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Variations in Chlorophyll and Carotenoid Contents in Tungro Infected Rice Plants

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Abstract

Sporadic tungro virus disease is considered as one of the most devastating viral diseases of rice and causes significant yield losses to sustainable annual rice productions in the world. An experiment was conducted to find out the influence of tungro disease on the chlorophyll and carotenoid content in fourteen rice cultivars of three groups viz., susceptible - MTU 1010, TN-1, IR 64, Tapaswini and IR 50; moderately resistant - Swarna, RP Bio 226, BPT 5204, Swarnadhan and Nidhi; and resistant rice cultivars - Tamphaphou , TKM 6, IRTN 51 and Vikramarya. The virus isolate induced a considerable alteration in the pigment content by always exhibiting lesser amounts of chlorophyll 'A', chlorophyll 'B', total chlorophyll and carotenoid contents in the leaves of susceptible rice cultivars as compared to moderately resistant and resistant cultivars. Considerable reduction in seed weight has been observed in the susceptible rice cultivars compared to moderately resistant and resistant cultivars due to virus infection.

Keywords: Rice tungro virus disease (RTD); Chlorophyll; Carotenoids

Introduction

Oryza sativa L. belonging to the family *Germaine* is considered as one of the most important cereal crops as it holds the key for household food and nutritional security. However, in major rice- growing south-eastern countries, many rice viruses have been posing a major threat to sustainable rice production. Of these, rice tungro virus disease is wide spread and occurred in epidemic form in several states of India and gained considerable importance during the last 50 years. Tungro disease is caused by co-infection of two different viruses, Rice tungro bacilliform virus (RTBV, genus Tungro virus, family Caulimoviridae) a pararetrovirus with a double stranded DNA genome, and Rice tungro spherical virus (RTSV, genus Waikavirus, family Secoviridae), a plant picorna virus with a single – stranded (+)-sense RNA genome. Both these viruses are transmitted in a semipersistant manner by green leafhopper vector *Nephotettix virescens* (Distant) and some other leafhopper species [1,2]. The most conspicuous symptoms of rice plants infected with both RTSV and RTBV are stunting and yellow to orange discoloration of the leaves [3].

A feature common to virus infection in plants, may be reduction in the number of chloroplasts in mesophyll [4], apart from a frequent involvement of the color change in most of the plants showing that chlorophyll content is either not synthesized at the same rate as in healthy plants or some amount of chlorophyll is destroyed as a consequence of infection. Therefore, measurement of chlorophylls and carotenoids which are regarded as essential pigments of higher plant assimilatory tissues could provide the basis to understand the physiological status of a plant. Hence the present study was aimed at studying the influence of rice tungro virus on leaf pigment content (chlorophyll A and B, carotenoids), and yield in terms of seed weight in susceptible, moderately resistant and resistant rice cultivars.

Materials and Methods

The present study follows the system developed by Heinrichs [5] at IRRI, for mass rearing of green leaf hopper vector. The seedlings of rice cultivar Taichung Native-1 T(N)1, were grown in pots to 45 days old plants and maintained in water resistant, insect proof wooden or mylar rearing cages under glass house conditions at IIRR in order to raise the disease specimens of tungro. About 500-1000 adult non-viruliferous colonies of GLH (*Nephotettix virescens*) were collected from the rice field at IIRR with the help of a sweep net and maintained in cages with 45 days old healthy feeder plant for 2-3 days oviposition (28 ± 2°C, >95% RH) followed by emergence of nymphs and their development into adults. The newly emerged adult vector population was then allowed to feed on rice tungro virus complex infected 45 days old susceptible T(N)1 rice seedlings for 24 hours to generate viruliferous GLH. These viruliferous green leafhoppers were then used for inoculation of healthy T (N) 1 rice seedlings @ 2-3 insects/seedling in pots under insect proof cages and were allowed for expression of symptom development for about 2-3 weeks. Thus rice tungro virus disease was maintained by repeating the process several times.

