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Impact of Infection on the Content of Primary Metabolites in *Pisum sativum* L. and *Solanum lycopersium* Leaves

Renu Kumari*[1], Divya Fageria[2], Kusum Kurdiya[3] and R. A. Sharma[4]

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A B S T R A C T

Phyto-pathogens negatively affect the physiological properties and phytochemical compounds of plants, as a result, the productivity of plants decreases. In this study, primary metabolites including protein, carbohydrates, lipid, phenol, chlorophyll, and carotenoids were identified in infectious leaf parts of *Pisum sativum* L. and *Solanum lycopersium* L. plants and compared with healthy leaves individually. The results suggested that the pathogen of pea and tomato significantly affects the primary metabolites when compared with healthy leaves. It is concluded that phyto-pathogens caused a prominent agricultural impact on *Pisum sativum* and *Solanum lycopersium*.

***Key words*:** Phyto-pathogens, *Pisum sativum*, *Solanum lycopersium*, Primary metabolites, Agricultural impact

Vegetables are important food crops that play a key role in improving diets as well as restoration of some micronutrient deficiencies, especially in underdeveloped nations around the world [1]. Vegetable has been reported to reduce some certain diseases associated with the heart, eye, digestive tract, and cancerogenic diseases [2]. Previous reports showed that several factors greatly reduce vegetable production. Among these limiting factors are pathogenic diseases which were observed to cause serious destruction on vegetables [3-4]. Due to the intake of infected vegetables and fruits, the death rate is continuously rising [5]. It has been estimated that each year, more than one-fifth part of the total world's crop production is damaged by phyto-pathogens [2]. Based on disease symptoms, infected organ, infected plant type, and the type of phytopathogen, plant diseases are classified into two types: infectious (biotic) diseases, which are caused by eukaryotes, prokaryotes, parasitic higher plants, viruses/viroids, nematodes, and protozoa, and non-infectious (abiotic) diseases, which are caused by different extreme environmental conditions [6]. Plant pathogens may affect the composition of plant populations [7-8] and in extreme cases, cause the local extinction of host species [9].

Pisum sativum L. (pea) belongs to the leguminous family with other important pulse crops like a fava bean, lentil, and chickpea, which is a temperate-region crop. Pulse crops have the capacity for nitrogen fixation and thus reduce global reliance on synthetic fertilizers. Among pulse crops, the pea is second only to common bean in terms of area of growth and tons of production. Pea is an important source of protein; carbohydrate; fiber; and micronutrients such as folates, iron, zinc, selenium, and carotenoids that play a significant role in human nutrition [10-17]. According to the statistics for the year 2021 in India, the total pea cultivation area is about 781 ha, production is 2.193 tons and the yield is 28.079 hg/ha [18]. But diseases are the most important biotic constraints affecting global pea production [19], causing yield losses that range from a small percentage to complete crop loss depending on location and environmental conditions. Pea pathogens including fungal pathogens such as *Aphanomyces euteiches*, *Peronospora viciae*, *Peyronellaea pinodes*, *Phoma medicaginis*, *Erysiphe pisi*, *Fusarium oxysporum*, etc. [20] and pests such as *Etiella zinckenella*, *Myzus persicae*, *Lampedes boeticus*, *Tetranychus telarius*, *Phytomyza atricornis*, etc. [21] have been identified to cause various detrimental effects on Pea plant.

Solanum lycopersicum L. (Tomato), belongs to the Solanaceae family, originated in the Andean region of South America, is the second most cultivated vegetable crop throughout the world following the potato, with approximately 181 million tonnes from 5 Mha, according to the Food and Agriculture Organization Statistics [22]. It contains high carbohydrates, moisture, total lipids, and protein3.18%, 94.78%, 0.97% and 1.167%, respectively. Considering minerals tomato contains such as magnesium, calcium, phosphorus, and others along with total dietary fiber. Tomato contains a high amount of vitamin C showing the best source of this vitamin and considered a reasonable source for human nutrition. It also has a strong profile of thiamine, riboflavin, niacin, pantothenic acid, and vitamin B_6 [23]. Hence, tomato is one of the most important vegetable crops in the world with by-products including fresh market fruit and processed paste, juice, sauce, and powder [24]. It also has numerous pharmaceutical properties such as anti-oxidant activity [25]. During cultivation and post-harvest period, it can be affected by more than 200 diseases caused by different pathogens such as fungi, bacteria,

* **Renu Kumari**
✉ renumani0496@gmail.com

[1-4] Department of Botany, University of Rajasthan, Jaipur - 302 004, Rajasthan, India

phytoplasmas, viruses, and viroids throughout the world [26-27]. Numerous pathogens such as *Alternaria solani, Septoria lycopersici, Botrytis cinerea, Fusarium oxysporum strains, Verticillium dahlia* (fungal pathogens), *Clavibacter michiganensis, Pseudomonas syringae* (bacterial pathogens), Tomato Spotted Wilt Virus, Cucumber Mosaic Virus, Tomato Brown Rugose Fruit Virus, Tomato Mosaic Virus, etc. (viral pathogens) are hampered the tomato productivity [28]. As a consequence, this research was carried out to estimate the effect of infection on phytochemical compounds in leaves of pea and tomato plants.

