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Correlation of protein and ash content of rice genotypes with rice weevil *Sitophilus oryzae* (L.) infestation

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Abstract
The present study was carried out at Seed Research and Technology Centre, PJTSAU, Rajendranagar, Hyderabad during 2019-20. Chemical traits like protein and ash contents of twenty five rice genotypes were assessed to know their effect on *Sitophilus oryzae* infestation. It was revealed that ash content had significant negative effect on mean adult emergence (-0.42) and per cent seed damage (-0.47), while it had significant positive effect with mean development period (0.41). On the other hand, protein content had significant positive relationship with mean adult emergence (0.44), per cent seed damage (0.42) and negative with mean developmental period (0.28).

Keywords: PJTSAU, *Sitophilus oryzae*, rice weevil

1. Introduction

Rice is the most important and extensively grown food crop in the world and is the staple food for more than 60 per cent of world's population (Muthukumar, 2014) [1]. It occupies a pivotal role in the national food and livelihood security system. India is the leading producer following china as well as a major exporter of rice in the world (Viraktamath *et al*., 2011) [2].

Food grain production has been steadily increasing due to advances in production technology, but improper storage resulting in high losses due to various factors. Among various biotic factors, post-harvest losses caused due to insect pests has become one of the alarming problems worldwide leading to huge losses (30-40 per cent) of the food grains (Kumar and Kalita, 2017) [3]. Among various storage pests, *Sitophilus oryzae* is reported to be one of the major pests of stored cereals (including rice) causing heavy losses both quantitatively and qualitatively throughout the world (Arannilewa *et al*., 2002) [4]. (Alavi *et al*. 2012) [5] Compiled data on postharvest losses in rice value chains from different studies conducted by the FAO and reported 10-37 per cent losses in rice in Southeast Asia. The rice weevil can cause grain loss between 12 and 20 per cent and under favourable conditions, the extent of damage may go up to 80% (Tefera *et al*., 2013) [6].

Seed is the critical determinant of the agricultural production. Most of the small holder farmers produce and store their seeds (including paddy) for next season as they have limited accessibility to seed producing enterprises. Of the total seed requirement in the country, only less than 20 per cent good quality certified seeds are available to the farmers and remaining seed requirement is met up by farmers saved seeds (Ravindra *et al*., 2014) [7]. Nearly 30 per cent of such seeds are deteriorated during storage period due to insects, rodents and microorganisms. Insects and mites cause severe damage especially in warm and humid conditions (Parimala *et al*., 2013) [8]. Hence, post-harvest seed management is one of the crucial and vital components to prevent loss of the paddy seed during storage.

Most common method employed for controlling this pest is by the use of chemical insecticides. The widespread usage of the same has been evoking global concern due to associated environmental hazards, development of resistance and presence of residues in the food. Breeding for resistance to stored grain weevil holds significant promise as it is sustainable and does not involve any recurring costs and environmental hazards (Derera *et al*., 2001) [9]. Chemical constituents of the grains play a vital role in determining the relative resistance to insect pest attack (Ewedairo *et al*., 2015) [10]. Stored seeds may have high resistance to insect pests because of the lack of vital nutrients or the presence of compounds that adversely affect insect development (Warchalewski *et al*., 2002) [11].

An array of compounds found in seeds acts either additively or synergistically against insect pests (Birch *et al*., 1986) [12]. So, in the present investigation, role of ash and protein content of 25 genotypes on weevil infestation was studied.

2. Materials and methods

Twenty-five rice genotypes were procured from different research stations of Telangana State. The selected genotypes, after procurement, were thoroughly cleaned by removing physical impurities if any and thereafter they were kept in an incubator at a temperature of 55^0C for four hours to kill the immature stages of insects if any without affecting the viability of the seeds (Singh, 1989) [13]. Thereafter moisture content of the test genotypes was standardized to near equilibrium by keeping the genotypes in the desiccator containing saturated solution of KOH for 21 days (Solomon, 1951) [14]. This pre-conditioned seed material was used in the experiment.