A total of fourteen rice genotypes divided into three groups were evaluated to determine the pigment content due to RTD infection in the present study. This included, susceptible - MTU 1010, TN-1, IR 64, Tapaswini and IR 50 ; moderately resistant - Swarna, RP Bio 226, BPT 5204, Swarnadhan and Nidhi; and resistant rice cultivars - Tamphaphou , TKM 6, IRTN 51 and Vikramarya. The seeds of rice cultivars were soaked overnight in petridishes. 3-4 days after germination ten seedlings/row of each cultivar were transplanted in plastic trays. After 9-10 days of transplantation five of the rice seedlings of each cultivar were inoculated by viruliferous green leafhopper vector, Hyderabad ecotype @ 5-6 adults/tiller by encaging individual seedlings by cellulate butyrate tubes for about 17-18 hours, inoculation access period (IAP) in a green house. The other five seedlings were kept as uninoculated control. After

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20 days when the plant indicated typical symptoms of RTD infection, disease score was recorded by adopting the standard evaluation method proposed by IRRI for rice. In the present study the selected rice cultivars were categorized based on RTD complex infection as: Resistant (0-30%, score -3), moderately resistant (31-60%, score - 5) and susceptible (61-100%, score - 7). After complete expression of visual symptoms in the treated plants, photosynthetic pigment content was studied in both control and infected plants of susceptible, moderately resistant and resistant rice cultivars [6].

Five replicates each for healthy and diseased rice leaf cultivars of susceptible, moderately resistant and resistant were prepared by excision of leaf from the plants grown in plastic trays. These were brought to the laboratory in polythene bags, lined with moist filter paper inside. The leaves were washed thoroughly in running cold water for 5 min and then cut into small pieces and used for the quantitative determination of chlorophyll A, chlorophyll B, total chlorophyll and carotenoids in a whole pigment extract of RTD infected and healthy leaves comprising of fourteen rice cultivars. Leaf chlorophyll pigments were extracted using cold 80% acetone and contents of chlorophyll and carotenoids were determined spectrophotometrically using Spectrascan UV 2600, Toshniwal Instruments Pvt. Ltd., and India. Leaf chlorophyll pigments were estimated by the method described by Lichtenthaler and Wellburn [7]. The results in tables are indicated as arithmetical means and ± standard error of mean has been performed.

Results and Discussion

Chlorophyll content

Significant differences were observed in leaf chlorophyll content ($p<0.001$), carotenoid content ($p<0.001$), seed weight ($p<0.001$) between healthy and infected samples and among genotypes (Table 1). The interaction was also found to be significant (Table 1). Chlorophylls and carotenoids are essential pigments of higher plant assimilatory tissues and responsible for variations of color from dark-green to yellow. Larcher [8] opined that chlorophyll content is one of the indices of photosynthetic activity. Further, the role of Chlorophyll A and B in the metabolic activities of the plant finally to the yield is a well-known phenomenon. The current study demonstrated a drastic reduction in chlorophyll 'A' (Table 2a and Figure 1), chlorophyll 'B' (Table 2a and Figure 2) and total chlorophyll (Table 2b and Figure 3) in susceptible rice cultivars compared to moderately resistant and resistant cultivars.

The reduction in chlorophyll 'A' content was high in susceptible rice cultivars, MTU 1010 (91.33%) followed by TN-1 (84.11%), IR 64(72.22%), Tapaswini (64.81%) and IR 50(63.6%). This was followed by moderately resistant rice cultivars, Swarna (54.2%), RP Bio 226(52.98%), BPT 5204(43.17%), Swarnadhan (41.76%), and Nidhi (40.7%). Resistant rice cultivars, *viz.,* Tamphaphou (24.27%), TKM 6(19.23%), IRTN 51(17.18%), showed comparatively less reduction in chlorophyll A with the least value observed in Vikramarya (12.5%). The findings of the study can be correlated with the earlier reports of Ramiah et al.[9] who presented the reduction in chlorophyll content in many host plants infected with different viruses such as virus infected leaves of *Cucurbita pepo, Abelmoschus esculentus* and *Glycine max*. Further, Dante et al. [10] pointed out decreased chlorophyll (total 'a' and 'b') contents in leaves of soybean infected with yellow mosaic virus.

