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Role of total phenolic compounds in inducing hypersensitive reaction against PNRSV in peach

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Abstract
Most plants produce a broad range of secondary metabolites that are toxic to pathogens, either as part of their normal programme of growth and development or in response to biotic stress and respond with a wide array of complicated procedures which use constitutive and induced phenolic substances affecting the level of susceptibility or resistance of the host plant. Detailed studies were conducted on interactions between PNRSV and peach trees resulting in necrotic shot holes to have a better understanding of how an increase in the expression of endogenous phenolic compounds could influence resistance against PNRSV in peach trees. The studies concluded that as the temperature rises the total polyphenolic content (TPC) decreases which ultimately leads to an increase in the virus concentration in prunus necrotic ringspot virus (PNRSV) infected peach trees whereas healthy tree showed no such effect of temperature on the concentration of PNRSV.

Keywords: phenolic, compounds, hypersensitive

Introduction
India produces all stone fruits including peach, plum, apricot and cherry in considerable quantity that are mainly grown in the North-Western part of India. Peach (*Prunus persica* L.) is probably the most important stone fruit among all these and is grown worldwide. In Himachal Pradesh, due to its wider adaptability, peach can be grown in the entire hilly zone particularly in the districts of Sirmour, Solan, Kullu, Kangra, Mandi and Shimla with a total area of 5076 hectares with a production of 8045 metric tonnes (FAO, 2017) [1]. Like other fruit crops, this crop is also known to be a victim of many plants pathogens like fungi, bacteria and viruses. Such pathogens on infection in plant tissues particularly local and resistant (hypersensitive) infection show a common shift in the metabolic pattern that includes accumulation of an array of secondary substances (Phenolics, flavonoids, coumarins, terpenoids, steroids etc.). This change in spectrum of secondary substances is mainly a response to either infectious agent or to a physiological stimuli and stresses.

Besides playing a vital role in the normal development of healthy plants, temperature is also a key factor in determining the nature of plant pathogen interactions. Any major shift in environmental conditions, particularly the ambient temperature is bound to have an impact not only on plants but also on pathogens and consequently plant diseases (Li *et al*. 2013[2]; Suzuki *et al*. 2014 [3]; Ashoub *et al*. 2015 [4]). Varying temperature regimes are expected to have a direct impact on biochemical constituents in both healthy and infected plants and the most pronounced effect can be visualized in total phenolic content (TPC).

Polyphenols are plant secondary metabolites derived from the phenylpropanoid and polyketide biosynthetic pathways that features more than one phenolic ring in their basic chemical structure. Polyphenols bear antioxidant and antimicrobial property (Quideau, 2013 [5]). Accumulation of polyphenolic compounds in and around local lesions in the plant is a well-established feature of the hypersensitivity response. Naturally existing antibiotic compounds occurring endogenously in healthy plants represent inbuilt chemical barriers to protect the plants against attack by a wide range of fungal and bacterial pathogens. However, virus infection leads to necrosis of cells at and adjacent to the site of infection, where virus movement is often restricted which leads to a systemic infection, usually in the absence of necrosis and the reason behind this distinction is the significant change in the concentration of polyphenols due to virus infection. With many changes in virus-infected plants (respiration and photosynthesis), their magnitude is often related to the severity of the symptoms produced and is greatest when the tissues become necrotic (Goodman *et al*. 1986) [6]. Keeping in view the crucial role of polyphenols in imparting resistance against plant virus infection, an experiment was conducted to compare the levels of polyphenols in leaves of healthy and

PNRSV infected peach cv. July Elberta trees. Different steps followed for conducting this experiment are presented in this paper.

Material and Methods

Extraction of Polyphenols

Samples drawn from PNRSV positive trees marked earlier were brought to the laboratory. Twenty grams each of homogenized leaves, bark, petals and fruits were extracted with 80 percent aqueous methanol (200ml, methanol-water, 80:20, v/v) at room temperature for 8 hours using an electric orbital shaker. The residues and the extracts were separated by filtering through a filter paper and the residues obtained were re-extracted twice with a fresh portion of extraction solvent. The extracts recovered from three extractions were combined and excess of the solvent distilled-off in a vacuum rotary evaporator at 45 °C. The semi-solid extracts obtained were quantitatively transferred to the extraction solvent and preserved at 4 °C until used for further experiments.

Determination of Total Phenolic Content (TPC)

A colorimetric method, based on Folin-Ciocalteu reagent was used to appraise the amount of total phenolics. The process involved the mixing of crude extract (5 ml) diluted with distilled water and volume was made up to 50 ml. Further, 1 ml of diluted sample was taken and added 1.25 ml Folin-Ciocalteu reagent along with 2.5 ml Na2Co3 and final volume was made up to 25 ml with distilled water. It was further incubated at 40 °C for 20 minutes in hot water bath (Manzoor *et al*. 2012) [7]. It was then shaken on electric orbital shaker. The absorbance of the final mixture was monitored at 760nm against blank in ELISA plate reader (Microscan MS5608A) and polyphenols was calculated by using the formula:

$$\text{Polyphenol (\%)} = \frac{\text{Concentration of standard} \times \text{dilution} \times 100 \times \text{OD of sample}}{\text{OD of standard} \times \text{weight of sample taken} \times 1000}$$

Standard for the curve was made against tannic acid by diluting 200ppm solution in 25 ml distilled water. The graph was plotted on X-axis for optical density (OD) values of the sample against Y-axis for tannic acid solution from 0.2, 0.4, 0.6 up to 0.8, respectively.

