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Gibberellin sensitivity study shows key insights into the GA3 pathway in a semi-dwarf rice mutant of improved white Ponni

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Abstract
Improved White Ponni (IWP) is a popular rice variety in south India. We have developed WP-22-2, a semi-dwarf mutant of Improved White Ponni through gamma-ray induced mutation breeding. Gibberellin sensitivity of the mutant was studied by spraying 50 µM GA_3 hormone on ten days old seedlings and after three days, seedling height, 1st internode and 2nd leaf lengths were measured. The variances between the treatments were compared using Student's t-test. The IWP was 2.54 cm taller than the untreated mutant. The 1st internode length between the two varied with high significance (P = .003). The GA_3 treatment has increased the seedling height of the mutant 6.0 cm in three days. The reversion of plant height in the mutant showed that the mutations in the GA_3 pathway have caused defective gibberellin production which ultimately reduced the plant height in the mutant.

Keywords: semi-dwarf; GA3 pathway; gibberellin sensitive mutants; gamma-rays; mutation breeding

Introduction
Rice is an important crop in the world and is the staple food for almost 50 per cent of the world's population. Rice yields have increased throughout the world after the introduction of IR8 – a semi-dwarf rice cultivar. Rice varieties with semi-dwarfism were lodging resistant and fertiliser responsive due to which high yields were achieved with these varieties [1].
The IR8 was developed from Dee-Geo-Woo-Gen and the gene responsible for its semi-dwarfism was identified as an incomplete recessive gene d47. It was later found to be an allele of the gene *sd1* – termed as 'Green Revolution' gene [2]. The allele in Dee-Geo-Woo-Gen had 383 bp deletion in the Gibberellin 20 oxidase 2 locus which caused defects in gibberellin synthesis which ultimately reduced the plant height [3]. Similarly other alleles of the *sd1* gene were reported to have single nucleotide variations and deletions [1, 2, 4].
Plant height is controlled by many genes in rice. The gibberellic acid pathway is controlled by key regulators such as *ent-kaurene oxidase, ent-kaurene synthase* [5], *ent-copalyl diphosphate synthase* [6], GA20oxidase, GA3oxidase [7] and other genes. Defective alleles of these genes cause extreme dwarfism to semi-dwarfism in plants. Such mutants are usually sensitive to gibberellin and can revert back to original plant height [2]. There are mutants which are considered gibberellin-insensitive due to their inability to recover the plant height with external gibberellin applications. *GID1* (gibberellin insensitive dwarf1) causes gibberellin insensitive mutants. It encodes a soluble receptor for perceiving gibberellic acid [8].
Studying of the gibberellin sensitivity gives a key insight into the genes responsible for the reduced plant height. A mutant termed as WP-22-2 was developed from the popular south Indian rice variety Improved White Ponni (IWP) through gamma-ray irradiation [9]. The genotype is semi-dwarf with reduced days to flowering and high yield than the Improve White Ponni. To elucidate the mechanism of semi-dwarfism in this genotype, the gibberellin responsiveness of this mutant was studied by external application of gibberellin hormone.

Materials and methods
Plant material
The rice seeds were obtained from the Department of Plant Breeding and Genetics, Agricultural College and Research Institute, Killikulam, Tamil Nadu, India. Well matured rice seeds of Improved White Ponni (IWP) and WP-22-2 were sown (3 replications) in Phytajars containing enriched compost and soil mixture.

Gibberellic acid preparation

10 mg of GA_3 (SRL, India) was first dissolved in few drops of absolute ethanol. Then the solution was made up to 10 mL with sterile deionised water to make a 1 mg mL^{-1} solution. From the stock, 692 µL of GA_3 solution was made up to 40 mL with sterile water to get 50 µM solution.

GA_3 treatment of mutant

The 50 µM solution was sprayed on the leaves of the WP-22-2 with a hand sprayer. After three days, seedling height, primary internode length and second leaf length of the genotypes IWP, WP-22-2 (untreated) and WP-22-2 (GA3) were measured.

Statistical analysis

Student's t-test on the variances of the different treatments was performed on the observations to find the significance. The mean lengths were plotted in R [10] and gg plot 2 [11].

