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Growth and physiology of rice (*Oryza sativa* L.) under foliar-applied growth regulating substances and PPFM

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Abstract
Rice is the Asia's economically and culturally most essential food crop and its production is substantially improved by agro-techniques such as soil fertility, water use, and pest management. However, abiotic factors, especially soil moisture stress due to erratic rainfall the most important factors limiting rice productivity. Adoption of agronomic practices like foliar application of stress tolerance inducing substances is one of the best solutions for improving crop productivity. Field experiments were conducted at Annamalai University Experimental Farm, Tamil Nadu during "*Kuruvai" and "Nanvarai"* (Kharif and Rabi) seasons of 2021 and 2022 to study the effect of foliar application of stress tolerance inducing substances on the performance of rice under moisture stress condition. The experiment was laid out in randomized block design (RBD) and was replicated thrice with 12 treatment combinations including RDF, KNO_3, $ZnSO_4$, salicylic acid / gibberellic acid / humic acid / PPFM. Growth parameters were significantly influenced by application of drought tolerance inducing substances under moisture stress condition. Among the treatments, Recommended dose fertilizer (120 kg N, 40 kg P_2O_5 and 40 kg K_2O ha^{-1}) + acute soil drying + KNO_3 @ 2% and $ZnSO_4$ @ 0.5% at panicle initiation and 15 days after 1st spray + humic acid @ 0.2% at booting stage and 15 days after 1st spray recorded highest growth parameters *viz*., plant height, number of tillers hill^{-1}, root volume and physiological parameters like leaf area index (LAI), crop growth rate (CGR) and chlorophyll content index (CCI).

Keywords: Drought, growth hormones, leaf chlorophyll, pink-pigmented facultative methylotrophs, soil moisture stress

Introduction
Rice is the world's most important food crop and a primary source of staple food for more than half of the world's population. Rice is produced in about 120 countries worldwide, with a total harvested area of 160.59 million hectares with production of 742.54 million tonnes and productivity of about 4.59 tonnes ha^{-1} (USDA, 2021) [20]. In India, it is the staple food for more than 65 per cent of the population. In India, rice is cultivated in an area of 43.80 million hectares with production of 122.13 million tonnes and productivity of about 2.7 t ha^{-1} (MAFW, 2021) [13]. In Tamil Nadu, rice is grown with an area of 2.2 million hectares resulting in production of 8.65 million tonnes and with the productivity of 3.93 t ha^{-1}.

Abiotic stress negatively influences plant growth and development, leading to heavy yield losses in rice (Verma, 2016) [21]. Moisture stress due to delayed monsoonal rainfall or frequent drought is one of the major abiotic stresses that affect rice production in many regions. Recent trends in climate change have also predicted a further increase in drought intensity and severity, thus standardization of location specific technologies critical to sustain rice production. Further, under moisture stress situations water use efficiency is low and applied nutrients such as NPK are not efficiently utilized by rice.

Potassium plays a foremost role in translocation of carbohydrates, photosynthates, water relations and is involved in several biochemical and physiological processes that are considered very crucial for plant growth and yield (Zain and Ismail, 2016) [22]. Potassium application helped in alleviating the harmful effect of water-deficit stressand maintains higher leaf greenness, leaf relative water content, net photosynthetic rate, free proline content, grain yield and water productivity (Das *et al*., 21) [2].

Zinc foliar fertilization improved water use efficiency by increasing the osmolytes, maintaining membrane stability and improving stomatal conductance (Hassan *et al*., 2020) [8]. Foliarly applied Zn predominantly reduced the damaging impact of water stress by improving the plant status in the form of plant height, relative water content and gas exchange attributes (Sattar *et al*., 2022) [16].

Salicylic acid is known to induce a wide range of cellular defense reactions and could modulate the gene expression of plants responding to water stresses (Biswaset *et al*., 2019) [1]. Salicylic acid plays a pro-oxidant role and controls cellular redox homeostasis through the regulation of antioxidant enzyme activities under stress conditions (Emad *et al*., 2019) [4].

