



Improvement of grain filling in rice through foliar spray by nutrients and growth promoters

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Abstract

An investigation was carried during 2018 at Department of Rice, Tamil Nadu Agricultural University, Coimbatore. The objective of the experiment was to identify the suitable foliar nutrient/ growth promoter for enhancing grain filling in rice. Three rice genotypes, ADT (R) 49, RICE CO 52 and CO 50 were grown under different foliar treatments. The treatments are 2% DAP+ 1% KCl, 2% MAP+ 1% KCl, ZnSO₄ 0.5%, 6-Benzylaminopurine 30 ppm, Homobrassinolide 0.3 ppm, NAA100 ppm and 1-MCP 20 ppm and the control plots were sprayed with water. Foliar spray was taken up at two stages, pre-anthesis (just before heading) and post-anthesis (2 weeks after flowering or during grain filling stage). The grain filling characteristics of rice genotypes significantly varied with the different foliar nutrients. The grain filling duration (25.0 days) significantly increases with the treatment 6 - Benzylaminopurine (30 ppm) in the genotype Rice CO 52 and the grain filling rate (1.24 mg grain⁻¹ day⁻¹) was found to be higher in genotype in the genotype CO (R) 50 under the treatment T₃ - 2% MAP + 1% KCl. The hormone concentration in grains showed a significant increase with the different foliar application. High ABA and ethylene ratio was observed in the treatments. The treatment 1- MCP (20 ppm) had recorded the high ABA and ethylene ratio in superior grains in the genotype Rice CO 52 (7600.0) and in inferior grains (954.6) followed by the genotype ADT (R) 49 (4250.0 and 833.3) in the superior and inferior grains respectively. The maximum grain yield of 8536.7 kg ha⁻¹ was registered by CO (R) 50 with an increase of 19.2 per cent under the treatment of 1- MCP (20 ppm) over other treatments and genotypes. To conclude, foliar application of 1- MCP (20 ppm) and 6-Benzylaminopurine (30 ppm) at pre-anthesis and post anthesis stage to suppress ethylene production further strengthened the grain sink strength of inferior spikelets at same time development of more number of cells in endosperm by synthetic cytokinin (BAP) thereby registered better grain filling process and yield components in the rice genotype CO (R) 50.

Keywords: Rice, grain filling, ethylene, anthesis, spikelet

1. Introduction

Grain filling is the final stage in rice growth at which the fertilized ovaries develop into caryopses. The filling rate and extent determine the final weight of the rice grain and these factors are vital to grain yield and quality. Grain filling in rice is characterized by its duration and rate which exhibit significant genotypic variation in each crop season. There are many possible explanations for poor grain filling and low grain weight of the inferior spikelets, including low enzyme activity in the conversion of sucrose to starch, hormone imbalances (Wang *et al.*, 2015) [18], nutrients level, assimilate transportation barriers (Yang and Zhang, 2010) [20] and the differential expression of genes associated with cell growth and signal transduction (Mohapatra and Panigrahi, 2011) [11].

Starch in rice grains contributes to about 90% of the final dry weight of unpolished grain (Murata and Matsushima, 1975) [9]. In fact, the process of grain filling in rice is the process of starch accumulation. Photosynthates are transported from source organs (leaves, culms and sheaths) to grains in the form of sucrose and formed starch by a series of enzymatic reactions (Nakamura and Yuki, 1992) [13]. More recent work has shown that there is no clear

causative relationship between assimilate concentration and spikelet development in rice (Mohapatra *et al.*, 2000) [8]. So far, the intrinsic factors responsible for variations in grain filling between the superior and inferior spikelets remain elusive. It has been proposed that spikelet development may be mediated through endogenous hormones (Yang *et al.*, 2000, 2001) [24, 21] and a low ratio between promotive and inhibitory hormones in inferior spikelets may lead to their poor development (Naik and Mohapatra, 1999) [11].

Plant hormones have been shown to play a significant role in modifying grain filling progress and other various factors that regulate grain filling progress. A rapid increase of grain weight after flowering and fertilization in rice plants is closely related to the level of endogenous plant hormones. The level of endogenous hormones rapidly increases after heading and flowering. There is a rapid increase in grain weight after fertilization reaching a peak within two weeks followed by a rapid decrease, suggesting that endogenous plant hormones play an important role in increasing sink capacity of rice.

