

**Basis of resistance against rice hispa,
Dicladispa armigera (Olivier) in paddy**

THESIS

By

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(A-2019-30-032)

Submitted to



**CHAUDHARY SARWAN KUMAR
HIMACHAL PRADESH KRISHI VISHVAVIDYALAYA
PALAMPUR – 176 062 (H.P.) INDIA**

in

Partial fulfilment of the requirements for the degree

of

**MASTER OF SCIENCE IN AGRICULTURE
(DEPARTMENT OF ENTOMOLOGY)
(ENTOMOLOGY)**

2022

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CERTIFICATE – I

This is to certify that the thesis entitled “**Basis of resistance against rice hispa, *Dicladispa armigera* (Olivier) in paddy**” submitted in partial fulfilment of the requirements for the award of the degree of **Master of Science (Agriculture)** in the discipline of **Entomology** of CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur is a bonafide research work carried out by **Ms. Shivani Thakur (A-2019-30-032)** daughter of **Smt. Shakuntla Devi** and **Sh. Pratap Singh** under my supervision and that no part of this thesis has been submitted for any other degree or diploma.

The assistance and help received during the course of this investigation have been fully acknowledged.

Place : Palampur
Dated : 1 January, 2022

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CERTIFICATE- II

This is to certify that the thesis entitled “**Basis of resistance against rice hispa, *Dicladispa armigera* (Olivier) in paddy**” submitted by **Ms. Shivani Thakur** (Admn. No. A-2019-30-032) daughter of Sh. Pratap Singh to the CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur in partial fulfilment of the requirements for the degree of **Master of Science (Agriculture)** in the discipline of **Entomology** has been approved by the Advisory Committee after an oral examination of the student in collaboration with an External Examiner.

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ACKNOWLEDGEMENTS

This thesis has been both fascinating and immensely enjoyable to compose. I'd like to thank a number of people who contributed to the finished version of my work in many different ways.

To begin, I thank God, the Almighty, for blessing upon me good health, fortitude, inspiration, zeal, limitless internal strength and favorable circumstances, to face and pass through all odds successfully at this juncture.

*With an overwhelming sense of legitimate pride and genuine obligation, I seize this rare opportunity to express my deep sense of gratitude, indebtedness and personal regards to **Dr. Ajai Srivastava** Professor, Department of Entomology, CSKHPKV, Palampur, the chairman of my advisory committee for opening my way to new horizons. I am immensely grateful to him for his genuine guidance, unceasing interest, valuable knowledge, technical advice, providing each and above all his affectionate way of dealing with the things throughout the course of my investigation. I will remain indebted to him for pruning my personality and giving new dimension in the scientific field through his analytical scientific outlook. I owe to him more than I could care to admit. I am very much thankful to him for picking me up as a student at the critical stage of my M. Sc. Words fails me to express my appreciation to his support, generous care. He was always beside me during the happy and hard moments to push me and motivate me.*

*It is my sole prerogative to place on record my indebtedness and everlasting gratitude to the members of my advisory committee **Dr. PK Sharma** (Deptt of Entomology), **Dr. Rajan Katoch** (Deptt of Biochemistry), **Dr. Sangita Sood** (Dean's nominee)(Deptt of Food Science, Nutrition and Technology) and **Dr. Nageswer Singh** (Retd.) (Deptt of Biochemistry) for their inspiring guidance, critical assessment of the manuscript and advice for its betterment.*

*I also thank to **Dr. RS Chandel** (Head), **Dr. AK Sood**, **Dr. PC Sharma**, **Dr. Surjeet Kumar**, **Dr. KS Verma**, **Dr. Anjana Thakur** and **Dr. Sharmistha Thakur** for their suggestions and kind cooperation during the period of investigation.*

Thanks are due to staff of laboratory Seema Manjul of Department of Entomology, CSK Himachal Pradesh Krishi Vishwavidyalaya for her extended help. My special thanks to the office staff Suresh Rana Sir, Rajesh Pathania ji, Jagbir bhai of Department of Entomology for their helping hand during course of study. I also extend my gratitude to the field staff for their immense support during the whole course of investigation.

*I must commend the individuals who mean a lot to me, my parents, **Sh. Pratap Singh** and **Smt. Shakuntla Devi**, for trusting in me and encouraging me to follow my dreams. I thank you for your selfless love, care and sacrifices in crafting my life. I would never be able to repay my parents for their love and affection. I take this precious moment to express my deep sentiment and indebtedness to my grand parents and younger brother **Ashutosh** whose affection, moral support and help led me to achieve my destination successfully.*

*I am indebted to all my friends **Kavita**, **Mayur**, **Shiwani**, **Himanshu**, **Pallavi**, **Pratibha**, **Priya**, **Arushi**, **Shalika**, **Manisha**, **Manjeet**, **Davinder**, **Ilyas** and **Shrutam** who always stood by my side to assist me at every step and to provide me mental, moral and social support. I appreciate the whole hearted co-operation extended by my seniors **Vikas Tandon sir**, **Dr. Suman Sanjta**, **Dr. Abhishek**, **Dr. Saurabh**, **Himanshu sir**, **Vasu sir**, **Ekta Ma'am**, **Nitika ma'am** and **Neerja ma'am** for their help during the course of investigation and thesis writing.*

*I am also thankful to **Ajit Sir**, **Jony bhaiya** and all the respected teachers and staff of RWRRC, Malan for their helping hand.*

Acknowledgements are inherently endless & incomplete, and I request indulgence from many friendly & helpful people whom I could not name here.

All are not mentioned, but none is forgotten. Needless, to say, errors and omissions are mine.

Place: Palampur

Dated: 1 January, 2022

(**Shivani Thakur**)

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LIST OF ABBREVIATIONS USED

| Sr.No. | Abbreviation | Meaning |
|--------|-----------------|--------------------------------|
| 1. | % | Per cent |
| 2. | / | Per |
| 3. | @ | At the rate of |
| 4. | > | Greater than |
| 5. | < | Lesser than |
| 6. | = | Equal to |
| 7. | µg | Microgram |
| 8. | °C | Degree Celsius |
| 9. | CD | Critical Difference |
| 10. | ml | Millilitre |
| 11. | mm | Millimetre |
| 12. | mg | Milligram |
| 13. | viz., | Namely |
| 14. | et al. | Et alii (and other) |
| 15. | g | Gram |
| 16. | l | Liter |
| 17. | cm ² | Square centimeter |
| 18. | i.e. | <i>id est</i> , in other word |
| 19. | m | Metre |
| 20. | M | Molar |
| 21. | N | Normal |
| 22. | nm | Nano meter |
| 23. | NS | Non-significant |
| 24. | Fig. | Figure |
| 25. | Df | Degree of freedom |
| 26. | etc. | Et cetara |
| 27. | Kg | Kilogram |
| 28. | r | Correlation coefficient |
| 29. | RWRC | Rice and Wheat Research Centre |
| 30. | CRD | Completely Randomized Design |
| 31. | Conc. | Concentrated |

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Title of thesis : Basis of resistance against rice hispa, *Dicladispa armigera* (Olivier) in paddy
Name of the student : Shivani Thakur
Admission Number : A-2019-30-032
Major discipline : Entomology
Minor discipline : Biochemistry
Degree : M.Sc.
Date of submission of thesis : 1 January 2022
Number of pages in thesis : 63
Major Advisor : Dr. Ajai Srivastava

ABSTRACT

The present investigations entitled “Basis of resistance against rice hispa, *Dicladispa armigera* (Olivier) in paddy at CSK Himachal Pradesh Krishi Vishwavidyalaya, Rice and Wheat Research Centre, Malan during the kharif season of 2020 and 2021 on some selected rice genotypes under greenhouse conditions. The seven genotypes of rice used were Nagardhan, Sukaradhan, HPR 2613, HPR 2617, Pusa 1121, W1263, and TN-1(susceptible check). They were obtained from the harvest of previous season. Various biophysical and biochemical parameters and their role in antixenosis and antibiosis mechanisms of resistance were studied. The studies on antixenosis showed that the number of settled adults, oviposition and grub damage was significantly less on resistant genotypes W1263, Pusa 1121 and Nagardhan. Longer leaf length and plant height were recorded in resistant genotypes. The grub damage was significantly correlated with flag leaf length ($r = 0.71$). The flag leaf length is also significantly correlated with adult settling behavior ($r = 0.26$). The antibiosis studies showed that total grub duration (8.8 to 11.2 days) in W1263, Pusa 1121 and Nagardhan was significantly lower than the other genotypes. Significantly lower grub weight (3.80 to 5.04 mg) and survival (73.06 to 87.81 %) and pupal weight (4.90 to 5.96 mg) was recorded in resistant genotypes. Pupal duration, adult emergence and adult longevity were at par in various genotypes. Amount of total sugars (10.00 to 19.00 mg/g), reducing sugars (3.73 to 7.86 mg/g), starch (61.33 to 84.00 mg/g), free amino acids (138.00 to 274.00 $\mu\text{g/g}$), and total proteins (7.46 to 9.50 %) was significantly less while phenol (1.20 to 4.16 mg/g) and crude fibre (11.40 to 79.63 %) content was higher in the W1263, Pusa 1121 and Nagardhan. Grub duration has a significantly negative correlation with total sugars ($r = -0.78$), reducing sugar ($r = -0.92$), free amino acid ($r = -0.90$). Phenol content was negatively correlated with grub survival ($r = -0.84$) and pupal weight ($r = -0.94$) and positively correlated with grub duration ($r = 0.83$). Crude fibre had a significantly positive correlation with grub duration ($r = 0.72$) and pupal duration ($r = 0.76$) and a negative correlation with grub survival ($r = -0.77$) and pupal weight ($r = -0.75$). Similarly, adult emergence also had a negative correlation with phenols ($r = -0.95$) and crude fibre ($r = -0.92$) and positive correlation with other biochemical factors.

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1. INTRODUCTION

Rice (*Oryza sativa* L.) is a staple food for more than 60 per cent of the world population (Anonymous 2017a). World production of paddy was reported to be 496.40 million metric tons in 2019. More than 90 per cent of the world's rice is grown in Asia. India is the second largest producer with production of 177.6 million tonnes under the total area of 43.70 million hectares in 2019. India is the largest exporter of rice in the world and is especially leading in its export of basmati rice. The total area under rice in Himachal Pradesh is about 80,000 hectares with a production of 121.4 thousand tonnes and productivity of 1546 kg ha⁻¹. Rice is usually cultivated in ten of the twelve districts of the State except Kinnaur and Lahaul & Spiti with Kangra and Mandi districts alone accounting for 71.2 per cent of area and 69.7 per cent of production.

Rice is attacked by several insects at different growth stages starting from seedling upto dough stage. More than 100 species of insects are known to attack this crop and 20 of these are of economic importance (Pathak and Dhaliwal 1981). Some major insect pests of rice include rice hispa (*Dicladispa armigera*), rice stem borer (*Scirpophaga incertulas*), rice leaf folder (*Cnaphalocrocis medinalis*), brown planthopper (*Nilaparvata lugens*), green leafhopper (*Nephotettix virescens*), white backed planthopper (*Sogatella furcifera*), whorl maggot (*Hydrellia sasakii*) and caseworm (*Nymphula depunctalis*) etc.

The rice hispa, *Dicladispa armigera* (Olivier) (Coleoptera: Chrysomelidae), which was initially believed to be a sporadic paddy pest, is rapidly emerging as a serious pest. It is now a major insect-pest of rice in Southern Asia and Australia, more particularly in Bangladesh, India and Nepal (Polaszek et al. 2002). In India, it has also gained major pest status particularly in north, north-eastern, eastern and central regions (Deka and Hazarika 1996; Hazarika et al. 2005). In Himachal Pradesh, the activity of rice hispa was recorded by Thakur et al. (1979), Choudhary et al. (2002) and Sharma and Srivastava (2008). Extensive loss is resulted due to the damage caused at vegetative growth stage of the plant (Islam 1989). The paddy yield attributing characters like plant height, tiller number, grain number per panicle and

grain yield are affected due to infestation. Losses are insurmountable to the growing paddy crop (Nath and Dutta 2002; Dutta and Nath 2003). Extent of losses may extend up to 28% in India (Nath and Dutta 2002).

D. armigera adults are shiny, bluish black beetles with short spines covered all over their body and measuring upto 4 to 5 mm in length. Adults in general live upto 45-78 days and males are short-lived as compared to females. There are 4 to 5 generations in a year on an average (Sen and Chakravorty 1970). Both adults and grubs of hispa feed on leaves and mainly attack during early vegetative stage of the crop. The damaged areas appear as white streaks parallel to the midrib and white translucent patches can be seen as a result of grub feeding. In severe epidemics, the leaves dry up and the crop presents a scorched appearance. Yield losses of upto 28 per cent is observed in India (Nath and Dutta 1997), however, it was as high as 100 per cent in transplanted post flood in Assam (Hazarika et al. 2005).

Various chemicals have been recommended for controlling the incidence of rice hispa but they are not very beneficial to the crop as well as to all the organisms. Therefore, some measures such as biological control, biopesticides as well as the use of genetic resistance are proved to be the most effective and safe. The role of insect resistance in rice germplasm had been an integral part of success in 'Green Revolution' and it resulted in increased rice production (Alagar et al. 2007). Resistance in the plants has been associated with biophysical parameters (Coley 1983), antifeedents and toxic substances (Otieno et al. 1985).

Artificial screening of some lines against hispa has led to the identification of some promising lines over the years. Keeping in view the importance of biophysical and biochemical mechanisms responsible for resistance; these plant mechanisms can be used in the rice crop in order to have lower level of hispa population. This will be an effective, economical and environment friendly method of pest management.

Therefore, the objective of the present studies undertaken is to analyse these lines for the mechanism of resistance in terms of biophysical and biochemical factors. In the light of the above, the present studies have been planned with the following objectives:-

1. To study antixenosis and antibiosis mechanisms of resistance in selected rice genotypes against *Dicladispa armigera*.
2. To study the role of biophysical and biochemical factors in selected rice genotypes against *Dicladispa armigera*.

2. REVIEW OF LITERATURE

2.1 Taxonomy and Literature

Dicladispa armigera (Olivier) is a species of leaf beetles belonging to the order Coleoptera and family Chrysomelidae. It is native to Southeast Asia that is also known as the "rice hispa."

Bangladesh (Alam 1967), India, and Laos are among the nations where *D. armigera* is known to be widespread. Bhutan, Cambodia, China, Indonesia, Iran, Korea, Malaysia, Myanmar, Nepal, Pakistan, Philippines, Sri Lanka, Taiwan, Thailand, Vietnam, and Papua New Guinea all include *D. armigera*, however it is not widely distributed. Extensive loss is resulted due to the damage caused at vegetative growth stage of the plant (Islam 1989). Losses are insurmountable to the growing paddy crop are (Nath and Dutta 2002; Dutta and Nath 2003). Extent of loss may extend up to 28% in India (Nath and Dutta 2002).

2.2 Life history of rice hispa

There are four stages in the life of rice hispa viz. egg, larva, pupa and adult. There are usually five larval instars. The female beetle begins depositing eggs three to four days after emergence and does so for a month. In a lifetime, a single female can lay up to 300 eggs. Eggs are inserted singly into the leaf tissues near the leaf tips. In around 5-7 days, they will hatch. The grubs begin feeding on the leaf's mesophyll and reach full maturity in around 15 days.

