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RESEARCH ARTICLE

Effect of Cowpea golden mosaic virus infection on leghaemoglobin in nodules of cowpea (*Vigna unguiculata*)

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Abstract Pulses are a great source of proteins but pulse crops are heavily damaged by various pathogens in agricultural fields. Cowpea (*Vigna unguiculata*) is an important leguminous crop belonging to the family fabaceae cultivated in all part of India and worldwide for green pods as pulse. Being leguminous crop the roots of plants have nodules which help in nitrogen fixation and improving fertility of soil. A variety of viruses are disseminated by seeds of infected plants to long distances. Localized spread from weeds or seed-infected crop plants is sometimes accomplished by vectors. The extent of crop damage is usually intrinsic part of plant genetic susceptibility to viruses (and virus complexes) or its sensitivity to infection. Virus infection adversely affects all the parts of plant including nodules of roots. Present study deals with the leghaemoglobin content and number of Rhizobium in root nodules of Cowpea Golden Mosaic Virus infected plant. It was found that infected plant's nodule have lowered amount of leghaemoglobin content and they harbor less number of bacteria when compared with healthy control plants and this adversely affects nitrogen fixation by infected plant.

Keywords: Cowpea, Cowpea Golden Mosaic Virus, Nodulation, Leghaemoglobin

Introducation

Cowpea [*Vigna unguiculata* (L.) Walp] is a grain legume grown in pockets of Punjab, Haryana, Delhi, and West UP and some areas of Rajasthan, Karnataka, Kerala, Tamilnadu, Maharashtra and Gujarat. Cowpea is also known as Black eye pea or southern pea. This crop is drought hardy in nature; it can be used as food, feed, fodder, forage, green manure and Soil improving cover crop. Cowpea is highly nutritious, and a good source of quality protein, with protein content of about 22-24% with essential amino acids such as lysine, leucine and phenylalanine. Cowpea is used as intercrop for its ability of nitrogen fixation; it can be sown by pairing the rows of main crops and taking one or two rows of cowpea in between two paired rows of either of pigeonpea, maize and sorghum.

Among the various pathogens effect of viruses can be devastating and they are major constraint in yield of crop and nitrogen fixing ability of cowpea. Cowpea golden mosaic virus affects all aspects of metabolism and growth of cowpea plant.

The survey was conducted for studying occurrence and severity of disease during March-April and August - September (twice in a year) in peri-urban areas of Gorakhpur villages. Pusa Komal variety was found to be susceptible to virus disease and showed yellow mosaic and chlorotic mottle symptoms on leaves and general retardation in growth was seen in all diseased plants.

Materials and methods

To estimate the yield losses, cowpea seedling were raised in thirty earthern pots, with three seedlings in each pots. Five of the pots were left un-inoculated while, remaining pots were inoculated with the cowpea golden mosaic virus by feeding whitefly under controlled conditions. It was observed that the Cowpea Golden Mosaic Viruses was the major constraints in increasing its production and wide spread cultivation in Gorakhpur region.

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The plants were inoculated at different time interval to assess the effect of infection on Cowpea plant and different criteria were taken to study the impact of infection viz - Number of flowers and Pods, pod size / Plant, Number of seeds / pod, Seed Size / Plant, Seed weight (100 grain), Yield / plant and Percent decrease over healthy plants and observations are presented in Table 1.

It was observed that virus infection has decreased the number of flowers, pods, seed, and length of pod and weight of seed in comparison to their healthy counterparts (Table 1). Reduction in the number of seeds flowers, pod size and weight was more is early inoculated plants than the mid and late inoculated ones.

Virus infection adversely effects number, size and weight of nodules. Virus infection has influenced the nodulation ability in cowpea. Nodules of infected cowpea plants have reduced number, fresh weight and volume than their comparable healthy plants. The percentage of reductions was increased with the age of the plants. Number, fresh weight and nodules also increased with increased age of the plants both in healthy and infected plants. (Shail pande 2015)

Present study deals with effect of virus on leghaemoglobin content of nodule. In plants infected with cowpea golden mosaic virus the nodule colour was not showing striking difference from healthy control plant but hemoglobin content was significantly different from control healthy plant.

For estimation of leghaemoglobin content, nodules from healthy and diseased plants of cowpea variety Pusa Komal were collected from plants grown in green house conditions in an insect proof chamber, where usual precautions were taken to keep the plants free from insects and nematod infection. Nodules were collected on 21, 28, 35, 42, & 49th day after inoculation with cowpea golden mosaic virus by feeding whitefly under controlled conditions. Nodules from healthy and diseased were carefully picked from roots after washing roots, then collected nodules were washed again using 30 mesh screen. Then nodules were dried and stored in plastic bags for freezing before extraction of leghaemoglobin from nodules. Then frozen nodules were crushed in 5ml of 0.1 N KOH, then centrifuged at10000 rpm for 10 minutes, supernatant was taken and $Na_2S_2O_4$ was added for reduction, after 10 minutes of reaction, reading of supernatant was analysed for spectrophotometry at 537, 557, 577 nm method reported By Tu, ford and Grau (1970). Similarly blank was prepared without nodule extract. Value of leghaemoglobin content was calculated by following formula

Leghaemoglobin content in O.D. = OD557 – ½ (OD537 + OD577)

Results and disscussions

Cowpea plants infected with cowpea golden mosaic virus showed a general decrease in all aspects: number of trifoliates, flowers, pods, seeds per pod and seed weight. Nodules of infected plants were less in number, size and weight as compared tp healthy control plant.