Cytokinins are the key regulators of plant growth and development, and function in many processes such as division of cells, biogenesis of chloroplasts, differentiation of the bud and root, initiation and growth of shoot meristem, tolerance to stress, and senescence.

Many studies have investigated the mechanism of filling and the differences between the two types of (primary and secondary) spikelets in rice varieties with different genetic backgrounds, such as rice varieties with large and small panicles; however, little is known about the cause of poor filling in inferior spikelets in rice varieties with similar genetic backgrounds. The current investigation aimed to study the filling of rice grains; and changes in the levels of various hormones, namely, abscissic acid (ABA) and ethylene, in both types of spikelets of rice varieties during the filling stage and to then identify whether the hormones regulate these variations in filling between the different types of spikelets and yield. With this background, we observed the effects of chemical regulators/nutrients on the hormonal levels in the grains and on their filling characteristics. There is considerable demand for further increase in the rice yield, and improving the filling of rice is now a prominent challenge.

2. Materials and methods

2.1. Rice materials and growth conditions

Three rice genotypes, ADT (R) 49, Rice CO 52 and CO (R) 50 were grown under different foliar treatments. The field experiment was carried out in western agro climatic zone of Tamil Nadu at 11°N latitude and 77°E longitude with an altitude of 426.72 m above Mean Sea Level under the natural light of a temperature in the range of about 32 °C and 22 °C using a randomized block design with three replications. The treatments are 2% DAP+ 1% KCl, 2% MAP+ 1% KCl, ZnSO₄ 0.5%, 6-Benzylaminopurine 30 ppm, Homobrassinolide 0.3 ppm, NAA100 ppm and 1-MCP 20 ppm and the control plots were sprayed with water. Foliar spray was taken up at two stages, pre-anthesis (just before heading) and post-anthesis (2 weeks after flowering or during grain filling stage). Five plants were collected from each treatment and replications for recording observations on grain filling traits and yield and yield components.

2.2. Grain filling rate and duration

Grain filling duration and grain filling rate was calculated as described by Yang *et al.* (2001) ⁽²¹⁾. The number of days from the initiation of the grain filling in the superior grains to the base of the apical grains is the grain filling duration and is expressed in days. Grain filling rate is the ratio of the single grain weight to the grain filling duration which states that the rate of grain filled per day and expressed as mg grain⁻¹ day.

2.3. Measurement of ABA and ethylene concentration

The endogenous hormones ABA and ethylene concentration in seeds during physiological maturity were estimated in the two best performing genotypes ADT (R) 49 and Rice CO 52 in both superior and inferior grains by the High Performance Liquid Chromatography (HPLC) and the Gas Chromatography Mass Spectrometry (GCMS). Ethylene production by the rice grains was determined according to Beltrano *et al.* (1994) ^[1] with modifications and Abscissic acid (ABA) content was measured by High Performance Liquid Chromatography (HPLC) as described by Yang (2007) ^[21].

2.4. Yield and yield components

For assessing the relationship between yield and its components, the following parameters were recorded at harvest. The details of the method for estimating each character was given by Yoshida, 1972 ^[25]. In each treatment, five plants were selected, labelled and the number of tillers producing panicles, total number of spikelets panicle⁻¹ and number of filled grains in a panicle was recorded. After initiation of flowering, the weight of the panicle of a primary tiller, single grain weight in apical and basal branches of the panicle in each plot were taken at 7 days interval as 7 DAF, 14 DAF, 21 DAF, 28 DAF and 35 DAF (Days After Flowering). The mean of the five panicle weight was expressed as g panicle⁻¹ and the single grain weight was expressed as mg grain⁻¹. The total number of spikelets panicle⁻¹, number of primary and secondary branches panicle⁻¹, number of spikelets in primary and secondary branches. The grain density obtained by using the formula (panicle length/grain number) and is expressed in cm and grains from each plot were sun dried, weighed and adjusted to 14 per cent moisture content and the grain yield was expressed as kg ha⁻¹. Data on different parameters analyzed during the research work were subjected to an analysis of variance depends on randomized block design with three replications given by (Gomez and Gomez, 1984) ^[5].

