Response of Different Wheat Cultivars (*Triticum aestivum* L.) to Biotic Stress

Uzma Kafeel, Urfi Jahan and Fareed A. Khan

Plant Ecology & Environment Laboratory (51), Department of Botany,
Aligarh Muslim University, Aligarh, Uttar Pradesh- 202002, India.
Corresponding Author Email: uzma0964@gmail.com.

ABSTRACT

Aphids are severe insect pests that inflict substantial damage to wheat crop. Earlier studies mostly focused on the susceptibility/resistance of wheat cultivars to specific aphid species. However, research on wheat physiology and stress responses to aphids are few. In the present study, the impact of wheat aphid, *Schizaphis graminum*, on physiology of three cultivars of *Triticum aestivum* L. (wheat), viz, PBW 621 (C1), DPW 62150 (C2), and HD 2967 (C3) have been studied. The aphid induces differential alterations in the growth and physiology of the wheat plant to fulfill its nutrition requirement. Our results indicated that all three cultivars were affected by aphid infestation. *S. graminum* harmed wheat physiology by reducing growth of cultivars and inducing stress. Present findings not only expand our existing understanding of insect-plant interactions but also advocate developing novel techniques for boosting agricultural plant resistance and tolerance to phloem-feeding insects.

Keywords: *Biotic stress, Aphid infestation, Wheat, Photosynthetic pigments, Proline, Sugar*

INTRODUCTION

Aphids are a significant category of agricultural insect pests that cause crop damage by damaging photoassimilates and vectoring various lethal plant viruses. Aphid herbivory causes host plants to evolve a variety of defense mechanisms, resulting in qualitative and quantitative modifications in plant yield due to morphological and metabolic changes (Shree *et al.,* 2021). The distribution of aphids throughout the host plants, as well as the influence on plant growth and defense mechanisms, has long sparked the interest of ecologists. Aphids are regarded serious pests in agriculture, causing major crop losses (Naikoo *et al.,* 2021, Dar *et al.,* 2017). Cereal aphids are the most damaging commercial wheat pests (Guan *et al.,* 2022). The wheat aphid, *Schizaphis graminum*, is one of the aphids that infest wheat in India and cause substantial crop losses (Singh *et al.,* 2022). Its style-like mouthpart feeds mainly on the wheat leaves and stem, injects saliva into plant leaves and sucks its phloem sap from sieve components. During feeding, the aphid injects a phytotoxin, which alters wheat physiology and stimulates defense responses (Goggin *et al.,* 2017). Aphid salivary enzymes destroy leaf chloroplasts, causing white, yellow, purple, or red-purple longitudinal streaks on the leaves of afflicted plants (Farmer 2014). In sensitive cultivars, chlorophyll deprivation affects yields by up to 50% (Cartelat *et al.,* 2005). According to research on plants' responses to herbivory, aphid infestations in wheat crops prevented chlorophyll production (Rehman *et al.,* 2014). Aphids induce cell damage in certain plants' leaves, stems, and inflorescences, as well as abnormal carbon partitioning and shoot loss, all of which harm photosynthetic pigments, leaves, and plant bulk (Dar *et al.,* 2017; Naikoo *et al.,* 2019; Naikoo *et al.,* 2020; Kafeel *et al.*, 2023). Aphid infection decreased the number of proteins, photosynthetic pigments, and other growth-promoting factors in wheat plants (Foyer & Nocter 2013). Reports suggest aphids suck on cell sap, which prevents chlorophyll biosynthesis (Heng-Moss *et al.,* 2003), changes water status (Simpson *et al.,* 2012), decreases plant growth, especially in the

case of shoots (Rehman *et al.,* 2014), interferes with mineral uptake and metabolism genes' activity. Studies show that aphids affect nutrient availability and quality in their host plants (Cao *et al.,*2018). Much research focuses on wheat cultivar susceptibility/resistance to specific aphid species, but information on wheat physiology and stress responses to aphids is limited. The present study examined the behavior and interactions of *S. graminum* on fresh mass, photosynthetic pigments, sugar, and proline concentration of wheat. *S. graminum* was chosen as the aphid because it is a parasitic insect that feeds voraciously on wheat plants. It belongs in the order Hemiptera and the superfamily Aphidoidea. This study assessed the impact of *S. graminum* (wheat aphid) on the growth and physiology of three different *Triticum aestivum* L. cultivars.