MATERIALS AND METHODS

1) *Collection of plant materials*

Pisum sativum L. and *Solanum lycopersium* L. was collected as experimental plant material from Ashok Nagar village near by Jhunjhunu, Rajasthan in India. The selected areas are known to vegetables growing areas and has suitable environment for the production of vegetables specially *Pisum sativum* and *Solanum lycopersicum.* Both infected and non-infected leaves of both experimental plants were isolated for estimations of primary metabolites content.

2) *Biochemical analysis*

Proteins

Protein content was evaluated by Lowry *et al.* [29] method in both infected and non-infected leaves of both *P. sativum* and *S. lycopersium*. 0.05gm samples (infected and non-infected) were homogenized individually with 10ml TCA (10%). The homogenized samples were centrifuged at the speed of 10000 rpm for 10 minutes tentatively. The supernatant was used to determine protein concentration. 0.5ml of each sample was diluted with distilled water up to 1ml. Then 5 mL of a freshly prepared alkaline solution that prepared by mixing 50 mL of 2% Na_2CO_3 in 0.1 N NaOH and 1 mL of 0.5% $CuSO_4.5H_2O$ in 1% Sodium potassium tartrate was added and incubated at 37°C for 10 minutes. After incubation 0.5mL of Folin-Ciocalteau (FC) reagent was added and vortexed properly. The absorbance was read at 750nm by spectrophotometer after 30 minutes of incubation. Bovine serum albumin (1mg/ml) was used to prepare the standard curve. Blank has all reagents except sample.

Total soluble sugars

Total Soluble Sugars (TSS) were estimated by using the phenol-sulphuric acid method of Dubois *et al.* [30]. 0.05gm samples (infected and non-infected) were homogenized with 20 mL of ethanol 80% with the help of mortar-pestle. Each sample was centrifuged at the speed of 12,000 rpm for 15 minutes tentatively and the supernatants were collected separately for TSS estimation. 0.5ml of each supernatant was diluted up to 1ml with distilled water.1 mL of 5% phenol was added to each sample and mixed completely. 5ml conc.H_2SO_4 was added gently in each tube and kept on ice for cooling. Then tubes were allowed to stand for 20 minutes on a water bath at 26-30°C. Glucose (100µg/mL) was used as standard. Blank has all reagents except sample. The intensity of the yellow color was measured at 490nm.

Starch

0.05gm samples (infected and non-infected) were homogenized 20 mL of ethanol80% by using mortar-pestle. Each sample was centrifuged at the speed of 12,000 rpm for 15 minutes tentatively and the pellets were separated individually to performed a starch test. Each pellet was suspended in 5 mL of 52% perchloric acid, followed by 6.5 mL of water was added to each sample and the mixture was shaken vigorously for 5 minutes. Later, above mentioned Dubois *et al.* [30] were followed with 1ml of each sample to calculate starch concentration.

Lipids

Lipid contents were measured by using Jayaram [31] method. 0.1 gm each sample was homogenized with 10 mL distilled water, thereafter 30 mL of chloroform and methanol (2:1) was added. The mixture was thoroughly mixed and left overnight at room temperature in dark for complete extraction. Later, 20 mL of chloroform mixed with 2 mL of water were added and centrifuged. Two layers were separated, the lower layer of chloroform, which contained all the lipids, was carefully collected in the pre-weighed glass vials. The chloroform layers dried in vacuo and weighed. Each treatment was repeated thrice and their mean values were calculated.

Phenols

Total phenol content in each sample was estimated by the spectrophotometer method of Bray and Thorpe [32]. The 0.2gm leaf samples were macerated with 10 mL of 80% ethanol. The mixtures were centrifuged at the speed of 15000 rpm for 15 minutes and 0.5 ml supernatant were collected separately was made up to 1 ml with 80% ethanol. 1mL of Folin-Ciocalteau (FC) reagent and 2 mL of 20% sodium carbonate solution was added and the mixture was vortexed properly. The samples were placed in boiling water for 1 min and cooled under running water. These reaction mixtures were diluted to 25 mL by adding distilled water and optical density was read at 750 nm against a blank. Gallic acid (100µg/mL) was used to draw the standard curve.