3. Results and Discussion

3.1. Grain Filling Rate and Grain Filling Duration

Grain filling in rice is characterized by its duration and rate which exhibit significant genotypic variation in each crop season. Grain filling rate and duration of the grain-filling period differed significantly among varieties. Cho *et al.* (1988) ⁽²⁾ also divided rice grain filling duration into three phases; lag phase of 5 days from heading, linear increasing phase of 5-20 days after heading and late filling period thereafter. The duration of the grain-filling period ranged from 12 to 21 days. It was generally longer in the large-grain varieties and shortest in varieties whose grain weight was less than 15 mg per grain. In this study, the significant influence of foliar treatments on grain filling characteristics of rice genotypes was shown (Fig. 1). The treatment 1-MCP (20 ppm) showed 22%, 25% and 26% increase in the genotypes ADT (R) 49, Rice CO 52 and CO (R) 50 respectively. The grain-filling rate ranged from 0.99 to 2.59 mg. grain⁻¹.day⁻¹. The highest grain filling rate was observed in the treatment 1- MCP (20 ppm) which was about 27% increase over control.

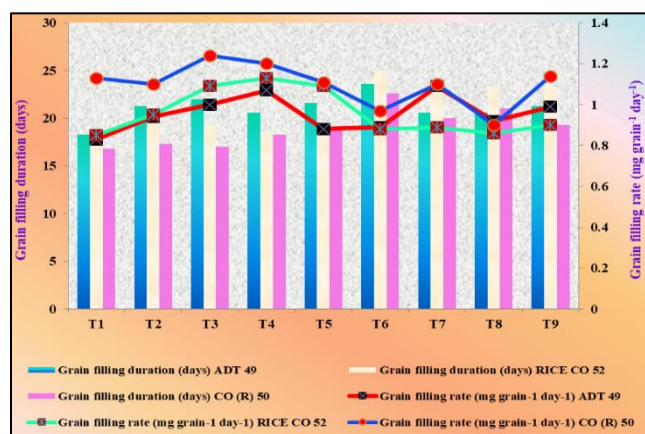


Fig 1: Effect of Nutrients and Plant growth promoters on grain filling characteristics

Grain filling duration (days) and Grain filling rate ($\text{mg grain}^{-1}\text{day}^{-1}$)

Similar results were reported by Panda *et al.* (2016) [16] reported that the decrease in the ethylene evolution as a result of decrease in the activity and expression of the ethylene biosynthesising enzymes could also be a reason for improvement of grain filling after 1- MCP application. Reduction in the level of ethylene evolution helped retention of more chlorophyll in the flag leaf of 1- MCP treated plants compared to the control plants suggesting that the foliar application was effective on rice in diminishing action of ethylene. Although, 1- MCP is an ethylene action inhibitor, repressed ethylene action might have down-regulated its synthesis ultimately.

Grain filling period was defined as the duration from anthesis to physiological maturity. The date of anthesis was determined when the anthers extruded in 50% of the panicle in the field and the date of physiological maturity was the day when the grain attained its maximum weight. In our study, the maximum grain filling duration was observed in the treatment of 6-Benzylaminopurine (30 ppm) in three rice genotypes (ADT (R) 49-23.6 days, Rice CO 52 - 25.0 days, and CO (R) 50- 22.6 days) followed by the treatment T₉ - 1- MCP (20 ppm) in all the genotypes (22.0,23.6 and 21.0).

Present results were supported by Guohui Zhu *et al.* (2011) reported that the grain-filling rate is increased with the decrease in ABA levels and reached a maximum when the ABA level was maintained in a moderate range, such as 0.5–1.5 $\text{nmol g}^{-1}\text{DW}$. The above results was also supported by Naik and Mohapatra (2000) [20].

3.2. ABA (ng. g^{-1}) and Ethylene ($\text{nmol. g}^{-1}\text{DW h}^{-1}$) content in superior and inferior grains

Grain filling process in rice was closely associated with the changes in hormone contents (Yang *et al.*, 2003) [24]. The increase in ABA contents at the end of grain filling and its rapid fall during maturation have leads to an assumption that ABA plays an important role in dry matter accumulation (Travaglia *et al.*, 2007) [19]. A higher ratio of ABA and ethylene in rice spikelets would be required to enhance the grain-filling rate. Spikelets in the lower part of the panicle that are confined in the flag leaf sheath for a longer period may be subjected to the inhibitory action of ethylene more than those in the upper part of the rice panicle. Ethylene accumulation in the boot of the flag leaf sheath significantly decreased grain filling in rice (Mohapatra and Mohapatra, 2006) [8].