MATERIALS AND METHODS

The experiment was conducted in a natural setting in a net house at the Aligarh Muslim University's Department of Botany in Aligarh, India. *Triticum aestivum* cultivars viz, PBW 621, DPW 62150, and HD 2967 were chosen for the experiment and assigned the designations C1, C2, and C3, respectively. For germination, each cultivar's healthy and viable seeds were surface sterilized with a 5 percent sodium hypochlorite (NaOCl) solution (v/v) for 15 minutes (Hamer, 1993). They were then thoroughly rinsed with double distilled water (DDW). Later seeds were planted in clay pots (Diameter=25 cm) with 4.0 kg compost-mixed sandy loam soil (1:3 ratio). When seedlings had four to five leaves, they were thinned, and five seedlings with similar growth vigor were kept in each container and given frequent waterings. 50 aphids (*Schizaphis graminum*) were infected onto each of the three chosen cultivars at 45 days after sowing (DAS). Each treatment was reproduced four times, and a completely random block design was used to arrange them. On each cultivar, aphid population was tallied on 60, 75, and 90 DAS, and a population mean was computed (Fig. 1). Each cultivar's fresh weight, photosynthetic pigments, sugar content, and proline content were measured at 90 DAS.

1. Plant fresh weight:

One wheat plant with healthy roots was removed carefully from the earthen pots. The plants were gently stirred in a water container to loosen the dirt and then washed under running water. Blotting sheets were used to dry the wet plants. Three plants of each cultivar were weighed for fresh mass (at 90 DAS) on an electronic balance (AH220A, AHN Germany).

2. Proline:

The proline content of fresh leaves was measured using the technique published by Bates *et al.* (1973). 300 mg of fresh leaf tissues were mixed with 5.0 ml of 3% sulphosalicylic acid in a mortar. After passing the homogenate through a Whatman No. 1 filter paper, it was blended with 2.0 ml of freshly made acid ninhydrin reagent and 2.0 ml of glacial acetic acid. The process was stopped after an hour of heating in a boiling water bath by moving the test tubes to an ice bath. After violently shaking the reaction liquid for 20-30 seconds, toluene was added. The toluene layer was aspirated and reheated at room temperature. The absorbance of the red color was measured at 520 nm using a UV-VIS spectrophotometer against a blank for the reagent (T70 UK). The amount of proline in the sample was calculated using a standard curve prepared from pure proline and represented on a fresh weight basis.

$$\mu \text{ moles } \mathbf{Proline} \ (per\ g \text{ tissue}) = \frac{\mu g\ Proline\ (ml^{-1}) \times Toluene\ (ml)}{115\ (\text{M.W.})} \times \frac{5}{\text{Sample}\ (g)}$$

M.W= molecular weight of proline.

3. Sugar:

The total sugars were calculated using the Dubois *et al.* (1956) approach. A test tube containing 30.0 mg of fresh leaf tissue and 10 ml of 80 percent ethyl alcohol (80 ml ethyl alcohol + 20 ml DDW) was cooked in a water bath. For 20 minutes, the homogenate was centrifuged at 1500g. Up to 10 mL of 80% ethyl alcohol was used to make the supernatant. A total of 4.0 mL of cold anthrone reagent and 1.0 mL of ethanolic extract were mixed. The mixture was vigorously mixed before being placed in a pot of boiling water for 10 minutes. A spectrophotometer was used to measure the absorbance at 620 nm after cooling under running water (T70 UK). Using known glucose concentrations, a standard curve was prepared. A glucose standard was used to calculate the overall sugar content.

4. Total chlorophyll and carotenoid:

Arnon's (1949) approach was applied to assess the total chlorophyll and carotenoids content in fresh leaves. 100 mg of fresh leaves were crushed in 10 mL of 80% acetone using a mortar and pestle. The suspension was decanted and filtered through a Whatman filter paper 1 onto a Buchner funnel. The optical density (OD) of the solution was measured using a spectrophotometer (UV-1700, Japan) at 645 and 663 nm for estimating chlorophyll and 480 and 510 nm for carotenoid. The total chlorophyll and carotenoid content was calculated using the following formula:

Total chlorophyll = $20.0(OD_{645}) + 8.02 (OD_{663}) \times \frac{V}{w \times 1000}$

Total carotenoid = $7.5(OD_{480}) — 1.49 (OD_{510}) \times \frac{V}{d \times w \times 1000}$ mg g^{-1} FW

Where OD= optical density of the extract at different wavelengths, V= final volume of chlorophyll extract in 80% acetone, W= fresh weight of leaf tissue (g), and d= length of light path (1 cm).