Chlorophyll and carotenoid

Photosynthetic and accessory pigments like Chlorophyll-a, b, and total chlorophyll contents along with carotenoids were estimated by Arnon [33] method. 1 gm leaves samples were collected and chilled in a deep freezer (-20°C). The pigments were extracted by homogenizing the small pieces of leaves in 80% acetone and then centrifuged at 10,000 rpm for 10 min. The supernatant obtained was used for pigments estimation. The absorbance of the supernatant was recorded at 663 nm, 646 nm, 480 nm, and 510 nm. 80% acetone was used as blank. The amounts of the pigments were calculated by the following formula:

$$\text{Total chlorophyll (µg/ml)} = 20.2\ (A_{645}) + 8.02\ (A_{663})$$
$$\text{Carotenoids (µg/ml)} = 7.6\ (A_{480}) - 1.49\ (A_{510})$$

Whereas, A_{645}, A_{663}, A_{480}, A_{510} are the absorbance at 645 nm, 663 nm, 480 nm, and 510 nm wavelengths. The results were calculated and expressed in gm.

RESULTS AND DISCUSSION

Protein

As shown in (Table 1-2), the protein concentration was significantly increased in both infected pea and tomato leaves. Healthy pea leaves showed a 1.76gm protein level that was increased up to 2.66gm after infection. Similarly, the amount of protein in infectious tomato leaves was calculated at 2.49gm which was higher than non-infected leaves (1.71gm). Similar observations were also reported for begomovirus-infected pumpkin/bitter gourd and for potyvirus-infected plum, where an increase in the level of total proteins was caused by a virus

infection, respectively [34-36]. A phytochemical study by Chen *et al.* [37] revealed that the protein level increased by 39.6% in virus-infected *Passiflora edulis* fruits as compared with healthy fruits. A non-significant reduction was observed in the protein content of mung bean leaves infected with a nematode [38]. The leaves of coconut infected by spiraling whitefly and sooty mold showed an increase in the soluble protein (114%) and amino acid content (126%) when compared to the non-infected leaves [39]. Host nutrition can play a key role in the outcome of pathogen infections in the host since it is critical for immune defense and resistance to pathogens. Poor nutrition, in particular protein or sugar depletion, is a major factor in high incidence and host mortality due to infectious diseases. Thus, the increased total proteins may be implicated in pathogen defense [37]. Earlier reports also described that the increasing levels of proteins helped the plants in maintaining their growth under various stressful environments [40-41] that supported the protein results of this study.

Carbohydrates

The total soluble sugar and starch contents were declined in both pea and tomato leaves after infection. 0.16 gm TSS and 1.94gm starch in non-infected pea leaves was reduced in infected leaves that were 1.14gm TSS and 1.70gm starch, respectively. In tomato leaves, 0.12gm TSS and 1.89gm starch were observed in healthy leaves. The amount of TSS and starch was recognized at 0.09gm and 1.65gm with infected tomato leaves that were less than healthy leaves. Lan *et al.* [42] also reported a 38.3% decrement in total sugar contents in *Passiflora edulis* leaves infected with the virus when compared with virus-free leaves. Khalil *et al.* [43] reported that virus-infected tomato plants caused a considerable decrease in total soluble sugar; insoluble sugar and carbohydrate contents in stem and plant leaves respectively as compared with control. Tomato yellow leaf curl virus causes definite changes in physiological functions of virus-infected plants resulting in a reduction of the main components of the plant viz; Plant growth, photosynthetic pigments, total carbohydrate contents, and Mg^{++} ions. However, the study of Chen *et al.* [37] on phytochemical compounds 19.1% increment in sugar contents in virus-infected *Passiflora edulis*fruits in comparison to-infected fruits. The 25% higher total sugar content was also reported in spiraling whitefly and sooty mold infected coconut leaves as compared to healthy leaves by Arun *et al.* [39]. In Arooj *et al.* [44] study, non-viral infected tomato leaves showed no color while infected leaves showed dark grey to black stains after being immersed in iodine and reactions. Their observation confirmed the presence of starch in infected leaves and absence in non-infected leaves. The amount of starch increased significantly in *Citrus sinensis* leaves infected with Candidatus Liberibacter asiaticus. According to [45-46], the carbohydrate level can be higher after pathogenic infection because it acts as a signaling molecule to induce a defense mechanism. Moreover, the sugar molecules have a crucial involvement in the osmotic adjustment mechanisms where it acts as a compatible solute [47].