The hormone ethylene and ABA contents in superior and inferior grains of current study was depicted in Fig. 2 showed the ABA concentration is higher in the superior grains than the inferior grains and the ethylene concentration was also higher in the inferior grains than the superior grains. In concordance with that the present study, the ABA concentration was higher in the superior grains of the genotype Rice CO 52 (34.0 ng. g^{-1}) than the genotype ADT (R) 49 and the low ABA concentration was observed in the control in both the genotypes. Zhu *et al.* (2011) [28] also had similar observation of present study. Obviously; it needs further study on the regulatory role of ABA in grains during grain filling. Similarly, the ethylene concentration was high under control in the inferior grains of the rice genotypes ADT (R) 49 - $0.053 \text{ nmol. g}^{-1}\text{DW h}^{-1}$ and Rice CO 52 - $0.042 \text{ nmol. g}^{-1}\text{DW h}^{-1}$

¹ than the treated plants. The ethylene inhibitor 1- MCP (20 ppm) has shown high ABA and ethylene ratio.

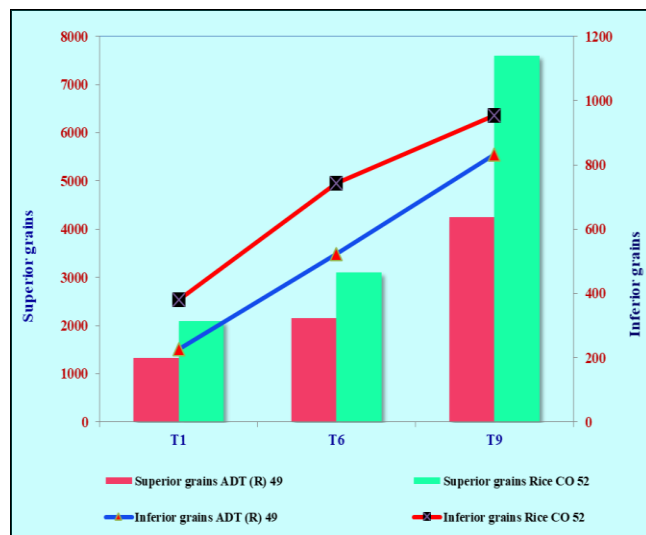


Fig 2: Effect of Nutrients and Plant growth promoters on ABA and Ethylene ratio

The increase in ABA concentration is due to the application of 1- MCP. This 1-Methylcyclopropene (1- MCP) is an anti-ethylene compound that hinders the action of ethylene by occupying its receptor sites (Sisler and Serek, 1997) [17]. Similar results were reported by Kato *et al.* (1993) [7] that ABA content in large-sized grains was higher than that in small-sized grains during rice grain filling and ethylene concentration was higher in the spikelets in the lower part of the panicle than those in the upper part of the rice panicle and the similar results were reported by Panda *et al.* (2009) [16]. Present results demonstrated that grain filling rate was not only correlated with the concentration of ABA and ethylene, but also correlated with the ratio of ABA to ethylene ratio.

3.3. Yield and Yield components

Grain weight is the product of rate and duration of grain filling (Yang *et al.* 2008) [25] Grain weight of a panicle is the product of average weight per grain and grain number. Generally, the larger the grain size, the smaller is the grain number. Similarly, the single grain weight of the apical grains which was depicted (Fig. 3a) and basal grains (Fig. 3b) shows the same trend and the panicle weight and the single grain weight were recorded higher in the treatment 1- MCP (20 ppm). This might be due to the ethylene production was high at early stage of grain development and grain filling improved when the adverse action of ethylene was blocked by 1- MCP application. Application of 1- MCP has been reported to prevent ethylene effects in several plants including rice. The end of grain filling, defined as the point where the grain growth curve had reached 95% of the maximum value. Likewise, the grain weight increased till it attains maximum grain dry weight. These results confirm previous reports that the grain-filling rate and the duration of the grain-filling period based on whole panicle tend to be higher in large-grain rice varieties by Sasahara *et al.* (1982) [17]. Yang *et al.* (2003) [24] reported that cell numbers in the rice endosperm play a dominant role in determining grain weight.