2.1 Sexing and mass multiplication

The test insect was mass multiplied on paddy BPT 5204 and freshly emerged seven days old adults were used for screening studies. The male and female sexes of the weevils were recognized according to the characters described by Halstead (1963) [15] i.e weevils having relatively long rostrum with narrow punctures along the rostrum arranged in regular rows and not touching each other were characterized as females. Whereas, males are characterized by having short rostrum with wide punctures along the rostrum. These are large and irregular, not in a row and often touching each other.

2.2 Screening

Ten grams of seed was taken and placed in small plastic tubes (7.5 cm x 5 cm) with tiny punctures on the lid (under three replications). The test insects (eight females and four males) were introduced into each tube to infest ten grams seeds of each test genotype (Gbaye and Ajiye, 2016) [16]. They were incubated at a temperature and relative humidity of 26 ± 2^0C and 70±5 per cent, respectively. The weevils were allowed to oviposit in the seeds for two weeks and then removed. The plastic tubes were kept back in incubator till the F1 adults emerge.

2.2.1 Mean adult emergence

The number of adults that emerged from each replication of the treatments were counted daily and discarded from the respective tubes until they cease to emerge. The mean adult emergence was worked out by pooling the data.

2.2.2 Mean development period:

The mean development period of test insect was worked out as per Howe (1971) [17].

$$D = \frac{\sum (A \times B)}{C}$$

Where, A = Number of adults emerged on nth day, B = 'n' days required for their emergence, C = Total number of adults emerged during experimental period, D = Mean developmental period (days).

2.2.3 Per cent seed damage

The number of damaged seeds by the weevil in each replication of the treatments was counted at the end of the experiment and converted into per cent damaged seeds.

2.3 Ash content (per cent)

The ash content of the test genotypes was calculated as per AOAC (1984) [18] and expressed in percentage. In this analysis, 5g of finely ground sample of test genotypes was taken and initially charred in the silica crucible. After complete charring, the crucibles were kept in a muffle furnace at 600^0C for 2 hours. Thereafter, the final weight of the sample was deducted from the initial weight and converted into percentage.

$$\text{Ash (per cent)} = \frac{\text{Weight of the ash}}{\text{Initial weight of the sample}} \text{X } 100$$

2.4 Crude protein content

Initially Nitrogen content of grain samples was determined as per micro-kjeldahl method as suggested by AOAC (1995) [19] using Kelplus auto analyser. Initially, 0.2g of sample was digested in presence of 2g of catalyst mixture (copper sulphate and potassium sulphate in 1:5 ratio) and 10 ml of conc. sulphuric acid at 420^0C for 2 hours. After cooling, the distillation was carried out in auto distillation system (loaded with 4% boric acid and 40% sodium hydroxide). The distillate obtained was titrated against 0.1N HCL till appearance the of pink colour. The per cent nitrogen was calculated as follows.

$$N_2\,(\%) = \frac{\text{(Titre value of sample - Titre value of blank) X 14 X Normality of HCl (0.1) X 100}}{\text{Weight of sample X 1000}}$$

The crude protein content was calculated by multiplying the nitrogen percent obtained with the factor 6.25 (Mariotti *et al*., 2008) [20] and expressed in percentage.

2.5 Statistical analysis

The data obtained was analyzed for ANOVA (5% probability level) following completely randomized design by using INDOSTAT statistical software. Percentage data obtained was subjected to angular transformation.

3. Results and Discussion

3.1 Mean adult emergence

The mean number of adults that emerged from various test genotypes ranged from 3.00 to 17.33 (Table 1). Significantly less number of adults had emerged from JGL 3844 (3.00) followed by JGL 1798 (4.00), MTU 1001 (4.00), RNR 2458 (4.00) and MTU 1010 (4.00) which were on par with MTU 7029 (4.37). Adult emergence recorded in KNM 118 (6.00) and RDR 7555 (6.67) were on par with each other. Whereas, significantly highest number of adults had emerged from JGL 11118 (17.33) followed by RNR 18833 (16.00). The adult emergence in rest of the genotypes varied from 7.00 to 12.67.

