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Research Paper

Resistance in Paddy Genotypes against *Sitotroga cerealella* (Oliv.) (Lepidoptera, Gelechiidae)

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ABSTRACT

The Angoumois grain moth, *Sitotroga cerealella* (Oliv.) is one of the principal causes of the loss of paddy grains in storage, which indirectly affects the food security. Paddy genotypes exhibit varying degree of susceptibility and was found by laboratory studies, using Dobie's susceptibility index. Though significant differences were observed in susceptibility, no genotype was completely immune and highly susceptible to the infestation, but some genotypes showed moderate resistance. Qualitative analysis was done for six paddy genotypes, three each from moderately resistant and susceptible category to estimate the starch, protein, total phenol, amylose contents and amylase inhibitor activity. Moderately resistant genotype viz., CB 14528, CB 16146, TM 12202 possessed higher amount of starch (73.83 - 70.50 mg/g), phenol (0.76 - 0.60 µg/g), amylose (25.79 - 24.15%) and lower amount of protein (3.33 mg/g - 4.27 mg/g) and amylase inhibitor activity (IC 50) (28.0 - 50.18 µg/mL) compared to susceptible ones. The phytochemicals present in selected moderately resistant (CB 14528) and susceptible genotypes (AD 13299) were analyzed using TD GC-MS. The above results were analyzed to know the cause of resistance in paddy genotypes against this notorious pest.

***Key words*:** *Sitotroga cerealella*, Paddy genotypes, Dobie's susceptibility index, Qualitative analysis, Phytochemicals

Rice (*Oryza sativa* L.) is the most widely consumed staple food in the world. It accounts for about of 60 to 70 per cent of the body calorie intake of the consumers [1]. It is commonly known as 'the Global grain'. About 90 per cent of the world's rice is grown and consumed in Asia. Among Asia, India has the largest area under rice of 43.5 m ha [2]. A huge amount of storage capacity, 354.07 lakh MT is available with (Food Corporation India) FCI, which is very prone to attack of storage insect [3] and expecting heavy losses when proper management was not followed.

The common biological agent for the loss and deterioration of stored paddy are beetles and moths. Among the 18 species of insects infesting stored paddy in India, only six species viz. Angoumois grain moth, *S. cerealella* (Oliv.); Lesser grain borer, *Rhyzopertha dominica* (Fabr.); Rice weevil, *Sitophilus oryzae* (Linn.); Wheat weevil, *Sitophilus granarias* (Linn.); Rice moth, *Corcyra cephalonica* (Staint.) and saw-toothed grain beetle, *Oryzaephilus surinamenasis* (Linn.) cause significant losses in stored rice. Among these six insects, Angoumois grain moth, *S. cerealella* (Oliv.) (Gelechiidae: Lepidoptera) is the most destructive pest of stored paddy [4]. The larvae of the moth utilize the entire grain for development by feeding on the embryo and endosperm, as well as cause an indirect damage to grain from subsequent attack by secondary insect pests. These damage leads to considerable quantitative and qualitative losses and the infested seeds become unable to germinate [5].

The qualitative parameters of grains are closely associated to susceptibility of the moth. The paddy variety with low amylose content tends to be more susceptible to *S. cerealella* [6]. The ash content had no significant correlation with resistance. It was also reported that the most resistant maize variety had increased phenolic content and low amylose content [7]. An inverse correlation between the moth emergence, per cent weight loss and the per cent fat content of the grains were observed by [8]. The protein, fat, and carbohydrate contents in grains are responsible for grains susceptibility to insects. The high protein content had increased the susceptibility of the moth to wheat grains [9]. The amount of "silica" present in the bract of paddy grains and amount of damage caused by the moth was negatively correlated. It was also reported that amylose content was higher in the resistant paddy varieties compared to susceptible varieties [10].

Being a destructive pest, different physical, mechanical and chemical methods were deployed, however none was found to be effective. One of the safe and sound methods is the use of resistant varieties to control the moth. Several studies reveal the use of resistant varieties of host plants have a good potential as an alternative to chemical control, as they focus on changing the development and survivorship of the pests [11-12]. Although resistant paddy varieties have been identified, but the study on relation of the resistance with the biochemical and phytochemical nature of the South Indian

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paddy varieties are limited. This study was conducted to find out the resistant varieties in paddy under South Indian condition against *S. cerealella* and to analyze the biochemical and chemical volatile difference in the resistant and susceptible varieties of paddy. These findings would be helpful in breeding and cultivation of the resistant cultivars to effectively control the pest at storage conditions.