Data analysis and calculation: To substantially differentiate values from one another, the data were presented as mean, standard error (n = 4) and were subjected to one-way ANOVA and Duncan's multiple range test. Analyses performed using version 23 of the SPSS program (IBM SPSS Statistics, Chicago, IL, USA).

RESULTS AND DISCUSSIONS

According to the study, cultivar PBW 621 was the most susceptible to aphid herbivory due to the plant's relatively significant high aphid population followed by DPW 62150 and HD 2967 (Fig. 1) . Plant biomass is used to monitor the effects of various biotic stress on plants. In the present study, aphid infestation impacted plant fresh mass of all the cultivars (Fig. 2). Plant fresh mass of cultivars reduced in relation to the escalating aphid population (Fig. 1&2), and these findings are similar to those reported by Van der Westhuizen *et al.,* (1997), Rehman *et al.,* (2014) and Bevacqua *et al.,* (2016). Fresh mass of cultivars highly correlated with the aphid population (Fig. 3). With a high aphid population, the cultivar PBW 621 proportionately supplied huge volumes of phloem sap (water and photosynthates) to herbivorous animals at the expense of the host plant's biomass (Fig. 2).

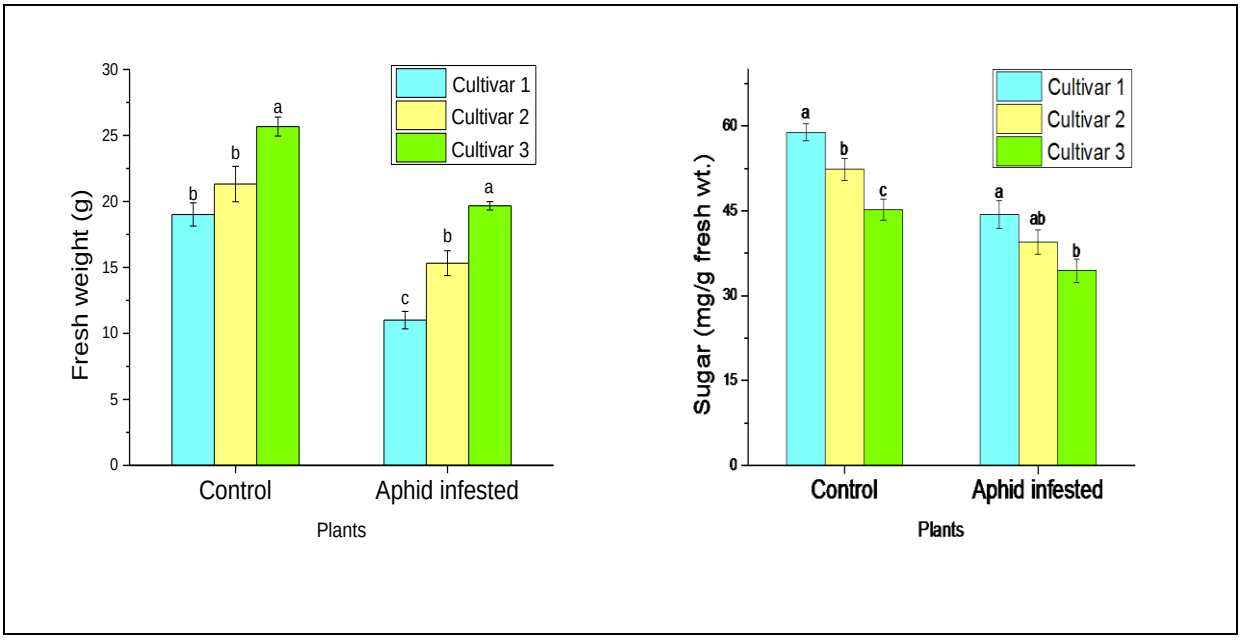
Plant sugar concentration has a significant role in determining how host plants and aphids interact. Sugars are frequently the primary determinants of an aphid's acceptance of a plant (Lemoine *et al.,*2011). Aphid infestation decreased sugar content of all cultivars. The pattern of aphid populations and sugar content were similar (Fig.1 & 2). The sugar content was negatively correlated with the aphid population (Fig. 3). As aphids prefer to suckle on the phloem sap of plants that are rich in sugar and mineral nutrients, PBW 621's high sugar content may be a causative aspect for high aphid infestation. Reports of Giordanengo *et al.,* (2010), Cao *et al.,* (2018), and Nalam *et al.,* (2019), provide additional support for these conclusions.

