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# Response of a Collection of Pumpkin Breeding Lines to Viruses

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## Abstract
Virus diseases are important problem for production of pumpkin in the world. Among the diseases, watermelon strain of *Papaya ringspot virus, Zucchini yellow mosaic virus*, *Cucumber mosaic virus* and *Watermelon mosaic virus* have been reported occurring in field growing pumpkin. To elucidate resistance response of pumpkin, twenty six pumpkin breeding lines were evaluated in the experimental field of Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU), Salna, Gazipur, to assess their response to viral diseases and horticultural qualities under natural condition during November 2010 to May 2011. Based on the visual observation of the test lines, seven (Pk13-1-1, Pk20-2-1, Pk02-2-1, Pk19-4-1, Pk54-4-12, Pk01-10-9-4 and Pk106) were showed highly resistant, four (Pk31-2-4, Pk07-4-7, Pk04-7-12-3 and BARI mistikumra 2) resistant, four (Pk55-2-2, BARI mistikumra1,Pk101 and Pk107) moderately resistant, 10 moderately susceptible and one (Pk05-1-2) susceptible against pumpkin viruses. Virus incidence and severity (Percent disease index) of test lines ranged from 0.0 to 79.9% and 0.0 to 83.3%, respectively. Four viruses were detected in those of symptomatic pumpkin leaves by Double antibody Sandwich ELISA (DAS-ELISA) techniques. Out of 26 test lines, five (Pk55-2-2, Pk05-1-2, BARI mistikumra1, BARI mistikumra 2 and Pk101) were positive to *Papaya ringspot virus* (PRSV-W); five (Pk05-4-1, Pk05-8-2, Pk75-1, Pk07-4-7 and Pk102) *Zucchini yellow mosaic virus* (ZYMV), two (Pk34-4-3 and Pk67-1-9) *Cucumber mosaic virus* (CMV), and only one (Pk105) *Watermelon mosaic virus* (WMV2). ELISA technique for assessment of virus response showed 13 lines were completely free from all of these four poty viruses. On the other hand, visual assessment technique for virus response demonstrated seven lines were completely free from viruses. It is interesting to note that visual assessment indicated six lines showed virus infection but serologically they were free from viruses suggesting these lines might be infected by other poty viruses. Significant variation was observed in the breeding lines for horticultural qualities relative to yield, yield contributing characters, fruit skin color, fruit shape. Considering virus reaction and horticultural qualities, 15 lines (Pk13-1-1, Pk20-2-1, Pk02-2-1, Pk19-4-1, Pk54-4-12, Pk01-10-9-4, Pk31-2-4, Pk04-7-12-3, Pk07-4-7, Pk55-2-2, BARI mistikumra1, BARI mistikumra 2, Pk101, Pk106 and Pk107) were selected for their improvement for virus disease resistance.

## 1. Introduction

Pumpkin (*Cucurbita moschata* Dutch. ex Poir under family Cucurbitaceae) is a very

popular vegetable in many tropical and sub-tropical countries. In Bangladesh, it ranks next to brinjal and radish in area under cultivation 25,235 ha and production 3, 41,000mt (BBS 2012). It is very nutritious due to high content of vitamin A and can play a vital role in meeting the vegetable shortage and nutritional problem.

Viruses are the most important pathogens of cucurbits (cucumber, water-melon, melon and pumpkins) belonging to the Cucurbitaceae family. More than 30 infectious viruses causing destructive symptoms and considerable economic losses were reported on these plants (Zitter *et al.* 1996). Their occurrence, spreading, intensity of infection and destructiveness depend on complex interrelations between the virus, its host plant, the vectors and the environment. Due to virus infection carotenoid levels of pumpkin reduces in host plants as reported by Sreenivasulu *et al.* (1989) and Muqit (1995), which is the most important quality of pumpkin.

Viral diseases cause important economic losses throughout the world. Most commercial pumpkin varieties are susceptible to the viral pathogens. So far there is a no resistance recommended variety of pumpkin in Bangladesh. Potyviruses form the largest and the most economically significant group of plant viruses (Riechmann *et al.* 1992). Severe losses in pumpkin production areas are due to potyvirus infection, including *Watermelon mosaic virus* (WMV), *Zucchini yellow mosaic virus* (ZYMV) and *Papaya ringspot virus* (PRSV) (Davis and Mizuki 1987, Somowiyarjo *et al* 1993).

Spraying of chemicals to prevent the buildup of vector population is not very effective because a single viruliferus vector is enough to cause infection. It is neither possible nor feasible to apply the pesticides continuously because of health hazard, phytotoxicity, environmental pollution and huge cost. So, to develop resistant varieties is one of the most promising methods to control viral diseases. Besides, control measures by cultural practices including the use of plastic mulches or oil sprays may provide temporary protection, but they are not sufficient to prevent significant economic loss. It was thus imperative to seek source of resistance to the disease in the form of *Cucurbita moschata*. The present investigation was conducted to search for sources of resistance to pumpkin viruses in pumpkin breeding lines.