Pupation lasts about 5 days and takes place inside the tunnels created by larvae. In about 20-25 days, the entire life cycle is completed. Adults have a maximum life span of roughly 80 days. The bug usually completes six life cycles in a year. In the absence of rice, the insects survive by eating graminaceous weeds.

2.3 Mechanism of resistance

Painter (1951) proposed three pathways for host plant resistance: non-preference, antibiosis, and tolerance. The word antixenosis was coined by Kogan and Ortman (1978) to replace the term non-preference. Antixenosis means that the plant is

shunned as a bad host because of certain characteristics that cause the insect to avoid the plant or variety for oviposition, food, shelter, and so on. Antixenosis has an effect on the host plant's insect settling, probing, and oviposition (Cohen 1997).

Antibiosis means toxic or other detrimental effect of one organism on another (Metcalf and Luckmann 1975).

2.3.1 Antixenosis

This mechanism provides some resistance to the rice against rice hispa. This insect in both the grub and adult stage thrives well on susceptible varieties whereas it shows non-preference towards the resistant varieties i.e less feeding and survival on them.

Dhaliwal et al. (1978) screened early maturing germplasm for their resistance to the insect-pest of rice. Rice hispa infected all the 62 lines, and none of them were fully free. However, with 2.0 to 3.0 damaged leaves per hill, five cultures, Bido, NTS-205, China 47, Roma, and Japan-57, were less susceptible. I-geo-tse, IARI-5829, TN-1, IR-1712-212-2, and URB-47, among the other five cultures, were more sensitive, with 6.7 to 8.3 damaged leaves per hill. With 3.3 to 6.4 damaged leaves per hill, the remaining 52 lines were moderately susceptible to the attack of rice hispa.

According to Thakur et al. (1979), HPU 71, HPU 734, HPU 741, T 23, CHINA 988, and HPU 2004 were found to be extremely susceptible to rice hispa, whereas HPU 731, HPU 8021, RT 42, Norin 18, and IR 579 were moderately infested.

Dhaliwal (1980) tested 334 rice varieties in Punjab for varietal differences in *D. armigera* tolerance and observed damage after 40 days of transplantation. Hispa infection was present in all of the varieties. IET 4109 had the least amount of infestation (5 damaged leaves per 10 slopes).

D. armigera caused 15.6–97 per cent leaf loss in 64 different rice cultivars studied; OR 165-94-1 and KAU 1945 were found to be the most resistant genotypes (Chand and Tomar 1984).

Karim and Razzaque (1989) devised a simple method for growing the chrysomelid *D. armigera* in the greenhouse and assessed 15 rice cultivars for resistance to this pest. All the varieties that were tested were susceptible.

Infestation underscores the paddy yield attributing characters like plant height, tiller number, grain number per panicle and grain yield. Affected deep-water rice plants can hardly tolerate the rising flood water level (Islam 1973; Khan 1989).

During kharif 1990, Singh et al. screened a total of 77 elite rice genotypes for resistance to the ephydrid *Hydrellia* sp. and the chrysomelid *D. armigera* in the field in Punjab, India. *Hydrellia* sp. caused the least damage on IR 9209-48-3-2 and UPR 82-1-7, whereas CR 192-9-1, PR 107, and TKM 6 were least preferred by *D. armigera* for oviposition and feeding.

Subramanian et al. (1990) made fecundity table of rice leaf folder for two successive generations on cultivars with broad leaves (MTU 2067) and narrow leaves (BPT 5204). The rate of increase was greater on MTU 2067 (0.04) as compared to BPT 5204 (0.026) and population of the insect increased twice on MTU 2067 in just 17 days whereas on BPT 5204 it took 26 days. The trend was same in both the generations and this indicates the preference of the leaf folder for egg laying on broader leaf than the narrower leaf cultivars.

Laskar et al. (2008) evaluated various rice germplasms of different durations against leaf folder and correlate the infestation of the insect pest with morphological parameters like leaf length and leaf width and found positive correlation between leaf folder infestation and leaf width ($r = 0.68$) and negative correlation between infestation and leaf length ($r = -0.37$).

Hazarika and Dutta (1991) screened 96 local cultivars in field when hispa populations were at their peak. 15-20 per cent leaf damage was observed in varieties such as Malbhog, Pokikoli, Silguti, Gajep Sali 1 and Sarusokuu while the control Borsali suffered 100 per cent infestation.

Dutta and Hazarika (1992) at Titabar (Assam) screened 50 summer and 17 winter cultivars for resistance to hispa (*D. armigera*). 11 summer entries were considered as suitable donors of resistance as they were found to be moderately

resistant (11-20% leaf damage). For hispa endemic areas, Mala and Govind proved to be best due to them being high-yielding varieties.

Dutta and Hazarika (1994) studied the relative susceptibility of 50 rice cultivars to artificial infestations with *D. armigera* in Assam. The varieties Mala, Garem, Bijer 3 and Bengaubisi, were recommended for Assam and were rated less susceptible (<20% leaf damage). Saket 4, a highly susceptible variety was also recommended.

Increased activity of the brown plant hopper, *Nilaparvata lugens* on rice variety IR 64 over that observed on varieties IR 22 and IR 62 was shown to be due to the chemical composition of the surface wax (Woodhead and Padgham 1998).

The varieties BR25 and BR7 are less susceptible than most of the modern rice varieties cultivated in Bangladesh (Anonymous 1999).

Rao et al. (2002) conducted screening experiment on several rice cultivars and examined various morphological characters. Positive correlation was found between leaf width ($r = 0.857$) and leaf infestation but no correlation was found with plant height. They also revealed relationship between leaf texture and relative infestation signifying that resistant lines had rough leaf texture that provide resistance to feeding by the leaffolder. Cultivars with damage rating 1-3, mainly had rough texture.

At Gerua, Assam, Rath (2002) screened forty six Sikkim rice cultivars for resistance to hispa, as well as one susceptible (TN-1) and one resistant check (ARC 5764). The least damaged (6.53-9.51 per cent leaf damage and damage score 1) cultivars by the pest were Kalanonia and Phaudel along with resistant check ARC 5764.

Rehman et al. (2005) carried out studies on the mechanism of resistance in rice to leaffolder, *Cnaphalocrocis medinalis*: a major pest of rice in Pakistan.

Xu et al. (2010) studied 15 japonica lines and 2 indica rice lines. Most of the lines were in damage leaf scale (DLS) 3 and 5 and TN-1, Tangjing 9358 and Line 91 SP showed susceptibility (DLS 7 to 9).

Prasad and Prasad (2011) conducted a field experiment at Ranchi with seven rice entries in protected and unprotected conditions. Overall, the results showed that among the seven rice genotypes studied, protected crops had a lower prevalence of rice hispa than unprotected crops of the same varieties.

Punithavalli et al. (2011) studied the effect of biophysical characters viz. plant height, leaf blade length and width on 10 cultivars and 2 wild genotypes. The resistant and wild genotypes were taller (105.7-151.7 cm) and had more leaf length (45.3-57.0 cm) as compared to susceptible ones which had less plant height (65.0-86.7 cm) and leaf length (35.7-42.0 cm). Leaf blade width was recorded less in resistant genotypes (0.7-1.1 cm) than susceptible ones (1.1-1.4 cm). Maximum emergence was recorded in TN-1 (37.1 %) and IR36 (33.3%) and minimum in TKM6 (6.7%). Study also concluded that leaf feeding preference by larvae was higher on second leaf from top > first from top > third from top > fourth from top and fifth from top.

Rashid et al. (2013) reported the rice varieties IRRI-6, KSK-282, DR-83, Basmati-370, Basmati-385 and Super Basmati for leaf folder resistance. IRRI-6 was the most resistant with least infestation (14.50%) followed by KSK-282 (15.99%) and DR-83 (19.12%). Basmati-370, Basmati-385 and Super Basmati were susceptible to leaf folder.

Akhter et al. (2015) studied the infestation of rice leaf folder on twenty three genotypes. Out of these, KSK-459 was found to be the most resistant (49.00-58.71 %) followed by PK 8649 (51.94-58.87 %). Cultivars KSK-454, KSK-453, KSK-462 and KSK-460 (check) were found to be more susceptible than other cultivars.

Tabari et al. (2016) studied the two categories of resistance, antixenosis and antibiosis in ten popular and diverse rice genotypes of different origin against the striped stem borer, *Chilo suppressalis*. Significant differences were found between genotypes for the number of egg masses, number of eggs, preference index, larval and pupal weight, larval development time, larval survival rate, larval mine length, and leaf trichome density. It was found that the rice genotypes Novator, A7801, and Nemat had the more pronounced antixenosis-type resistance, whereas AB1 and Shirodi had better antibiosis-type resistance. Interestingly, the rice genotype AN-74

for which Nemat is the parental line showed both types of resistance and could be effectively used in an integrated pest management of the rice striped stem borer.

2.3.2 Antibiosis

In Bihar, India, Haque et al. (1987) conducted field cage tests to investigate the resistance of 10 elite rice lines to *D. armigera*. One hundred hispa adults were released on the 15 days old plants and several antixenosis and antibiosis parameters such as percentage of damaged leaves, number of eggs laid, number of grubs, and grub survival percentage were recorded. Type 3, a scented type from Uttar Pradesh, was the pest's least favourite. TCA 4, IET 6263, and Rajendra Shan 201 were among the most susceptible lines. Pest attack was severe in Madhubani.

Salim (1988) carried out detailed studies on whitebacked planthopper, *Sogatella furcifera*, a major insect-pest of rice in Pakistan and various other rice growing countries. He used insect resistant IR 2035 and susceptible TN-1 rice cultivars in culture solution with different levels of K and Fe. The quantity of allelochemicals in IR 2035 was higher than susceptible TN-1 plants when level of K was higher and vice-versa.

Under screen house conditions, Sharma et al. (2014) studied the survival and development of rice hispa, *D. armigera*, on four distinct rice cultivars viz., taraori basmati, HRK 126, IR 64, and HKR 47. The larval duration (12.50 and 13.65 days), pupal period (7.77 and 8.63 days) and total life period (25.27 and 26.46 days), was longest on HKR 47 making it a susceptible variety and shortest on Taraori basmati making it a resistant variety.

Oyetunji et al. (2014) studied antibiosis mechanisms of resistance in African rice against African rice gall midge *Orseolia oryzivora* (Harris and Gagne). Various biochemicals such as phenols, terpenoids, monoterpenoids and salicylic acid were found to be the reason of antibiosis mechanism in the three rice cultivars, *Oryza glaberrima* and there was negative correlation between amount of biochemicals and tiller infestation.

Krisnawati et al. (2017) studied antibiosis mechanism in soybean against *Spodoptera exigua*. It was concluded that the larval weight was maximum in the

susceptible variety G511 H/Anj-1-2 (0.715 g) followed by G511 H/Anj-7-1 (0.613 g) and it was minimum in the resistant check G100H (0.26 g). Similarly, pupal weight was maximum in G511 H/Anj-1-2 (0.278 g) and minimum in G100H (0.161 g).

2.3.3 Biochemical factors

Biochemical factors consist of certain chemicals in the form of sugars, amino acids, proteins, phenols, salicylic acid etc. and these chemicals affect the insect physiology and behaviour.

a) Phenols

Phenols act as defensive substances in the form feeding deterrent against herbivores as well as microorganisms (Usha Rani and Jyothsna 2010). To initiate the defense mechanism against herbivorous insects in plants, phenols are oxidised in the presence of peroxidase (POD) and polyphenol oxidase (PPO). They get oxidised and produce quinones which are actually toxic to insects (Bhonwong et al. 2009). These quinones also get attached to the leaf protein and decrease the protein digestion in insects (Duffey and Stout 1996).

Umamaheshwari et al. (2006) recorded highest phenol content in resistant line IR-54742-22-19-3R (22.52 ppm) among all lines as compared to genotype TN-1 (11.5 ppm).

Punithavalli et al. (2013a) observed various biochemicals viz. total phenols, ortho-dihydroxy phenols and proteins which were analyzed in several susceptible and resistant genotypes. Total phenols content in healthy leaves ranged from 3.48-10.83 mg/g, whereas in leaf folder infested leaves, it varied from 4.31-14.08 mg/g. Also, resistant genotypes had higher phenol content i.e. *Oryza minuta* and Ptb33 that was 9.86 and 10.83 mg/g in uninfested leaves and 12.94 and 14.08 mg/g in infested leaves, respectively. The similar trend was found in ortho-dihydroxy phenol and its amount was 3.87, 3.66 and 3.87 mg/g in resistant genotypes TKM6, *Oryza rhizomatis* and LFR 831311 but was in low amount i.e. 2.29, 2.34 and 2.29 mg/g in TN-1, IR 36 and Pusa basmati, respectively.

The effect of phenol concentration on infested leaf area by leaf folder was studied by Vanitha et al. (2015). She reported that amount of total phenol was

higher in resistant genotypes (2.55-3.93 mg/g) and less increase in susceptible ones (1.44-1.86 mg/g).

Deepa et al. (2016) concluded that at different growth stages total phenols, silica and o-dihydroxy phenols content was more in resistant genotypes but sugar content was higher in susceptible genotypes. Total phenols were found to be three time higher in resistant genotype HKR 47 (10.46 mg/g) than susceptible check TN-1 (3.887). Silica content was lowest in susceptible check TN-1 (9.00%). It was concluded that leaf folder resistant lines had higher phenolic content than susceptible lines. There was also an overall increase in the amount of phenol in infested plants than healthier plants but the increase was more in resistant genotype Ptb33 (0.62-2.31g/g) and less in susceptible genotypes TN-1 (0.21-0.76g/g) and Jaya (0.27-0.91 mg/g) (Dharshini and Gowda 2014).

b) Total and reducing sugars

At low concentration sugars acts as feeding stimulants and at high concentration they act as toxic compounds to insects. According to Knapp et al. (1966) and (Kalode and Pant 1967) higher sugar content was found in the susceptible parts of plants. There was a positive correlation between damage caused by white backed planthopper with sugar, and chlorophyll content (Rath et al. 1998).

Chandramani (2003) found that plants supplied with lignite fly ash, biofertilizer, neem cake and FYM had higher phenols, less amino acids and less total sugars and therefore were more resistant.

Mahadeva (2011) reported significant decrease in the amount of total sugars, reducing sugars and soluble proteins in the infested leaves of Mulberry after infestation of thrips. The highest decrease in reducing sugar content was in S54 genotype (31.68%) but lowest in MR2 (9.23%).

Praveen et al. (2013) reported that total soluble proteins and total soluble sugars in maize leaves and stem was lower in resistant cultivars while it was higher in susceptible cultivars and as a result they act as feeding stimulant for the maize borer. On the other hand, total phenols and tannins were found to be higher

in resistant cultivars than in susceptible ones. Therefore, both the phenols and tannins are associated with anti-feedent and deterrency or repellency actions against the maize borer, *Chilo partellus*.

Various biochemical constituents, viz. total sugars, proteins and reducing sugars were analyzed in several rice lines in healthy as well as BPH infested plants (Dharshini and Gowda 2014). Total protein content was found to be higher in TN-1 (5.02mg/g) and Jaya (5.62) than Ptb33 (1.63). There was decrease in the amount of total protein content in the infested individuals than healthier one. Similarly, total sugars and nitrogen concentration was lower in resistant while higher in susceptible genotypes. Also, reducing sugars were in higher amount in TN-1 (31.79 mg/g) and Jaya (34.81 mg/g) but very little amount in Ptb33 (7.87 mg/g) and Raibhog (10.17 mg/g) and decrease in amount of reducing sugar after infestation was observed in all the genotypes. High phenolics and less amount of total sugars in shoot tips of resistant varieties of rice has antibiotic affect on gall midge, *Orseolia oryzae* (Peraiah and Roy 1979).