Proline plays a crucial defensive role in stressed plants. Numerous plant species that thrive under various types of stresses exhibit proline buildup as a defense mechanism and has an important protective role in stressed plants (Rehman *et al.,*2014.; Dar *et al.,*2016; Naikoo *et al.,*2019; El-Beltagi *et al.,* 2020; Naikoo *et al.,* 2020., Kafeel *et al.,* 2022). The statistically analyzed data on the proline content of the three cultivars show that proline content on cultivars increased in proportion to the aphid population at both observed growth stages (Fig. 2). Similar outcomes were reported in studies of Kaur *et al.* (2017), Florencio-Ortiz *et al* (2018), Manghwar *et al.,* (2020) and Rashwan (2021). Proline content of cultivars revealed a strong positive relationship with aphid population (Fig. 3). The intense stress generated by aphid infestation may have harmed membrane permeability, resulting in water shortage stress conditions and excessive proline buildup (Florencio-Ortiz *et al.,* 2018).

Pigment concentration is associated with healthy symptoms and plant production, photosynthetic pigments are consistently used to quantify the influence of diverse biotic and abiotic stressors because to their unique function in photosynthesis and the economics of green cells (Naikoo *et al.,*2021). The differences in photosynthetic pigments (total chlorophyll and carotenoid concentrations) produced by aphid infestation on each of the selected cultivars were statistically examined, and the results are shown in Fig. 2 and Fig. 3. The square correlation coefficient of total chlorophyll and carotenoid levels, like fresh mass and sugar content, shows substantial negative linear relationships with a rise in aphid population (Fig. 3). The results are similar to the findings of Nikolova & Georgieva (2018), Rostami *et al.,* (2020), Naikoo *et al.* (2021), and Othmen *et al.* (2022). The aphid infestation impaired total chlorophyll and carotenoid contents more severely in cv. PBW 621 and DPW 62150 than HD 2967.

Statistically examined data for the three selected cultivars have been summarized in figures (1, 2 and 3). According to the data, PBW 621 is the most susceptible and HD 2967 is the most resistant to the selected aphid species (*Schizaphis graminum*). The difference in growth and biochemical parameters of control and aphid infested plants of PBW 621 and HD 2967 was significant (Fig. 2). The percent increase in aphid population on the selected *T.aestivum* cultivars was linearly regressed against changes in specified parameters (Fig. 3). Fresh mass, total chlorophyll and carotenoid contents displayed strong negative relationships with an increase in aphid population however proline content had a positive correlation with the percent increase in aphid population, according to the square of the correlation coefficient.

Fig. 1. Mean aphid population on 3 cultivars of *Triticum aestivum,* PBW 621 (**C1**), DPW 62150 (**C2**) and HD 2967 (**C3**). Data: mean ± SE; (*n* = 4). According to DMRT, different small letters at each growth phase demonstrate statistically significant variation at $p < 0.05$.