The study discovered that chlorophyll 'B' content varied to a large extent in RTD susceptible, moderately resistant and resistant cultivars. Greater reduction in chlorophyll 'B' has been observed in the susceptible rice cultivars as compared to moderately resistant and resistant rice cultivars. Among the fourteen rice cultivars, Vikramarya showed least reduction (12.43%)in the pigment chlorophyll 'B' , followed by TKM 6(23.81%), IRTN 51(27.12%) and Tamphaphou (29.53%) as compared to moderately resistant rice cultivars RP Bio 226(55%), BPT 5204(45.13%), Swarna (43.9%), Swarnadhan (42.98%) and Nidhi (42.19%). Whereas drastic reduction in the pigment content was observed in the susceptible rice cultivars MTU 1010(81%), followed by TN (1)(75%), IR 64(72.41%), IR 50(71.68%) and Tapaswini (63.33%).

The present study coincides with the findings of Bhavani et al. [11], who revealed the negative effects of viral infection, on sunflower (*Helianthus annus L.*) leaves. They reported that chlorophyll a, chlorophyll b, total chlorophyll and chlorophyll a/b ratios were all low in virus infected leaves as compared to healthy leaves. Furthermore the study undertaken by Charitha Devi and Radha [12] indicated a significant loss of total chlorophyll, chlorophyll a and chlorophyll b in the leaves of cucumber plants infected with cucumber mosaic virus as compared to their corresponding healthy ones. The data clearly indicated that the meager loss of chlorophyll in the resistant rice cultivars Vikramarya, TKM 6**,** IRTN 51 and Tamphaphou confirms the high degree of resistance towards both the vector and the pathogen.

The study presents a drastic reduction in total chlorophyll in the susceptible rice cultivars TN-1 (83.22%), followed by MTU 1010 (76%), IR 64 (72.3%), IR 50 (65.68%) and Tapaswini (64.44%). This decrease in total chlorophyll in the susceptible rice cultivars seemed to be high when compared to moderately resistant cultivars *viz.,* Nidhi (41.1%), Swarnadhan (42.06%), BPT 5204(43.68%), Swarna (51.97%) and RP Bio 226(53.51%) and resistant cultivars Tamphaphou (25.67%), TKM 6(20.59%), IRTN 51(19.6%) and Vikramarya (12.48%) indicating a clear reduction in pigment content in susceptible rice cultivars when compared to resistant cultivars.

Studies carried out by Funayama-Noguchi and Terashima [13], demonstrated that the net increase in Chlorophyll content per whole leaf stopped in the virus-infected *Eupatorium makinoi* leaves when their lamina lengths were about half of the maximum value, which was probably due to inhibition of chlorophyll synthesis. When plants suffer from biotic stress, they often turn yellow. The results of this study suggest that chlorophyll metabolism may be susceptible to biotic stress.

Earlier reports of Sheffield, Smith and Bawden [14-16] considered that virus infection destroyed the chlorophyll of leaves producing chlorosis. This might be attributed to the stimulation of chlorophyllase which attack chlorophyll and inhibit chloroplast development. Hence the function of chloroplast seems to be directly affected by a decline in chlorophyll content. Furthermore, Xiang et al. [17] and Kong et al. [18] opined that direct or indirect interactions of RNA virus-encoded proteins with the chloroplastic proteins contribute significantly to viral pathogenesis. Moreover, the chloroplast's dual roles, as the home of photosynthesis and a hub of defense response [19,20] make them attractive targets for viral pathogens as evidenced by the cases of *Tobacco mosaic virus* (TMV), *Plum poxvirus* and *Potato virus Y*.

Carotenoid level

It has also been reported that the viral pathogen has significantly reduced the carotenoid level (Table 2b and Figure 4) in the susceptible cultivars MTU 1010(59.195%), followed by TN-1(55.02%), IR 64(41.28%), Tapaswini (40.85%) and IR 50(37.58%). The results indicate a considerably less loss of carotenoids in Swarna (34.2%), RP Bio 226 (32.3%), Swarnadhan (27.93%), Nidhi (26.98%) and BPT 5204 (25.96%) with a least reduction observed in the resistant cultivars Vikramarya (14.89%), IRTN 51 (17.65%), TKM 6 (20.3%) and Tamphaphou (23.11%).

