescence of leaves. In present study, it was evident that leaf area was reduced by drought up to 36.21 per cent. Kumar *et al.* (2012) already reported that leaf surface area was reduced under water stress condition in tomato.

As observed in the present study, PPFM (2%) increased the leaf area up to 17.71 per cent under drought condition when compared to control (Table 1). Increment of leaf area by PPFM might be due to increased compatible osmolytes like proline, which maintain the plant water potential ultimately turgor. Sivakumar *et al.* (2017) reported that the foliar application of PPFM (2%) increased the proline content by 11.34 per cent in tomato which responsible for water potential maintenance in plant. Exogenous application of salicylic acid counteracts the drought stress inhibiting plant growth and delays water loss and leaf rolling ultimately maintaining leaf area (Kadioglu *et al.*, 2011).

Transpiration rate determines the significance of various physiological processes along with their impact on biomass production and yield. The transpiration rate highly reduced under drought stress conditions and lower transpiration rate

recorded in control (24.17) compared to the absolute control (36.03). Makela *et al.* (1999) reported that the transpiration rate affected by water limitation in tomato plants. PPFM (2%) recorded the highest transpiration rate of 32.12 mmol H<sub>2</sub>O m<sub>2</sub> s<sup>-1</sup> which is on par with brassinolide (31.86) and salicylic acid treatment (31.32) (Table 2). The similar findings were observed by Khan *et al.* (2003), photosynthetic rate, stomatal conductivity and transpiration rate could also be enhanced by salicylic acid application. The positive effect of PPFM might be due to the increment of osmolytes like proline and enhance the water uptake and maintained the water status of the plant. The increment of water status in terms of RWC by PPFM (2%) was observed by Sivakumar *et al.* (2017) in tomato under drought condition which supports present findings.

Total chlorophyll content of the plant influences the photosynthetic rate and thereby alters the biomass production and yield. The drought-induced alterations in leaf chlorophyll content could be due to impaired biosynthesis or accelerated pigment degradation.

In the present study, total chlorophyll content decreased up to 45% under drought condition. Chlorophyll content in terms of SPAD value decreased under moisture stress condition was reported by Lipika Das *et al.* (2016). Present study corroborated with the earlier findings.

Among the PGRs, BAP recorded the highest total chlorophyll content (2.31 mg g<sup>-1</sup>) followed by PPFM (2%) treatment (2.15 mg g<sup>-1</sup>) and brassinolide (1.82 mg g<sup>-1</sup>) compared to control (1.42 mg g<sup>-1</sup>) (Fig. 1). The higher chlorophyll 'a' and 'b' contents were observed in absolute control (1.93 and 0.79 mg g<sup>-1</sup>) when compared to control (0.98 and 0.44 mg g<sup>-1</sup>).

Among the PGRs, BAP showed higher chlorophyll 'a' and 'b' followed by PPFM (2%). The positive effect of BAP on chlorophyll content might be due to its action of anti-senescence property and protect the chlorophyll under drought condition. Synkova *et al.* (1997) found that the cytokinin delays leaf senescence and can directly influence chlorophyll and photosynthetic protein synthesis and degradation. The improvement in chlorophyll content by PPFM under drought may be due to the phytochromes, and it has direct relations and interactions with cytokinin and gibberellins. Enhancement of chlorophyll content by seed inoculation and foliar spray of *Methylobacterium* was reported by Meenakshi and Savalgi (2009) in soybean. Madhaiyan *et al.* (2004) observed higher photosynthetic activity in rice by *Methylobacterium* might be due to enhancement of chlorophyll concentration. These two earlier studies support the present investigation.

Chlorophyll Stability Index (CSI) is an indicator of the stress tolerance capacity of the plants and is a measure of integrity of membrane (Murthy and Majumder, 1962). A higher CSI helps the plants to withstand stress through better availability of chlorophyll, leading to increased photosynthetic rate, more dry matter production and higher productivity.

Kilen and Andrew (1969) observed a high correlation between CSI and drought tolerance in corn. In the present study, brassinolide recorded the highest CSI of 78.66 per cent which is on par with salicylic acid (76.9%), BAP (76.2%) and PPFM (2%) treatment (75.33%) (Table 2).