Deepa et al. (2016) found that total sugar content was minimum in Thogai Samba (7.409 mg/g) and maximum in TN-1 (16.578 mg/g) and concluded that susceptible genotypes contain higher sugar concentration than resistant genotypes. The sugar content was analyzed at different stages of plant (20, 40 and 60 days after planting) and sugar content generally reduced with increase in age.

c) Proteins and amino acids

Sinha et al. (2005) interpreted that total soluble protein content decreased in all tested varieties due to infestation of rice leaffolder. Edwards and Wratten (1983) told that quantity of proteins responsible for defense and repair increases after attack of insect.

Punithavalli et al. (2013a) observed that total protein content varied from 6.23 to 8.08 mg/g in uninfested leaves and 6.11 to 7.61 mg/g in infested ones. After the leaffolder infestation, a decrease in total protein quantity in all cultivars was observed which was 5.77 per cent in TN-1 and 1.33 per cent in case of TKM6. Vanitha et al. (2015) studied the effect of various biochemicals, viz. amino acids and protein content on the damaged leaf area and reported that amino acids and

protein content were higher in susceptible cultivars but lower in resistant one. Also, the relative damaged leaf area was positively correlated with total amino acids ($r = 0.858$) and crude proteins ($r = 0.931$).

d) Fibre content

Fibre content in plants mainly includes cellulose, hemicellulose and lignin content and major component of cell wall. A dicot plant contains 30 per cent hemicellulose, 30 per cent cellulose, 35 per cent pectins and 1-5 per cent proteins and a monocot generally contain 55 per cent hemicellulose, 25 per cent cellulose and 10 per cent pectin (Cosgrove 1997).

Cellulose is the most abundant polymer in nature. It is synthesized by cell membrane proteins and is the major component of cell wall and highly stable (Taylor 2008). It is the polymer of glucose with β (1-4) linkage. Hemicellulose differs from cellulose as it is an amorphous and soluble in acid or alkaline solutions. Lignin is the most resistant polymer in nature and influence plant growth by providing structural support and resistance to abiotic and biotic stresses. After abiotic stress or insect injury, one of the responses includes activation of the phenylpropanoid metabolism pathway that results in production of lignin, which reinforces the cell wall (Douglas 1996; Dixon and Paiva 1995; Boerjan et al 2003). Lignin deposition after insect injury has been reported in many plants such as tobacco (Lagrimini 1991) and *Arabidopsis* (Delessert et al. 2004; Howe and Schaller 2008). Plant tissues having more lignin content are tougher and less palatable to the insect than that of parts having less lignin content. Lignin deposition affects the insect not only by making the tissues tough but also by the production of toxic compounds like quinones and peroxides which are produced by enzyme phenoloxidase that is required for the polymerization of lignin. The defense mechanism of plant fibres against insects acts either as pre-ingestion to reduce feeding or as post-ingestion to decrease the nutrition value. In the former mechanism, insect feeding is reduced due to the hardened cell wall and in the latter mechanism, the digestion and nutrition value of the plant is affected by phenolic compounds and therefore cause anti-nutritional effect (Brodeur-Campbell et al. 2006; Schroeder et al. 2006).

Lignin content in maize leaves was analyzed before and after the infestation of European corn borer and it was found that lignin content increased in the infested leaves from 34.4 to 39.3 g/kg in non-Bt variety and 31.6 to 33.6 g/kg in Bt variety but there was decrease in lignin amount in the stem (Yanni et al. 2011). Ordas et al. (2002) selected four inbred lines of maize, 2 of which (EP39 and A509) were resistant and two (EP47 and EP42) were susceptible to the corn borer. The resistant lines had higher amount of lignin and xylose in the pith than susceptible lines.

Hedin et al. (1984) investigated that inbred lines that were found to be resistant to Southwestern corn borer, *Diatraea grandiosella* (Dyar) had higher amount of hemicellulose and crude fibre amount was negatively correlated with larval damage.

Similarly, Hedin et al. (1996) revealed that maize genotypes having higher amount of hemicellulose were less susceptible to fall armyworm, *Spodoptera frugiperda* (Smith).

3. MATERIALS AND METHODS

The present study entitled “Basis of resistance against rice hispa, *Dicladispa armigera* (Olivier) in paddy” was conducted on some selected rice genotypes under screen house conditions at CSK Himachal Pradesh Krishi Vishwavidyalaya, Rice and Wheat Research Centre (RWRC), Malan during the kharif season of 2020 and 2021. This research Centre is geographically situated at an elevation of 961 meters above mean sea level. It is located at a latitude of 32°07.180 North and a longitude of 76°25.065 East. The experimental farm area falls in the mid-hill sub-humid zone-II of Himachal Pradesh agro-climatically and is characterized by humid temperate climate and has acidic soils.

A. MATERIALS

3.1 Test plants/ Varieties

The seven genotypes of rice used were Nagardhan, Sukaradhan, HPR 2613, HPR 2617, Pusa 1121, W1263 (resistant check), and TN-1 (susceptible check). They were obtained from the harvest of previous season.

B. METHODS

3.1 Raising test plants

The stock culture of hispa was maintained in net house on paddy as well as in cages. The hispa beetles were initially collected from the farmer’s field with the help of sweep net method and some were separately raised on potted plants (30-45 days old plant seedlings) and raised for sorting out sexes of the insect.

3.2 Resistance parameter

3.2.1 Antixenosis resistance

3.2.1.1 Settling behaviour of adults

The tested genotypes were transplanted in small plastic pots 30 to 40 days after sowing and kept under net house in five replications. Each of the five replications was placed under small nylon mesh net for no choice test and all the genotypes were covered separately. A large number of adults were released on 50-day old plants with 14 adults in each cages. The number of male and female adults will be counted 4, 8, 12, 24, 48, 72 hrs after release. The plants will be disturbed after each count for reorientation of the insects.

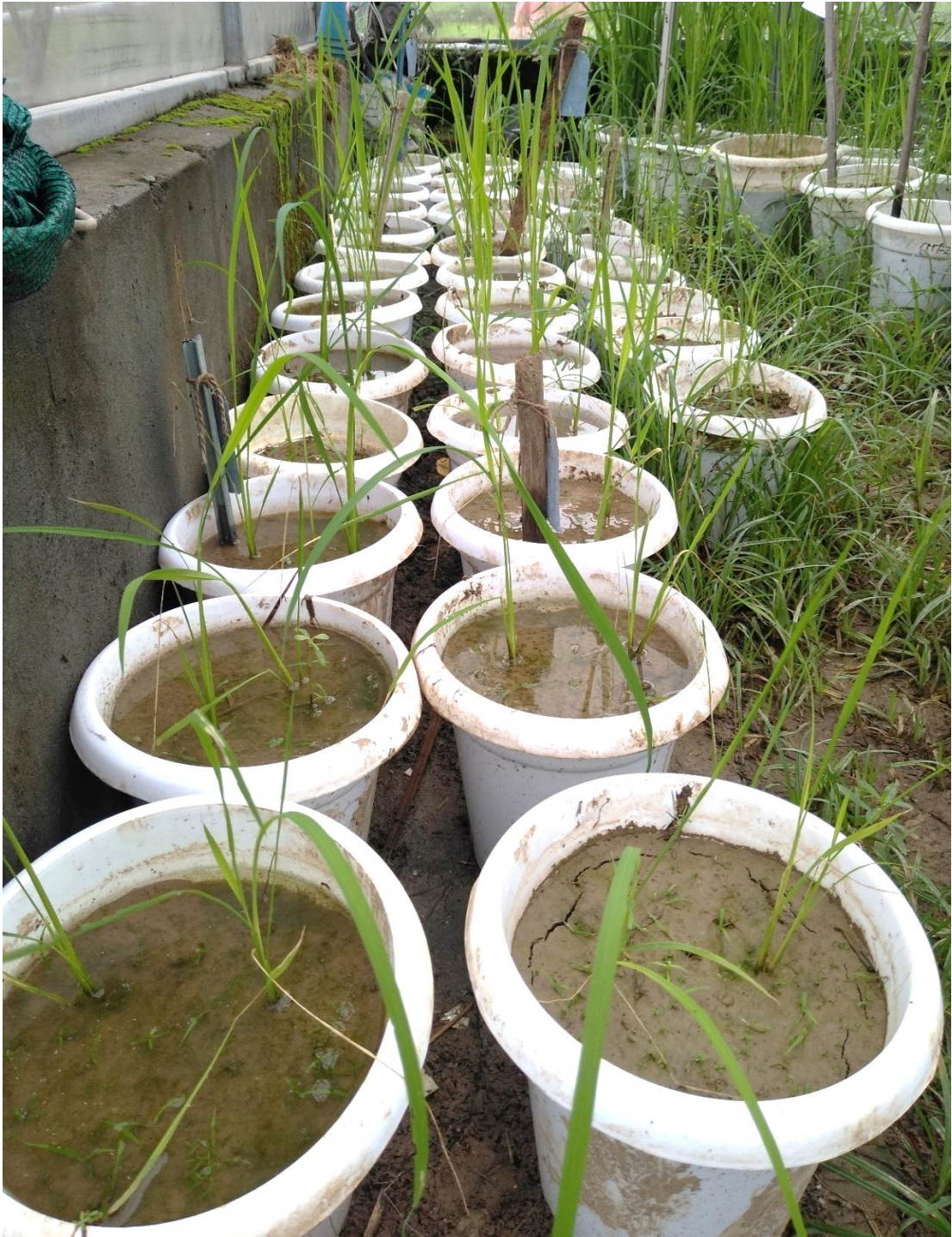


Plate 3.1 Potted transplanted plants raised as test plants for the study of antixenosis and antibiosis mechanism



Plate 3.2 Plants kept in mesh net cages for studying the adult settling, oviposition and leaf infestation experiment

3.2.1.2 Ovipositional preference

The tested genotypes from above experiment were examined for ovipositional preference. Three to five days after releasing the adults, the caged nets were opened and the number of eggs was counted on each genotype. Then next day onwards the number of grubs emerging were observed and counted to estimate the hatched and unhatched eggs percentage.

3.2.1.3 Feeding area

Feeding area was examined after the grubs fed individually on 50-day old plant covered with the nylon mesh net cage. The grubs when fully developed leave the slit and then damaged leaf area was calculated by drawing the leaf on millimetre graph paper using butter paper.

3.2.2 Biophysical characters

Different biophysical characters of test genotypes like flag leaf width, leaf length, plant height, were studied on 50 days old plants for determining their role in antixenosis.

3.2.3 Antibiosis resistance

3.2.3.1 Grub duration

Five plants of each genotype were transplanted in plastic pots after 30 to 40 days of sowing. Ten newly hatched grubs were released on 40-day old plants. These plants were enclosed with nylon mesh net cages. The grubs were observed daily and the total time taken by them to pupate on different genotypes was recorded in days.

3.2.3.2 Final grub weight and grub survival

The final instar grubs just before pupation were weighed on an electric balance and the number of grubs that pupate successfully out of the total were counted on each genotype and larval survival was calculated.

3.2.3.3 Pupal weight and duration

Pupae were collected from each genotype which were then weighed on an electric balance. They were then observed daily and the total time taken for them to transform into an adult were recorded in days.

3.2.3.4 Adult emergence

The number of adults emerged from the pupae from different genotypes was recorded.

3.2.3.5 Adult longevity

The adults which emerged after raising were observed daily and their longevity was recorded in number of days.

3.2.4 Biochemical factors

Different biochemical constituents namely phenols, total soluble sugars, reducing sugars, non-reducing sugars, starch, free amino acids, proteins and fibre content were observed in the infested and uninfested plants of the 50 days and 70 days old genotypes.

3.2.4.1 Total phenols

Total phenols were determined by using Folin-Ciocalteu reagent method (Malick and Singh 1980).

Extraction

For the estimation of total phenols, the leaves of ninety-day old rice genotypes were collected and oven dried at 40-50°C for 4-6 hrs. One gram of dried leaf sample was taken in a 50 ml volumetric flask and 20 ml of 80% ethanol is added to it. It was heated on a boiling water bath for 10 minutes. This solution was then cooled and then grounded in pestle and mortar. After that it was filtered with the help of a muslin cloth. The filtrate is then reextracted with 5 ml of 80% ethanol and was then filtered with the help of Whatman filter paper no. 1. The alcohol is then evaporated into dryness from the supernatant. 1 ml of distilled water is added to the content after evaporation.

Reagents: Folin-Ciocalteu reagent (diluted in 1:1 with distilled water)

Saturated Na_2CO_3 (10 g/50 ml distilled water)

Procedure

0.2 ml of extract was pipetted into a test tube and then 1 ml of FC reagent was added to it. Then 2 ml of 20% Na_2CO_3 was added and the solution was shaken vigorously and then it was heated for 1 minute in a water bath after which a blue color appears. It was then cooled under running tap water. After 1 hour absorbance of blue color was recorded at 650 nm against a blank reagent with the help of a spectrophotometer. Concentration of total phenols was determined with the help of catechol standard curve.

3.2.4.2 Total sugars

Total sugars were determined by Anthrone method (Yemm and Willis 1954).

Extraction

1 g of moisture free sample was taken and grinded with 50 ml 80 % ethanol in pestle and mortar. The sample was then filtered through Whatman filter paper No. 1. The supernatant collected was then used for estimation of total soluble sugars. The filtrate was boiled till the volume is reduced to half and then volume is made up by adding 98 ml of distilled water. Now 1 ml of saturated lead acetate solution along with a pinch of sodium oxalate crystal was added to the filtrate and mixed properly. The solution turned milky which was then filtered with the help of Whatman filter paper No. 1.

Reagents: Anthrone reagent

Procedure

0.1 ml of extract was pipetted into a test tube and the volume was made up to 1 ml by adding distilled water. Then 4 ml of anthrone reagent was added to the extract. The content was then vortexed and then kept in a boiling water bath for 10-15

minutes. It was then cooled under running tap water and then the absorbance was measured at 625 nm against a reagent blank. The concentration of total sugar was calculated from glucose standard (100-1000 µg) run simultaneously.

3.2.4.3 Reducing sugars

Reducing sugar was determined by using DNS (dinitro salicylic acid) method given by Miller (1972).

Reagents:

DNS reagent: Dissolve 1 g of DNS, 0.2 g (200 mg) of crystalline phenol and 0.05 g (50 mg) of sodium sulphite in 100 ml of 1% NaOH solution by stirring.

40% Rochelle salt solution (sodium-potassium tartrate solution).

Procedure

1 ml of alcohol-free sugar extract was pipetted into a test tube and the volume was made up to 3 ml by adding distilled water. 3 ml of DNS reagent was added to the extract and mixed. It was then heated in a boiling water bath for 5 minutes. After the color was developed, 1 ml of 40% Rochelle salt was added to the warm solution and mixed. It was then cooled under running tap water. The absorbance was then measured at 510 nm against a blank. The concentration of reducing sugar was calculated from a standard glucose graph (100-1000 µg) run alongside.