Lipid

The amount of lipid was recorded higher in both infected leaves of pea and tomato. The lipid content of healthy leaves of pea was 3.33gm that was 2 fold increase (6.66gm) after infection. Likewise, the infected tomato leaves were exhibited 5.41gm lipid content that was higher as compared to non-infected leaves (3.26gm). This result is in contrast to some reports including cucumber mosaic virus-infected *Passiflora* edulis. Lan *et al.* [42] reported that total fat contents were decreased by 35.0% in *Passiflora edulis* leaves infected with the virus as compared to virus-free leaves. The % loss of fat content was observed in 60.33% in cowpea leaf infected by *Colletotrichum destructivum*in comparison with non-infected leaf [48]. Chen *et al.* [39] 2018 reported that the total fat contents decreased by 21.6% in virus-infected *Passiflora edulis* fruits compared with virus-free *P. edulis* fruits. Nutrition levels of the host play some vital roles during the battle against invading pathogens [42]. Thus, the enhancement of the total lipid contents explains the positive influence on pea and tomato plants to trigger defense mechanisms by infection.

Phenol

The concentration of phenol in non-infected leaves was 5.15gm but it was 4.75 gm in infected pea leaves. Non-infected tomato leaves were showed 4.87gm phenol while 2.98gm was recorded with infected leaves. Consequently, in both the experimental plant leaves the phenol content was diminished after infection. On the other hand, total phenols increased in the leaves of mung beans infected with nematode, reported by Ahmed *et al.* [38]. The total phenol contents were significantly higher in the virus-infected *Passiflora edulis* fruits, with a 19.1% increase over the healthy *P. edulis* fruits [37]. Ming *et al.* [49] also revealed 362.5%, 263.1%, and 274% increment in phenol content in roots, pseudo-stems, and leaves, respectively of *Fusarium oxysporum* infected banana plant compared to the healthy plants. The results of Arooj *et al.* [44] revealed that phenolic compound in virus-infected tomato leaves at maximum temperature increased whereas at the minimum temperature it is decreased. In 1969, Kosuge [50] explained that total phenol levels increased during the early stages of infection but later declined. Phenols may serve as defense compounds against pathogens. Early increases in phenol caused by pathogen invasion trigger the transcription of messenger RNA that codes for phenylalanine ammonia-lyase (PAL); increasing amounts of PAL in the plant brings about the synthesis of phenolic compounds [51]. Lower levels of phenols during the later stages are linked to the oxidation of phenols by polyphenol oxidase (PPO) [52].

Total chlorophyll and carotenoid

In this study, the total chlorophyll and carotenoid contents in fresh leaves of *P. sativum* were recognized at 0.087gm and 0.066gm, respectively that were reduced up to 0.034gm and 0.021gm in infected leaves of a pea. Similarly, results were obtained with infected tomato leaves. Total chlorophyll was 0.073gm and 0.029gm in non-infected and infected leaves of *S. lycopersium*. Carotenoids levels were 0.051gm and 0.017gm in non-infected and infected tomato leaves. Ultimately the reduction in total chlorophyll and carotenoid concentration in infected tomato leaves was 39.73% and 33.33%, respectively. Recently a similar reduction in Chl. a, b, and carotenoid contents were observed in the virus-infected leaves of Datura plant, studied by Fayziev *et al.* [53]. The amount of chlorophyll a in the healthy *D. stramonium* leaves was 9.98 mg/g, whereas it was 2.88 mg/g and 2.65 mg/g in the leaves of weak and moderate infected plants with an ordinary isolate of potato virus X respectively. Chlorophyll b was 5.16 mg/g in leaves of healthy plants, but ordinary (PVC-UZ 214) isolate reduced Chlorophyll b content until 2.25 and 2.21 mg/g in weak and moderately infected *D. stramonium* leaves respectively. The carotenoid content was 2.24 mg/g in healthy plants, but it was 0.44 and 0.41 mg/g in the leaf of the *D. stramonium* plant, which is weak and moderately infected with the ordinary (PVXO-UZ 214) isolate of the virus respectively. The content of β-carotene was 23.23, 20.13, 14.60, 3.37 µg/g FW in 24^{th} leaves, 21^{th} leaves, 18^{th} leaves, and red

fruits, respectively [54]. β-carotene content in 24th leaves was 6.89 times greater than that in red fruits. β-carotene and lutein, major carotenoids in green leafy vegetables, are potent antioxidants [55].

