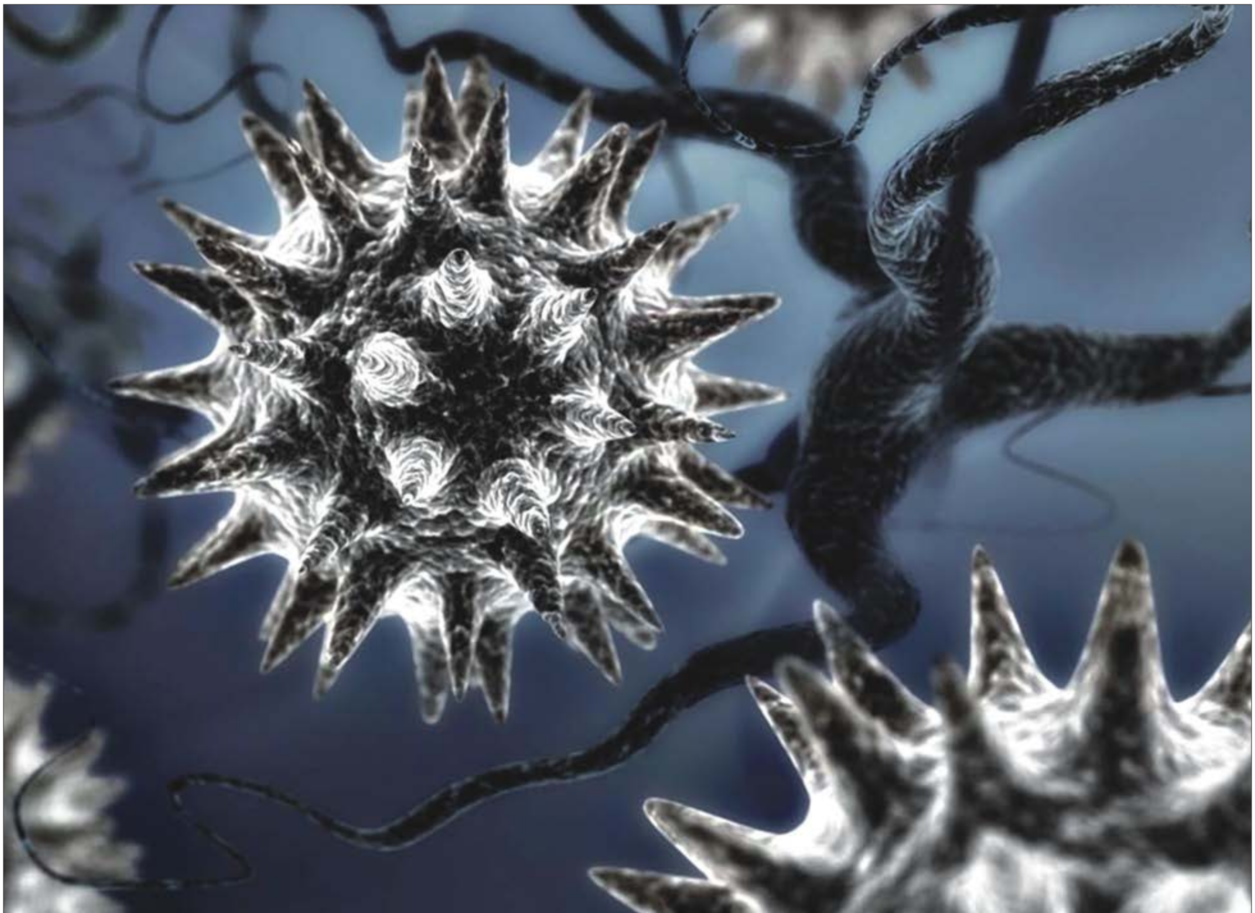

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Influence of Soybean Mosaic Virus Infection on Carbohydrate Content in Nodule of Soybean (*Glycine max* L. Merr.)

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Abstract: The present investigation was conducted to see the effect of Soybean Mosaic Virus (SMV) infection on carbohydrate content in nodules of soybean. Observation made on the carbohydrate (reducing sugar, non-reducing sugar and starch) content of nodules in soybean mosaic virus infected soybean indicated the reduction of all the contents in comparison to nodules in the healthy plants. Reducing sugar ranged from 2.0 to 3.39 mg g^{-1} dry weight in healthy and in diseased it ranged from 1.86 to 3.00 mg g^{-1} dry weight and in non – reducing sugar ranged from 1.4 to 2.75 mg g^{-1} dry weight and in healthy and in diseased it ranged from 1.3 to 2.45 mg g^{-1} dry weight. The results were significant in the case of non-reducing sugar. The reducing and non-reducing sugars were estimated by the colorimetric method. The alcohol extract was clarified and residue left on the filter paper was returned to the extracting flask, dried at 65°C and preserved for starch analysis.