Results and Discussions

The ten days old seedlings of WP-22-2 were treated with 50 µM GA_3 solution. After three days the seedling height, 1st internode length and 2nd leaf length were measured. Comparison between the IWP, WP-22-2 (untreated) and WP-22-2 (GA3) showed key morphological differences.

Intercalary meristem cell division and elongation are the major causes for internodal elongation in rice and flaws in these processes severely affect the plant height. Many studies in dwarf mutants suggest defects in gibberellic acid pathway reducing the cell division [12].

The height difference between the two untreated types IWP and WP (untreated) was clearly visible within 13 days. The seedling height was a combination of the 1st internode and the 2nd leaf. In 13 days, 2.54 cm length difference in seedling height was observed between the IWP and WP-22-2 (untreated) which were highly significant ($P = 0.001$) (Table 1 & 2; Fig 1 & 2). The 1st internode length between the two genotypes varied with a length difference of 1.6 cm and with high significance ($P = 0.003$). The second leaf length between the two genotypes was non-significant. The comparison shows that the seedling height was primarily determined by the 1st internode elongation since the 2nd leaf length between the two was non-significant. This can be compared with previous reports suggesting the prevention of internode elongation is considered an important phenomenon in dwarf mutants of rice [13].

The overall seedling length showed high rate of increase in GA_3 treated WP-22-2. The WP-22-2 (GA3) showed an increase of 6.0 cm compared to the untreated plants. Similarly 1.36 cm increase in 1st internode length and 4.64 cm increase in 2nd leaf length was observed in the WP-22-2 (GA3) in comparison to the untreated WP-22-2. These results show that the mutant is highly sensitive to the externally applied GA_3.

The comparison of the WP-22-2 (GA3) and IWP show that the mutant had attained the original plant height after GA_3 application. The 1st internode of WP-22-2(GA3) has elongated to a comparable length as Improved White Ponni (non-significant in the t-test) while the 2nd leaf length has surpassed the IWP (3.7 cm higher than the IWP). This further validates the earlier reports suggesting the defects in the GA_3 pathway caused by mutations in dwarf mutants are usually responsive to external GA_3 [4]. These results further state that the mutation could be in the genes controlling the GA_3 pathway and especially the *sd1*.

As the WP-22-2 mutant is lodging resistant due to the semi-dwarfism, it is advantageous over the Improved White Ponni. The preliminary studies show that the mutant is gibberellin sensitive and the mutations that have caused defective gibberellin production. The genes that control the trait could be narrowed down to the GA_3 pathway; however, genome level analyses would further validate this claim.

Table 1: Mean internode and second leaf lengths of IWP, WP-22-2 (untreated) and WP-22-2 (GA3 treated)

	Trait	Mean (cm)	Standard Error
IWP	1st internode	6.96	0.29
	2nd leaf	11.20	0.54
	Seedling height	18.16	0.34
WP-22-2 (untreated)	1st internode	5.36	0.09
	2nd leaf	10.26	0.46
	Seedling height	15.62	0.43
WP-22-2 (GA3)	1st internode	6.72	0.41
	2nd leaf	14.90	0.99
	Seedling height	21.62	0.97

Table 2: Student's t-test for variances of first internode and II-leaf lengths

	Test	t-stat	p value (two-tail)
Seedling height	IWP vs. WP-22-2 (untreated)	4.61	.001**
	WP-22-2 (untreated) vs. WP-22-2 (GA3)	-5.62	.000**
	IWP vs. WP-22-2 (GA3)	-3.35	.004**
1st internode	IWP vs. WP-22-2 (untreated)	5.21	.003**
	WP-22-2 (untreated) vs. WP-22-2 (GA3)	-3.23	.005**
	IWP vs. WP-22-2 (GA3)	0.48	.638NS
2nd leaf	IWP vs. WP-22-2 (untreated)	1.33	.219NS
	WP-22-2 (untreated) vs. WP-22-2 (GA3)	-4.26	.000**
	IWP vs. WP-22-2 (GA3)	-3.28	.004**

**Significant at 1% level of significance; NS- non significant

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