Under the low soil moisture tension regime (10 kPa), the application of GA_3 promoted photo-assimilation and their translocation towards the developing spikelets (Pal *et al*., 2020) [14]. Humic acid could improve plant resistance to mitigate the abiotic drought damages. Humic substances enhance plant growth and stress tolerance through better uptake of water and nutrients (Hasanuzzaman *et al*., 2021) [7].

Pink-Pigmented Facultative Methylotrophs (PPFMs) are known to improve plant growth by adopting various mechanisms *viz*., nitrogen fixation and nodule formation, phosphate solubilization, plant growth regulators production (auxins, cytokinins, gibberellic acid), production of urease enzyme, vitamin B_{12} production, synthesis of siderophores. Methylotrophic bacteria adapt to survive in stress conditions such as low nutrient, drought and high temperature by producing biofilm, aggregate formation and producing ultraviolet-protecting compounds (Sivakumar *et al*., 2021) [19].

With this background, a field experiment was conducted to study the effect of foliar application of stress tolerance inducing substances on the growth and physiological attributes of transplanted rice under moisture stress.

2. Materials and Methods

Field experiments were conducted at Annamalai University Experimental Farm, Annamalai Nagar, Tamil Nadu to study the effect of foliar application of drought tolerance inducing substances on the performance of transplanted rice under moisture stress conditions. The study was conducted during "*Kuruvai" and "Nanvarai"* (Kharif and Rabi) seasons of 2021 and 2022. The experimental field is geographically located at 11º 24'N latitude and 78º41'E longitude and at an altitude of +5.79 meter above the mean sea level (MSL).

The weather of study area is moderately hot. The crop received a rainfall of 598 mm and was distributed over 19 rainy days. The maximum temperature ranged from 33.4 to 38.2 °C with a mean temperature of 36.4 °C. The minimum temperature ranged from 23.7 to 27.5 °C with a mean of 25.8 °C. The relative humidity ranged from 68 to 87 per cent with a mean of 79 per cent. The soil is clay loam and low in available nitrogen (224 kg ha^{-1}), medium in available phosphorus (18.37 kg ha^{-1}) and potassium (209 kg ha^{-1}).

The experiment was laid out in randomized block design (RBD) and was replicated thrice with 12 treatments *viz*., T_1 - Farmer's practice with acute soil drying (control); T_2 - Recommended dose fertilizer (RDF) with normal irrigation + water spray; T_3 - RDF + acute soil drying; T_4 - RDF + acute soil drying + KNO_3 @ 2% + $ZnSO_4$ @ 0.5% at panicle initiation and 15 days after 1st spray; T_5 - RDF + acute soil drying + salicylic acid @ 200 ppm at booting stage and 15 days after 1st spray; T_6 - RDF + acute soil drying + gibberellic acid @ 40 ppm at booting stage and 15 days after 1st spray; T_7 - RDF + acute soil drying + humic acid @ 0.2% at booting stage and 15 days after 1st spray; T_8 - RDF + acute soil drying + pink pigment facultative methylotrophs (PPFM) @ 2% at booting stage and 15 days after 1st spray; T_9 - RDF + acute soil drying + KNO_3 @ 2% + $ZnSO_4$ @ 0.5% at panicle initiation and 15 days after 1st spray + salicylic acid @ 200 ppm at booting stage and 15 days after 1st spray; T_{10} - RDF + acute soil drying + KNO_3 @ 2% + $ZnSO_4$ @ 0.5% at panicle initiation and 15 days after 1st spray + gibberellic acid @ 40 ppm at booting stage and 15 days after 1st spray; T_{11} - RDF + acute soil drying + KNO_3 @ 2% + $ZnSO_4$ @ 0.5% at panicle initiation and 15 days after 1st spray + humic acid @ 0.2% at booting stage and 15 days after 1st spray and T_{12} - RDF + acute soil drying + KNO_3 @ 2% + $ZnSO_4$ @ 0.5% at panicle initiation and 15 days after 1st spray + PPFM @ 2% at booting stage and 15 days after 1st spray. A short duration rice variety ADT 45 was used as test crop for the experiment.