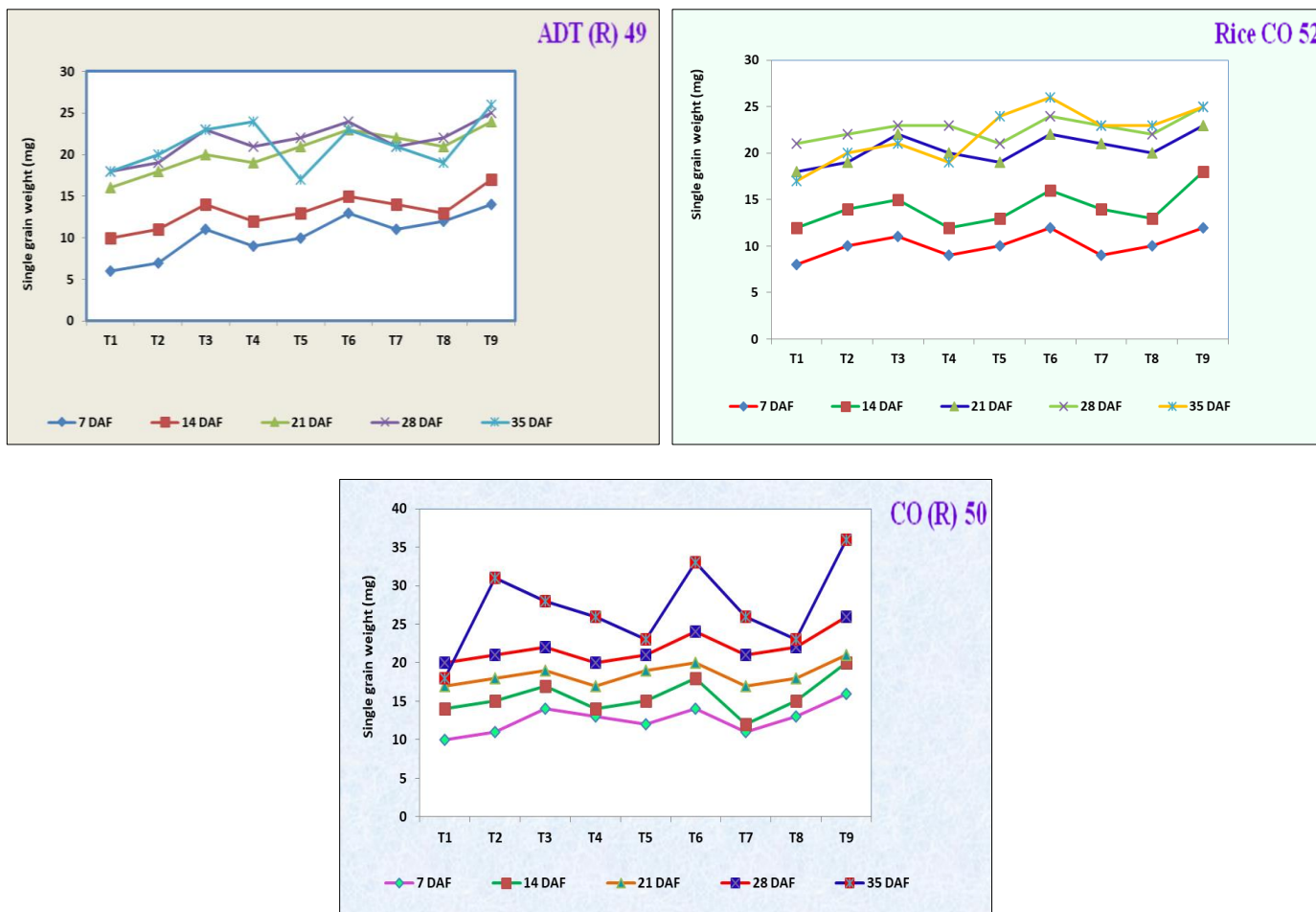
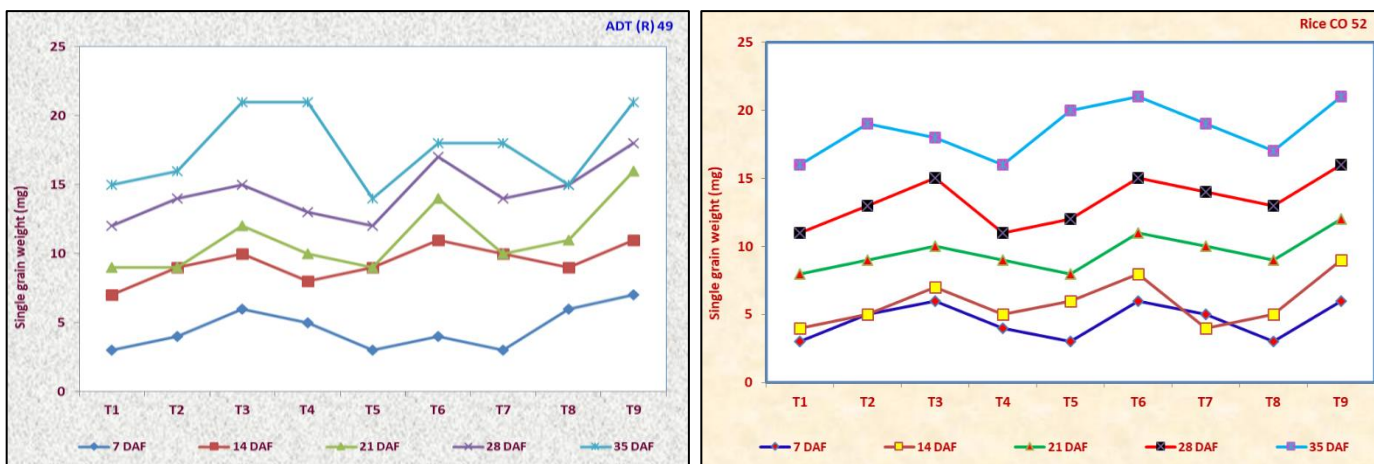