3.2 Mean development period (days)

The mean development period of test insect observed in various genotypes ranged from 35.84 to 43.83 days (Table 1). The shortest mean development period was recorded in JGL 11118 (35.84 days) which was on par with RNR 18833 (37.47 days). While, it took maximum time for the adults to emerge in MTU 1001 (43.83 days) which was on par with JGL 3844 (43.48 days), MTU 7029 (43.32 days), RNR 2458 (43.16 days), JGL 1798 (42.56 days) and MTU 1010 (42.10 days). While in the rest of the genotypes, it varied from 37.90 to 41.66 days.

3.3 Seed damage (per cent)

Seed damage in rice genotypes ranged from 1.38 to 10.82 per cent (Table 1). Lowest seed damage was observed in JGL

3844 (1.38%) which was on par with JGL 1798 (1.69%) and RNR 2458 (1.76%). Per cent seed damage in MTU 7029 (2.25%) was on par with MTU 1010 (2.56%) and MTU 1001 (2.56%). While significantly highest seed damage was observed in JGL 11118 (10.82%) followed by RNR 18833 (8.62%). Seed damage in the rest of the genotypes varied from 3.81 to 7.67 per cent.

3.4 Ash (per cent)

The ash content of various test genotypes ranged from 1.10 to 1.79 per cent. (Table 2 and figure 1). Lowest ash content was recorded in JGL 384 (1.10%) which was on par with RNR 1446 (1.18%), RNR 15048 (1.19%) and RNR 10754 (1.20%). Whereas, it was highest in RDR 7555 (1.79%) which was on par with JGL 3844 (1.75%) and MTU 7029 (1.71%). Ash content in RNR 18833 (1.61%) was on par with RNR 2458 (1.57%), KNM 118 (1.57%), JGL 1798 (1.53%) and RNR 2465 (1.52%).

Ash content in rest of the genotypes varied from 1.29 to 1.50 per cent *viz*., BPT 5204 (1.29%) and RDR 763 (1.30%), JGL 18047 (1.32%), JGL 24423 (1.35%), JGL 3855 (1.35%), JGL 11727 (1.39%), JGL 11470 (1.40%), JGL 11118 (1.41%), RDR 355 (1.42%), JGL 3828 (1.42%), MTU 1010 (1.47%), MTU 1001 (1.49%) and JGL 17004 (1.50%).

From the results, it is evident that genotypes with less adult emergence, less seed damage and high developmental period recorded good amount of ash *viz*., JGL 3844 (1.75%), MTU 7029 (1.71%), RNR 2458 (1.57%), JGL 1798 (1.53%), MTU 1001 (1.49%) and MTU 1010 (1.47%). High ash content in grains might leave some toxic effect on insect interfering with digestion and making the variety non- preferable for feeding, growing and adult emergence (Padmasri, 2018) [21]. Similarly, Ramakrishna (2002) [22] reported that, ash content in maize had significant negative correlation with adult emergence of *S. oryzae*. On the other hand, highly infested genotypes *viz*., RNR 18833 and JGL 11118, although possessed good amount of ash (1.61% and 1.41%, respectively), the greater infestation of them might be due to good amount of crude protein content and/or some other factors.

3.5 Crude protein (per cent)

Crude protein content was significantly lowest in MTU 7029 (6.18%) followed by JGL 11470 (6.93%) which was on par with JGL 3828 (6.99%), RDR 355 (7.06%), JGL 3855 (7.12%) and MTU 1001 (7.31%). While, highest protein content was recorded in RNR 18833 (8.72%) which was on par with JGL 17004 (8.62%), JGL 3844 (8.62%) and JGL 11118 (8.37%) (Table 2 and figure 2).