3.2.4.4 Starch

Starch was determined using Anthrone reagent method (Yemm and Willis 1954).

Reagents:

Anthrone reagent: 0.2 g of anthrone in 100 ml of ice-cold sulphuric acid 52% of perchloric acid.

Extraction

The residue left after filtration from extraction is used for determination of starch. 0.2 g of residue was taken in a small 50 ml beaker and 5 ml of water was added to it. 6.5 ml of 52% perchloric acid (add 270 ml of 72% perchloric acid to 100 ml of distilled water) was added to the beaker. The content was first stirred continuously for 5 minutes and then intermittently for next 15 minutes. 20 ml of distilled water was added to the content and centrifuged for 15 minutes @ 3250 rpm. The supernatant was then poured into a 100 ml volumetric flask. Now the same extraction was repeated with the time extending up to 30 minutes. Now the content in the flask was washed in their own respective flask and the volume was made up to 100 ml with distilled water. The content in the flask was then filtered with Whatman filter paper No. 1 and the first 5 ml of the filtrate was discarded.

Procedure

0.05 ml of supernatant was pipetted out into a test tube and the volume was made up to 1 ml with distilled water. Then 4 ml of anthrone reagent was added to it. The solution was then heated for 5-8 minutes in hot water bath and then it was cooled under running tap water giving a green to dark green color to it. The absorbance was then checked @630 nm with help of spectrophotometer.

3.2.4.5 Total protein

The total protein method was analyzed using micro-Kjeldahl method.

Reagents:

Conc. H₂SO₄

Digestion mixture: Potassium sulphate: Copper sulphate in the ratio 10:1.

40% Sodium hydroxide

40% Boric acid

0.1N Standard HCl

Methyl red indicator

Procedure

The procedure for protein determination consists of two steps which are digestion and distillation which is carried out in Kjeldahl unit. 1 g of powdered sample was transferred into distillation tube. Then 3 g of digestion mixture was added to it. Then 10 ml of Conc. H_2SO_4 was added to the tube and then the content was kept in the digestion assembly of Kjeldahl unit for 3 hrs. After digestion, the content was kept for cooling before distillation process. Distillation was done in the distillation unit of the Kjeldahl's apparatus and titration is done side by side with standard 0.1 N HCl.

3.2.4.6 Free amino acids

Free amino acids were determined using standard protocol given by Jayraman (1981)

Extraction

0.2g of sample was extracted with 80% ethanol. It was centrifuged @ 5000 rpm for 20 minutes.

Reagents:

Reagent A – 0.2M Acetate buffer (pH 5.5)

Reagent B – 2 g of ninhydrin was dissolved in 25 ml acetone and volume was then raised to 50 ml with acetate buffer (pH 5.5, 0.2M)

Reagent C – Standard Glycine

Procedure

0.2 g of sample was extracted using 80% ethanol and the extract was centrifuged @ 5000 rpm for 20 minutes. 0.4 ml aliquot of the extract was taken in a test tube and the volume was made up to 4 ml with distilled water. Then 1 ml of Ninhydrin solution was added to it and the test tube with content was kept in hot

water bath for 15 minutes. It was then cooled and 1 ml of 50% ethanol was then added to it. The absorbance of blue colour was then checked at 550 nm.

3.2.4.7 Fibre

Reagents:

0.255 ± 0.005 N Standard H₂SO₄

0.313 ± 0.005 N Standard NaOH

Procedure

2 g of sample was boiled with 200 ml of H₂SO₄ for 30 minutes. It was then filtered through muslin cloth and washed with boiling water until washings were free of acid. The residue was then boiled with 200 ml of NaOH for 30 minutes. It was then again filtered through muslin cloth and washed with 25 ml of boiling H₂SO₄, three 50 ml portions of water and 25 ml of alcohol. The residue was then transferred to pre-weighed ashing dish (W1, g). The residue was dried for 2h at 130 ± 2°C, cooled in a desiccator and weighed (W2, g). It was then ignited for 30 minutes at 600 ± 15°C. Finally, it was cooled in a desiccator and reweighed (W3, g).

4. RESULTS AND DISCUSSIONS

The basis of resistance and mechanisms were studied against rice hispa, *Dicladispa armigera* (Guenee) in the selected seven genotypes of rice viz., HPR 2613, HPR 2617, Sukaradhan, Nagardhan, Pusa 1121, W1263 (resistant check) and TN-1 (susceptible check) under the screen house conditions at Rice and Wheat Research Centre, Malan of CSKHPKV, Palampur during the kharif season of 2020 and 2021. The following are the results regarding various parameters of plant resistance.

4.1 Antixenosis resistance

Under antixenosis studies, the various genotypes showed significant differences among various genotypes in various parameters.

4.1.1 Settling behaviour of adults

The number of settled adults on different genotypes varied significantly during observations at different time intervals. The adults were initially counted 4 hours after release. The least number of adults were found on W1263 (1.20) and Pusa 1121 (1.20) which were statistically at par with each other and the highest number were settled on TN-1 (2.4) (Table 4.1). After 8 hours of release, the number of settled adults differed slightly on different genotypes. The least number of adults was found on W1263 (1.0) followed by HPR 2613 (1.60), HPR 2617 (1.60) and Pusa 1121 (1.60) which were statistically at par with each other. Highest number of adults were settled on TN-1 (2.80) followed by Sukaradhan (1.80) and Nagardhan (1.80) which were at par with each other (Table 4.1). The number of settled adults differed significantly 24 hours after release and the lowest number of adults were recorded on W1263 followed by HPR 2613 (1.40), HPR 2617 (1.40) and Pusa 1121 (1.40) which were at par with Nagardhan (1.60). The highest number of settled adults was recorded on TN-1 (2.00) and Sukaradhan (2.00). A similar trend was observed 48 and 72 hours after release. Similarly, after 72 hrs the least number of settled adults were recorded on W1263 (1.20) and HPR 2617 (1.20) which were at par with Nagardhan (1.40), Pusa 1121

(1.40) and HPR 2613 (1.60) but significantly lower than TN-1 (2.40) and Sukaradhan (2.40) (Table 4.1).

The number of adults settled on different genotypes differed significantly. Overall, the pooled mean values showed that least number of adults were settled on W1263 (1.12) followed by HPR 2617 (1.40), Pusa 1121 (1.44), Nagardhan (1.52) and HPR 2613 (1.56) which did not differ significantly and were at par with each other. The highest average number of adults were found on TN-1 (2.40) followed by Sukaradhan (2.12) which were at par with each other (Table 4.1).

Table 4.1. Settling behaviour of *Dicladispa armigera* adults on rice genotypes

| Genotype | Number of settled adults/plant (mean) after | | | | | | |
|-------------------|---|------|---------------|------|------|-------------|-------------------------|
| | 4 h | 8 h | 24 h | 48 h | 72 h | Pooled mean | Per cent settled adults |
| HPR 2613 | 1.80 | 1.60 | 1.40 | 1.40 | 1.60 | 1.56 | 52.00 |
| HPR 2617 | 1.60 | 1.60 | 1.40 | 1.20 | 1.20 | 1.40 | 70.66 |
| Sukaradhan | 2.00 | 1.80 | 2.00 | 2.40 | 2.40 | 2.12 | 80.00 |
| Nagardhan | 1.40 | 1.80 | 1.60 | 1.40 | 1.40 | 1.52 | 46.66 |
| Pusa 1121 | 1.20 | 1.60 | 1.40 | 1.60 | 1.40 | 1.44 | 37.33 |
| W1263 (RC) | 1.20 | 1.00 | 1.00 | 1.20 | 1.20 | 1.12 | 50.66 |
| TN-1 (SC) | 2.40 | 2.80 | 2.00 | 2.40 | 2.40 | 2.40 | 48.00 |
| CD(p=0.05) | | | (0.26) | | | | |

Means within a column followed by the same letter are not significantly different at $p \leq 0.05$ according to LSD

4.1.2 Oviposition preference

The ovipositional preference on selected genotypes was recorded by counting the total number of eggs on each genotype and per cent hatched eggs. The number of eggs differed significantly from each other on different genotypes. Highest number of eggs were laid on TN-1 (55.2) followed by Sukaradhan (42.4) and HPR 2617 (41.6) and these were at par with each other (Table 4.2). The lowest number of eggs were laid on the resistant check W1263 (27.0) followed by the genotype HPR 2613 (29.2) and both were at par with each other (Table 4.2).

Table 4.2. Oviposition preference and per cent hatching of *Dicladispa armigera* on rice genotypes

| Genotype | Total fecundity (number) (mean) [#] | Per cent hatched eggs (mean) [^] |
|--------------------|---|--|
| HPR 2613 | 29.2 (5.49) | 72.85 (58.77) |
| HPR 2617 | 41.6 (6.52) | 80.32 (63.75) |
| Sukaradhan | 42.4 (6.58) | 75.38 (60.26) |
| Nagardhan | 33.0 (5.82) | 69.96 (56.78) |
| Pusa 1121 | 32.0 (5.73) | 66.52 (54.65) |
| W1263 | 27.0 (5.28) | 64.18 (53.44) |
| TN-1 | 55.2 (7.49) | 86.96 (68.82) |
| CD (p=0.05) | (0.39) | (5.00) |

[#]Figures in parentheses are the mean of $\sqrt{n+1}$ transformations

[^]Figures in parentheses are the mean of arc sine $\sqrt{\text{percentage}}$ transformations

Mean within a column followed by the same letter are not significantly different at $p \leq 0.05$ according to LSD

Similarly, different genotypes also recorded significantly different per cent hatched eggs. Highest per cent hatched eggs were recorded on susceptible check TN-1 (86.96%) and it was at par with HPR 2617 (80.32%) but higher than Sukaradhan (75.38%) (Table 4.2). The lowest per cent hatching was recorded on W1263 (64.18%) followed by Pusa 1121 (66.52%) and Nagardhan (69.96%) and these were significantly at par with each other and with HPR 2613 (72.85%) (Table 4.2).

These results are in corroboration with Liao and Chen (2017) that ovipositional preference was less on resistant plants as compared to susceptible plants. These results are also supported by Suresh (1992) that oviposition and per cent hatched eggs were less on W1263 and *Oryza minuta*. Likewise, Ramchandran and Khan (1991) also provided that egg laying on susceptible IR31917-45-3-2 was much higher than resistant *O. brachyantha* and only 5 per cent of total oviposition was on *O. brachyantha* but 73 per cent on IR31917-45-3-2. Similarly, study conducted by Dakshayani et al. (1993) reported that maximum number of egg laying in per cent of total was on susceptible variety TN-1 (18.8) and on resistant Ptb12 only 5.6 per cent egg laying was done.

4.1.3 Damaged leaf area

The damaged leaf area after grub feeding differed significantly among selected genotypes. The minimum feeding area was observed in W1263 (3.38 cm²) followed by Pusa 1121 (4.20 cm²), Sukaradhan (4.46 cm²) and HPR 2617 (4.70 cm²) which were significantly similar to each other. The maximum leaf area was infested in TN-1 (7.62 cm²) followed by Nagardhan (6.62 cm²) and HPR 2613 (5.42 cm²) and these genotypes were significantly at par with each other (Table 4.3).

The per cent damaged leaf area was also significantly different in selected genotypes and the highest per cent leaf area was consumed in TN-1 (37.49%) followed by HPR 2613 (24.15%) which were at par. HPR 2617 (21.75%), Nagardhan (20.64%) and Pusa 1121 (19.99%) were significantly similar to each other. The lowest per cent damaged leaf area was recorded in W1263 (13.12%) which was at par with Sukaradhan (15.19%) (Table 4.3).

The results are in accordance with study of Punithavalli et al. (2013b) that per cent damage by leaffolder was highest in TN-1 (46.3%) and lowest in TKM6 (12.3%). Also, Dakshayani et al. (1993) investigated that leaf area consumed by fifth instar larvae of leaffolder was maximum in TN-1 (330.0 mm²) and much lower in resistant check Ptb12 (160.9 mm²) and TKM6 (210.7 mm²) and there was significant difference in these varieties.

Table 4.3. Infested leaf area by the grubs of *Dicladispa armigera* in selected rice genotypes

| Genotype | Infested area (cm²) (mean) | Per cent infested area# (mean) |
|-------------------|--|---|
| HPR 2613 | 5.42 | 24.15 (29.42) |
| HPR 2617 | 4.70 | 21.75 (27.78) |
| Sukaradhan | 4.46 | 15.19 (22.93) |
| Nagardhan | 6.62 | 20.64 (27.00) |
| Pusa 1121 | 4.20 | 19.99 (26.55) |
| W1263 | 3.38 | 13.12 (21.23) |
| TN-1 | 7.62 | 37.49 (37.74) |
| CD(p=0.05) | (0.27) | (0.87) |

#Figures in parentheses are the mean of arc sine $\sqrt{\text{percentage}}$ transformations

Means within a column followed by the same letter are not significantly different at $p \leq 0.05$ according to LSD



Plate 4.1 Infested leaf area by rice hispa, *Dicladispa armigera*

4.2 Biophysical factors

Various biophysical factors such as plant height, length and width of flag leaf were recorded to find out their role in antixenosis resistance.

4.2.1 Morphological parameters

The various morphological parameters such as plant height, flag leaf length and width differed significantly in the selected genotypes. The flag leaf length varied significantly among the genotypes ranging from 23.34 to 38.64 (Table 4.4). The length of flag leaf was highest in Sukaradhan (38.64) which was significantly similar to HPR 2617 (38.24), Nagardhan (33.98) and TN-1 (32.42). W1263 (23.34) had the lowest leaf length among the genotypes and it was at par with HPR 2613 (26.12) and Pusa 1121 (27.30) (Table 4.4).

Table 4.4. Morphological parameters in rice genotypes

| Genotype | Flag leaf length (cm) (mean) | Leaf width (mm) (mean) | Plant height (cm) (mean) |
|-------------------|---|-----------------------------------|-------------------------------------|
| HPR 2613 | 26.12 | 7.60 | 40.4 |
| HPR 2617 | 38.24 | 8.84 | 49.0 |
| Sukaradhan | 38.64 | 9.06 | 61.6 |
| Nagardhan | 33.98 | 6.42 | 48.4 |
| Pusa 1121 | 27.30 | 8.04 | 51.8 |
| W1263 (RC) | 23.34 | 11.20 | 61.2 |
| TN-1 (SC) | 32.42 | 8.60 | 41.8 |
| CD(p=0.05) | (1.93) | (0.51) | (3.58) |

Means within a column followed by the same letter are not significantly different at $p \leq 0.05$ according to LSD

Laskar et al. (2008) reported negative correlation between infestation of leaf and length of flag leaf.

The width of flag leaf varied from 6.42 to 11.20 mm and was minimum in Nagardhan (6.42 mm) followed by HPR 2613 (7.60 mm) and Pusa 1121 (8.04 mm) (Table 4.4). The leaves were found to be broadest in W1263 (11.20 mm) followed by

Sukaradhan (9.06 mm) which was at par with HPR 2617 (8.84 mm) and TN-1 (8.60 mm).

The plant height of different genotypes ranged from 40.4 cm in HPR 2613 to 61.6 cm in Sukaradhan (Table 4.4). The genotype HPR 2613 (40.4 cm) was the shortest and was at par with TN-1 (41.8 cm). The genotypes Pusa 1121 (51.8 cm), HPR 2617 (49 cm) and Nagardhan (48.4 cm) were at par with each other. Sukaradhan (61.6 cm) was the tallest genotype and was at par with W1263 (61.2 cm) (Table 4.4).