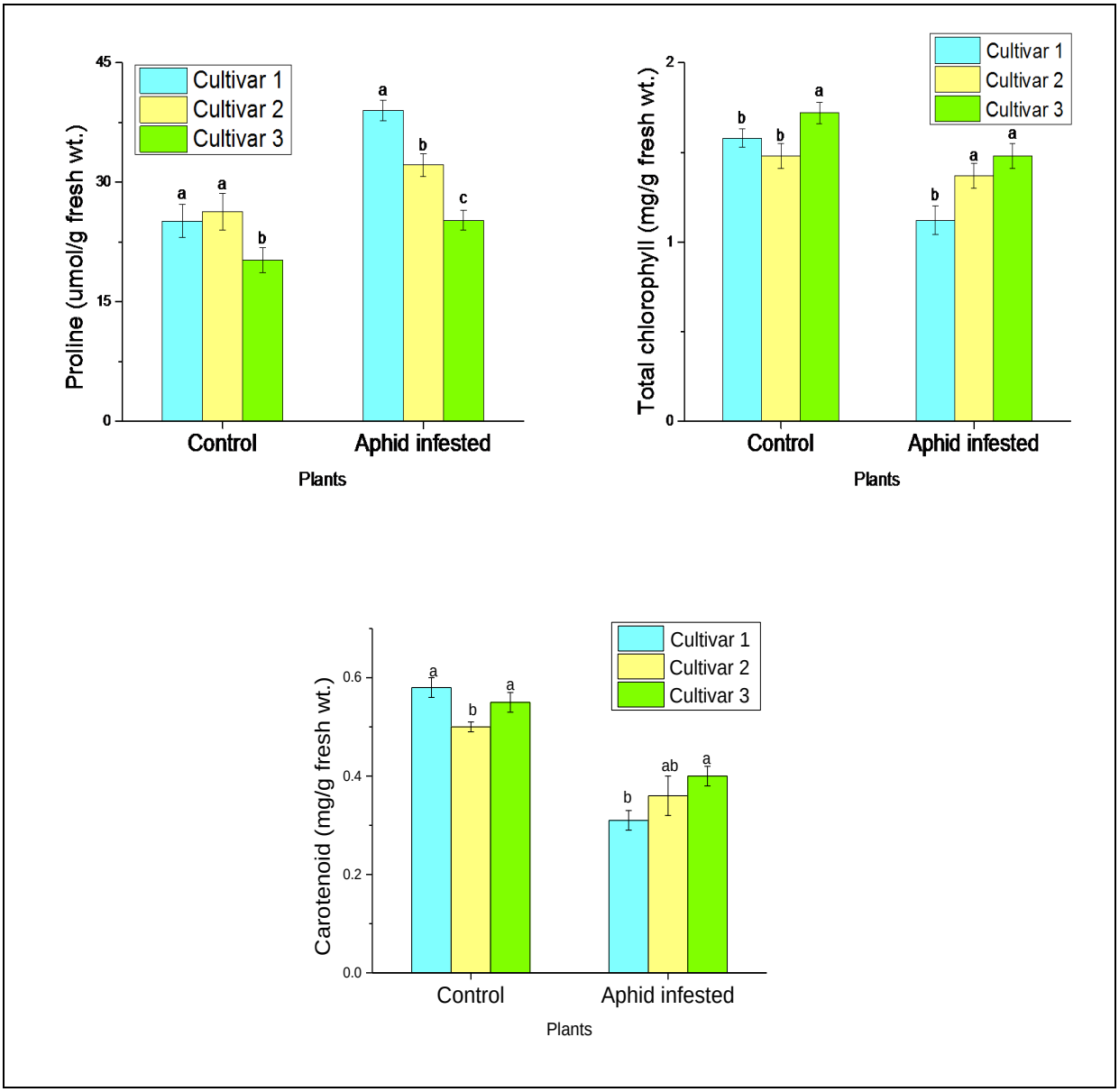


Fig. 2. Plant fresh mass, sugar, proline, total chlorophyll and carotenoid content of 3 cultivars of *Triticum aestivum,* PBW 621 (**C1**), DPW 62150 (**C2**) and HD 2967 (**C3**) at 90 days after sowing (**DAS**). Data: mean ± SE; (*n* = 4). According to DMRT, different small letters at each growth phase demonstrate statistically significant variation at $p < 0.05$.