Table 1 Primary metabolites content (gram) in fresh and infected leaves of *Pisum sativum*

Plant material	Protein	TSS	Starch	Lipid	Phenol	Total chlorophyll	Carotenoids
Fresh leaves	1.76±0.590	0.16±0.081	1.94±0.363	3.33±0.260	5.15±0.130	0.087±0.006	0.066±0.013
Infected leaves	2.66±0.188	0.14±0.040	1.70±0.110	6.66±0.252	4.75±0.443	0.034±0.007	0.021±0.003

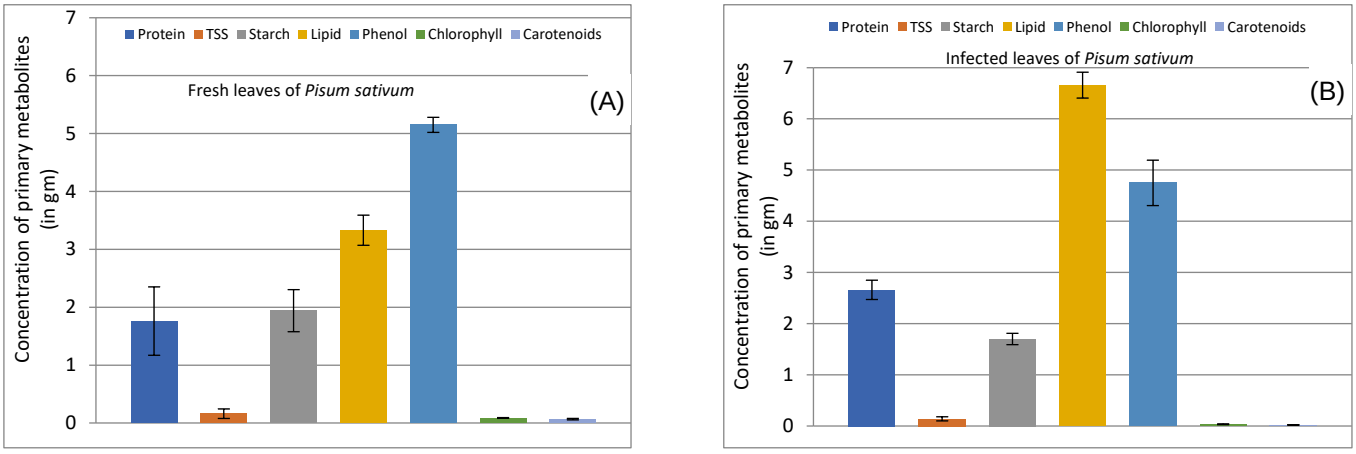

Fig 1 Primary metabolites content (gram) in (A) fresh and (B) infected leaves of *Pisum sativum*

Table 1 Primary metabolites content (gram) in fresh and infected leaves of *Solanum lycopersium*

Plant material	Protein	TSS	Starch	Lipid	Phenol	Total chlorophyll	Carotenoids
Fresh leaves	1.71±0.17	0.12±0.044	1.89±0.106	3.26±0.327	4.87±0.410	0.073±0.039	0.051±0.028
Infected leaves	2.49±0.303	0.09±0.045	1.65±0.202	5.41±0.545	2.98±0.442	0.029±0.008	0.017±0.002