Protein content in rest of genotypes varied from 7.37% to 8.18% *viz.*, MTU 1010 (7.37%), RNR 1446 (7.53%), JGL 1798 (7.56%), RNR 2458 (7.56%), JGL 11727 (7.59%), JGL 384 (7.65%), KNM 118 (7.65%), RDR 763 (7.78%), BPT 5204 (7.84%), RNR 10754 (7.84%), RDR 7555 (7.87%), RNR 2465 (7.90%), JGL 18047 (8.06%), RNR 15048 (8.15%) and JGL 24423 (8.18%).

Protein content was comparatively higher in the genotypes with greater adult emergence, seed damage and shorter developmental period *viz.*, RNR 18833 (8.72%) and JGL 11118 (8.37%). This shows that, high protein content in these genotypes might have made them highly preferred host for growth and development of rice weevil resulting in greater adult emergence and seed damage, while opposite was true in case of least preferred ones *i.e* MTU 7029 (6.18 %), MTU 1001 (7.31%), MTU 1010 (7.37%), JGL 1798 (7.56%) and RNR 2458 (7.56%). Although, least preferred genotype *i.e* JGL 3844 (8.62%) recorded high protein content, the least preference might be attributed to high ash content and/or others factors

Table 1: Relative preference of rice genotypes against *Sitophilus oryzae.*

S. No.	Treatment	Mean adult emergence	Mean development peiod	Seed damage (%)
1	JGL 384	09.67	40.68	5.11 (13.07)
2	JGL 1798	04.00	42.56	1.69 (7.47)
3	JGL 3828	07.00	38.62	3.89 (11.37)
4	JGL 11470	08.00	39.11	5.73 (13.85)
5	JGL 3855	11.67	40.55	5.84 (13.98)
6	JGL 11727	07.00	38.65	5.03 (12.97)
7	JGL 11118	17.33	35.84	10.82 (19.20)
8	JGL 17004	11.67	40.09	7.57 (15.97)
9	JGL 18047	11.00	39.39	7.52 (15.91)
10	JGL 24423	08.00	41.16	5.91 (14.07)
11	JGL 3844	03.00	43.48	1.38 (6.74)
12	KNM 118	06.00	41.66	3.84 (11.30)
13	RNR 18833	16.00	37.47	8.62 (17.07)
14	RNR 10754	11.67	38.10	6.63 (14.92)
15	RNR 15048	08.67	41.17	5.49 (13.55)
16	MTU 7029	04.37	43.32	2.25 (8.62)
17	MTU 1001	04.00	43.83	2.56 (9.21)
18	RDR 7555	06.67	40.80	3.81 (11.25)
19	RDR 763	12.67	37.90	7.67 (16.08)
20	RDR 355	08.00	40.59	5.91 (14.07)
21	RNR 1446	10.37	39.11	6.30 (14.53)
22	RNR 2458	04.00	43.16	1.76 (7.62)
23	RNR 2465	07.67	40.59	4.03 (11.57)
24	*MTU 1010	04.00	42.10	2.56 (9.20)
25	**BPT 5204	09.33	40.26	6.90 (15.23)
	SEm±	0.26	0.62	0.21
	CD (P= 0.05)	0.73	1.76	0.60
	CV (%)	5.28	2.66	2.89

*Resistant check
**Susceptible check
Figures in parentheses are angular transformed values

Table 2: Chemical traits assessed in rice genotypes.