The RDF schedule of 120 kg N, 40 kg P_2O_5 and 40 kg K_2O ha^{-1} was adopted except for the control treatment. Two hand weedings were given on 20 and 35 days after transplanting. As per the treatment schedule, the treatment plots T_2 was directly irrigated with the help of pipeline, and maintained the field at saturated condition, whereas, in rest of the treatments, acute moisture stress was induced. Field water tube made of perforated PVC pipe having 40 cm length and 15 cm diameter was used to monitor the receding water depth on the field. Acute water stress created deep hairline cracks in the rice field, at that time the water level in the water tube goes to a depth of 12 cm. In the mean-time the soil moisture content of the soil was 23 per cent, and at this point irrigation was given.

The leaf Chlorophyll Content Index (CCI) was measured by using SPAD meter (CCM-200 plus) in all the treatments with a collective of five samples in each treatment followed by 3 replications during heading and 50 per cent flowering stage (75 DAT). The CGR explain the dry matter accumulated per unit land area per unit time, expressed as g m^{-2} day^{-1}. It was calculated by using the following formula as suggested by Enyi (1962) [5]. The experimental data pertaining to the crop were statistically analyzed as per the procedure suggested by Gomez and Gomez (1984) [6]. For obtaining significant results, the critical differences were worked out at 5% level of probability to draw statistical conclusions.

3. Results and Discussion

The plant height was significantly influenced by the foliar application of drought tolerance inducing substances. There was no significant difference observed between the treatments at tillering stage. However, after the foliar application, there was a significant difference was noticed between the treatments. Among them, Recommended Dose of Fertilizer (RDF) + acute soil drying + KNO_3 @ 2% + $ZnSO_4$ @ 0.5% at panicle initiation stage and 15 days after 1st spray + humic acid @ 0.2% at booting stage and 15 days after 1st spray (T_{11}) was recorded the maximum plant height of 77.22 and 96.34 cm at flowering and harvesting stages, respectively. Likewise, significantly higher root volume of 41.28 cc at flowering stage was noticed in treatment $T_{11.}$ The increased in the plant height was 6.29 per cent, 37.84per cent and 41.01per cent at tillering, flowering and harvest stages, respectively when compared to the control (Fig.1). The per cent increase in no. of tillers $hill^{-1}$ was 43.02per cent over control at flowering stage.

Among the treatments, significantly higher CCI value of 14.22 and 18.20 at heading and 50 per cent flowering stage, respectively were noticed in treatment T_{11} (recommended dose fertilizer (RDF) + acute soil drying + KNO_3 @ 2% + $ZnSO_4$ @ 0.5% at panicle initiation stage and 15 days after 1st spray + humic acid @ 0.2% at booting stage and 15 days after

1st spray). The higher values of the LAI (6.76) and crop growth rate of 5.86 and 6.31 g m^{-2} day^{-1} at 30-60 DAT and 60-90 DAT was found in recommended dose fertilizer (RDF) + acute soil drying + KNO_3 @ 2% + $ZnSO_4$ @ 0.5% at panicle initiation stage and 15 days after 1st spray + humic acid @ 0.2% at booting stage and 15 days after 1st spray (T_{11}).