4.3 Antibiosis resistance

For the study of antibiosis various parameters were observed in selected genotypes and significant differences were recorded among them.

4.3.1 Grub duration and grub survival

The total grub duration was recorded and significant variations were observed in these parameters. The total grub duration was maximum in W1263 (11.2 days) that was at par with Sukardhan (10.8 days) and TN-1 (10.8 days). It was also significantly similar to Pusa 1121 (10.2 days) and HPR 2617 (10.2 days). The grub duration was minimum in Nagardhan (8.8 days) and it was significantly similar to HPR 2613 (9.4 days) (Table 4.5). Khan et al. (1989) reported variation in the total larval duration on the various genotypes and wild rice races and susceptible check IR36 recorded the minimum larval duration (20.0 days) while *Oryza australiensis* recorded the maximum duration (25.3 days) followed by *O. nivara* (23.3 days), *O. perennis* (22.6 days) and TKM6 (22.2 days).

The grub survival was calculated by counting the number of pupae in each replication of each genotype and maximum survival was noticed in the susceptible check TN-1 (87.81 %) and it was significantly similar to Sukaradhan (85.03 %), HPR 2613 (86.60 %) and HPR 2617 (81.01 %). The genotypes Pusa 1121 (78.21 %) and Nagardhan (78.14 %) were at par with each other and also significantly similar to W1263 (73.06 %) (Table 4.5).

4.3.2 Pupal duration, Adult emergence and Adult longevity

The pupal duration was recorded maximum in W1263 (6.6 days) and minimum in TN-1 (4.4 days). The per cent emerged adults was maximum in the

susceptible genotype TN-1 (87.81 %) and was minimum in W1263 (53.16 %) (Table 4.5). Not much significant difference was observed among different genotypes. The least adult longevity was recorded in Nagardhan (18.2 days) and the maximum duration was observed in Sukaradhan (20.4 days) (Table 4.5).

Table 4.5. Total grub duration, per cent pupation, pupal duration, adult emergence and adult longevity of *Di cladispa armigera* in paddy

| Genotype | Total Grub duration (days)[#] (mean) | Per cent pupation[#] (mean) | Pupal duration (days) (mean) | Adult emergence (%) (mean) | Adult longevity (days) (mean) |
|-------------------|--|---|---|---------------------------------------|--|
| HPR 2613 | 9.4 (7.84) | 83.60 (66.33) | 5.0 | 70.69 | 18.4 |
| HPR 2617 | 10.2 (18.61) | 81.00 (64.28) | 5.4 | 80.70 | 18.4 |
| Sukaradhan | 8.8 (17.24) | 85.03 (67.24) | 5.4 | 77.68 | 20.4 |
| Nagardhan | 10.8 (19.16) | 78.14 (62.15) | 5.2 | 63.98 | 18.2 |
| Pusa 1121 | 10.8 (19.16) | 78.21 (62.18) | 6.0 | 64.39 | 19.0 |
| W1263 (RC) | 11.2 (19.53) | 73.06 (58.85) | 6.6 | 53.16 | 19.2 |
| TN-1 (SC) | 10.2 (18.61) | 87.81 (69.71) | 4.4 | 84.70 | 19.2 |
| CD(p=0.05) | (0.98) | (4.29) | (0.84) | (8.45) | NS |

Figures in parentheses are the means of $\sqrt{n+1}$ transformation

Means within a column followed by the same letter are not significantly different at $p \leq 0.05$ according to LSD



(A)



(B)



(C)



(D)

Plate 4.2 (A) Eggs, (B) Grub, (C) Pupa and (D) Adult stages of rice hispa

4.3.4 Final grub weight and pupal weight

The final grub weight was recorded maximum in TN-1 (5.04 mg) which is the susceptible check followed by Sukaradhan (4.66 mg) which was at par with HPR 2613 (4.64 mg). Pusa 1121 (4.40 mg) was at par with HPR 2617 (4.34 mg). It was recorded minimum in W1263 (3.80 mg) which was at par with Nagardhan (3.82 mg). Similarly, Pupal weight was maximum in Nagardhan (5.98 mg) and minimum in Pusa 1121 (4.90 mg) (Table 4.6). Khan et al. (1989) also reported variation in pupal weight varied from 9.9 mg in *O. perennis* to 23.7 mg in *O. australienesis*.

Table 4.6. Final grub weight and pupal weight of *Dicladispa armigera* on rice genotypes

| Genotype | Grub weight (mg) (mean) | Pupal weight (mg) (mean) |
|-------------------|----------------------------|-----------------------------|
| HPR 2613 | 4.64 | 5.08 |
| HPR 2617 | 4.34 | 5.58 |
| Sukaradhan | 4.66 | 5.96 |
| Nagardhan | 3.82 | 4.98 |
| Pusa 1121 | 4.40 | 4.90 |
| W1263 | 3.80 | 5.00 |
| TN-1 | 5.04 | 5.94 |
| CD(p=0.05) | (0.17) | (0.24) |

Means within a column followed by the same letter are not significantly different at $p \leq 0.05$ according to LSD

4.4 Biochemical factors

Various biochemicals such as total sugars, reducing sugars, starch, phenols, proteins, free amino acids and crude fibre were analyzed in selected genotypes in 50 and 70 days old plants.

4.4.1 Total soluble sugars, reducing sugars and starch

Total sugar contents differed significantly among genotypes in 50 days old plants. It was maximum in the susceptible check TN-1 (21.00 mg/g) followed by Nagardhan (19.33 mg/g) which was significantly similar to HPR 2613 (19.00 mg/g) (Table 4.7). It was minimum in the resistant check W1263 (14.00 mg/g) followed by Pusa 1121 (15.00 mg/g) which was significantly similar to HPR 2617 (16.66 mg/g) (Table 4.7).

Rice hispa infestation resulted in a significant decrease in sugar content in all the genotypes and the minimum sugar content was recorded in W1263 (10.00 mg/g) followed by Pusa 1121 (12.33 mg/g). The maximum amount of sugar content was recorded in TN-1 (19.00 mg/g) followed by Nagardhan (17.00 mg/g) which was at par with HPR 2613 (16.83 mg/g). Sukaradhan (15.90 mg/g) is significantly similar to HPR 2617 (14.33 mg/g) (Table 4.7). The maximum per cent decrease of total sugar content after infestation was observed in W1263 (28.57%) followed by Pusa 1121 (17.80%) and HPR 2617 (13.98%) and the minimum decrease was observed in TN-1 (9.52%) (Fig. 4.1).

Table 4.7. Total sugars content (mg/g dry weight) before and after infestation by *Dicladispa armigera* in the leaves of 50 and 70 days old rice genotypes

| Genotype | 50 days old plants | | | 70 days old plants | | |
|-------------|------------------------|----------|-------|------------------------|----------|-------|
| | Uninfested | Infested | Mean | Uninfested | Infested | Mean |
| HPR 2613 | 19.00 | 16.83 | 17.91 | 17.93 | 16.33 | 17.13 |
| HPR 2617 | 16.66 | 14.33 | 15.50 | 14.33 | 12.66 | 13.50 |
| Sukaradhan | 18.00 | 15.90 | 16.95 | 17.00 | 16.00 | 16.50 |
| Nagardhan | 19.33 | 17.00 | 18.16 | 16.33 | 13.66 | 15.00 |
| Pusa 1121 | 15.00 | 12.33 | 13.66 | 14.90 | 13.26 | 14.08 |
| W1263 (RC) | 14.00 | 10.00 | 12.00 | 13.30 | 10.33 | 11.81 |
| TN-1(SC) | 21.00 | 19.00 | 20.00 | 18.00 | 17.40 | 17.70 |
| MEAN | 17.57 | 15.05 | | 15.97 | 14.23 | |
| CD (p=0.05) | Genotype | 1.33 | | Genotype | 1.00 | |
| | Infestation | 0.71 | | Infestation | 0.53 | |
| | Genotype x Infestation | NS | | Genotype x Infestation | NS | |

Means within a column followed by the same letter are not significantly different at $p \leq 0.05$ according to LSD

In 70 days old plants, the minimum amount of total sugars was in W1263 (13.30 mg/g) followed by HPR 2617 (14.33 mg/g) which was at par with Pusa 1121 (14.90 mg/g). The maximum amount of total sugars was observed in TN-1 (18.00 mg/g) which was at par with HPR 2613 (17.93 mg/g) and Sukaradhan (17.00 mg/g) followed by Nagardhan (16.33 mg/g). Similarly after infestation, the maximum amount of total sugar was recorded in TN-1 (17.40 mg/g) followed by HPR 2613

(16.33 mg/g) which was at par with Sukaradhan (16.00 mg/g). The minimum amount was recorded in W1263 (10.33 mg/g) followed by Pusa 1121 (13.26 mg/g) which was at par with Nagardhan (13.66 mg/g) (Table 4.7). After infestation the highest per cent decrease was observed in W1263 (22.33%) followed by Nagardhan (16.35%) and the lowest was observed in TN-1 (3.33%) (Fig. 4.1)

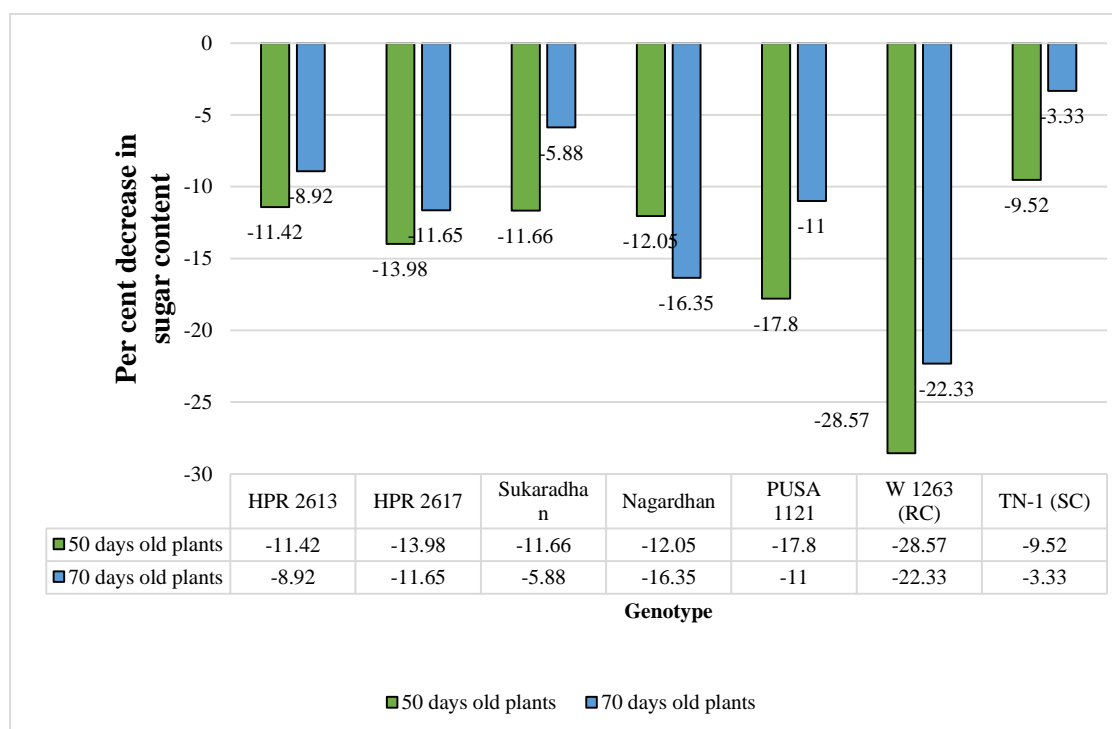


Fig 4.1. Per cent reduction in total sugar content of 50 and 70 days old infested plants as compared to uninfested plants

Similarly, the reducing sugar content in 50 days plants was highest in TN-1 (8.33 mg/g) which was at par with Sukaradhan (8.03 mg/g) followed by HPR 2613 (7.50 mg/g). It was lowest in W1263 (4.43 mg/g) which was at par with Pusa 1121 (4.93 mg/g) followed by Nagardhan (6.63 mg/g) which was at par with HPR 2617 (6.76 mg/g) (Table 4.8). The per cent decrease after infestation was maximum in W1263 (15.8%) and lowest in Pusa 1121 (4.05%) (Fig. 4.2).

In 70 days old plants the maximum sugar content was observed in TN-1 (7.66 mg/g) which was at par with HPR 2613 (7.56 mg/g) and Sukaradhan (7.40 mg/g). The minimum sugar content was observed in W1263 (4.06 mg/g) which was at par with Pusa 1121 (4.30 mg/g). Similarly after infestation Reducing sugars was highest in TN-1 (7.36 mg/g) which was at par with HPR 2613 (7.06 mg/g) and Sukaradhan (7.03

mg/g) (Table 4.8). The per cent decrease in reducing sugars was lowest in HPR 2617 (3.8%) and highest in W1263 (25.36%) (Fig. 4.2).

The reduction in the amount of total and reducing sugars after infestation could be due to the activation of various enzymes related to the resistance mechanism especially the enzymes of phenyl propanoid pathway that results in the conversion of sugars to phenols and other secondary metabolites which in turn enhances the resistance mechanism of the plant. The results are according to the findings of Vanitha et al. (2015) who stated that the susceptible lines with higher per cent damaged leaves had higher amount of total sugars and lines with less per cent leaf damage had lesser total sugar amount and a correlation of $r = 0.88$ was found between leaf infestation and sugar content. The damage in variety Jaya was 23.94 per cent and it had sugar content of 6.28 mg/g but in NP-218 the per cent leaf damage was 1.31 with sugar content of 2.22 mg/g.