The positive role of PPFM on CSI might be due to the protection of chlorophyll under stress condition through cytokinin production. Kannan *et al.* (2017) reported that application of PPFM increased the CSI under moisture stress condition in cotton.

Foliar application of PPFM (1 ml/lit) registered higher chlorophyll stability index than the control in cardamom and can be a good choice for the organic growers under drought situation was reported by Sathyan *et al.* (2018).

The positive role of brassinolide on CSI might be due to the fact that it may help membrane integrity by enhancing the level of the antioxidant system (Arora *et al.*, 2008). Application of salicylic acid increases the accumulation of Ca<sup>+2</sup> which can maintain membrane integrity (Khan *et al.*, 2010) and ameliorates the impact of abiotic stress through improving antioxidant system (Yusuf *et al.*, 2008). These studies are supported the present investigation.

Superoxide dismutase (SOD) is an effective scavenging enzyme which protects the plant against super oxide damage which is produced by drought induced oxidative stress. In the present study, SOD activity was enhanced by foliar spray of PPFM and PGRs under drought. Higher SOD activity was registered by 2 per cent PPFM (358.45) followed by brassinolide (356.88) and salicylic acid (351.12) (Fig. 2).

In the present study, SOD activity was increased up to 45% under drought condition compared to absolute control. SOD activity was enhanced gradually with increasing concentration of PEG induced drought was observed by Narendra Kumar *et al.* (2017). The positive effect of PPFM on SOD might be due to the enhancement of scavenging enzymes activity as defense mechanism under drought.

Green (1992) demonstrated that the biochemical study of all *Methylobacterium* strains was producing catalase, which is an efficient antioxidant enzyme. The involvement of brassinosteroid in the regulation of reactive oxygen species metabolism is evident as they can induce and regulate the expression of certain antioxidant genes and increase the activities of key antioxidant enzymes, including superoxide dismutase, peroxidase and catalase (Ogweno *et al.*, 2008).

Foliar application with salicylic acid decreased the lipid peroxidation and increased the antioxidant enzyme SOD activity in tomato plants grown under salt stress (He and Zhu, 2008). These studies are supported the present investigation.

Number of fruits per plant was lower in control (13.0) compared to absolute control (21.0). Comparing the PGRs and PPFM, PPFM (2%) treatment (19.0) and brassinolide (18.0) significantly recorded more number of fruits followed by salicylic acid (17.0). Same trend was followed as that of fruit yield also. Comparing the treatments, absolute control significantly recorded the highest value of 674.10 g plant<sup>-1</sup> and control recorded the least value of 386.30 g. Among the PGRs, PPFM (2%) documented significantly superior fruit yield of 477.90 g which is on par with brassinolide (464.50 g) followed by salicylic acid (445.60 g) (Table 3).

The positive effect of PPFM on fruit yield might be due to the protection of plant under drought condition by cytokinin synthesis. PPFM might induce the cytokinin is an anti-senescence hormone which protects plant against drought.

Kannan *et al.* (2017) registered that the foliar application of PPFM increased the cotton yield under moisture stress condition. The present study confirms the early findings of PPFM (2%) increased the fruit yield in tomato (Sivakumar *et al.*, 2017) under drought condition.

Brassinosteroids stimulated both growth and yield of tomato plants under field conditions as observed by Vardhini and Rao (2001). Seed treatment or foliar application of chemicals like kinetin, salicylic acid (Karlidag *et al.*, 2009) may increased yield of different crops might be due to enhanced photosynthetic rates, leaf area and plant dry matter production (Khan *et al.*, 2003). Gudesblat and Russinova (2011) also explained brassinosteroid ameliorates the adverse effects of drought stress and thereby increase the yield of the crop. The present investigation is in agreement with these findings. However, 2% PPFM is showed its supremacy on yield under drought compared other PGRs. The same result of foliar application of PPFM (1%) found highly effective than brassinolide (0.1 ppm) in sustaining the productivity through mitigating the ill-effects of water stress in rice was reported by Ajaykumar and Murali (2018).

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