Chlorophyll A	**DF**	**sum of squares**	**Mean Squares**	**F Value**	**P Value**
Rep	1	0.033	0.033		
Genotype	13	20.2523	1.5579	26.78	0.000
Treatment	1	14.4841	14.4841	248.98	0.000
Genotype X Treatment	13	7.709	0.593	10.19	0.000
Error	27	1.5707	0.0582		
Total	55	44.0491			
	Grand mean	1.6461			
	CV	14.65			
Chlorophyll B	DF	sum of squares	Mean Squares	F Value	P Value
Rep	1	0.00112	0.00112		
Genotype	13	1.68232	0.12941	48.57	0.000
Treatment	1	1.46254	1.46254	548.96	0.000
Genotype X Treatment	13	0.48568	0.03736	14.02	0.000
Error	27	0.07193	0.00266		
Total	55	3.7036			
	Grand mean	0.5123			
	CV	10.07			
Total Chlorophyll	DF	sum of squares	Mean Squares	F Value	P Value
Rep	1	0.0463	0.0463		
Genotype	13	29.7768	2.2905	36.28	0.000
Treatment	1	25.1518	25.1518	398.33	0.000
Genotype X Treatment	13	9.804	0.7542	11.94	0.000
Error	27	1.7049	0.0631		
Total	55	66.4838			
	Grand mean	2.1584			
	CV	11.64			
Carotenoids	DF	sum of squares	Mean Squares	F Value	P Value
Rep	1	0.0569	0.0569		
Genotype	13	7.9608	0.61237	53.22	0.000
Treatment	1	1.432	1.432	124.46	0.000
Genotype X Treatment	13	1.1973	0.0921	8	0.000
Error	27	0.3107	0.01151		
Total	55	10.9576			
	Grand mean	0.7726			
	CV	13.88			
Seed weight	DF	sum of squares	Mean Squares	F Value	P Value
Rep	1	0.305	0.305		
Genotype	13	351.262	27.02	10106.3	0.000
Treatment	1	215.698	215.698	80677.3	0.000
Genotype X Treatment	13	38.996	3	1121.96	0.000
Error	27	0.072	0.003		
Total	55	606.334			
	Grand mean	10.5			
	CV	0.49			

Table 1: Analysis of variance for leaf pigment content and seed weight.

The results are in agreement with the previous findings of Mali et al. [21], who suggested that significantly higher content of total phenols, increase in free proline accumulation and less decrease in total chlorophyll 'a', 'b', carotenoids, soluble carbohydrate and starch were characteristic in yellow mosaic virus resistant moth bean genotype as compared to susceptible genotype. Similar conclusions has been reached by Hemida [22], who demonstrated a gradual decline in the photosynthetic pigments (chlorophylls a, b and carotenoids), water soluble carbohydrates, total soluble proteins and total free amino acids in the leaves of two host plants (*Vicia faba* and *Phaseolus vulgaris*) inoculated with bean yellow mosaic virus for 4, 12 and 20 days when compared with their corresponding healthy plants.

Certain characteristics such as leaf area, chlorophyll content and flow of nutrients in the plants are generally influenced by a reduced photosynthetic rate of the plant caused due to virus infection, leading to a gross loss in quality and quantity of the crop. This information

	Observations	Chlorophyll A (1a)			Chlorophyll B (1b)		
		Healthy	Diseased	Mean % reduction	Healthy	Diseased	Mean % reduction
TN-1	R1	3.4	1.15	84.11 ± 16.092	0.4	0.07	75 ± 7.16
	R2	4.28	0.07		0.44	0.14	
MTU 1010	R1	1.7	0.54	91.33 ± 5.13	0.91	0.24	81 ± 6.76
	R2	1.3	0.28		1.09	0.14	
IR 64	R1	1.49	0.36	72.22 ± 3.525	0.53	0.14	72.41 ± 1.495
	R2	1.57	0.49		0.34	0.1	
Tapaswini	R1	3.25	1.09	64.81 ± 1.68	1.04	0.36	63.33 ± 2.03
	R2	3.2	1.18		1.06	0.41	
IR 50	R1	1.58	0.58	63.6 ± 0.28	0.56	0.15	71.68 ± 1.52
	R2	1.66	0.6		0.57	0.17	
Swarna	R1	1.38	0.76	54.2 ± 6.835	0.39	0.26	43.9 ± 10.08
	R2	1.61	0.61		0.43	0.2	
RP BIO 226	R1	1.97	0.77	52.976 ± 9.575	0.63	0.29	55 ± 1.08
	R2	1.39	0.81		0.57	0.25	
BPT 5204	R1	1.46	0.93	43.167 ± 6.28	0.51	0.34	45.13±10.755
	R2	1.76	0.9		0.62	0.28	
Swarnadhan	R1	1.78	1.03	41.76 ± 0.366	0.59	0.35	42.98 ± 2.24
	R2	1.86	1.09		0.62	0.34	
Nidhi	R1	1.77	1.11	40.7 ± 3.065	0.58	0.36	42.19 ± 3.89
	R2	2.21	1.25		0.7	0.38	
Vikramarya	R1	3.2	2.79	12.5 ± 0.305	0.91	0.79	12.43 ± 0.78
	R2	3.28	2.88		0.86	0.76	
TKM 6	R1	1.86	1.46	19.23 ± 1.915	0.62	0.47	23.81 ± 0.375
	R2	1.98	1.63		0.64	0.49	
Tamphaphou	R1	1.98	1.48	24.27 ± 0.945	0.73	0.53	29.53 ± 2.095
	R2	2.14	1.64		0.76	0.52	
IRTN 51	R1	2.52	2.02	17.18 ± 2.645	0.84	0.63	27.12 ± 2.015
	R2	2.75	2.35		0.93	0.66	