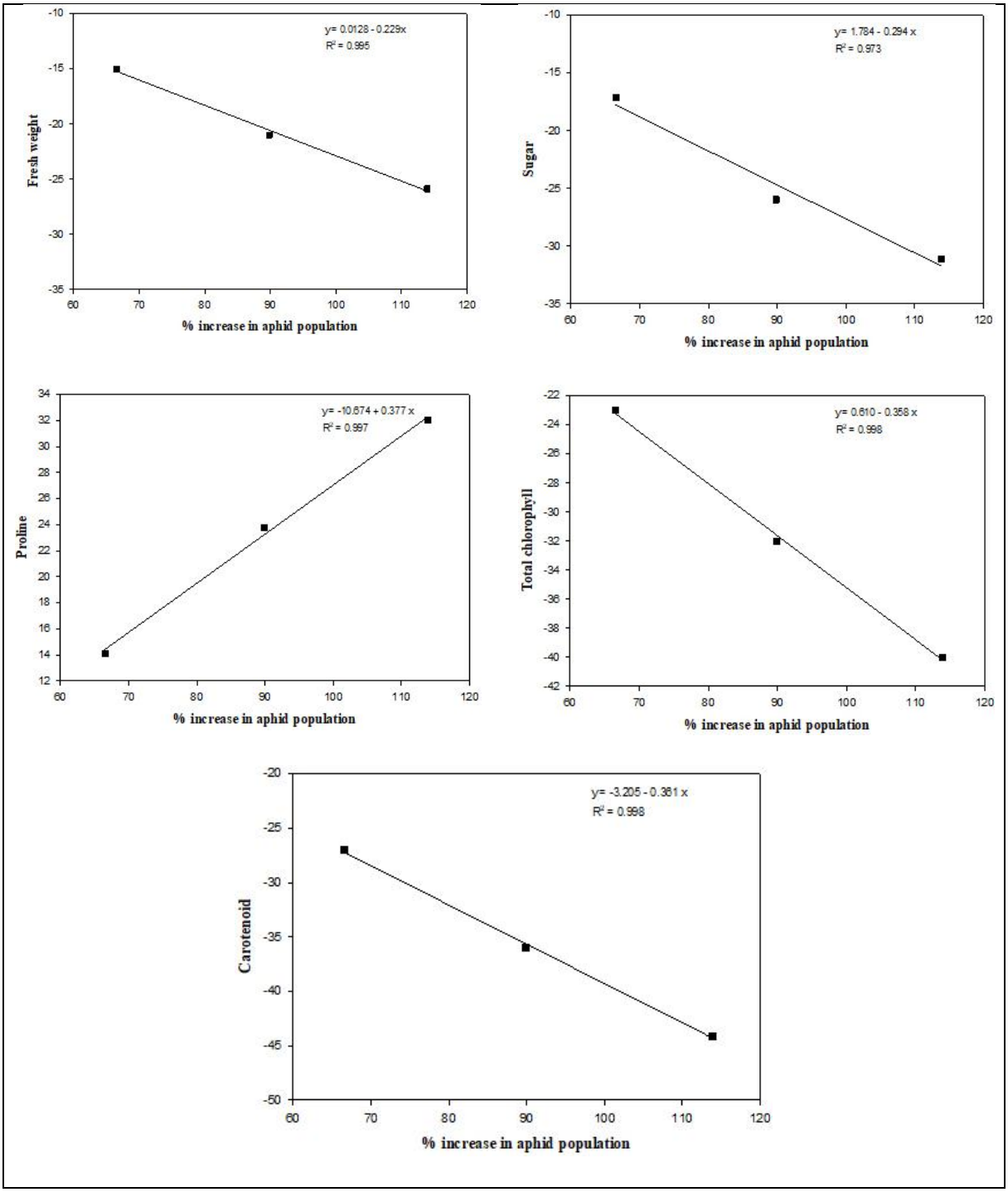


Fig. 3. The regression line depicts the coefficient of correlation between percent nonconformity in chosen parameters and percent increase in aphid population of three *Triticum aestivum* cultivars, PBW 621 (C1), DPW 62150 (C2), and HD 2967 (C3) (bottom to top, respectively). All cultivars were originally infected with 50 aphids per plant, and the aphid increase was determined at 90 DAS. The percentage increase in aphid population on each cultivar was considered as the independent variable (X-axis), whereas selected parameters were treated as the dependent variable (Y-axis).

CONCLUSION

Schizaphis graminum affected the physiology of wheat cultivars by lowering the amount of fresh plant mass and photosynthetic pigments. In addition, the aphid altered plant nutrition metabolism by lowering sugar concentrations. In addition, the aphid induced a stress response in wheat cultivars as indicated by their elevated proline level. All of these data imply that aphid changed the development and physiological characteristics of its host in order to satisfy its nutritional requirements. These findings not only expand our existing understanding of insect-plant interactions, but they also support the development of innovative strategies to increase wheat output resistant to phloem-feeding insects. In correlation research, the dose-response relation is a basic approach. The response within one species to varied aphid levels establishes relationships solely at the level of the particular species. The inherited resistance, defense methods, growth and yield potential of the cultivars of a same species vary. The treatment of three cultivars of wheat plant with aphids and the correlation of cultivar-response factors produce a reaction at the community level.

ACKNOWLEDGMENTS

The authors would like to thank the Chairperson of the Department of Botany, Aligarh Muslim University, Aligarh for providing the necessary facilities for this study.