The LAI during tillering and floweringwere39.93per cent and 94.25per cent, respectively over control. The crop growth rate during 30-60 DAT and 60-90 DAT were 266.25per cent and 29.04per cent, respectively over control. The enhanced growth might be due to synergistic and cumulative effects of combined use of NPK fertilization and foliar application of growth regulating substances. The positive effect of initially applied N, P_2O_5 and K_2O enhanced the plant height, number of tillers $hill^{-1}$, DMP and LAI. It could be due to the fact that the timely added fertilizers meet the immediate nutrient requirement of the rice crop in the early growth stages by enhancing the availability of nutrients in the rhizosphere soil. These macro nutrients are the key constituents of chlorophyll biosynthesis, protein, nucleic acid and other constituents, which vigorously enhance the growth of rice.

Nitrogen plays a vital role in plant growth and stimulating porphyrin molecules present in important metabolic compounds such as chlorophyll and cytochrome pigments necessary for photosynthesis and respiration. It affects the organic structure, physiological characteristics and biomass synthesis and distribution towards plant parts, and has the greatest effect on dry matter production (Khalofah *et al*., 2021; Schmierer *et al*., 2021) [11 & 17]. Phosphorus is found in essential biomolecules, including nucleic acids, ATP and phospholipids, which play a crucial role in root development, tillering, biomass accumulation, reproduction and ultimately yield (Jiaying *et al*., 2022) [10]. Potassium plays an essential role in plant functions, needed for osmo-regulation, enzyme activation, regulation of cellular pH, cellular cation-anion balance, regulation of transpiration by stomata, and the transport of the products of photosynthesis (Dreyer *et al*., 2017) [3].

Foliar-applied humic substances encourage plant growth by mechanisms similar to those involved in root application.Under stress situation humic acid significantly stimulated enzyme activity, encouraged photosynthesis, stimulate cell division and cell elongation, absorbance and transport of nutrients and assimilates, enhancing carbohydrateprotein synthesis, rubisco and hormone-like activity. It also stimulated growth by promoting the osmotic adjustment ability and antioxidant capacity of plants. Thus, enhanced current and reserve carbohydrates production during the reproductive stageboost CGR (Irani *et al*., 2021; Shen *et al*., 2020) [9 & 18].

The chlorophyll content index of rice was increased at 43.50 per centheading stage and 70.80per cent at 50 per cent flowering stage (Fig.2). During moisture stress, the NPK nutrition along with foliar application of growth regulating substances such as KNO_3, $ZnSO_4$ and humic acidincreased the CCI. As an agronomic trait, the CCI is closely related to the optimum availability of photosynthates, chlorophyll and nutrients in the leaf. The CCI value of plants can be used as an illustration to quantify the health conditions of plant growth. The relationship between CCI and chlorophyll has been found to be linear and can be used as a decision support tool for N-fertilization of crops. Hence, higher CCI value demonstrated the effectiveness of the treatment and the plants effectively utilized the available nutrients for their metabolic activities. Studies indicated that CCI was significantly and positively correlated with grain yield and harvest index (Liu *et al*., 2019; Rahbari *et al*., 2021) [12 & 15].

Table 1: Effect of foliar application of drought tolerance inducing substances on growth attributes of rice