Fig 3a: Response of Nutrients and Plant Growth Promoters on Growth of Single grain weight (mg) in apical branches after flowering at days Interval in different rice genotypes



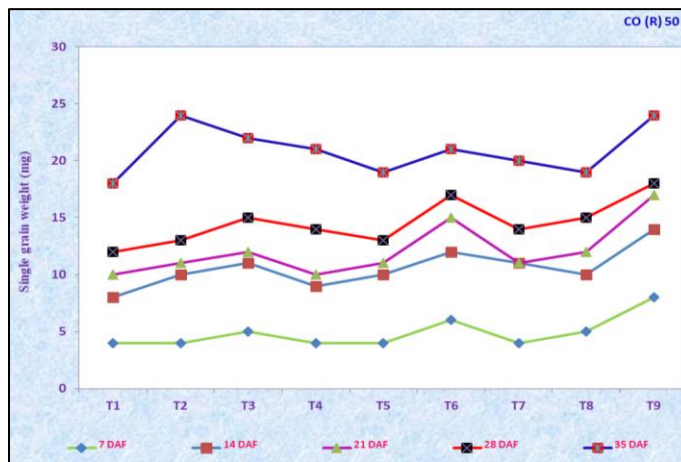


Fig 3b: Response of Nutrients and Plant Growth Promoters on Growth of Single grain weight (mg) in basal branches after flowering at 7 days interval in different rice genotypes

In the present study, regarding the spikelet fertility, the treatment 1- MCP 20 ppm had recorded the highest spikelet fertility percentage in all the genotypes (ADT (R) 49 – 93.2%, Rice CO 52 – 94.9% and CO (R) 50 – 95.6%). The increment in the spikelet fertility is not only due to the application of an ethylene inhibitor, but also by the application of nitrogenous fertilizers and potassium. Similar findings were reported by Ehsanullah *et al.* (2001) [4]. The grain density had recorded the highest in the genotype CO (R) 50 which was about 23% under the treatments 2% DAP + 1% KCl, ZnSO₄ 0.5% and 6–Benzylaminopurine (30 ppm) as depicted (Fig. 4).

influenced by different foliar nutrients and growth promoters (Fig. 5), the treatment 1-MCP (20 ppm) had observed the highest grain yield (8536.7 kg ha⁻¹) in the genotype CO (R) 50 which was about 36% increase over control. The treatment 6 – Benzylaminopurine (30 ppm) had also recorded the next higher grain yield (8100 kg ha⁻¹) under the same genotype. It increased grain yield about 32% over control. The increment in the grain yield is due to the application 1- MCP, delays leaf senescence and thereby provides assimilates to the sink for a longer grain filling period and increases the grain weight and thus higher yield. The treatment homobrassinolide also significantly increased 27% grain yield over control. Similar results were reported by Nagarayan (1999) [12] that foliar sprays of diammonium phosphate (DAP) and KCl, one at panicle initiation and the other at 10% flowering may increase yields up to 0.75 t ha⁻¹. Similar results were also found by Chunfeng Zheng *et al.* (2016) [3] that the foliar application of 6-BAP resulted in increased grain yields.