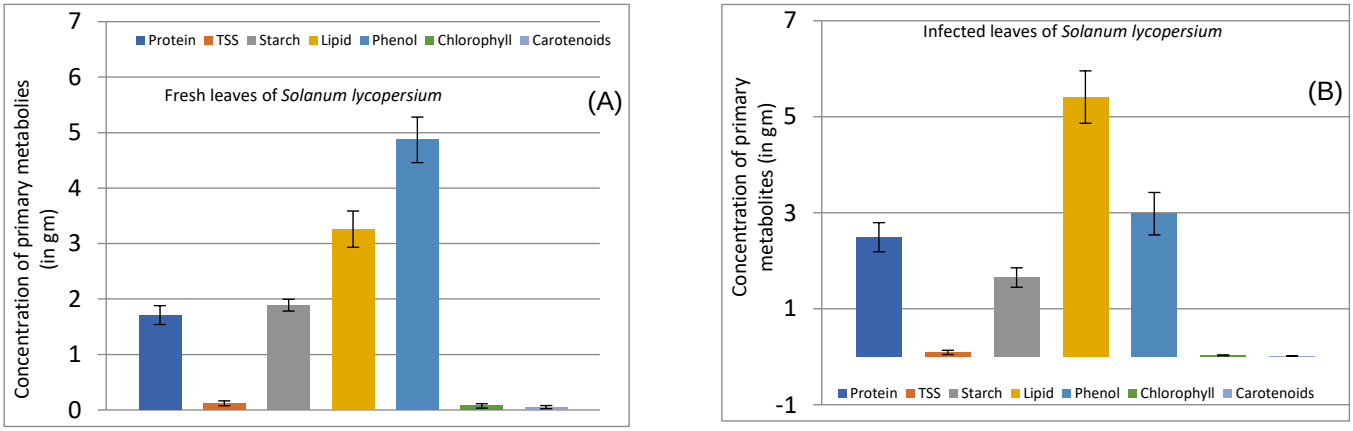

Fig 2 Primary metabolites content (gram) in (A) fresh and (B) infected leaves of *Solanum lycopersium*

CONCLUSION

Pea and tomato crops are very important vegetables that have more medicinal values as well as nutritional values. But pathogenic infections cause several adverse effects on plant nutrients and their yields. Therefore, in the present study, the effects of infection on primary metabolites of both pea and tomato leaves were identified. Results confirmed that the infections caused by pathogens altered the amounts of primary metabolites. This assessment has significant importance to understand the effects of infection on the biochemical changes in plants. It will also be helpful to increase awareness in farmers to use eco-friendly bio-fertilizers to prevent pathogenic infection with increasing plant productivity.

LITERATURE CITED

1. FAO. 2004. *Fruit and Vegetables for Health*. Proceedings of the Joint FAO/WHO Workshop. Kobe, Japan. pp 1-46.
2. Kunjwal N, Srivastava RM. 2018. *Insect Pests of Vegetables*. *In*: (Eds) Omkar. Pests and Their Management. pp 163-221.
3. Jipanin J, Rahman A, Jaimi JR, Phua P. 2001. Management of Pesticide use on vegetable production: Role of Department of Agriculture Sabah. 6th SITE Research Seminar. pp 13-14.