Key words: Sucrose, SMV, legumes, nitrogen fixation, amino acid, protein

INTRODUCTION

Soybean (*Glycine max* L. Merr.) an important leguminous crop possesses nodules on its root as miniature nitrogen fixing factories. It has been in cultivation in China and Japan for thousands of years. Soybean is being cultivated in the northern hilly regions from Kashmir to Nagaland in parts of Punjab and Central India in small packet primarily as pulse crop. In recent years soybean cultivation has assumed much important in hills and tarai- bhabar regions of Uttar Pradesh, Madhya Pradesh, Punjab and Maharashtra. View from oil and protein contents (40% protein and 20% oils) soybean is far superior to rice, wheat, maize, groundnut and other pulses probably the best and cheaper source of proteins. Soybean mosaic virus (SMV) is an aphid- and seed-transmitted virus that infects soybean (*Glycine max* L. Merr.) plants and causes significant yield losses. Seed-borne infections are the primary sources of inoculum for SMV infections. Transmission of SMV through seed ranged from 0 to 43% and isolate-by-soybean line interactions occurred in both transmission rates and percentages of mottled seeds (Domier *et al*., 2007). More than one SMV strains may be present in the same soybean field and plants may be infected simultaneously by multiple strains. Interaction among different strains may be complementary or antagonistic. Recently, Chen *et al*. (2004) reported the reactions of soybean to simultaneous double inoculation with different SMV strains and demonstrated that mosaic strains predominated over necrotic strains and both predominated over avirulent strains. Virus diseases of soybean have become increasingly prevalent and damaged this crop in different parts of the world. In India,

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three different virus diseases affect this crop (Anonymous, 1971), of which Soybean mosaic is widely prevalent in Uttar Pradesh. Despite comprehensive changes virus diseases induce in host physiology (Bawden, 1964). The changes in carbohydrate content in virus infected plant tissues have been reported by several workers (Sultana *et al.*, 1998; Mali *et al.*, 2000; Shalitin and Shmuel, 2000; Adomako and Hutcheon, 2008; Lehrer *et al.*, 2007; Goodman *et al.*, 2008; Singh and Shukla, 2009). Thind *et al.* (2005) and Matthews (1970) pointed out that some viruses appear to have effect on carbohydrates in the leaves, while others may alter both their rate of synthesis and rate of translocation. Soybean crop is important for oils and protein contents it can play a vital role in bringing nutritional gap and improving the health of our people. Various carbohydrate fractions viz., reducing sugars, non - reducing sugars and starch were estimated.

MATERIALS AND METHODS

The experiment was done during March 2009 to September 2009 at H.C.P.G. College, Varanasi, India. Two lots of 30 plants were taken. One lot of 30 plants was inoculated with SMV at first trifoliate stage while the second lot of 30 plants was kept as control. The plants were raised in an insect proof glass house, which was fumigated regularly to keep it free from insects. The environmental conditions, of glass house were photoperiod 11 L: 13D; temperature 25-30°C during day and 20-25°C during night throughout the experimental period. Nodules from both healthy and diseased plants were harvested separately on 20, 30, 40, 50, 60 and 90 days after inoculation. For measurement of the carbohydrate fractions, nodules from five plants in each treatment at each interval were taken and averages expressed as mg/g dry weight of the material. Nodule samples were dried at 65°C until constant weight, powdered and stored in a dessicator. One gram of material was extracted in different grades of alcohol starting from 80% and followed by 60, 40, 30% and finally in water. The initial extraction in 80% alcohol was accompanied by boiling over water bath using a reflex condenser for half an hour and the extraction was continued over night at room temperature (28-30°C). The supernatant was decanted into a flask. The residue left was further extracting in lower grades of alcohol two drops of toluene were added to prevent possible microbial activity. After last extraction in water, the solution was filtered through whatman No. 42 filter paper. The alcohol extract was clarified according to the method described in AOAC (1965) and used for estimation of sugars. The residue left on the filter paper was returned to the extracting flask, dried at 65°C and preserved for starch analysis. The reducing and non-reducing sugars were estimated by the colorimetric method of Somogyi (1952) using Nelson's chromogenic reagent. The starch was estimated according to the method described by McCready *et al.* (1950).