MATERIALS AND METHODS

Mass culturing of S. cerealella

The method of mass culturing developed by [10] was followed throughout the experiment. The stock culture of Angoumois grain moth was maintained by obtaining adult moths from the Paddy Breeding Station (PBS), Tamil Nadu Agriculture University, Coimbatore. About 20 pairs of adults were released into a plastic jar containing 500 g of the medium (paddy). Initially it was kept horizontally for obtaining maximum surface area for egg laying. After one week the adults were removed. The new batch of adults emerged after one month. The newly emerged moths were captured as suggested by [13] and used for screening of paddy genotypes.

Screening of paddy genotypes

Ten ART and MLT paddy genotypes maintained at the Paddy breeding Station, Tamil Nadu Agricultural University were selected for screening. Screening to assess the damage by Angoumois grain moth was carried out under no choice method. The screening procedures as mentioned by [13] and [14]. The D.S.I was assessed as described below to categorize the genotypes in to different susceptibility groups.

D.S.I

DSI was calculated based on the number of moths emerged in each test variety and mean developmental period. The susceptibility index was calculated as given by [15]. The resistant and susceptible varieties were sorted out based the D.S.I as:

DSI	Classification
0 to 4.0	Resistant
4.1 to 7.0	Moderately resistant
7.1 to 10.0	Susceptible
>/= 10.1	Highly susceptible

Grain damage and content loss

To calculate the percent damage, 100 grains of each variety of paddy was randomly collected from each jar and number of damaged grains were counted by observing the hole of larval entrance under simple microscope and the visible damage of grains. Then damage per cent was calculated by using the formulae mentioned by [13]. After the complete emergence of adults, the total weight of grains from each jar or variety was recorded separately. Then the per cent grain content loss was measured as given by [13].

Biochemical analysis

The biochemical analysis was carried out for the selected resistant and susceptible varieties.

Protein content

The protein content was analyzed as suggested by [16].

Total phenols

Total phenols was analysed as suggested by [17.

Amylose content

The amylose content was estimated given by [18].

Starch content

The starch content was analyzed as suggested by [19].

Amylase inhibition

Amylase inhibition was estimated as given by [20].

Statistical analysis

All data obtained were subjected to Analysis of Variance (ANOVA). The data on grain damage and content loss were subjected to arcsine transformation. The treatment means were compared by Duncan Multiple Range-Test (DMRT). The package used for the entire analysis was SPSS version 16.00 was developed by IBM Corporation with critical difference at P=0.05 and interpreted.

TD GC-MS analysis for identification of phytochemicals in resistant and susceptible paddy genotypes

The phytochemicals present in resistant and susceptible paddy genotypes were identified through TD GC/MS (GC Clarus 500 Perkin Elmer analysis). Chemical compounds were identified by using a column Elite-5MS (100% Dimethyl poly siloxane), 30 × 0.25 mm × 0.25 μm df equipped with GC clarus 500 Perkin Elmer GC/MS. The turbo mass-gold-perkin-Elmer detector was used. The carrier gas flow rate was 1 ml per min, split 10:1, and injected volumes were 3 μl. The column temperature was maintained initially at 110°C at the rate of 10°C/min-No hold followed by increases up to 280°C at the rate of 5° C /min-9 min (hold). The injector temperature was 250°C and this temperature was held constant for 36 min. The electron impact energy was 70 eV, Julet line temperature was set at 2000°C and the source temperature was set at 200°C. Electron impact (EI) mass scan (m/z) was recorded in the 45-450 aMU range. Using computer searches on the NIST Ver.2005 MS data library and comparing the spectrum obtained through GC/MS, the compounds present in the sample were identified.

RESULTS AND DISCUSSION

Screening of paddy varieties

The paddy genotypes were categorized as resistant and susceptible based on the D.S.I. Among the ten genotypes, none was completely immune to the attack of *S. cerealella*. The moderate resistance were shown by CB 14 528, CB 16 146, CB 15 541, TM 12202, CB 15 714 and TNTRH 55 genotypes, whereas susceptibility were observed in AD 12205, CB 15 144, CB 12 132 and AD 13299 genotypes. No genotype was completely susceptible to the attack of the moth based on the susceptibility index (Fig 1).