Results

Peach cv. July Elberta trees were serologically indexed and infected trees were marked for drawing samples during different months of the year. Total phenolic content (TPC) was estimated throughout the year and relative concentration of PNRSV was recorded for both infected and healthy peach trees. A critical analysis of the values recorded for TPC and OD revealed that the temperature has a pronounced effect on TPC as the phenolic content followed a decreasing trend with a rise in temperature and vice-versa in case of fall in temperature as indicated by the values presented in Table 1. It was also observed that total phenolic content had a significant effect on the concentration of PNRSV in infected trees as an increase in TPC resulted in the masking of symptoms whereas a decrease in TPC led to the development of necrotic shot holes in leaves. The data presented in Table 1has also been depicted in Fig1 and Fig 2.

Table 1: Effect of temperature on total phenolic content and virus concentration in healthy and infected peach CV. July Elberta trees

Month	Temperature (^{0}c)	Total Polyphenolic Content (%)	O.D. value (A_{405}nm) Healthy Infected
January	10.4	1.447	0.098 0.201
February	15.6	1.296	0.103 0.237
March	21.2	0.233	0.100 0.859
April	27.1	0.273	0.087 0.955
May	37.7	0.224	0.093 1.112
June	29.6	0.486	0.108 0.950
July	28.5	0.504	0.090 0.256
August	27.9	0.663	0.076 0.203
September	26.6	0.732	0.088 0.180
October	25.4	0.883	0.101 0.305
November	20.6	0.986	0.076 0.289
December	17.1	1.201	0.087 0.299

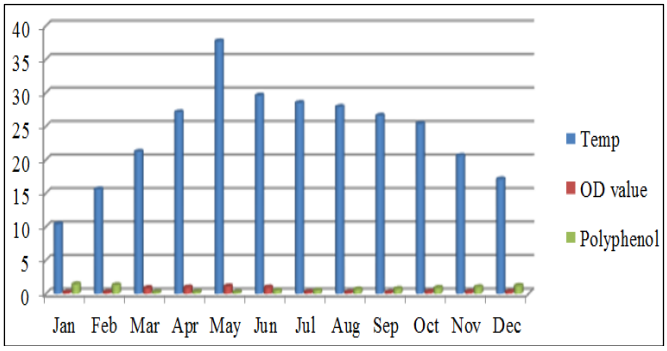

Fig 1: Relative total phenolic content and virus concentration in PNRSV infected peach cv. July Elberta As influenced by temperature

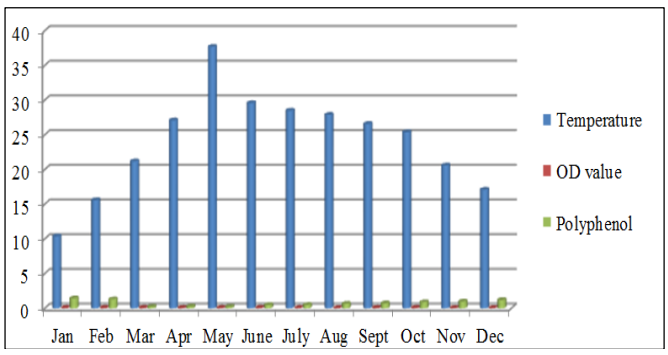

Fig 2: Relative total phenolic content and virus concentration in healthy peach cv. July Elberta as influenced by temperature

Discussion

Phenolic compounds not only result in upscaling of resistance level in virus infected plants but also contribute in enhancing the mechanical strength of host cell walls in the formation of physical barriers that prevent the spread of systemically spreading pathogens particularly viruses (Ngadze *et al*. 2012; Singh *et al*. 2014) [8, 9]. In the present investigations, the amount of total phenols was much higher during winter

months when the virus concentration was relatively low whereas during spring and early summer, necrotic shot hole symptoms were prominent and the virus concentration was at its peak. At this time, total phenolic content was almost negligible in infected peach trees. Similar trends have been reported by a number of workers in many crops (Tanguy and Martin, 1972; Meena *et al*. 2008; Siddique *et al*. 2014; Steplowska *et al*. 2015) [10, 11, 12, 13]. Higher temperatures resulted in more rapid replication of PNRSV in peach trees and facilitated the spread of the virus by weakening the plant defense response as indicated by very low levels of polyphenols in the infected trees. A number of workers also made such observations from various parts of the world (Zhang *et al*. 2012; Kiraly *et al*. 2008; Prasch and Sonnewald, 2013) [14, 15, 16].

Conclusion

Higher temperature causes a rapid growth rate with corresponding symptom development and initial accumulation of virus concentration which reduces after a certain period as the temperature lowers down. In the present study, total phenolic content was significantly higher in the leaves of healthy trees as compared to PNRSV infected peach trees. Alteration in the level of phenolic compounds in peach trees led to change in susceptibility to the virus. It can thus be concluded that reduced levels of polyphenols have a significant effect on the development of necrotic shot holes which are a direct consequence of an increase in the concentration of virus.

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