Healthy and infected nodule samples with the age of the plants were analysed. Although general increase in leghaemoglobin content is evident in both healthy and infected plants. It was found that nodules of healthy plants had higher leghaemoglobin content than those of diseased ones. Tu, Ford and Grau (1970) reported that the nodules of healthy soybean plants had the higher leghaemoglobin content than those of Soybean mosaic virus diseased nodules.

Tobbaco Ring Spot Virus-infected and

Figure1: Effect of CpGMV infection on Leghaemoglobin content in healthy and diseased nodules of *Vigna unguiculata*

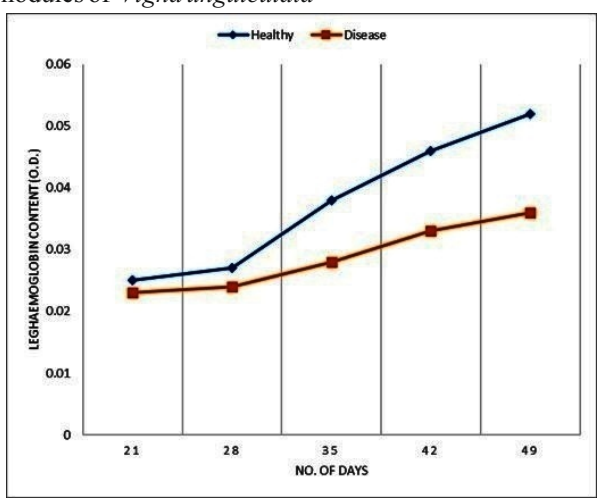

Table 1-Effect of CpGMV infection on the yield of Cowpea

Sl. No.	Changes observed	Healthy Control	Early Inocul.	%decrease over healthy	Mid Inocul-ation	%decrease over healthy	Late inocul.	%decrease over healthy
1.	Average Number of flowers/plant	19.95	7.15	64.16%	14.85	25.56%	16.05	19.54%
o2.	Average Number of Pods/Plants	17.20	5.75	66.56%	10.65	38.08	12.35	28.19%
3.	Average Number of Seeds/Pods	5.95	2.85	52.10%	3.45	42.01%	4.30	27.73%
4.	Average length of pod/plant	3.15	1.10	65.07%	2.05	34.92%	2.55	19.04%
5.	Average Size (Volume) of seed/plant (ml.)	0.28	0.15	46.42%	0.19	32.14%	0.22	21.42%
6.	100 grain weight	23.5	14.2	39.57%	17.8	24.25%	20.2	14.04%

non-infected soybeans grown in the glasshouse were studied for various aspects of growth including leghemoglobin content, which showed 3% decrease during the bloom and early pod stage but in later stages of pod-fill and mature-pod stage leghaemoglobin content increased by 25% reported by Orellana *et al.* (1978).

Rao *et al.* (1987) and Rao and Shukla (1988) reported lesser leghaemoglobin content in pea root nodules infected with cucumber mosaic virus and sesbania mosaic virus, respectively. Urdbean Mosaic Virus infection lowered the leghaemoglobin content in the nodules of *Vigna mungo* plants grown from *Rhizobium* treated seed reported by Singh *et al.* (1985).The effect of common bean mosaic virus infection on leghaemoglobin content and nitrogenase activity was studied by Chaudhary *et al.* (1987) on mungbean, and it was found that both nitrogenase activity and leghaemoglobin content decreased due to virus infection. Sharma and Varma (1988) observed similar results, wherein leghaemoglobin content was reduced by Cowpea chlorotic spot virus infection on cowpea plants. Patil and Sayyad (1991) under took the study of leghaemoglobin content in cowpea nodules as influenced by virus-rhizobium interactions. They reported that virus infection reduced the leghaemoglobin content substantially. They also reported that virus *Rhizobium* interactions have reduced the leghaemoglobin content in the nodules of cowpea plant.

Gomaa *et al.* (2006) studied effect of Broad bean mottle virus on faba bean and reported that number, size and leghaemoglobin content was reduced in Broad Bean Mosaic Virus inoculated plants. Taiwo *et al.* (2014) studied interactive effect of *Rhizobium* inoculation and viruses such as Cowpea aphid borne mosaic virus, Southern bean mosaic virus, Cowpea yellow mosaic virus and Black eye cowpea mosaic virus on cowpea cultivars and observed that difference in nodule number and parameters were not significantly different from control except in black eye cowpea mosaic virus infected plants who showed 55-66% reduced nodulation with or without rhizobia inoculation.

The decrease in leghaemoglobin content in nodules of virus infected cowpea plant is probably due to metabolic disturbance in the host plant caused by Cowpea Golden Mosaic Virus infection reported by Manil (1958). It is also possible that interaction between the virus and *Rhizobium* affected the leghaemoglobin content in the Cow-Pea plant.

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