Grain damage and content loss

The grain damage and weight loss are both related parameters. The grain damage was directly proportional to the grain weight loss. The moderately resistant genotype CB 14528 had a lower grain damage of 5.00 per cent and grain weight loss of 2.57 per cent. The susceptible genotype (AD 13299) possessed the highest loss of 18.24 and 47.67 per cent both in terms of grain weight and grain damage respectively. The susceptible index is positively correlated with the grain damage and content loss (Fig 2). Since the susceptible genotype has higher moth emergence, it should also suffer from higher weight loss [21]. Similar results i.e., the susceptible genotype suffered higher weight loss and damage compared with resistant genotype were also reported by [8]. In the results obtained by the [8] it was reported that the susceptible variety Basmati- Pak showed highest weight loss of 25.4% compared with the resistant genotype, Basmati 370 where the weight loss was 6.2%.

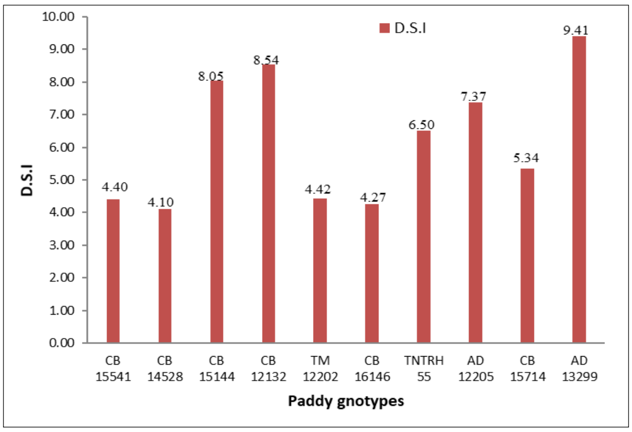

Fig 1 D.S.I of different paddy genotypes

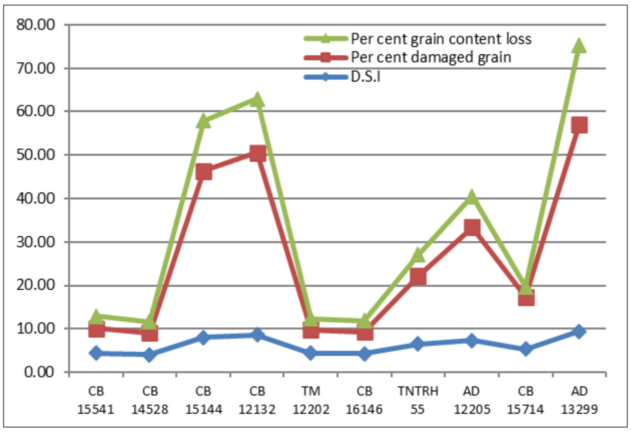

Fig 2 Relation of susceptibility index with the grain damage and grain content loss

Biochemical analysis

The biochemical analysis were carried out in the selected genotypes i.e., three each from the moderately resistant and susceptible categories. It is evident that the quantitative differences in the biochemical constituents are one among the reasons contributing to the resistance in paddy genotypes. The highest starch content was observed in the moderately resistant paddy genotype CB 14528 (73.83 mg/g) and the least was observed for the AD 13299 (61.00 mg/g). In the other moderately resistant genotypes also the starch content was more compared with the susceptible genotypes (Fig 3).

The protein content in the moderately resistant paddy genotype, CB 14528 (3.33 mg/g) was less compared to that of in the susceptible paddy genotype, AD 13299 (7.87mg/g) (Fig 3). The degree of resistance has usually been attributed to nutritional factors such as lipid, protein and amylose content [22]. There was a positive correlation between the susceptibility and the protein content which was also reported by [8]. But these results were not in conformity with the work of [10] who reported that the resistant varieties have high protein content. This variation may be due to the inherent nature of the varieties.