REFERENCES

Arnon, D. I. 1949. Copper enzymes in isolated chloroplasts, polyphenoxidase in Beta vulgaris with the folin phenol reagent. Journal of Biological Chemistry, **193**: 265-75.

Bates, L. S., Waldren, R. P. and Teare, I. D. 1973. Rapid determination of free proline for water-stress studies. Plant and soil, **39**(1): 205-207.

Bevacqua, D., Grechi, I., Génard, M. and Lescourret, F. 2016. The consequences of aphid infestation on fruit production become evident in a multi-year perspective: insights from a virtual experiment. Ecological Modeling, **338:** 11-16.

Cao, H. H., Zhang, Z. F., Wang, X. F. and Liu, T. X. 2018. Nutrition versus defense: Why *Myzus persicae* (green peach aphid) prefers and performs better on young leaves of cabbage. *PloS one*, **13**(4): e0196219.

Cartelat, A., Cerovic, Z. G., Goulas, Y., Meyer, S., Lelarge, C., Prioul, J. L. and Moya, I. (2005). Optically assessed contents of leaf polyphenolics and chlorophyll as indicators of nitrogen deficiency in wheat (Triticum aestivum L.). Field crops research, **91**(1): 35-49.

Dar, M. I., Naikoo, M. I., Rehman, F., Naushin, F. and Khan, F. A. 2016. Proline accumulation in plants: roles in stress tolerance and plant development. In Osmolytes and plants acclimation to changing environment: emerging omics technologies pp. 155-16. Springer, New Delhi.

Dar, M. I., Green, I. D., Naikoo, M. I., Khan, F. A., Ansari, A. A. and Lone, M. I. 2017. Assessment of biotransfer and bioaccumulation of cadmium, lead and zinc from fly ash amended soil in mustard–aphid–beetle food chain. Science of the total environment, **584**: 1221-1229.

Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. T. and Smith, F. 1956. Colorimetric method for determination of sugars and related substances. *Analytical chemistry*, **28**(3): 350-356.

El-Beltagi, H. S., Mohamed, H. I. and Sofy, M. R. 2020. Role of ascorbic acid, glutathione and proline applied as singly or in sequence combination in improving chickpea plant through physiological change and antioxidant defense under different levels of irrigation intervals. Molecules, **25**(7): 1702.

Farmer, E. E. 2014. Leaf defence. OUP Oxford.

Florencio-Ortiz, V., Sellés-Marchart, S., Zubcoff-Vallejo, J., Jander, G. and Casas, J. L. 2018. Changes in the free amino acid composition of Capsicum annuum (pepper) leaves in response to Myzus persicae (green peach aphid) infestation. A comparison with water stress. PloS one, **13**(6): e0198093.

Foyer, C. H. and Noctor, G. 2013. Redox signaling in plants. Antioxidants & Redox Signaling, **18**(16): 2087-2090.

Giordanengo, P., Brunissen, L., Rusterucci, C., Vincent, C., van Bel, A., Dinant, S. and Bonnemain, J. L. 2010. Compatible plant-aphid interactions: how aphids manipulate plant responses. *Comptes rendus biologies*, **333**(6-7): 516-523.

Goggin, F., Quisenberry, S. S. and Ni, X.2017. 14 Feeding Injury. Aphids as crop pests, 303.

Guan, W., Edwards, M. G., Gatehouse, J. A. and Gatehouse, A. M. 2022. Responses of Wheat (*Triticum aestivum*) to Grain Aphid (*Sitobion avenae*) Infestation and Mechanical Wounding Using a cDNA Subtractive Library Approach. *Agricultural Sciences,* **13**(6): 715-740.

Hamer, A. 1993. Dynamics of fungal growth in stored grain.

Heng-Moss, T. M., Ni, X., Macedo, T., Markwell, J. P., Baxendale, F. P., Quisenberry, S. S. and Tolmay, V. (2003). Comparison of chlorophyll and carotenoid concentrations among Russian wheat aphid (Homoptera: Aphididae)-infested wheat isolines. *Journal of Economic Entomology,* **96**(2): 475-481.

Kafeel, U., Jahan, U., Raghib, F. and Khan, F. A. 2022. Global Importance and Cycling of Nanoparticles. In The Role of Nanoparticles in Plant Nutrition under Soil Pollution pp. 1-20. Springer, Cham.