4. Zurina M, MohdRoff MN, Azizan A, Idris A. 2015. Factors influencing farmers in Cameron Highlands to use insecticide in cabbage cultivation. *Jr. Agric. Environ. Sci*. 15: 1095-1101.
5. Hionas T. 2019. Fruit and vegetable intake linked to declining global health for your diary. https://www.soci.org/news/general-news/fruits.
6. Horsfall JG, Cowling EB. 1980. *Plant Disease*. 3rd Eds. Academic Press, 1980 New York, NY.
7. Gilbert GS. 2002. Evolutionary ecology of plant diseases in natural ecosystems. *Annu. Rev. Phytopathol*. 40: 13-43.
8. Pagán I, Fraile A, García-Arenal F. 2016. Evolution of the interactions of viruses with their plant hosts. *In*: Virus Evolution: Current Research and Future Directions. (Eds) Weaver, S.C., Denison, M., Roossinck, M., Vignuzzi, M. Caister Academic Press: Poole, U.K. pp 127-154.
9. Alexander HM, Mauck KE, Whitfield AE, Garrett KA, Malmstrom CM. 2014. Plant-virus interactions and the agro-ecological interface. *Eur. Jr. Plant Pathology* 138: 529-547.
10. Patterson CA, Maskus H, Dupasquier C. 2009. *Pulse Crops for Health*. Cereals Food World. 54: 108-113.
11. Roy F, Boye JI, Simpson B.K. 2010. Bioactive proteins and peptides in pulse crops: Pea, chickpea and lentil. *Food Research International* 43: 432-442.
12. Jha AB, Arganosa G, Tar'an B, Diederichsen A, Warkentin TD. 2013. Characterization of 169 diverse pea germplasm accessions for agronomic performance, mycosphaerella blight resistance and nutritional profile. *Genetic Resource and Crop Evolution* 60: 747-761.
13. Jha AB, Tar'an B, Diapari M, Warkentin TD. 2015. SNP variation within genes associated with amylose, total starch and crude protein concentration in field pea. *Euphytica* 206: 459-471.
14. Gali KK, Liu Y, Sindhu A, Diapari M, Shunmugam ASK, Arganosa G, Daba K, Caron C, Lachagari RVB, Tar'an B. 2018. Construction of high-density linkage maps for mapping quantitative trait loci for multiple traits in field pea (*Pisum sativum* L.). *BMC Plant Biology* 18: 172.
15. Gali KK, Sackville A, Tafesse EG, Lachagari RV, Mcphee K, Hybl M, Mikic A, Smykal P, Mcgee RJ, Bustin J. 2019. Genome-wide association mapping for agronomic and seed quality traits of field pea (*Pisum sativum* L.). *Front. Plant Science* 10: 1538.
16. Dissanayaka DN, Gali KK, Jha AB, Reddy Lachagari VB, Warkentin TD. 2020. Genome-wide association study to identify single nucleotide polymorphisms associated with Fe, Zn, and Se concentration in field pea. *Crop Science* 60: 2070-2084.
17. Jha AB, Warkentin TD. 2020. Biofortification of pulse crops: Status and future perspectives. *Plants* 9: 73.
18. Anonymous. 2021. FAO. https:// www.fao. org/ faostat/.
19. Warkentin TD, Smýkal P, Coyne CJ, Weeden N, Domoney C, Bing, DJ, Leonforte A, Xuxiao Z, Dixit GP, Boros L. 2015. Pea. In Grain Legumes; Springer: New York, NY, USA. pp 37-83.
20. Jha AB, Gali KK, Alam Z, Lachagari VBR, Warkentin TD. 2021. Potential Application of genomic technologies in breeding for fungal and oomycete disease resistance in pea. *Agronomy* 11: 1260.
21. Yadav A, Singh V, Yadav A, Kumar A, Singh H. 2018. Effect of dates of sowing on the incidence of pea stem fly, *Ophiomyia phaseoli* (Tryon) on pea in Rajasthan. *Bull. Env. Pharmacol. Life Sci*. 7(10): 71-74.
22. FAOSTAT. 2019. Available online: http://www.fao.org/faostat/en/#data/QC (accessed on 12 September 2021)
23. Perveen R, Suleria HAR, Anjum FM, Butt MS, Pasha I, Ahmad S. 2015. Tomato (*Solanum lycopersicum*) carotenoids and Lycopenes chemistry; metabolism, absorption, nutrition, and allied health claims-A comprehensive review. *Critical Reviews in Food Science and Nutrition* 55(7): 919-929.
24. Rigano MM, De Guzman G, Walmsley AM, Frusciante L, Barone A. 2013. Production of pharmaceutical proteins in solanaceae food crops. *Int. Jr. Mol. Sci*. 14: 2753-2773.
25. Pellegrini N, Riso P, Porrini M. 2000. Tomato consumption does not affect the total antioxidant capacity of plasma. *Nutrition* 16: 268-271.
26. King KC, Lively CM. 2012. Does genetic diversity limit disease spread in natural host populations? *Heredity* 109: 199-203.
27. Singh VK, Singh AK, Kumar A. 2017. Disease management of tomato through PGPB: Current trends and future perspective. *3 Biotech* 7: 255.
28. Panno S, Davino S, Caruso AG, Bertacca S, Crnogorac A, Mandi´c A, Noris E, Mati´c S. 2021. A review of the most common and economically important diseases that undermine the cultivation of tomato crop in the Mediterranean basin. *Agronomy* 11: 2188.
29. Lowry OH, Rose HN, Broug J, Farr AL, Randall RJ. 1951. Protein measurement with the Folin-phenol reagent. *Jr. Biol. Chem*. 193: 265-275.
30. Dubois M, Gills KA, Hamilton JK, Rebers PA, Smith F. 1951. Colorimetric method for determination of sugars and related substances. *Anal. Chemistry* 28: 350-356.
31. Jayaraman J. 1981. *Laboratory Manual in Biochemistry*. Wiley Eastern Limited, New Delhi. pp 96-97.
32. Bray HG, Thorpe WV. 1954. Analysis of phenolic compounds of interest in metabolism. *Meth. Biochem. Analysis* 1: 27-52.
33. Arnon DI. 1949. Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. *Plant Physiology* 24: 1-15.
34. Raj SK, Khan MS, Singh R, Kumari N, Prakash D. 2005. Occurrence of yellow mosaic geminiviral disease on bitter gourd (*Momordica charantia*) and its impact on phytochemical contents. *Int. Jr. Food Sci. Nutrition* 56: 185-192.
35. Jaiswal N, Singh M, Dubey RS, Venkataramanappa V, Datta D. 2013. Phytochemicals and antioxidative enzymes defence mechanism on occurrence of yellow vein mosaic disease of pumpkin (*Cucurbita moschata*). *3 Biotech* 3: 287-295.
36. Usenik V, Kastelec D, Stampar F, VirscekMarn M. 2014. Effect of Plum pox virus on chemical composition and fruit quality of plum. *Jr. Agr. Food Chemistry* 63: 51-60.
37. Chen S,Yu N, Yang S, Zhong B, Lan H. 2018. Identification of Telosma mosaic virus infection in *Passiflora edulis* and its impact on phytochemical contents. *Virology Journal* 15: 168.
38. Ahmed N, Abbasi MW, Shaukat SS, Zaki MJ. 2009. Physiological changes in leaves of mungbean plants infected with *Meloidogyne javanica*. *Phytopathol. Mediterr*. 48: 262-268.