RESULTS

Reducing sugar (Fig. 1) ranged from 2.0 to 3.39 mg g^{-1} dry weight in healthy and in diseased it ranged from 1.86 to 3.00 mg g^{-1} dry weight and in non-reducing sugar (Fig. 2) ranged from 1.4 to 2.75 mg g^{-1} dry weight and in healthy and in diseased it ranged from 1.3 to 2.45 mg g^{-1} dry weight content of nodules of both healthy and diseased samples increased throughout the experimental period. The sugar content (reducing and non-reducing) in nodules of diseased plant is less than the healthy ones. This reduction is significant in case of the non- reducing sugar but non significant in the case of the reducing sugar (both at 5% level statistically).

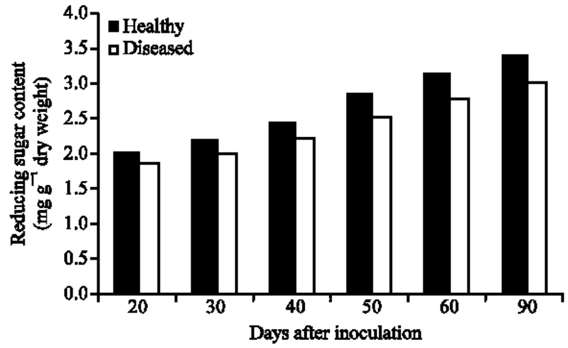


Fig. 1: Effect of Soybean mosaic virus infection on Reducing sugar content in nodules of soybean

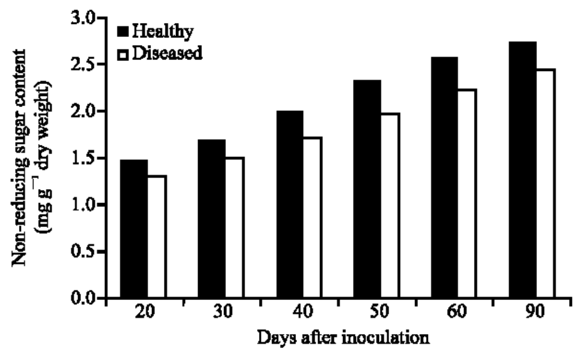


Fig. 2: Effect of Soybean mosaic virus infection on Non-Reducing sugar content in nodules of soybean

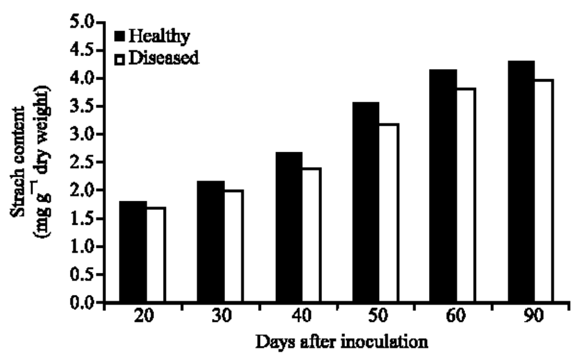


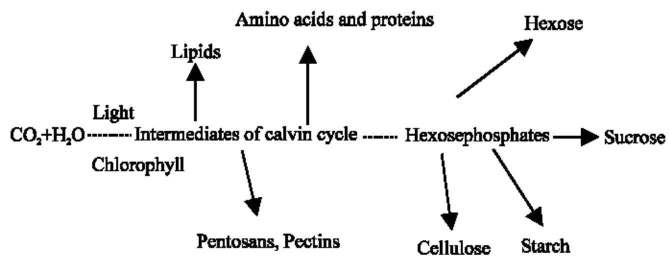
Fig. 3: Effect of Soybean mosaic virus infection on starch content in nodules of soybean

The starch content of nodules in both healthy and diseased samples increased with the age of the plant (Fig. 3) but the amount was always less in diseased samples (1.7 mg g^{-1} dry weight to 3.98 mg g^{-1} dry weight) than healthy ones (1.81 mg g^{-1} dry weight to 4.29 mg g^{-1} dry weight) throughout the experimental period. The findings are not significant when analyzed statistically (at 5% level).

In the present investigation various carbohydrate fractions (reducing sugar, non-reducing sugar and starch) studied show that the infection by SMV depleted the reducing sugar, non-reducing sugar and starch from the nodule tissues of the infected soybean plants.