Table 2a: Alteration in the amount of pigment content (1a) chlorophyll A, chlorophyll B; (1b) total chlorophyll and carotenoids (1c) in susceptible, moderately resistant and resistant rice cultivars induced by rice tungro virus infection.

Entry	Observations	Total Chlorophyll			Carotenoids (1c)		
		Healthy	Diseased	Mean % reduction	Healthy	Diseased	Mean% reduction
TN-1	R1	3.8	1.22	83.22 ± 13.83	2.17	1	55.02 ± 1.01
	R2	4.72	0.21		2.61	1.15	
MTU 1010	R1	2.61	0.78	76 ± 6.16	0.85	0.34	59.195 ± 0.055
	R2	2.39	0.42		0.89	0.355	
IR 64	R1	2.02	0.5	72.3 ± 3.07	0.53	0.31	41.28 ± 0.205
	R2	1.91	0.59		0.56	0.33	
Tapaswini	R1	4.29	1.45	64.44 ± 1.76	0.76	0.42	40.85 ± 3.62
	R2	4.26	1.59		0.88	0.55	
IR 50	R1	2.14	0.73	65.68 ± 0.195	0.84	0.46	37.58 ± 8.775
	R2	2.23	0.77		0.65	0.47	
Swarna	R1	1.77	1.02	51.97 ± 8.96	0.66	0.45	34.2 ± 2.025
	R2	2.04	0.81		0.92	0.59	
RP BIO 226	R1	2.6	1.06	53.51 ± 6.655	0.96	0.64	32.3 ± 1.285
	R2	1.96	1.06		0.65	0.45	

BPT 5204	R1	1.97	1.27	43.68 ± 7.445	0.47	0.38	25.96 ± 6.215
	R2	2.38	1.18		0.57	0.39	
Swarnadhan	R1	2.37	1.38	42.06 ± 0.285	0.54	0.39	27.93 ± 0.16
	R2	2.48	1.43		0.57	0.41	
Nidhi	R1	2.35	1.47	41.1 ± 3.27	0.62	0.48	26.98 ± 4.335
	R2	2.91	1.63		0.64	0.44	
Vikramarya	R1	4.11	3.58	12.48 ± 0.41	1.57	1.38	14.89 ± 2.67
	R2	4.14	3.64		1.72	1.42	
TKM 6	R1	2.48	1.93	20.59 ± 1.55	0.74	0.6	20.3 ± 1.3
	R2	2.62	2.12		0.79	0.62	
Tamphaphou	R1	2.71	2.01	25.67 ± 0.155	0.93	0.81	23.11 ± 8.7
	R2	2.9	2.16		1.32	0.92	
IRTN 51	R1	3.36	2.65	19.6 ± 1.46	0.8	0.64	17.65 ± 2.22
	R2	3.68	3.01		0.9	**0.76**	

Table 2b: Total chlorophyll and carotenoids.