Table 4.8. Reducing sugars content (mg/g dry weight) before and after infestation by *Dicladispa armigera* in the leaves of 50 and 70 day old rice genotypes

| Genotype | 50 days old plants | | | 70 days old plants | | |
|----------------|---------------------------|----------|------|---------------------------|----------|------|
| | Uninfested | Infested | Mean | Uninfested | Infested | Mean |
| HPR 2613 | 7.50 | 6.66 | 7.08 | 7.56 | 7.06 | 7.31 |
| HPR 2617 | 6.76 | 6.26 | 6.51 | 6.30 | 6.06 | 6.18 |
| Sukaradhan | 8.03 | 7.82 | 7.81 | 7.40 | 7.03 | 7.21 |
| Nagardhan | 6.63 | 5.83 | 6.23 | 6.23 | 5.33 | 5.78 |
| Pusa 1121 | 4.93 | 4.73 | 4.83 | 4.30 | 3.83 | 4.06 |
| W1263(RC) | 4.43 | 3.73 | 4.08 | 4.06 | 3.03 | 3.55 |
| TN-1(SC) | 8.33 | 7.86 | 8.10 | 7.66 | 7.36 | 7.51 |
| MEAN | 6.66 | 6.10 | | 6.21 | 5.67 | |
| CD (p=0.05) | Genotype | 0.27 | | Genotype | 0.22 | |
| | Infestation | 0.14 | | Infestation | 0.11 | |
| | Genotype x Infestation | NS | | Genotype x Infestation | 0.31 | |

Means within a column followed by the same letter are not significantly different at $p \leq 0.05$ according to LSD

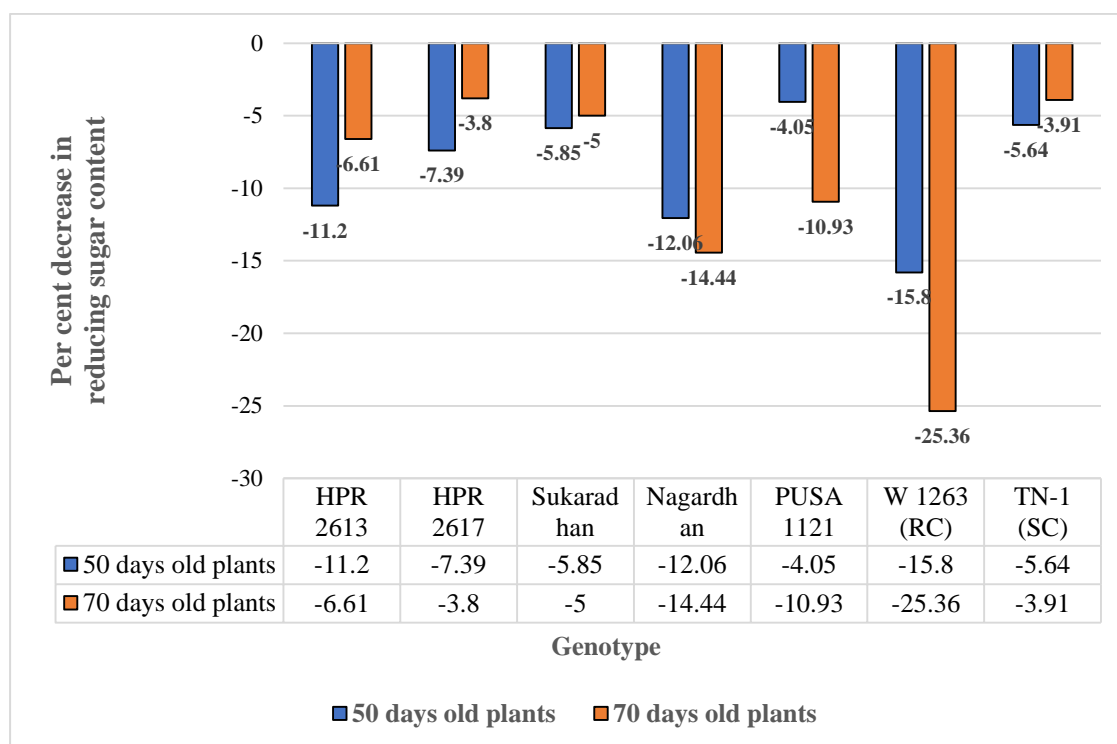


Fig 4.2. Per cent reduction in reducing sugar content of 50 and 70 days old infested plants as compared to uninfested plants

Starch content in 50 days old plant was highest in Sukaradhan (86.33 mg/g) which was at par with Nagardhan (82.33 mg/g) followed by HPR 2617 (76.66 mg/g) which was at par with TN-1 (76.00 mg/g) and HPR 2613 (75.66 mg/g) (Table 4.9). After infestation the amount of starch was maximum in Sukaradhan (84.00 mg/g) which was at par with Nagardhan (81.00 mg/g). The minimum infestation was recorded in W1263 (61.33 mg/g) which was at par with Pusa 1121 (61.45 mg/g) followed by HPR 2613 (74.33 mg/g), HPR 2617 (74.66 mg/g) and TN-1 (75.33 mg/g) which were at par with each other (Table 4.9). The per cent decrease in starch content was maximum in W1263 (5.46%) and minimum in TN-1 (0.88%) (Fig. 4.3).

Similarly, in 70 days old plants the starch content was maximum in Sukaradhan (76.33 mg/g) which was at par with Nagardhan (75.66 mg/g) followed by HPR 2613 (68.00 mg/g), TN-1 (65.33 mg/g) and HPR 2617 (64.66 mg/g) which were at par with each other. The minimum amount of starch was recorded in W1263 (56.00 mg/g) which was at par with Pusa 1121 (57.33 mg/g) (Table 4.9). After infestation the maximum amount of starch was recorded in Sukaradhan (74.00 mg/g) which was at par with Nagardhan (72.00 mg/g) and the minimum amount was recorded in W1263

(52.66 mg/g) which was at par with Pusa 1121 (53.33 mg/g) (Table 4.9). The per cent decrease in starch content was highest in Pusa 1121 (6.97%) and lowest in TN-1 (1.11%) (Fig. 4.3)

Table 4.9. Starch content (mg/g dry weight) before and after infestation by *Dicladispa armigera* in the leaves of 50 and 70 day old rice genotypes

| Genotype | 50 days old plants | | | 70 days old plants | | |
|----------------|---------------------------------------|----------|-------|---------------------------------------|----------|-------|
| | Uninfested | Infested | Mean | Uninfested | Infested | Mean |
| HPR 2613 | 75.66 | 74.33 | 75.5 | 68.00 | 66.50 | 67.83 |
| HPR 2617 | 76.66 | 74.66 | 74.66 | 64.66 | 62.66 | 62.66 |
| Sukaradhan | 86.33 | 84.00 | 85.16 | 76.33 | 74.00 | 74.16 |
| Nagardhan | 82.33 | 81.00 | 81.66 | 75.66 | 72.00 | 73.83 |
| Pusa 1121 | 62.00 | 61.45 | 61.83 | 57.33 | 53.33 | 55.33 |
| W1263(RC) | 65.33 | 61.33 | 64.33 | 56.00 | 52.66 | 54.33 |
| TN-1(SC) | 76.00 | 73.33 | 74.66 | 65.33 | 64.60 | 63.66 |
| MEAN | 74.90 | 73.04 | | 66.19 | 62.90 | |
| CD (p=0.05) | Genotype | 2.38 | | Genotype | 2.82 | |
| | Infestation | 1.27 | | Infestation | 1.50 | |
| | Genotype x Infestation | NS | | Genotype x Infestation | NS | |

Means within a column followed by the same letter are not significantly different at $p \leq 0.05$ according to LSD

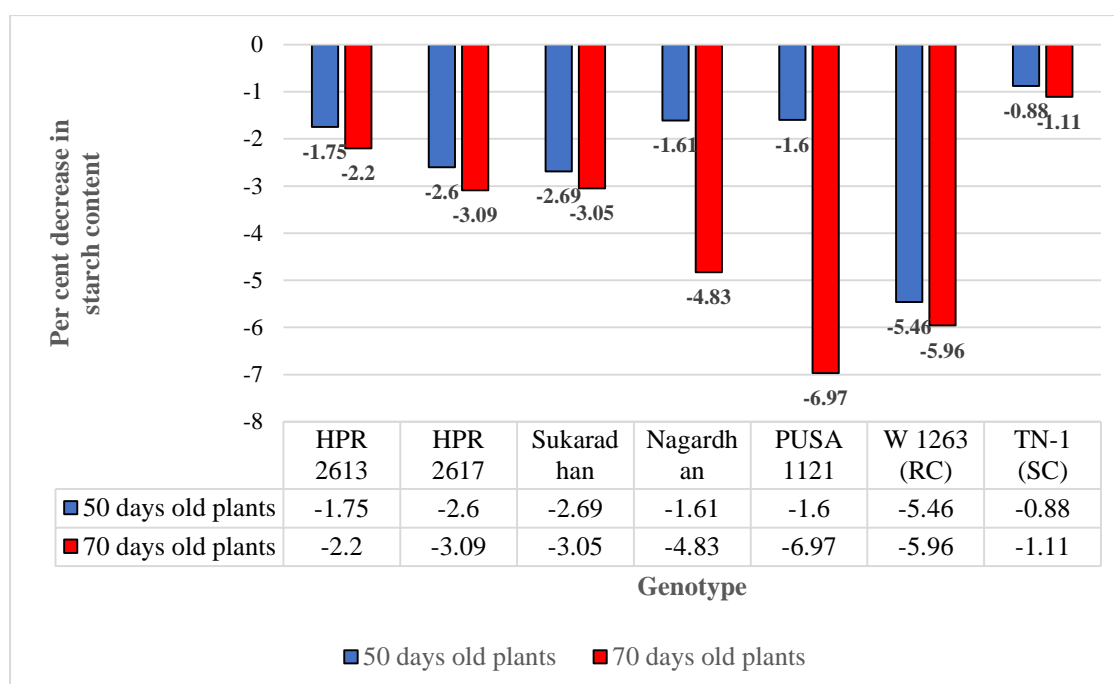


Fig 4.3. Per cent reduction in starch content of 50 and 70 days old infested plants as compared to uninfested plants

4.4.2 Free amino acids and total soluble protein

The free amino acids were present in 50 and 70 days old plants. The maximum amount of free amino acids present in 50 days old rice genotypes was recorded in TN-1 (281.66 $\mu\text{g/g}$) which was at par with Sukaradhan (265.00 $\mu\text{g/g}$) and HPR 2617 (203.00 $\mu\text{g/g}$). The minimum was recorded in W1263 (147.66 $\mu\text{g/g}$) followed by Pusa 1121 (177.33 $\mu\text{g/g}$) and Nagardhan (191.00 $\mu\text{g/g}$) which was at par with HPR2613 (199.00 $\mu\text{g/g}$) (Table 4.10). After infestation the amount of free amino acids was recorded maximum in TN-1 (274.00 $\mu\text{g/g}$) which was at par with Sukaradhan (237.33 $\mu\text{g/g}$) followed by HPR 2617 (197.33 $\mu\text{g/g}$). The minimum amount was recorded in W1263 (138.00 $\mu\text{g/g}$) followed by Pusa 1121 (155.66 $\mu\text{g/g}$) and Nagardhan (180.66 $\mu\text{g/g}$) which was at par with HPR 2613 (188.00 $\mu\text{g/g}$) (Table 4.10).

In 70 days old plants the concentration of free amino acids was lower than the 50 days old plants i.e. the amino acid content decreased in plants with maturity. The maximum amount was recorded in TN-1 (256.66 $\mu\text{g/g}$) which was at par with Sukaradhan (239.67 $\mu\text{g/g}$) followed by HPR 2617 (189.33 $\mu\text{g/g}$) and HPR 2613 (184.33 $\mu\text{g/g}$) which were at par with each other. The minimum amount was recorded in W1263 (125.33 $\mu\text{g/g}$) followed by Pusa 1121 (157.66 $\mu\text{g/g}$) and Nagardhan (171.00 $\mu\text{g/g}$) (Table 4.10). There was a reduction in the amount of free amino acids after infestation and the minimum amount was recorded in W1263 (125.00 $\mu\text{g/g}$) followed by Pusa 1121 (141.66 $\mu\text{g/g}$). Nagardhan (165.00 $\mu\text{g/g}$) was at par with HPR 2613 (167.33 $\mu\text{g/g}$) followed by HPR 2617 (180.00 $\mu\text{g/g}$). The maximum amount was recorded in TN-1 (249.66 $\mu\text{g/g}$) which was at par with Sukaradhan (221.33 $\mu\text{g/g}$) (Table 4.10). The highest per cent decrease was observed in Pusa 1121 (10.15%) and the minimum was observed in W1263 (0.26%) (Fig. 4.4). Free amino acids acts as a main source for growth, development and oviposition and also participate in protein synthesis and other biogenetic pathways (Douglas 2006). The reduction in amino acids after infestation is also due to the phenyl propanoid pathway in which they are used to make some secondary metabolites that provides plants with some resistance against abiotic and biotic stress.

Table 4.10. Free amino acid content ($\mu\text{g/g}$ dry weight) before and after infestation by *Dicladispa armigera* in the leaves of 50 and 70 day old rice genotypes

| Genotype | 50 days old plants | | | 70 days old plants | | |
|----------------|---------------------------------------|----------|--------|---------------------------------------|----------|--------|
| | Uninfested | Infested | Mean | Uninfested | Infested | Mean |
| HPR 2613 | 199.00 | 188.00 | 193.50 | 184.33 | 167.33 | 175.83 |
| HPR 2617 | 203.00 | 197.33 | 200.16 | 189.33 | 180.00 | 184.66 |
| Sukaradhan | 265.00 | 237.33 | 251.16 | 239.67 | 221.33 | 230.50 |
| Nagardhan | 191.00 | 180.66 | 185.83 | 171.00 | 165.00 | 168.00 |
| Pusa 1121 | 177.33 | 155.66 | 169.50 | 157.66 | 141.66 | 149.66 |
| W1263(RC) | 147.66 | 138.00 | 142.83 | 125.33 | 125.00 | 125.16 |
| TN-1(SC) | 281.66 | 274.00 | 263.83 | 256.66 | 249.66 | 244.66 |
| MEAN | 209.23 | 192.71 | | 189.14 | 176.14 | |
| CD (p=0.05) | Genotype | 6.14 | | Genotype | 6.73 | |
| | Infestation | 3.28 | | Infestation | 3.60 | |
| | Genotype x Infestation | 8.69 | | Genotype x Infestation | 9.52 | |

Means within a column followed by the same letter are not significantly different at $p \leq 0.05$ according to LSD

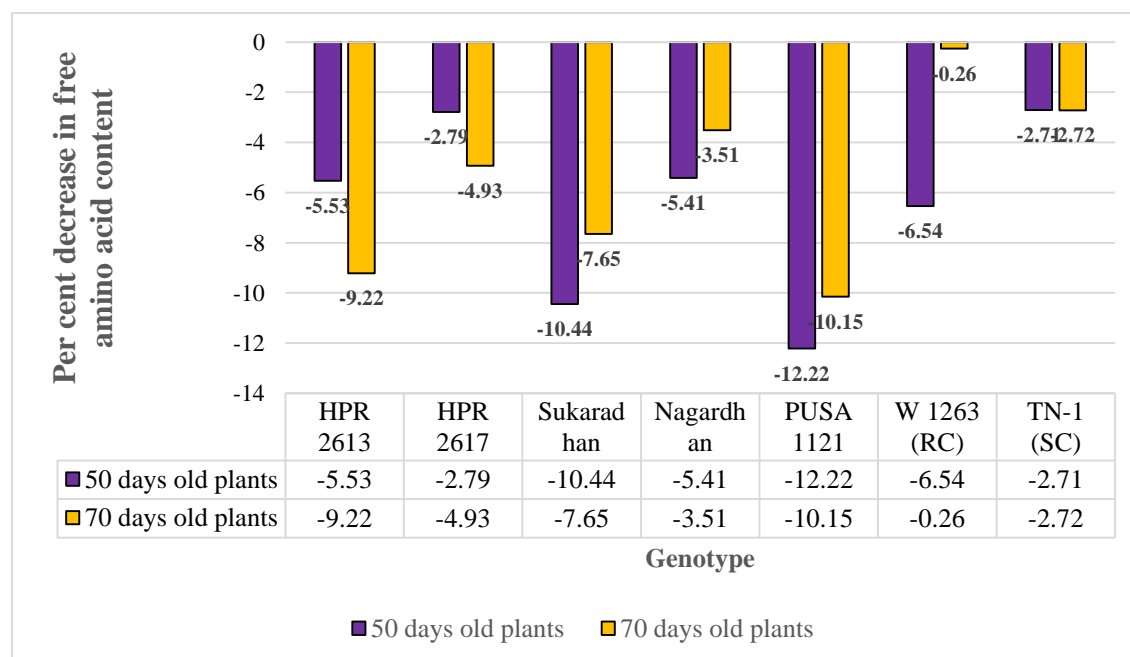


Fig 4.4. Per cent reduction in free amino acid content of 50 and 70 days old infested plants as compared to uninfested plants

The total soluble protein content in 50 days old uninfested plants varied from 7.80 to 10.30 mg/g (Table 4.11) and the maximum amount was recorded in TN-1 (10.30 mg/g) whereas minimum amount was recorded in W1263 (7.80 mg/g) and Pusa 1121 (7.80 mg/g) (Table 4.11). A reduction in protein content was observed in all the genotypes post infestation and the maximum per cent reduction was observed in HPR 2617 (15.81%) and the minimum in Pusa 1121 (2.56%) (Fig. 4.5)

In the 70 days old plants the highest amount of protein content was observed in TN-1 (8.73 mg/g) which was at par with HPR 2617 (8.66 mg/g) and Sukaradhan (8.33 mg/g). The minimum amount was observed in W1263 (5.96 mg/g) followed by Pusa 1121 (6.16 mg/g) and Nagardhan (7.66 mg/g) which was at par with HPR 2613 (7.83 mg/g). The per cent decrease was highest in HPR 2617 (21.94%) and it was lowest in Pusa 1121 (0.48%) (Fig. 4.5).