Treatment	Plant height (cm)			Tillers m^{-2}	Root volume (cc)
	Tillering	Flowering	Harvest		
T_1 - Farmer's practice with acute soil drying (control)	44.98	56.02	68.32	317.16	24.09
T_2 - Recommended dose fertilizer (RDF) with normal irrigation + water spray	45.56	60.28	74.28	344.73	27.85
T_3 - RDF + acute soil drying	45.28	58.17	71.18	328.59	25.99
T_4 - T_3 + KNO_3 @ 2% + $ZnSO_4$ @ 0.5% at panicle initiation and 15 days after 1st spray	45.82	62.43	77.36	360.10	29.68
T_5 - T_3 + salicylic acid @ 200 ppm at booting stage and 15 days after 1st spray	46.30	65.15	80.68	381.47	32.02
T_6 - T_3 + gibberellic acid @ 40 ppm at booting stage and 15 days after 1st spray	46.52	67.33	83.70	394.49	33.82
T_7 - T_3 + humic acid @ 0.2% at booting stage and 15 days after 1st spray	46.78	67.65	84.72	403.36	34.21
T_8 - T_3 + pink pigmented facultative methylotrophs (PPFM) @ 2% at booting stage and 15 days after 1st spray	46.07	62.95	80.22	374.96	31.50
T_9 - T_4 + salicylic acid @ 200 ppm at booting stage and 15 days after 1st spray	47.31	72.36	90.57	429.65	37.79
T_{10} - T_4 + gibberellic acid @ 40 ppm at booting stage and 15 days after 1st spray	47.59	74.75	93.49	441.73	39.55
T_{11} - T_4 + humic acid @ 0.2% at booting stage and 15 days after 1st spray	47.81	77.22	96.34	453.60	41.28
T_{12} - T_4 + PPFM @ 2% at booting stage and 15 days after 1st spray	47.05	69.99	87.62	417.01	36.01
S.Em±	0.12	0.70	0.92	3.77	0.57
CD (P=0.05)	NS	2.06	2.74	11.20	1.71

Table 2: Effect of foliar application of drought tolerance inducing substances on physiological attributes of rice

Treatments	Chlorophyll content index (CCI)		LAI	Crop growth rate (g m^{-2} day^{-1})	
	Heading stage	50% Flowering stage	Flowering stage	30-60 DAT	60-90 DAT
T_1 - Farmer's practice with acute soil drying (control)	9.91	10.89	3.48	1.60	4.89
T_2 - Recommended dose fertilizer (RDF) with normal irrigation + water spray	10.81	12.48	4.12	2.49	5.23
T_3 - RDF + acute soil drying	10.36	11.68	3.78	1.94	4.97
T_4 - T_3 + KNO_3 @ 2% + $ZnSO_4$ @ 0.5% at panicle initiation and 15 days after 1st spray	11.04	13.19	4.44	3.02	5.44
T_5 - T_3 + salicylic acid @ 200 ppm at booting stage and 15 days after 1st spray	11.87	14.75	5.11	3.60	5.64
T_6 - T_3 + gibberellic acid @ 40 ppm at booting stage and 15 days after 1st spray	12.37	15.53	5.39	4.08	5.78
T_7 - T_3 + humic acid @ 0.2% at booting stage and 15 days after 1st spray	12.39	15.59	5.49	4.23	5.80
T_8 - T_3 + pink pigmented facultative methylotrophs (PPFM) @ 2% at booting stage and 15 days after 1st spray	11.77	13.95	4.82	3.52	5.61
T_9 - T_4 + salicylic acid @ 200 ppm at booting stage and 15 days after 1st spray	13.34	17.18	6.17	5.10	6.13
T_{10} - T_4 + gibberellic acid @ 40 ppm at booting stage and 15 days after 1st spray	13.79	17.90	6.48	5.50	6.23
T_{11} - T_4 + humic acid @ 0.2% at booting stage and 15 days after 1st spray	14.22	18.20	6.76	5.86	6.31
T_{12} - T_4 + PPFM @ 2% at booting stage and 15 days after 1st spray	12.86	16.43	5.85	4.68	5.98
S.Em±	0.14	0.23	0.09	0.10	0.01
CD (P=0.05)	0.42	0.68	0.26	0.31	0.04