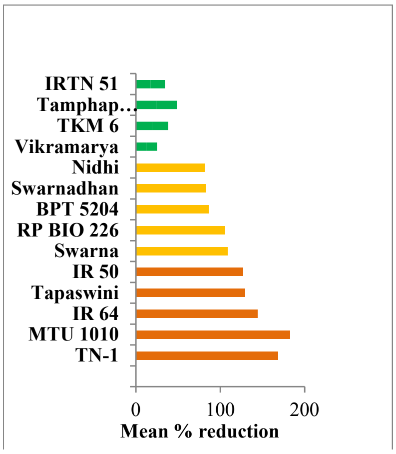

Figure 1: Graphical representation of leaf pigment content and yield component in terms of seed weight (chlorophyll A).

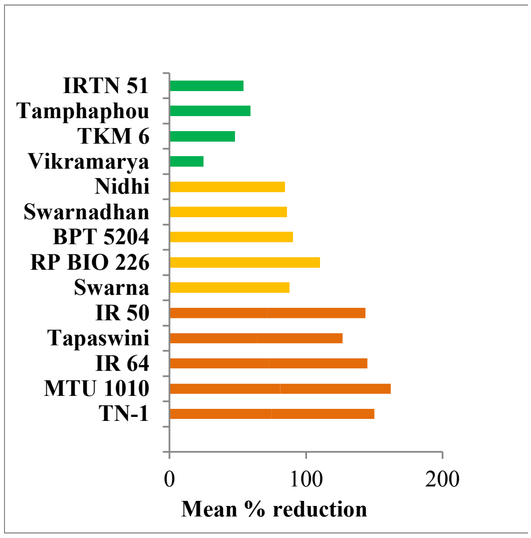

Figure 2: Graphical representation of leaf pigment content and yield component in terms of seed weight (cholorophyll B).

provides an essential tool to assess the economics of the disease control.

The findings of the study ultimately demonstrated that the virus infection affected the yield of the rice plants whereby significantly reduced seed weight has been found in the susceptible rice cultivars when compared to moderately resistant and resistant cultivars (Table 3 and Figure 5) which indicates 58.76% reduction in seed weight while in vikramarya it was showing 5.73%.

The findings are in agreement with the earlier observations of Singh et al. [23], whose studies elucidated that the Cucumber mosaic virus (CMV) significantly reduced total production and chlorophyll contents in infected leaves of cucumber as compared to healthy leaves. Similarly Bhavani et al. [11] concluded that the virus infection decreased the overall growth and yield of sunflower when compared to corresponding healthy plants.

Conclusion

Considering the economic importance of rice tungro virus disease, efforts are underway to develop tungro tolerant rice genotypes. The study has generally drawn the basic information on the physiological status of plant and primary productivity in terms of seed weight by measurement of photosynthetic pigments in susceptible, moderately

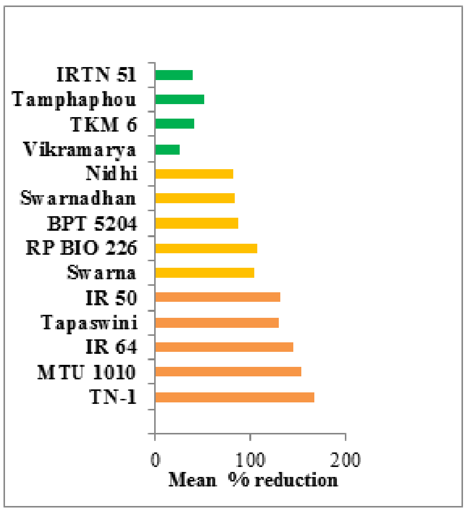

Figure 3: Graphical representation of leaf pigment content and yield component in terms of seed weight (total chlorophyll).

