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Some Changes and Transformation in Pea (Pisum Sativum L.) Infected With Mosaic Virus

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ABSTRACT:-

Peas are distributed worldwide. Both the balanced composition (protein 20–30%, starch 20–50%, sugars 4–10%) and the negligible amounts of deleterious compounds like protease inhibitors or lectins make pea a good source of animal and human nutrition. Since pea, like the other relevant grain legumes, has the ability to undergo symbiosis with Rhizobia, protein production can be several times higher in legumes as compared to cereals. In addition, pea may well become an “industrial crop” due to some unique features of its starch, which can serve as a raw material, e.g., biodegradable plastics. It can be expected that the acreage will increase when certain breeding objectives like pathogen resistance and stress tolerance are achieved.

INTRODUCTION

Virus infection alters the entire metabolism of the host plant by changing the biochemical processes. Carbohydrate, proteins, amino acids and phenols have received considerable attention in relation to resistance in plants against diseases. During host-pathogen interaction, amino acids may act as substrates for the pathogen (Fric, 1964, Titarenko et al., 1993). Increase in the carbohydrate constitution due to severity of the disease may serve as easily metabolized carbon source for the fungal pathogen (Patil et al., 1985, Jeun and Hwang, 1991). These effects are brought-about; possibly, through the virus-induced synthesis of new proteins by the host, some of which are biologically active substances and can interfere with the normal metabolism of the host. Effect of virus pathogen on vegetative growth on plant has been recorded by several workers (Bawden, 1959, John, 1963, Farakas and Solymosy, 1965, Srivastava, 1971, and Ram et al.1984).

Based on the above informations, attempts have been made to study the changes in total carbohydrate, protein, amino acid and phenolics in pea plants infected with pea mosaic virus.

The key argument against increasing the acreage for growing peas (which is desirable not only for economical but also for ecological reasons) is the relatively low yield stability. This, in turn, reduces the grower's confidence in pea as a crop

(Hebblethwaite et al. 1985). Being a multifactorial problem, strategies to improve yield stability necessarily have to be manifold and include:

1. Improvement of harvesting characteristics: e.g., improved standing ability, concentration of the pods at the top of the plant, more pods per node, more seeds per pod and, most important, reduced pod abortion due to stress.

2. Introduction of or selection for resistance against diseases like pea wilt (Fusarium oxysporum and F. solani), powdery mildew (Erysiphe polygoni) and diseases like seedling and foot rot (Aschocyta ssp.), bacterial blight (Pseudomonas syringae pv. pisi) and common root rot (Aphanomyces eutiches). Of particular and recent importance are resistances against certain viral pathogens (PSbMV, PEV, PSV).

3. Problems concerning more sustainable techniques in controlling weeds and strategies to implement traits essential for surviving climatic stresses.

Besides improving yield and yield stability, a second objective will be to optimize the protein and starch quality of pea. Objectives in this field will include:

1. Improvement of storage protein composition by increasing the amount of essential amino acids (by addition of codons, e.g. methionine, or the introduction of heterologous storage protein genes, e.g., 2S-albumin gene).

2. Increase the percentage of amylopectin in the starch.

It is noteworthy to acknowledge that through conventional plant breeding more than 1000 varieties have been established, but only a few are of commercial importance.

To solve some of the problems, genetic engineering may provide some new and promising methods which could speed up classical breeding to further improve pea as an important crop species.

Pea Tissue Culture/Regeneration in Vitro

A major prerequisite for the application of the different methods available for introducing new or foreign genes into a given crop or to select for desirable traits in vitro is the ability to regenerate fertile plants from complex tissue or isolated protoplasts. In a first report by Gamborg et al. (1974), intact plants were regenerated via organogenesis from a macerated cell mass derived from apical meristems. Kartha et al. (1974) used the complete shoot apical meristems and obtained a somewhat higher efficiency. Malmberg (1979) was able to regenerate pea plants via organogenesis from calli derived from epicotyl explants after a time-consuming tissue culture, however, with a low frequency. Only a few genotypes responded to this protocol. Other tissues used for regeneration were immature leaflets (Mroginski and Kartha 1981), mature embryo-derived callus (Hussey and Gunn 1984), node ex plants (Griga et al. 1986), immature embryos (Natali and Cavallini 1987), cotyledonary nodes (Jackson and Hobbs 1990), hypocotyl slices (Nielsen et al. 1991), and nodal thin layers (Nauerby et al. 1991). In contrast, reports on regeneration via somatic embryogenesis are rather limited (Kysely et al. 1987; Tetu et al. 1990) and immature zygotic embryos or apical meristems seem to be the only useful explant. No convincing report is available on the regeneration of pea plants from cell suspensions, although some embryo-like structures expressing storage proteins have been obtained (Bohmer and Jacobsen, unpubl.).

RIVIEW OF LITERATURE

Plant pathogens and insect herbivores can interact when they co-exist on the same host plant: they might compete directly for plant resources or interact indirectly via induced changes in plant morphology, physiology and the activation of plant defences. These ‘tripartite’ plant-insect-virus interactions are further complicated when the pathogen is obligately dependent on the insect for its transmission. The overall interaction between the pair of species is now a combination of facilitation of the pathogen by the vector and the varying reciprocal response in the insect, and can lie anywhere along a continuum between mutualism (+, +), commensal (+, 0) and contramensal (+, -) (Hodge and Arthur 1996). It can be envisaged that there would be evolutionary pressures on the pathogen not to be antagonostic towards its insect vector and that those pathogens that modified plant biology so as to improve vector performance would subsequently be more successful in terms of their own transmission.