## 2. Materials and Methods

### 2.1. Growing of Pumpkin Lines

The experiment was conducted during November 2010 to April 2011. The test entries consisting of 26 selected pumpkin lines were used in the present experiment. They were collected from Vegetable division, Horticultural Research Centre (HRC), Bangladesh Agricultural Research Institute (BARI), Gazipur. Twenty to twenty eight days old seedlings of the 26 test entries, earlier raised in polybags, were planted individually in pits of 45 cm x 45 cm x 40 cm sizes in unit plots of 2.0 m and 2.0 m. There were 78 plots with 3 replications and each plot contained 4 pits for each of the test entries. Standard cultural practices and recommended doses of fertilizers were applied. Bait traps were placed in field for controlling fruit flies (Nasiruddin and Karim 1992).

### 2.2. Measured Traits

The experiment was laid out in randomized complete block design (RCBD) with three replications. The virus incidence, severity of virus diseases in experimental fields was recorded through frequent investigation. Viruses were identified studying visible symptoms followed by serological test.

Numbers of plants in each plot showing virus disease-like symptoms were recorded and the disease incidence, which measures the extent of propagation of a disease within a given field (Agrios 2005), was also estimated by using the following formula:

$$\%\ \text{Disease incidence} = \frac{\text{Number of diseased plant (or parts)}}{\text{Total number of plants (or parts) observed}} \times 100\%$$

According to Begum and Khan (1996), the lines were graded as different degrees of susceptibility and resistant based on disease incidence viz. highly resistant (HR=0.0% disease incidence) resistant (R=0-25% disease incidence), moderately resistant (MR= 26-50% disease incidence), moderately susceptible (MS= 51-75% disease incidence) and susceptible (S= 76-100% disease incidence).

Disease severity was expressed in percent disease index (PDI). The PDI was computed by using a standard formula (Paper *et al.* 1996) as given below:

$$\text{PDI} = \frac{\sum \text{Disease grade x number of plants in grade}}{\text{Total number of plants x highest disease grade}} \times 100$$

The severity of different virus diseases of pumpkin was indexed on a 0-5 scale, where 0 = no visible symptoms, 1 = slightly mosaic on leaves, 2 = mosaic patches and/or necrotic spots on leaves, 3= leaves near apical meristem deformed slightly, yellow, and reduced in size; 4= apical meristem with mosaic and deformation, and 5= extensive mosaic and serious deformation of leaves, or plant dead (Xu *et al*. 2004). Fully ripen fruits were harvested and data on fruit yield, yield contributing characters, fruit skin color fruit shape were recorded.

### 2.3. Identification of Viruses

Pumpkin plants grown in the experimental field were checked at 55 days after transplanting. The recorded symptoms include mosaic, leaf curling, chlorosis, shoe-sting,

leaf distortion, fern leaf and smaller leaflets of plants. Individual plants showing visible symptoms of virus diseases were recorded. Photographs of the symptoms were taken and compared with standard literatures (Zitter *et al.* 1996). Viruses were identified by serological detection. Samples of virus infected leaves were transported to the lab and detected by DAS-ELISA (Double Antibody Sandwich Enzyme Linked Immunosorbent Assay). Four antisera were used for virus detection namely *Papaya ringspot virus* (PRSV-W), *Zucchini yellow mosaic virus* (ZYMV), *Watermelon mosaic virus* (WMV2) and *Cucumber mosaic virus* (CMV).

### 2.4. Statistical Analysis

Data were analyzed using MSTAT-C computer program. Wherever necessary, data were transformed following appropriate methods before ANOVA. Means were compared according to Duncan's Multiple Range Test (DMRT) at 5% level of significance.

## 3. Results and Discussion

### 3.1. Virus Incidence and Severity of Different Viruses in Pumpkin Breeding Lines

Data on the disease incidence and severity are presented in Table 1. Remarkable variations were found among different lines ranging from 0.0 to 79.9% and 0.0 to 83.3%, respectively under natural infected conditions.

Twenty six lines were tested in the present investigation and exhibited wide variations in their response to different virus incidence. No virus like symptom (0.0% disease incidence) was evident in seven lines viz. Pk13-1-1, Pk20-2-1, Pk02-2-1, Pk19-4-1, Pk54-4-12, Pk01-10-9-4 and Pk106 which were graded as highly resistant (HR). Four lines (Pk31-2-4, Pk07-4-7, Pk04-7-12-3 and BARI mistikumra 2) showed 12.3-23.2% virus incidence and were graded as resistant (R). Another four lines (Pk55-2-2, BARI mistikumra1, Pk101 and Pk107) showed 31.0-49.3% incidence and were graded as moderately resistant (MR). Ten lines (Pk05-8-2, Pk34-4-3, Pk75-1, Pk67-1-9, Pk37-1-4, Pk61-1-1, Pk05-7-11-8, Pk102 and Pk105) showed 50.8-72.2% virus incidence which was graded as moderately susceptible (MS). Only one line (Pk05-1-2) showed 79.9% virus incidence and was graded as susceptible to pumpkin viruses.