The total soluble protein content after infestation was higher in TN-1, Sukaradhan, HPR 2617 and Nagardhan (7.60, 7.00, 6.76 and 6.63 mg/g respectively) and it was lower in W1263, Pusa 1121 and HPR 2613 (5.86, 6.13 and 6.40 mg/g respectively) (Table 4.11).

The results are supported by the study conducted by Vanitha et al. (2015) that the total amino acid content was found lower in resistant genotypes and higher in susceptible ones. The highest amino acid amount was present in Jaya (3.28 mg/g) but the lowest was in NP-218 (1.08 mg/g) and positive correlation of $r = 0.85$ was found between leaf damage and amino acid content. Punithavalli et al. (2013b) also reported a decrease in the soluble protein content after infestation. They observed highest amount of soluble proteins in highly susceptible TN-1 (8.08 mg/g) followed by IR36 (8.05 mg/g) and low amount in Ptb33 (6.49 mg/g) and TKM6 (6.30 mg/g) in the healthy plants. After infestation, protein content decreased in all genotypes and protein amount in infested TN-1 (7.61 mg/g) was highest and lowest in TKM6 (6.21 mg/g).

Table 4.11. Total soluble protein content (mg/g dry weight) before and after infestation by *Dicladispa armigera* in the leaves of 50 and 70 day old rice genotypes

| Genotype | 50 days old plants | | | 70 days old plants | | |
|----------------|------------------------------|----------|------|------------------------------|----------|------|
| | Uninfested | Infested | Mean | Uninfested | Infested | Mean |
| HPR 2613 | 8.56 | 7.46 | 8.01 | 7.83 | 6.40 | 7.11 |
| HPR 2617 | 9.30 | 7.83 | 8.56 | 8.66 | 6.76 | 7.71 |
| Sukaradhan | 8.83 | 8.63 | 8.73 | 8.33 | 7.00 | 7.66 |
| Nagardhan | 8.43 | 8.10 | 8.26 | 7.66 | 6.63 | 7.15 |
| Pusa 1121 | 7.80 | 7.60 | 7.60 | 6.16 | 6.13 | 6.15 |
| W1263(RC) | 7.80 | 7.46 | 7.63 | 5.96 | 5.86 | 5.91 |
| TN-1(SC) | 10.30 | 9.56 | 9.93 | 8.73 | 7.60 | 8.16 |
| MEAN | 8.64 | 8.14 | | 7.61 | 6.64 | |
| CD (p=0.05) | Genotype | 0.33 | | Genotype | 0.43 | |
| | Infestation | 0.18 | | Infestation | 0.23 | |
| | Genotype x Infestation | 0.47 | | Genotype x Infestation | 0.61 | |

Means within a column followed by the same letter are not significantly different at $p \leq 0.05$ according to LSD

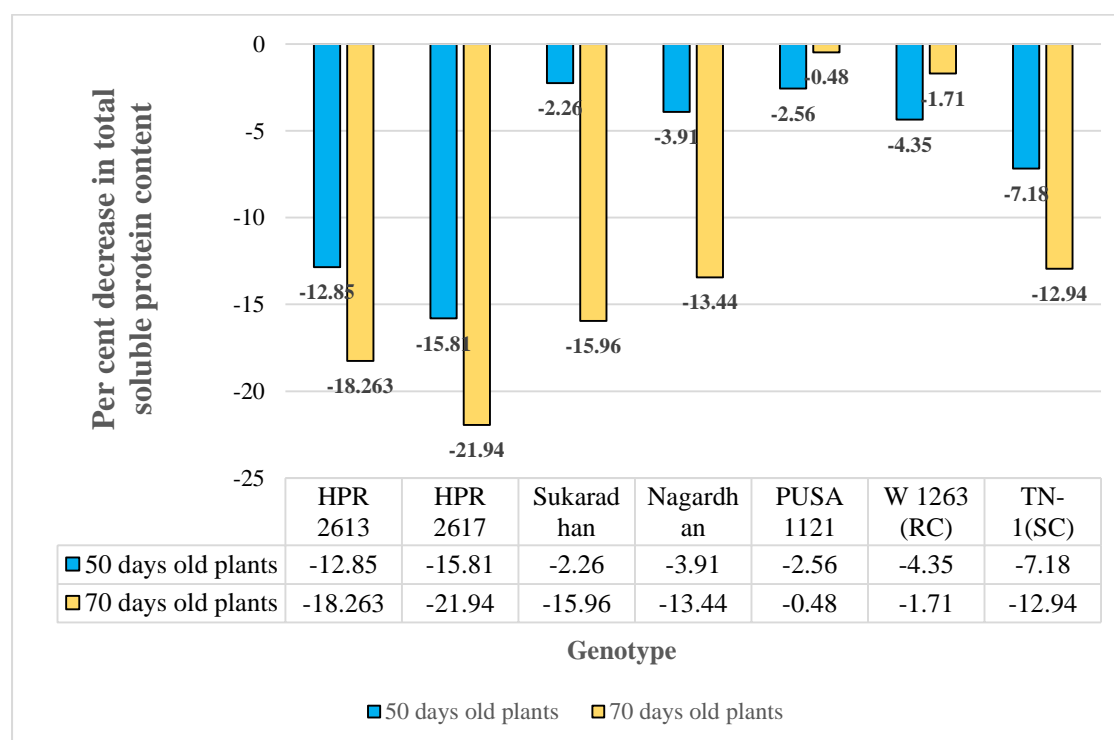


Fig 4.5. Per cent reduction in total soluble protein content of 50 and 70 days old infested plants as compared to uninfested plants

4.4.3 Total Phenols

The total phenol content differed significantly in case of both the uninfested and infested plants of 50 and 70 day old plants. In 50 days old plants phenol content was maximum in W1263 (2.90 mg/g) which was at par with Pusa 1121 (2.63 mg/g), Nagardhan (2.50 mg/g) and HPR 2613 (2.30 mg/g). It was minimum in TN-1 (0.93 mg/g) followed by HPR 2617 (1.60 mg/g) and Sukaradhan (1.63 mg/g) which were at par with each other (Table 4.12). There was an increment in phenol content after infestation and the maximum amount was recorded in W1263 (4.16 mg/g) followed by Pusa 1121 (3.40 mg/g) which was at par with Sukaradhan (3.16 mg/g). It was minimum in TN-1 (1.20 mg/g) which was at par with Nagardhan (1.80 mg/g) and HPR 2617 (1.96 mg/g) followed by HPR 2613 (2.80 mg/g) (Table 4.12). The per cent increase was highest in W1263 (43.45%) and it was lowest in Nagardhan (10.43%) (Fig 4.6).

In 70 days old plants the phenol content was highest in W1263 (3.63 mg/g) which was at with Pusa 1121 (3.40 mg/g), Nagardhan (3.33 mg/g) and HPR 2613 (3.20 mg/g). It was lowest in TN-1 (1.80 mg/g) followed by HPR 2617 (2.20 mg/g) and Sukaradhan (2.33 mg/g) which were at par with each other. Similarly, the highest phenol content post infestation was recorded in W1263 (4.46 mg/g) which was at par with Pusa 1121 (4.30 mg/g) and Nagardhan (4.03 mg/g) followed by HPR 2613 (3.76 mg/g). The lowest amount was recorded in TN-1 (2.20 mg/g) which was at par with HPR 2617 (2.76 mg/g) and Sukaradhan (2.83 mg/g) (Table 4.12). The maximum per cent increase was observed in Pusa 1121 (26.47%) and the minimum was observed in HPR 2613 (17.5%) (Fig. 4.6).

The increase in phenol content was due to the conversion of other biochemicals such as total and reducing sugars and amino acids into phenols through phenyl propanoid pathway and phenol is one of the product of this pathway that result in induced resistance in the plants after insect infestation. The results are supported by the study of Vanitha et al. (2015) that higher amount of phenols was present in resistant lines like XR-99982 (3.93 mg/g) and NP-2118 (3.06 mg/g) and lower amount was present in susceptible cultivars like Jaya (1.44 mg/g) and PAU3879-87-1-1 (1.77 mg/g).

Similarly, Punithavalli et al. (2013a) also concluded that TN-1 (3.48 mg/g) had the lowest amount of phenols among the tested genotypes and resistant wild genotype *Oryza minuta* (9.86 mg/g) and Ptb33 (10.83 mg/g) had higher amounts. They also reported the increase in phenol quantity in the leaves after infestation (12.94 mg/g in *O. minuta* and 14.08 mg/g in Ptb33) while in TN-1 phenol amount at induced level was 4.31 mg/g.

Deepa et al. (2016) also supported the results and reported that in plants, 20 days after transplanting the highest amount of phenols was recorded in moderately resistant cultivar HKR47 (9.956 mg/g) and lowest amount in susceptible TN-1 (3.441mg/g). However, 60 days after transplanting maximum amount of phenols were present in PB1460 (11.728 mg/g) and HKR47 (11.353 mg/g) was third from top following BG367-2 (11.536 mg/g) but still TN-1 had the minimum amount of 4.280 mg/g. They also observed the increase in amount of total phenols in rice leaves with increase in age.

Table 4.12. Total phenol content (mg/g dry weight) before and after infestation by *Dicladisa armigera* in the leaves of 50 and 70 day old rice genotypes

| Genotype | 50 days old plants | | | 70 days old plants | | |
|----------------|---------------------------------------|----------|------|---------------------------------------|----------|------|
| | Uninfested | Infested | Mean | Uninfested | Infested | Mean |
| HPR 2613 | 2.30 | 2.80 | 2.55 | 3.20 | 3.76 | 3.48 |
| HPR 2617 | 1.60 | 1.96 | 1.78 | 2.20 | 2.76 | 2.48 |
| Sukaradhan | 2.50 | 3.16 | 2.83 | 2.33 | 2.83 | 2.58 |
| Nagardhan | 1.63 | 1.80 | 1.72 | 3.33 | 4.03 | 3.68 |
| Pusa 1121 | 2.63 | 3.40 | 3.02 | 3.40 | 4.30 | 3.85 |
| W1263(RC) | 2.90 | 4.16 | 3.53 | 3.63 | 4.46 | 4.05 |
| TN-1(SC) | 0.93 | 1.20 | 1.06 | 1.80 | 2.20 | 2.00 |
| MEAN | 2.07 | 2.64 | | 2.84 | 3.48 | |
| CD (p=0.05) | Genotype | 0.29 | | Genotype | 0.31 | |
| | Infestation | 0.15 | | Infestation | 0.16 | |
| | Genotype x Infestation | NS | | Genotype x Infestation | NS | |

Means within a column followed by the same letter are not significantly different at $p \leq 0.05$ according to LSD

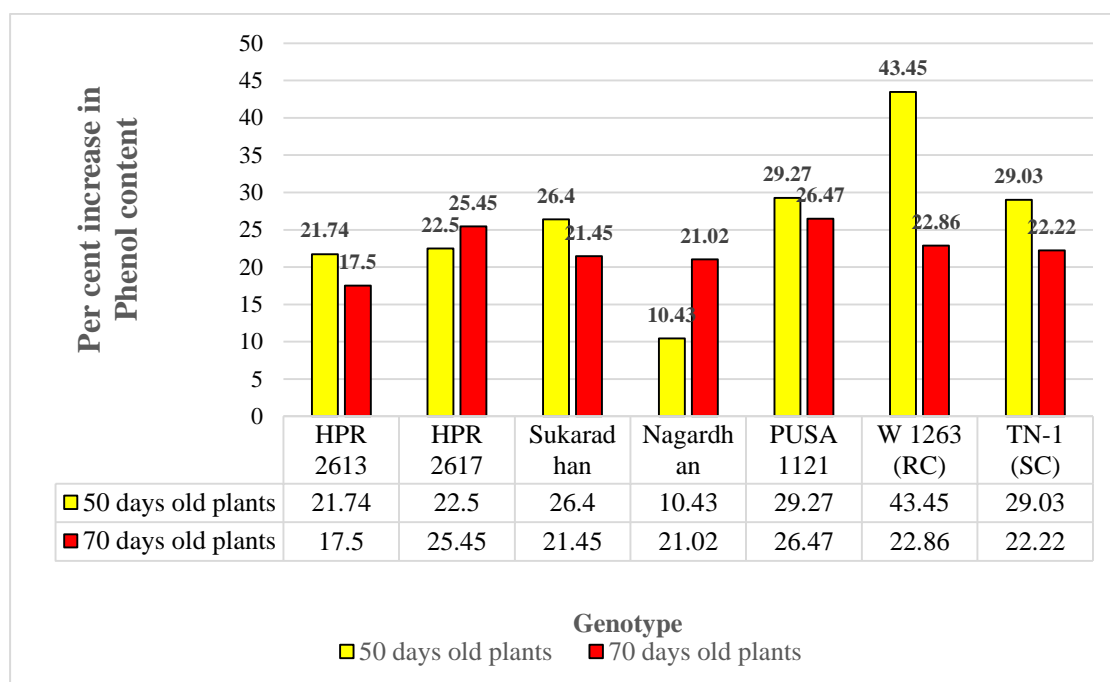


Fig 4.6. Per cent increase in phenol content of 50 and 70 days old infested plants as compared to uninfested plants

4.4.4 Crude fibre

The crude fibre content differed significantly in 50 and 70 days old plants of selected genotypes. In 50 days old plants the maximum amount of crude fibre content was recorded in Pusa 1121 (17.86 mg/g) which was at par with W1263 (16.96 mg/g) followed by Nagardhan (14.86 mg/g). The minimum amount was recorded in HPR 2617 (10.63 mg/g) which was at par with TN-1 (11.66 mg/g) followed by Sukaradhan (12.43 mg/g) and HPR 2613 (13.20 mg/g) (Table 4.13). After infestation an increment in fibre content was observed and the maximum amount was recorded in W1263 (19.63 mg/g) which was at par with Pusa 1121 (18.70 mg/g) followed by Nagardhan (16.13 mg/g). The minimum amount of fibre content was recorded in HPR 2617 (11.40 mg/g) followed by TN-1 (13.43 mg/g) and Sukaradhan (13.83 mg/g) which were at par with each other (Table 4.13). The per cent increase was observed maximum in W1263 (15.74%) and minimum in Pusa 1121 (4.7%) (Fig. 4.7).