Various estimates suggest that aphids account for the transmission of between 25-50% of the plant viruses disseminated by insects. A number of previous field and laboratory investigations have examined the responses of aphids to infected host plants . Aphids developing on virus-infected plants have been demonstrated to show reduced, improved or no change in

individual and/or population growth rates on infected plants, depending on the system examined. It is often found that the distribution of aphids exhibits a bias towards virus-infected plants although this is not always the case (see Castle et al. 1998). There are also reports of increased production of winged alate-form progeny on infected plants, a factor liable to enhance subsequent dispersal of the plant pathogen.

Pea enation mosaic virus (PEMV) is a widespread aphid-borne virus that infects a number of leguminous plants, causing stunting and deformation of the plant and mottling and curling of leaves, and the disease can result in severe crop losses (c. 50%) in beans and peas. PEMV consists of a symbiotic mutualism between an *Enamovirus* and *Umbravirus* and is transmitted by a number of aphid species in a circulative persistent (non-propagative) manner. The virus can be acquired during access feeding periods of only a few minutes, and after a latent period the aphids can inoculate new plants in bouts of stylet probing less than 30 seconds duration.

The pea aphid, *Acyrthosiphon pisum* , is responsible for the transmission of a number of viruses affecting legume field crops, including PEMV. *A. pisum* has previously been found to show varying responses to single and multiple virus infections of clovers, the response often being dependent upon the stage of infection and severity of disease symptoms . Previously, we examined the response of *A. pisum* to PEMV infection of *Vicia faba* L. and found that although the *A. pisum* showed clear preferences for settling on the yellow foliage of virus-infected plants there were no effects on their growth, reproductive output or production of winged progeny.

The outcome of many non-trophic interactions between pairs of species can be dependent upon the biotic and abiotic environmental conditions in which the interaction occurs. In particular, the occurrence of interspecific facilitation is often found to be more prevalent when conditions are marginal for at least one of the species involved, and some abiotic or biotic stress is ameliorated by one species to the benefit of the other. It has been suggested that plant pathogen-induced facilitation of insect herbivores is more likely to occur when the uninfected host-plant possesses high resistance or is in some way an inferior resource to the insects. *Vicia faba* L. is considered one of the highest quality host plants for *A. pisum* due to its low aphid resistance, and it is possible that virus-infection could not improve (or degrade) the resource sufficiently to induce observable changes in aphid performance.

MATERIALS AND METHODS

The effect of virus infection on certain aspects of changes of the host was studied. Ten days old seedlings of the test plants were taken into two groups of 120 each. The first group of plants was left as healthy control after inoculation with only neutral phosphate buffer, while those of the second group were inoculated with pea mosaic virus. Twenty plants of each group were harvested on 30,35,40 and 45 day of inoculation.

One gram of fresh leaves was macerated with 5 ml of chloroform, methanol and water in ratio 1:1:1. The extract was filtered, through Whatman filter paper No.1and the residue was extracted with 5ml. of extraction solvent. The extract was pooled together to obtain a volume of 10ml. extract. On keeping the mixture for some time the lower layer clearly settled down (chloroform layer) and upper layer (methanol & water layer) was separated. Then, the lower layer consisting of chloroform extract was further evaporated to dryness. The precipitate was redissolved in methanol, water (1:1) total phenolic content. Total phenol was estimated spectrophotometric ally by Prussion blue method at 700nm as modified by Graham (1992).

The upper layer was utilized for the estimation of total carbohydrate (Sadasivam and Manikam, 1996), total protein (Lowry et al., 1951 and Bergersen, 1980) and total amino acid (Yemm and Cocking, 1955). The solvent was evaporated to dryness under vacuum and redissolved in 5ml. of 0.6mM phosphate buffer (pH 6.2). From this extract carbohydrate, amino acid and protein were estimated as per the standard protocols (Danial, 1991).

SUMMARY

Primary and secondary metabolites viz.carbohydrates, proteins, amino acids and phenols have received considerable attention in relation to resistance in plants against diseases. Total carbohydrate, amino acid and protein in different parts of infected plants by pea mosaic virus and healthy plants were carried out. Root nodulation and root abnormality were also observed. Carbohydrate amount of healthy and infected leaves, stem and root increased, with the increase in growth of plants. Amino acid amount in different counter parts of diseased plants were higher as compared to healthy. Protein content was always higher in infected plant parts (leaf, stem and root) than their healthy counterparts, but maximum protein content was found in diseased leaves followed by root and stem) The results revealed, that pea mosaic virus infection was also found to reduce the number, size and fresh weight of root nodules. The number of secondary roots and nodules decreased significantly in diseased plants. The phenolic content decreased with the increase in infection in plants.

Some prior investigations into plant virus-aphid interactions have suggested that increased alate production on diseased plants is caused by physiological changes in the host plant, such as modification of nitrogen metabolism and changes in amino acid profile of the phloem sap. Poor nutrition seems an unlikely stimulus for alate production in the system used in this experiment, as the results of the aphid performance assays suggested that PEMV-infected peas were, if anything, superior hosts compared to control plants. Also, there was no increase in alate progeny when using a single founding *A. pisum* , indicating that infection of the plants *per se* (and any associated nutritional differences) did not directly induce production of winged forms. When multiple founding aphids were housed in clip cages the proportion of alate progeny on infected plants was almost double that observed on the controls. In terms of numbers of aphids, levels of crowding within the clip cages would be very similar in the control and PEMV-treated plants: the density of founding adults was equal, overall nymph production was not affected and any virus-induced increases in aphid size would only be slight within the short duration of the assay. Thus it appears that a combination of factors is required to produce the high numbers of alate progeny observed on the PEMV-infected plants, the effects of maternal crowding being somehow heightened when present in conjunction with host plant infection. The effects of crowding can be accentuated by higher contact rates resulting from increased restlessness of aphids, although this behavioural response was not examined explicitly

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