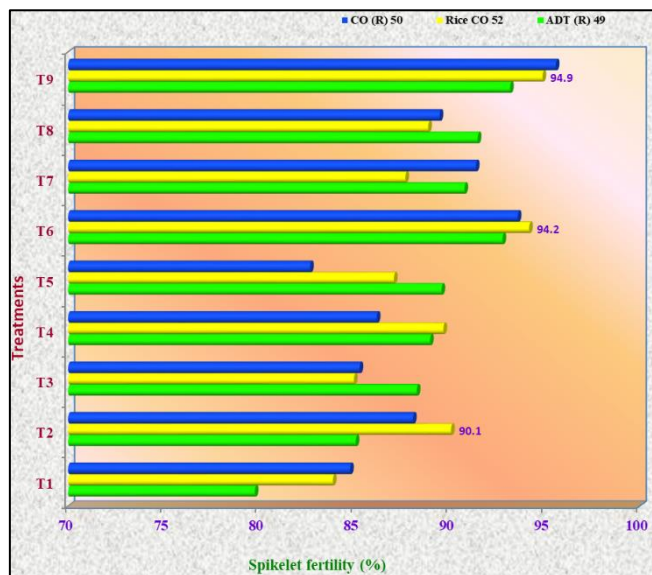


Fig 4: Response of Nutrients and Plant Growth Promoters on Grain yield (kg ha⁻¹) of rice genotypes

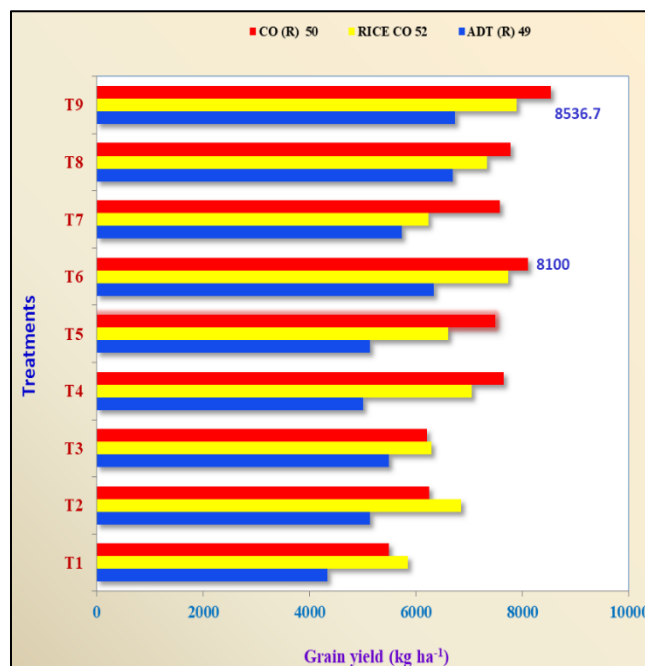


Fig 5: Effect of Nutrients and Plant Growth Promoters on Grain yield (kg ha⁻¹) of rice genotypes

The foliar application of nutrients and plant growth regulators showed significant influence on grain yield of rice. Grain yield in rice is composed of the number of grains and panicle grain sterility. In present study, the grain yield per plant was recorded highest in the treatment 1- NAA (100 ppm) and 2% DAP + 1% KCl (39.3 g) in the genotype Rice CO 52 which was about 36% increase in the genotype CO (R) 50 and 29% increase in the genotype Rice CO 52. Likewise, the grain yield (kg ha⁻¹)

4. Conclusion

Further investigation is needed to understand the cross-talk between ABA and ethylene and its response to abiotic stress and the relationship to grain filling process in rice by use of mutants or transgenic plants with an attenuated capacity to respond to or synthesize the growth regulators.

5. References

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