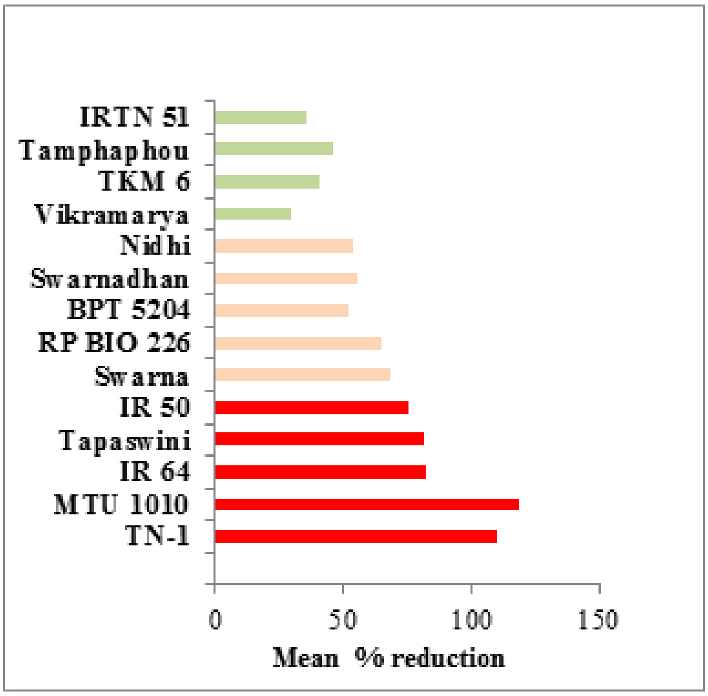

Figure 4: Graphical representation of leaf pigment content and yield component in terms of seed weight (carotenoids).

Entry	Observations	Seed Weight		
		Healthy	Diseased	Mean % reduction
TN-1	R1	8.73	3.6	58.76 ± 0.26
	R2	8.94	3.64	
MTU 1010	R1	8.97	3.7	58.75 ± 0.005
	R2	9.15	3.775	
IR 64	R1	12.48	5.88	52.88 ± 0.0005
	R2	12.67	5.97	
Tapaswini	R1	9.7	5.33	45.045 ± 0.0025
	R2	9.88	5.43	
IR 50	R1	11.58	6.4	44.8 ± 0.065
	R2	11.97	6.6	
Swarna	R1	13.03	8.6	33.99 ± 0.005
	R2	13.3	8.78	
RP BIO 226	R1	14.52	9.58	34.02 ± 0.005
	R2	14.7	9.7	
BPT 5204	R1	13.82	9.12	34.005 ± 0.0035
	R2	13.97	9.22	
Swarnadhan	R1	14.73	10.8	26.67 ± 0.015
	R2	14.82	10.87	
Nidhi	R1	14.61	10.71	26.57 ± 0.002
	R2	14.76	10.82	
Vikramarya	R1	14.88	14.03	5.73 ± 0.014
	R2	14.97	14.11	
TKM 6	R1	13.27	11.53	13.12 ± 0.01
	R2	13.48	11.71	
Tamphaphou	R1	9.57	7.9	17.44 ± 0.005
	R2	9.75	8.05	
IRTN 51	R1	13.3	11.55	13.15 ± 0.0035
	R2	13.39	11.63	

Table 3: Considerable loss of Seed weight (g) due to Rice tungro virus infection in the inoculated susceptible, moderately resistant and resistant rice cultivars compared to the control plants of same stage.

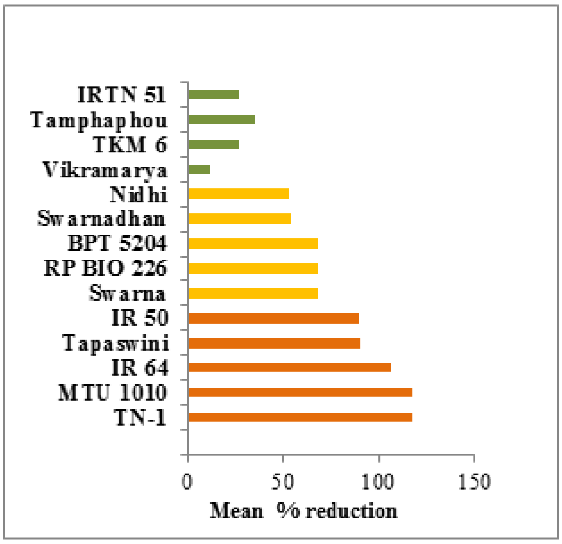

Figure 5: Graphical representation of leaf pigment content and yield component in terms of seed weight (seed weight).

resistant and resistant rice genotypes against tungro infection. It can be concluded that the meager loss of chlorophyll in the resistant rice cultivars confirms the high degree of resistance towards both the vector and the pathogen. Further it can be attributed that stimulation of chlorophyllase might contribute in destruction of chlorophyll by inhibiting chloroplast development in virus infected plants thereby affecting the productivity.

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