Kafeel, U., Jahan, U. and Khan, F. A. 2023. Role of mineral nutrients in biological nitrogen fixation. In Sustainable Plant Nutrition. Academic Press. 87-106

Kaur, H., Salh, P. K. and Singh, B. 2017. Role of defense enzymes and phenolics in resistance of wheat crop (*Triticum aestivum* L.) towards aphid complex. *Journal of Plant Interactions*, **12**(1): 304-311.

Lemoine, R., Camera, S. L., Atanassova, R., Dédaldéchamp, F., Allario, T., Pourtau, N. and Durand, M. 2013. Source-to-sink transport of sugar and regulation by environmental factors. *Frontiers in plant science,* **4**: 272.

Manghwar, H., Hussain, A., Ali, Q., Saleem, M. H., Abualreesh, M. H., Alatawi, A. and Munis, M. F. H. 2021. Disease severity, resistance analysis, and expression profiling of pathogenesis-related protein genes after the inoculation of Fusarium equiseti in wheat. Agronomy, **11**(11):2124.

Naikoo, M. I., Dar, M. I., Raghib, F., Jaleel, H., Ahmad, B., Raina, A. and Naushin, F. 2019. Role and regulation of plants phenolics in abiotic stress tolerance: An overview. Plant signaling molecules, 157-168.

Naikoo, M. I., Kafeel, U., Naushin, F. and Khan, F. A. 2020. Halophytes in India

and their role in phytoremediation. Handbook of Halophytes: From Molecules to Ecosystems towards Biosaline Agriculture, 1-21.

Naikoo, M. I., Raghib, F., Dar, M. I., Khan, F. A., Hessini, K. and Ahmad, P. 2021. Uptake, accumulation and elimination of cadmium in a soil-Faba bean (*Vicia faba*)-Aphid (*Aphis fabae*)-Ladybird (*Coccinella transversalis*) food chain. Chemosphere, **279**: 130522.

Nalam, V., Louis, J. and Shah, J. 2019. Plant defense against aphids, the pest extraordinaire. Plant Science, **279**: 96-107.

Nikolova, I. and Georgieva, N. 2018. Effect of biological products on the population of aphids and chemical components in alfalfa. Banat's *Journal of Biotechnology*, **9**(18).

Othmen, S. B., Boussaa, F., Hajji-Hedfi, L., Abbess, K., Dbara, S. and Chermiti, B. 2022. Effects of nymphal density (*Bactericera trigonica*) and feeding on photosynthetic pigments, proline content and phenolic compounds in carrot plants. European *Journal of Plant Pathology*, **163**(1): 51-59.

Rashwan, R. 2021. Susceptibility of Some Ornamental Plants to Aphid Infestation and Effects on Plant Biochemical Components and Morphological Structures. Arab Universities *Journal of Agricultural Sciences,* **29**(3): 913-923.

Rehman, F., Khan, F. A. and Anis, S. B. 2014. Assessment of aphid infestation levels in some cultivars of mustard with varying defensive traits. Archives of Phytopathology and Plant Protection, **47**(15): 1866-1874.

Rostami, E., Madadi, H., Abbasipour, H., Allahyari, H. and Cuthbertson, A. G. 2020. Pest density influences on tomato pigment contents: the South American tomato pinworm scenario. *Entomologia Generalis*, **41**(2).

Shree, P., Kumar, M. and Singh, D. K. 2021. Molecular and Biochemical Aspect of Insect-Plant Interaction: A Perspective for Pest Management. In *Plant-Pest Interactions: From Molecular Mechanisms to Chemical Ecology* pp. 417-436. Springer, Singapore.

Simpson, K. L. S., Jackson, G. E. and Grace, J. 2012. The response of aphids to plant water stress–the case of Myzus persicae and Brassica oleracea var. capitata. Entomologia Experimentalis et Applicata, **142**(3): 191-202.

Singh, B., Narang, D. and Chhuneja, P. 2022. Current Understanding of the Plant Defense Mechanism and Available Genetic Resources for Aphid Resistance in Wheat. In: Tanda, A.S. (eds) Molecular Advances in Insect Resistance of Field Crops. Springer, Cham. 293-314. https://doi.org/10.1007/978-3-030-92152-1_11

Van der Westhuizen, A. J. and Pretorius, Z. 1995. Biochemical and physiological responses of resistant and susceptible wheat to Russian wheat aphid infestation. Cereal Research Communications, 305-31.