39. Arun K, Janeeshma E, Job J, Puthur JT. 2021. Physiochemical responses in coconut leaves infected by spiraling whitefly and the associated sooty mold formation. *Acta Physiologiae Plantarum* 43(3): 1-13.
40. Agastian P, Kingsley SJ, Vivekanandan M. 2000. Effect of salinity on photosynthesis and biochemical characteristics in mulberry genotypes. *Photosynthetica* 389: 287-290.
41. Ferreira RB, Monteiro SARA, Freitas R, Santos CN, Chen Z, Batista LM, Teixeira AR. 2007. The role of plant defense proteins in fungal pathogenesis. *Mol. Plant Pathology* 8: 677-700.
42. Lan H, Lai B, Zhao P, Dong X, Wei W, Ye Y, Wu Z. 2020. Cucumber mosaic virus infection modulated the phytochemical contents of *Passiflora edulis*. *Microbial Pathogenesis* 138: 103828.
43. Khalil R, Bassiouny F, El-Dougdoug K, Abo-Elmaty S, Yousef M. 2014. A dramatic physiological and anatomical changes of tomato plants infecting with tomato yellow leaf curl germinivirus. *International Journal of Agricultural Sustainability* 10: 1213-1229.
44. Arooj S, Iftekhar Y, Mubeen M, Ullah MI, Sajid A, Ali S, Qudsia H. 2019. Effect of environmental factors on biochemical properties of tomato leaf curl virus infected leaves of tomato. *Pakistan Journal of Phytopathology* 31(1): 105-111.
45. Morsy MR, Jouve L, Hausman JF, Hofmann L, Stewart JM. 2007. Alteration of oxidative and carbohydrate metabolism under abiotic stress in two rice (*Oryza sativa* L.) genotypes contrasting in chilling tolerance. *Jr. Plant Physiology* 164: 157-167.
46. Morkunas I, Ratajczak L. 2014. The role of sugar signaling in plant defense responses against fungal pathogens. *Acta Physiol. Plant* 36: 1607-1619.
47. Nounjan N, Chansongkrow P, Charoensawan V, Siangliw JL, Toojinda T, Chadchawan S, Theerakulpisut P. 2018. High performance of photosynthesis and osmotic adjustment are associated with salt tolerance ability in rice carrying drought tolerance QTL: physiological and co-expression network analysis. *Fronts Plant Science* 9: 1135.
48. Amadioha AC, Nwazuo ED. 2019. Alterations of biochemical composition of leaf and stem of cowpea (*Vigna unguiculata* (L.) Walp.) by *Colletotrichum destructivum* O'Gara in Nigeria. *Journal of Experimental Agriculture International* 32(2): 1-7.
49. Ming FS, Razali Z, Somasundram C. 2021. Involvement of phenolic compounds and their composition in the defense response of *Fusarium oxysporum* infected Berangan banana plants. *Sains Malaysiana* 50(1): 23-33.
50. Kosuge T. 1969. The role of phenols in host response to infection. *Annual Review of Phytopathology* 7: 195-222.
51. Taiz L, Zeiger E. 2002. *Plant Physiology*. 3rd Edition, Sinaur Associates Inc, Sunderland, MA, USA. pp 290.
52. Mayer AM, Harel E. 1979. Polyphenol oxidases in plants. *Phytochemistry* 18: 193-215.
53. Fayziev V, Jovlieva D, Juraeva U, Shavkiev J, Eshboev F. 2020. Effects Of Pvxn-Uz 915 necrotic isolate of potato virus X On amount of pigments of *Datura Stramonium* leaves. *Journal of Critical Reviews* 7(9): 400-403.
54. Kim DS, Na H, Kwack Y, Chun C. 2014. Secondary metabolite profiling in various parts of tomato plants. *Kor. Jr. Hort. Sci. Technol*. 32(2): 252-260.
55. Jiménez-Escrig A, Jiménez-Jiménez I, Sánchez-Moreno C, Saura-Calixto F. 2000. Evaluation of free radical scavenging of dietary carotenoids by the stable radical 2,2-diphenyl-1- picryl-hydrazyl. *Jr. Sci. Food Agric*. 80: 1686-1690.