DISCUSSION

The reduction in carbohydrate content in nodules of diseased plants may be due to the reduction in carbohydrate content of leaves of diseased plant due to SMV infection. Leaf in the plant is considered as seat of carbohydrate synthesis and synthesized carbohydrates from it are transported to other parts of the plant like stem, root and nodules etc. Any disturbance in carbohydrate synthesis or its oxidation may directly influence the carbohydrate content in different parts of a plant including nodules. The product of photosynthesis in plants are the hexose, sucrose and starch. To maintain a



(Levitt, 1969) normal production of carbohydrate, it is essential that the various reactions shown in the scheme above proceed smoothly. In the photosynthesis scheme shown earlier, there is the synthesis of amino acids and proteins from the intermediary products. The necessary carbon skeletons for such products are derived from the intermediary products. It has been observed that the total nitrogen content is greater in SMV infected leaves than that of uninoculated healthy leaves (Table 1).

Due to virus infection, there is an increased demand for abnormal protein production required for the rapid synthesis of virus particles. For this purpose there has to be increased diversion of assimilated carbon towards protein synthesis resulting in the decreased production of carbohydrates in leaves. This reduction of carbohydrate directly influenced the carbohydrate content of nodules. The decrease in carbohydrate content in pigeon pea sterility mosaic virus affected plants was observed by Narayansawamy and Ramakrishnan (1965) and explained it on the basis of a possible retardation and or faster breakdown of carbohydrates due to increased respiration and conversion of carbohydrates into amino acids which are ultimately used for protein synthesis. Ryzhkov (1943) also observed that in tobacco leaves affected with TMV, accumulation of the virus coincided with an expenditure of carbohydrate and increased protein nitrogen and total nitrogen. Bolas and Bewley (1930) suggested that the action of the virus on the starch was the production of acids which (1) react with nitrogen to form protein, (2) attack the chlorophyll causing mottling and (3) affect respiration with formation of carbon dioxide. Jeyarajan and Ramakrishnan (1968) pointed out

Table 1: Total nitrogen content percent dry weight in leaves of soybean plants at various intervals

	Total nitrogen content after SMV inoculation (days)					
Treatment	20	30	40	50	60	90
Healthy	3.6	3.8	4.1	4.3	4.4	4.6
Diseased	3.9	4.2	4.5	4.7	4.8	5.0

that the infection by the virus not only reduced the rate of synthesis of starch but also enhances the breakdown of starch into sugars which after conversion into phosphate esters serve as substrates for the enhanced rate of respiration. Diener (1963) reported that the decreased photosynthetic activity, coupled with the increased respiratory rates generally observed in virus infected leaves, should lead to decreased concentrations of assimilate, i.e., carbohydrate. Thus it is assumed that the reduced carbohydrate content of leaves may be responsible for this reduced carbohydrate content of nodules in diseased plants. Another possibility is the hindrance in translocation of carbohydrate from leaves to nodules by SMV infection. Barton-Wright and McBain (1933) observed a reduced translocation of carbohydrate in para crinkle infected potato plants. In diseased nodules there is possibility of faster breakdown of available carbohydrate in amino acids. Starch, sucrose and reducing sugars were found to accumulate in infected tissues. Translocation of photosynthates (mainly as sucrose) to the stem and root system, as estimated by the overnight loss of carbohydrates from the leaves and by $^{14}CO_2$ tracer experiments, was as efficient in the infected plants as in the healthy (Adomako and Hutcheon, 2008). Starch and sucrose concentration in green leaf tops of mature (18 months old) was the highest in asymptomatic plants followed by symptomatic and then by virus-free plants. Carbohydrates increased during daytime by 5-20%. The carbohydrate concentration in leaves of young, 6 months old plants was much lower than in mature plants and it increased to 500% during daytime (Lehrer *et al*., 2007). Source leaves infected with CMV were characterized by high concentrations of reducing sugars and relatively low starch levels. The altered level of carbohydrates was accompanied by increased respiration and decreased net photosynthetic rates in the infected leaves (Shalitin and Shmuel, 2000). Carrot motley dwarf virus increased fructose, glucose and sucrose concentrations in carrot leaves, but in petioles and roots sucrose increased while fructose and glucose decreased: more sugar accumulated in plants infected late than early. Cereal Yellow Dwarf Virus (CYDV) increased fructose, glucose, sucrose and four fructosans in leaves of oats, an avirulent strain more than a virulent one. Virulent strains of BYV and CYDV apparently decrease photosynthesis more than avirulent ones (Goodman *et al*., 2008). The carbohydrate content was found to be lower in infected tissue (11.4, 9.3 and 8.2% for reducing sugars, non-reducing sugars and starch, respectively) in *Carica papaya* L. (Singh and Shukla, 2009).

CONCLUSION

The decrease in carbohydrate content in nodules of diseased plants may be due to the possible reduction and (or) faster breakdown of carbohydrates due to increased respiration and conversion of available carbohydrates into amino acids, which are ultimately used for synthesis of protein.

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