The phenol content was the highest in the moderately resistant paddy genotype CB 14528 (0.76 µg/g) while lowest in the susceptible genotype AD 13299 (0.35 µg/g) (Fig 3). The phenol content in the moderately resistant genotype was 2.17 times higher than that of the susceptible genotype. Phenol has always contributed in the induced resistance of host plants to insects. The higher phenol content was observed in the resistant variety compared to susceptible one in maize [13].

The amylose content in the paddy is an important constituent that decides the resistance and susceptibility. The moderately resistant paddy genotype, CB 14528 (25.79%) had higher amylose content compared with susceptible genotype, AD 13299 (21.49%). It can be concluded that the moderately resistant genotypes had higher amylose content than the susceptible genotypes (Fig 3). Similar findings were reported by [23] where in lowest survival from egg to adult of Angoumois grain moth was seen in the varieties having high amylose content. Paddy genotypes with low amylose content tend to be more susceptible to the infestation by Angoumois grain moth [6]. Contrary to the above findings, [24] reported the absence of any relationship between amylose content and infestation of rough rice by Angoumois grain moth, but the most susceptible paddy variety Tainan 3 has lowest amylose content.

The amylase inhibitor activity in the moderately resistant CB 14528 was 28.00 µg/ml indicating efficient amylase inhibition. AD 13299, which is a susceptible genotype, possessed less amylase inhibition as indicated by IC 50 value of 70.70 µg/ml (Fig 3). Amylase inhibitory activity is one of the lines of defense in plants to contain infests infestation. Higher activity is efficient in controlling insect infestation by inhibiting the starch degrading enzyme amylase. Amylase activity is required for the breakdown of starch to provide carbon source for the insect. Higher amylase inhibitory activity is indicated by low IC 50, confers resistance to infestation. The enzyme inhibitors impede digestion through their action on insect gut digestive α-amylases and proteinases, which play a key role in the digestion of grain starch and proteins [25].

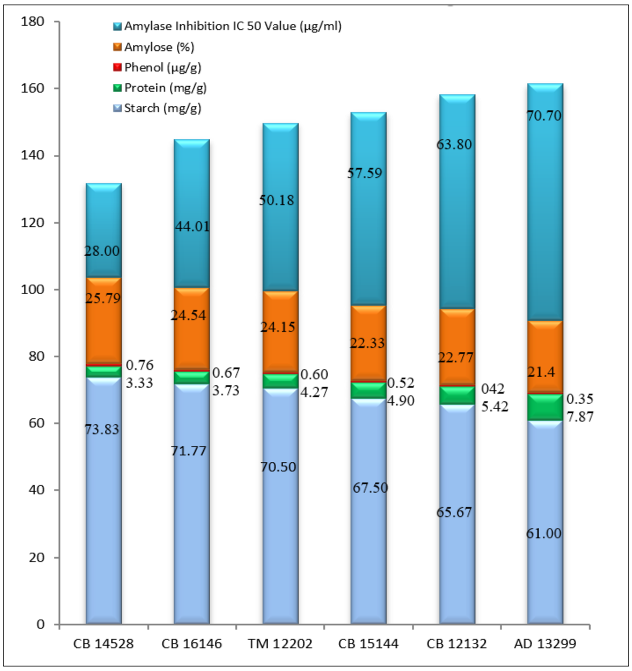

Fig 3 Biochemical constituents of selected paddy genotypes

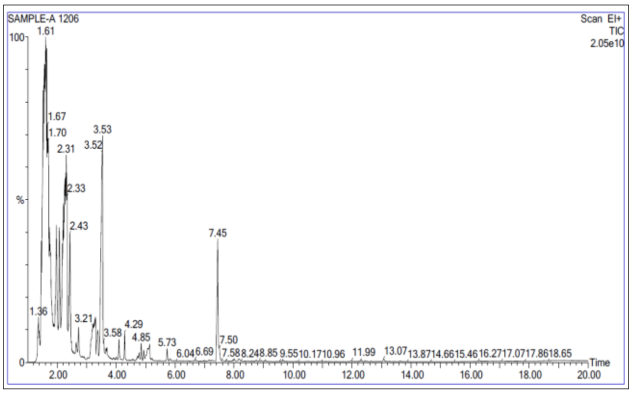

Fig 4 Gas chromatogram of the chemical constituents in moderately resistant genotype (CB 14 528)