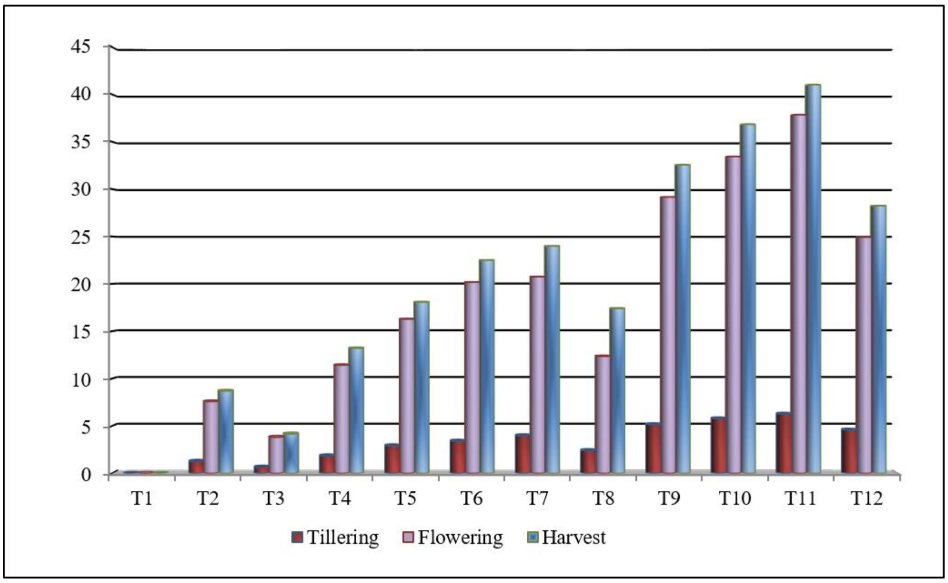

Fig 1: Effect of foliar application of drought tolerance inducing substances on per cent change in plant height (cm) at tillering, flowering and harvest stages

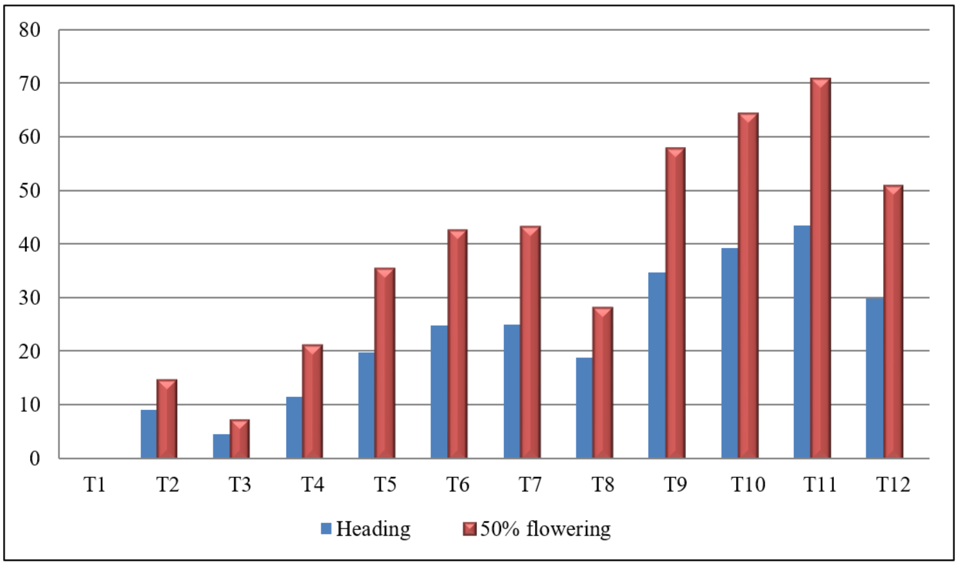

Fig 2: Effect of foliar application of drought tolerance inducing substances on per cent change in chlorophyll content index of rice at heading and 50% flowering stages

4. Conclusion

In nutshell, it could be concluded that under soil moisture stress conditions, application of 120 kg N, 40 kg P_2O_5 and 40 kg K_2O ha^{-1} along with KNO_3 @ 2% and $ZnSO_4$ @ 0.5% at panicle initiation and 15 days after first spray and humic acid @ 0.2% at booting stage and 15 days after first spray (flowering stage) holds promising agronomically sound best management technology for augmenting growth and physiological attributes of rice in North-Eastern Agro-Climatic Zone of Tamil Nadu.

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