In 70 days old plants, the highest crude fibre content was recorded in W1263 (20.06 mg/g) followed by Pusa 1121 (18.63 mg/g) and Nagardhan (16.03 mg/g). The lowest fibre content was observed in HPR 2617 (11.13 mg/g) followed by TN-1

(12.76 mg/g), Sukaradhan (13.43 mg/g) and HPR 2613 (14.73 mg/g). Similarly, after infestation the genotypes W1263, Pusa 1121, Nagardhan and HPR 2617 (21.16, 20.03, 17.66 and 15.80 mg/g respectively) had higher fibre content as compared to HPR 2617, Sukaradhan and TN-1 (12.40, 14.60 and 14.86 mg/g respectively) which had lower fibre content (Table 4.13).

The increment in fibre content post infestation is due to the activation of the defense mechanism via phenyl propanoid pathway that results in the formation of lignin which is the final product of this pathway.

Table 4.13. Crude fibre content (mg/g dry weight) before and after infestation by *Dicladispa armigera* in the leaves of 50 and 70 day old rice genotypes

| Genotype | 50 days old plants | | | 70 days old plants | | |
|----------------|-------------------------------|----------|-------|-------------------------------|----------|-------|
| | Uninfested | Infested | Mean | Uninfested | Infested | Mean |
| HPR 2613 | 13.20 | 14.16 | 13.68 | 14.73 | 15.80 | 15.26 |
| HPR 2617 | 10.63 | 11.40 | 11.01 | 11.13 | 12.40 | 11.76 |
| Sukaradhan | 12.43 | 13.83 | 13.13 | 13.43 | 14.60 | 14.02 |
| Nagardhan | 14.86 | 16.13 | 15.50 | 16.03 | 17.66 | 16.85 |
| Pusa 1121 | 17.86 | 18.70 | 18.28 | 18.63 | 20.03 | 19.33 |
| W1263(RC) | 16.96 | 19.63 | 18.30 | 20.06 | 21.16 | 20.62 |
| TN-1(SC) | 11.66 | 13.43 | 12.55 | 12.76 | 14.86 | 13.82 |
| MEAN | 13.94 | 15.33 | | 15.25 | 16.64 | |
| CD (p=0.05) | Genotype | 0.57 | | Genotype | 0.51 | |
| | Infestation | 0.31 | | Infestation | 0.27 | |
| | Genotype x Infestation | 0.82 | | Genotype x Infestation | NS | |

Means within a column followed by the same letter are not significantly different at $p \leq 0.05$ according to LSD

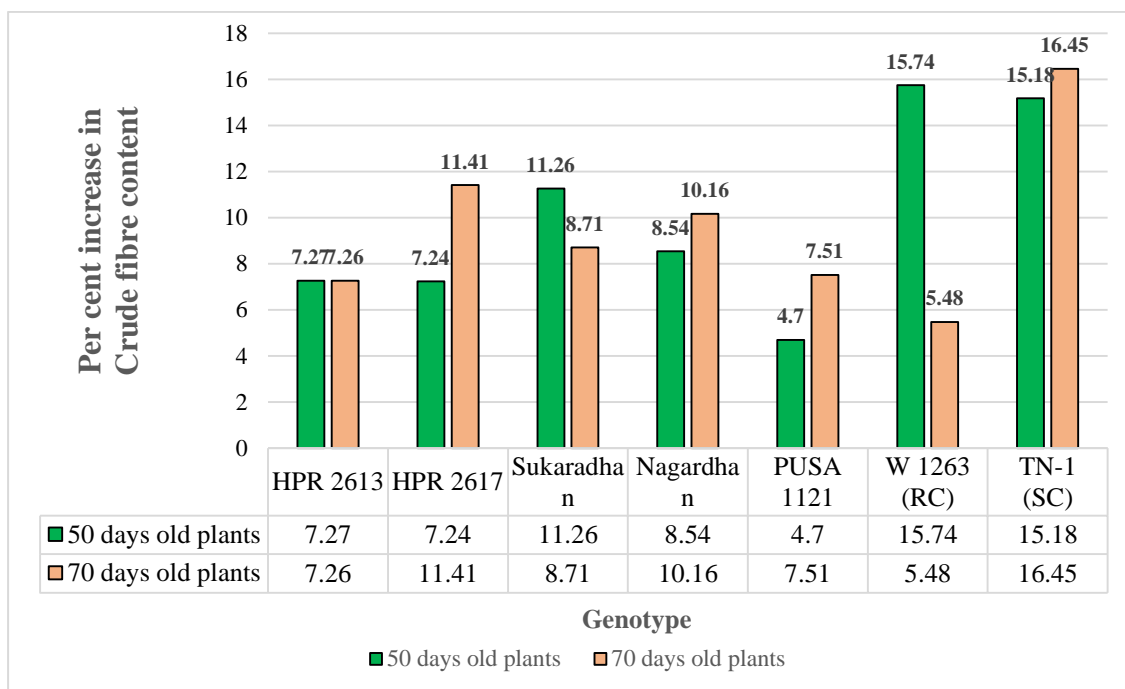


Fig 4.7. Per cent increase in crude fibre content of 50 and 70 days old infested plants as compared to uninfested plants

4.5 Pearson's correlation between antixenosis parameters and biophysical factors

The correlation between antixenosis parameters and biophysical factors can be observed in table 14. The settling behaviour of adults was positively significant with flag leaf length ($r = 0.71$) and it did not have any significant correlation with flag leaf width and plant height. The ovipositional behaviour did not have any significant correlation with flag leaf length width and plant height. Similarly, per cent hatched eggs also did not have any significant correlation with flag leaf length, width and plant height. Infested leaf area has a negative correlation with plant height ($r = -0.73$) and a positive correlation with flag leaf length. It does not have any significant correlation with flag leaf width (Table 4.14).

The correlation studies showed that antixenosis parameters like flag leaf length has a positive correlation with adult settling behavior and infested leaf area i.e. longer the leaf more the adults will settle on it and also more the infested leaf area.

Table 4.14. Pearson's correlation between antixenosis parameters and biophysical factors

| Characters | Adult settling behaviour | Ovipositional preference | Per cent hatched eggs | Infested leaf area |
|------------------|--------------------------|--------------------------|-----------------------|--------------------|
| Flag leaf length | 0.71* | 0.62 | 0.60 | 0.28* |
| Flag leaf width | 0.26 | -0.03 | -0.13 | -0.61 |
| Plant height | 0.39 | -0.26 | -0.50 | -0.73* |

*Significant at 5 per cent level of significance

4.6 Pearson's correlation between antibiosis parameters and biochemical factors

The grub duration was found to be negatively correlated with total sugars ($r = -0.78$), reducing sugars ($r = -0.92$), amino acids ($r = -0.90$) and proteins ($r = -0.81$) but was directly correlated with total phenols ($r = 0.83$) and crude fibre ($r = 0.72$). It means that greater the amount of phenols and crude fibre longer will be the grub duration whereas, if the amount of sugars, amino acids and proteins is higher it will shorten the grub duration (Table 15).

The grub weight was directly correlated to total sugars ($r = 0.89$), reducing sugars ($r = 0.94$), starch ($r = 0.74$) amino acids ($r = 0.91$) and proteins ($r = 0.89$). It was negatively correlated to total phenols ($r = -0.79$) and crude fibre ($r = -0.75$). Similarly, grub survival was positively correlated to total sugars ($r = 0.94$), reducing sugars ($r = 0.96$), amino acids ($r = 0.92$) and proteins ($r = 0.87$) but negatively correlated to total phenols ($r = -0.84$) and crude fibre ($r = -0.77$). On the contrary, pupal duration was negatively correlated with total sugars ($r = -0.78$), reducing sugars ($r = -0.84$), starch ($r = -0.79$), free amino acids ($r = -0.75$) and proteins ($r = -0.81$) but was positively correlated with total phenols ($r = 0.66$) and crude fibres ($r = 0.76$). The pupal weight was inversely correlated to total phenols ($r = -0.94$) and crude fibres ($r = -0.75$) and had a non significant correlation with total sugars and starch. The emergence percentage had a negatively significant correlation with total phenols ($r = -0.95$) and crude fibres ($r = -0.92$) (Table 4.15).

Table 4.15. Pearson's correlation between antibiosis parameters and biochemical factors

| Characters | Grub duration | Grub weight | Grub survival | Pupal duration | Pupal weight | Adult emergence |
|-------------------------------|----------------------|--------------------|----------------------|-----------------------|---------------------|------------------------|
| Total sugars | -0.78* | 0.89** | 0.94** | -0.78** | 0.58 | 0.73** |
| Reducing sugars | -0.92** | 0.94** | 0.96** | -0.84** | 0.82** | 0.89** |
| Starch | -0.65 | 0.74* | 0.59 | -0.79* | 0.48 | 0.50 |
| Free amino acids | -0.90** | 0.91** | 0.92** | -0.75* | 0.91** | 0.88** |
| Total soluble proteins | -0.81** | 0.89** | 0.87** | -0.81** | 0.85** | 0.89** |
| Total phenols | 0.83** | -0.79* | -0.84** | 0.66* | -0.94* | -0.95** |
| Crude fibre | 0.72* | -0.75* | -0.77* | 0.76* | -0.75* | -0.92** |

* Significant at 5 per cent level of significance

** Significant at 1 per cent level of significance

5. SUMMARY AND CONCLUSIONS

The rice hispa, *Dicladispa armigera* (Olivier) (Coleoptera: Chrysomelidae), which was initially believed to be a sporadic paddy pest, is rapidly emerging as a serious pest and it also causes significant yield losses. Both adults and grubs of hispa feed on leaves and mainly attack during early vegetative stage of the crop. The damaged areas appear as white streaks parallel to the midrib and white translucent patches can be seen as a result of grub feeding. The use of insecticides is considered as a conventional method to manage this pest but it causes environmental pollution, insecticidal resistance and also certain negative effects on the non-target pests. Therefore, host plant resistance is considered as one of the best ways to manage this insect pest as it is ecologically sound and also economically feasible. The study was conducted on some selected rice genotypes under screen house conditions at CSK Himachal Pradesh Krishi Vishwavidyalaya, Rice and Wheat Research Centre, Malan during the kharif season of 2020 and 2021. The seven genotypes of rice used were Nagardhan, Sukaradhan, HPR 2613, HPR 2617, Pusa 1121, W1263 (resistant check), and TN-1 (susceptible check). They were obtained from the harvest of previous season.

In this study, we recorded the two types of plant resistance mechanism i.e antixenosis parameters (settling behaviour of adults, ovipositional preference and damaged leaf area) and antibiosis parameters (grub duration and survival, final grub weight, pupal weight, pupal duration, adult emergence and adult longevity). Various morphological (flag leaf length, flag leaf width and plant height) and biochemical factors (total soluble sugars, reducing sugars, phenols, free amino acids, proteins and crude fibres) were also analyzed in the leaves to determine their role in resistance.

The number of adults settled, ovipositional preference and infested leaf area differed significantly among various genotypes. The highest number of settled adults was found on the susceptible check TN-1 (2.40) followed by Sukaradhan (2.12) and the least number was found on the resistant check W1263 (1.12) followed by HPR 2617 (1.40) and Pusa 1121 (1.44). The maximum number of eggs were laid on TN-1 (55.2) followed by Sukaradhan (42.4) which was at par with HPR 2617 (41.6) and the

least numbers were laid on W1263 (27.0) which was at par with HPR 2613 (29.2). A positively significant correlation was found between adult settling and flag leaf length ($r = 0.71$) which means that the number of settled adults was higher in longer leaves such as Sukaradhan (38.64 cm) which was at par with HPR 2617 (38.24 cm) and leaves were shorter in resistant genotypes like W1263 (23.34 cm) and Pusa 1121 (27.30 cm) etc. The infested leaf area was maximum in susceptible check TN-1 (7.62 cm^2) and minimum in resistant check W1263 (3.38 cm^2) followed by Pusa 1121 (4.20 cm^2). It was positively correlated with flag leaf length ($r = 0.28$) which means that longer the flag leaf greater will be the infested leaf area.

Similarly, in case of antibiosis mechanism, various parameters were studied and total grub duration differed significantly in various genotypes. The maximum total grub duration was recorded in susceptible check W1263 (11.2 days) and the minimum was recorded in Nagardhan (8.8 days). The grub weight was recorded maximum in TN1 (5.04 mg) and minimum in W1263 (3.80 mg) which was at par with Nagardhan (3.82 mg). The per cent grub survival was maximum in TN1 (5.04 mg) and minimum in W1263 (73.06%) followed by PUSA 1121 (78.21%) and Nagardhan (78.14%) which were at par with each other.

The total soluble sugar content in W1263 (10.00 mg/g and 10.33 mg/g in 50 and 70 days old plants), Pusa 1121 (12.33 mg/g and 13.26 mg/g in 50 and 70 days old plants) was less than the susceptible genotypes and there was a significant negative correlation between total sugars and grub duration ($r = -0.78$) and a significant positive correlation of total sugars with grub weight ($r = 0.89$) and per cent survival ($r = 0.94$). Reducing sugars also has a negative correlation with grub duration ($r = -0.92$) and a positive correlation with grub weight ($r = 0.94$) and grub survival ($r = 0.96$). Similarly, starch, free amino acids and proteins also has a negative correlation with grub duration ($r = -0.65$, $r = -0.90$ and $r = -0.81$ respectively) and positive correlation with grub weight and grub survival. But on the contrary, total phenols and crude fibres has a significantly positive correlation with grub duration ($r = 0.83$, $r = 0.72$) and negative correlation with grub weight and grub survival. The ones with positive correlation means that higher the phenols and fibre content greater will be the grub duration and vice versa.

Similarly, in case of pupal duration there is a negative correlation with total soluble sugar, reducing sugar, starch, free amino acids and proteins ($r = -0.78$, $r = -0.84$, $r = -0.79$, $r = -0.75$, $r = -0.81$, respectively) and a positive correlation with phenols and crude fibres ($r = 0.66$, $r = 0.76$). But in case of pupal weight it has a positive correlation with total sugars, reducing sugars, starch, free amino acids and proteins ($r = 0.58$, $r = 0.82$, $r = 0.48$, $r = 0.91$, $r = 0.85$, respectively) and it has a negative correlation with phenols and crude fibres ($r = -0.94$, $r = -0.75$).

The above studies indicated that different genotypes can affect settling, oviposition, feeding and other parameters of the rice hispa. The genotypes Pusa1121 and Nagardhan possessed a combination of antixenosis and antibiosis mechanism of resistance to *Dicladispa armigera*. These genotypes showed adverse effect on adult settling, total fecundity, grub feeding, grub duration, grub weight, grub survival and pupal weight. Reducing sugars, amino acids and proteins are found to be essential compounds to the insect they help in reducing developmental period and increasing the grub weight and survival however phenols and fibres adversely affect the hispa biology by increasing the developmental period thereby decreasing number of generations and also decreasing the grub and pupal weight and survival of the insect. The promising genotypes have lower sugars and protein and higher phenol and lignin content than the susceptible genotypes. These genotypes could be used for breeding purpose to make some resistant/moderately resistant varieties that could be used in IPM strategies. It would be effective and environmentally sound way for the management of rice hispa and would decrease the cost on insecticidal applications.

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