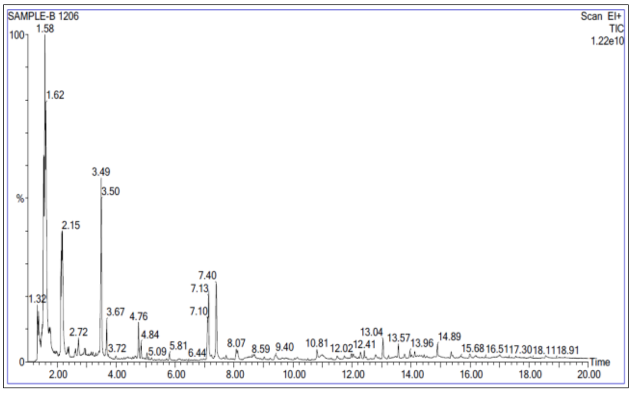

Fig 2 Gas chromatogram of the chemical constituents in susceptible genotype (AD 13299)

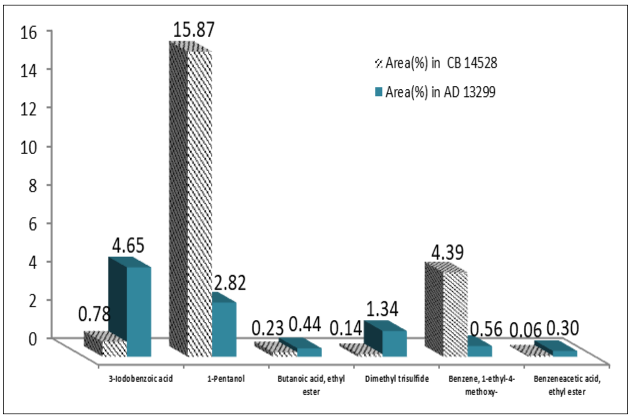

Fig 6 Chemical compounds identified both in moderately resistant and susceptible paddy genotypes

Chemical profile of moderately resistant and susceptible paddy genotypes

The volatile profiles of moderately resistant and susceptible genotypes analyzed with the help of TD GC-MS are depicted in (Fig 4-5) respectively. On comparison of the D.S.I and biochemical analysis, two promising moderately resistant (CB 14528) and susceptible (AD 13299) genotypes were selected for phytochemical analysis. Out of the total compounds in the chemical profile, six were common but at different concentrations in both moderately resistant, CB 14528 and susceptible genotype, AD 13299 (Fig 6).

In the moderately resistant genotype (CB 14528), the butyric acid 2-hydroxy-3-methyl- methyl ester showed maximum area of 19.02% which was absent in the susceptible genotype. In the susceptible genotype (AD 13299) the dominant chemical compound found was 1-Butanol (3-methyl- acetate) (10.61%), which was absent in the moderately resistant genotype. Out of six common compounds, the main chemical compounds viz., 1-Pentanol, dimethyl trisulfide were reported to show resistance against insect attack. Pentanol was found in higher concentration in the moderately resistant genotype. Dimethyl trisulfide was found to be higher in susceptible genotype compared to that in the moderately resistant genotype.

The presence of butyric acid 2-hydroxy-3-methyl-methyl ester in the moderately resistant variety may be one of the reasons for the resistance, since it is not present in the susceptible genotype. The presence of pentanol in the paddy grains were confirmed by [26]. It was also reported by [27] that the pentanol is an active organic compound produced by plants which elicit immunity against microbial pathogens and insect pest. It was also reported that 1-pentanol and the derivatives 2-pentanol and 3-pentanol induced plant systemic resistance.

Dimethyl trisulfide present in the susceptible genotype might have influenced the growth and development of *S. cerealella*. It was reported by [28]. The dimethyl trisulfide act as a strong attractant for the insects. Dimethyl trisulfide was used by saprophilous insects to locate breeding sites [29].

CONCLUSIONS

It is evident, from the qualitative analysis that the highest amount of starch, phenol and amylose and lowest amount of protein and amylase inhibition are some of the reasons for resistance. The chemical profiling shows that resistance is due to the quantitative difference in the main compounds like phenol and dimethyl trisulfide. Thus, these quantitative variations in different compounds finds advantageous in the breeding purpose to get a resistant variety, which is a safe and sound method for managing the *S. cerealella*.

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