S. No.	Name of the treatment	Ash content (per cent)	Crude protein (per cent)
1	JGL 384	1.10	7.65
2	JGL 1798	1.53	7.56
3	JGL 3828	1.42	6.99
4	JGL 11470	1.40	6.93
5	JGL 3855	1.35	7.12
6	JGL 11727	1.39	7.59
7	JGL 11118	1.41	8.37
8	JGL 17004	1.50	8.62
9	JGL 18047	1.32	8.06
10	JGL 24423	1.35	8.18
11	JGL 3844	1.75	8.62
12	KNM 118	1.57	7.65
13	RNR 18833	1.61	8.72
14	RNR 10754	1.20	7.84
15	RNR 15048	1.19	8.15
16	MTU 7029	1.71	6.18
17	MTU 1001	1.49	7.31
18	RDR 7555	1.79	7.87
19	RDR 763	1.30	7.78
20	RDR 355	1.42	7.06
21	RNR 1446	1.18	7.53
22	RNR 2458	1.57	7.56
23	RNR 2465	1.52	7.90
24	*MTU 1010	1.47	7.37
25	**BPT 5204	1.29	7.84
	SEm±	0.03	0.14
	CD (P= 0.05)	0.10	0.40
	CV (%)	3.54	2.54

*Resistant check

**Susceptible check

Table 3: Correlation coefficients between chemical traits (Crude protein and ash content) of test genotypes and biological parameters of *Sitophilus oryzae.*

Biological Parameters Chemical traits	Mean adult emergence (number)	Mean development period (days)	Seed damage (per cent)
Ash content (per cent)	-0.42*	0.41*	-0.47*
Crude protein (per cent)	0.44*	-0.28	0.42*

*****Significance at 5% level

**Significance at 1% level

Table 4: Chemical traits assessed in rice genotypes.

S. No.	Name of the treatment	Ash content (per cent)	Crude protein (per cent)
1	JGL 384	1.10	7.65
2	JGL 1798	1.53	7.56
3	JGL 3828	1.42	6.99
4	JGL 11470	1.40	6.93
5	JGL 3855	1.35	7.12
6	JGL 11727	1.39	7.59
7	JGL 11118	1.41	8.37
8	JGL 17004	1.50	8.62
9	JGL 18047	1.32	8.06
10	JGL 24423	1.35	8.18
11	JGL 3844	1.75	8.62
12	KNM 118	1.57	7.65
13	RNR 18833	1.61	8.72
14	RNR 10754	1.20	7.84
15	RNR 15048	1.19	8.15
16	MTU 7029	1.71	6.18
17	MTU 1001	1.49	7.31
18	RDR 7555	1.79	7.87
19	RDR 763	1.30	7.78
20	RDR 355	1.42	7.06
21	RNR 1446	1.18	7.53
22	RNR 2458	1.57	7.56
23	RNR 2465	1.52	7.90

24	*MTU 1010	1.47	7.37
25	**BPT 5204	1.29	7.84
	SEm±	0.03	0.14
	CD (P= 0.05)	0.10	0.40
	CV (%)	3.54	2.54

*Resistant check
**Susceptible check

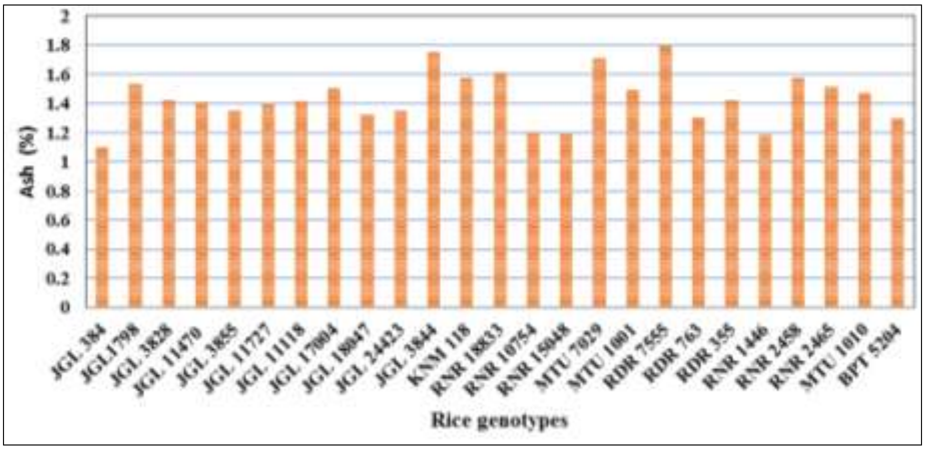

Fig 1: Ash contents in various rice genotypes

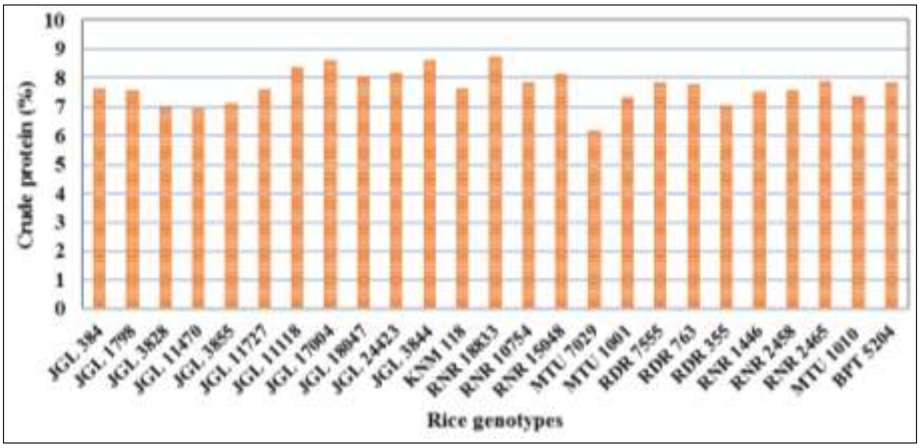

Fig 2: Crude protein content in various rice genotypes

Similar findings were reported by Muthukumar (2014) [1] who screened 56 paddy genotypes to assess the role of biochemical traits in imparting resistance or susceptibility to *Rhizopertha dominica*. It was revealed that the protein content had significant positive effect on the seed damage and weight loss caused due to *R. dominica*. Padmasri (2018) [21] reported that the least preferred maize genotype BH 412055 recorded less protein content (7.65%). Whereas, moderately susceptible genotypes *viz.*, CM 118 and DHM 117 recorded with high protein content of 12.22 and 11.69 per cent, respectively. Similarly, Murad and Batool (2017) [23] reported that wheat varieties with higher protein were more susceptible to *S. cerealella*, since in such varieties there was significantly higher number of adults emerged coupled with greater seed damage and percent weight loss.

4. Correlations
4.1 Ash content
Ash content had significant positive relationship with mean development (0.41). While, it had significant negative relationship with adult emergence (-0.42) and seed damage (-0.47) (Table 3). Similarly, Vishwamitra (2011) [24] reported significant negative correlation between ash content and adult emergence, seed damage and per cent weight loss due to *C. chinensis* in redgram varieties. Jyothsna (2015) [25] also reported a significant negative correlation between ash content in groundnut and adult emergence of *Caryedon serratus*. Aleksandra *et al*. (2018) [26] studied the behaviour of the angoumois grain moth in different grain substrates and revealed that ash content had significant negative effect on adult emergence (r= -0.73).

4.2 Protein content
Protein had non-significant and negative correlation with mean development period (-0.28). Whereas, it had significant positive relationship with adult emergence (0.44) and seed damage (0.42) (Table 3).
The results are in conformity with the findings of Murthy (1974) [27] who reported positive correlation between protein content of sorghum genotypes and adult emergence of *S. oryzae*. Yadav (2017) [28] also reported that, protein content had positive relationship with grain damage and per cent weight loss in wheat.

5. Conclusion
The genotypes containing good amount of ash and or less amount of protein *viz.*, JGL 1798, MTU 1001, RNR 2458, MTU 1010, MTU 7029, RDR 7555, KNM 118 and JGL 3844

can further be exploited for some other traits and the best traits can be incorporated in the breeding program to obtain resistant varieties.

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