No (0.0%) virus severity was noticed in the seven HR lines. Severity of virus diseases in terms of percent disease index (PDI) in other 19 test lines of pumpkin varied from 1.7 to 83.3%. Maximum PDI was observed in Pk05-1-2 (83.3%), which closely followed byPk75-1 (73.3%). On the other hand, the minimum PDI observed in BARI Mistikumra2 (1.7%) proceeded by Pk07-4-7 (2.5%). Out of 312 plants of 26 lines 131 plants were found healthy or completely free from virus infection.

The incidence of different cucurbit viruses in pumpkin and other cucurbits have been studied by several researchers (Masud 1995, Kristia *et al.* 2002, Strange *et al.* 2002, Papayiannis *et al.* 2005, Coutts and Jones 2005, Köklü and Yilmaz 2006, Bananej and Vahdat 2008, Masud *et al.* 2009). Results of the present study are in agreement with the findings of the previous authors studies.

***Table 1.*** *Virus incidence and severity in pumpkin breeding lines under natural condition.*

| Lines | Virus incidence (%)* | Reaction | Virus severity (PDI) | Number of healthy plants |
|---|---|---|---|---|
| Pk05-4-1 | 70.4 bc | MS | 70.0 | 2 |
| Pk31-2-4 | 21.9 h | R | 16.7 | 4 |
| Pk05-8-2 | 56.3 d | MS | 35.0 | 1 |
| Pk34-4-3 | 72.2 b | MS | 47.5 | 2 |
| Pk75-1 | 70.8 bc | MS | 73.3 | 1 |
| Pk67-1-9 | 70.4 bc | MS | 47.5 | 0 |
| Pk37-1-4 | 67.0 c | MS | 35.0 | 2 |
| Pk07-4-7 | 16.1 i | R | 2.5 | 5 |
| Pk13-1-1 | 0.0 k | HR | 0.0 | 12 |
| Pk20-2-1 | 0.0 k | HR | 0.0 | 12 |
| Pk02-2-1 | 0.0 k | HR | 0.0 | 12 |
| Pk19-4-1 | 0.0 k | HR | 0.0 | 12 |
| Pk55-2-2 | 49.3 e | MR | 19.2 | 4 |
| Pk61-1-1 | 69.1 bc | MS | 70.0 | 2 |
| Pk54-4-12 | 0.0 k | HR | 0.0 | 12 |
| Pk05-1-2 | 79.9 a | S | 83.3 | 0 |
| Pk04-7-12-3 | 23.2 h | R | 5.0 | 4 |
| Pk05-7-11-8 | 56.1 d | MS | 19.2 | 3 |
| Pk01-10-9-4 | 0.0 k | HR | 0.0 | 12 |
| BARI mistikumra1 | 31.0 g | MR | 13.3 | 3 |
| BARI mistikumra 2 | 12.3 j | R | 1.7 | 4 |
| Pk101 | 40.5 f | MR | 15.0 | 3 |
| Pk102 | 50.8 e | MS | 25.0 | 2 |
| Pk105 | 52.8 de | MS | 20.8 | 2 |
| Pk106 | 0.0 k | HR | 0.0 | 12 |
| Pk107 | 31.8 g | MR | 10.0 | 3 |
| CV (%) | 4.9 | | 17.7 | |
| LSD | 3.6 | | 8.5 | |

*ANOVA was performed after arcsine transformation (n+0.5) of original values. Values within the same column with a common letter(s) do not differ significantly (P=0.05). HR=Highly Resistant, R=Resistant, MR= Moderately Resistant, MS= Moderately Susceptible, and S=Susceptible

Results of the present investigation demonstrate that the incidence and severity of different viruses in the field varies depending on the test lines. The present investigation proves that some pumpkin lines tested were resistant against pumpkin viruses in natural condition and some were susceptible. Considering virus incidence and severity (PDI) 15 lines (Pk13-1-1, Pk20-2-1, Pk02-2-1, Pk19-4-1, Pk54-4-12, Pk01-10-9-4, Pk31-2-4, Pk04-7-12-3, Pk07-4-7**,** Pk55-2-2, BARI mistikumra1, BARI mistikumra2, Pk101, Pk106 and Pk107) may be selected for their improvement.

In Bangladesh, virus infections appeared to be detrimental for pumpkin. The use of virus-free pumpkin variety has been known for a long time to be the most importance for successful production of pumpkin (Saifullah 2003). The improvement of virus free variety will also have an impact in the development of efficient pumpkin production systems.

### 3.2. Serological Detection of Viruses

Serological detection results are presented in Table 2. Out of

26 pumpkin lines samples, 13 samples **(**Pk34-4-3, Pk55-2-2, Pk05-1-2, Pk07-4-7, Pk75-1, Pk67-1-9, Pk05-4-1, Pk05-8-2, BARI mistikumra1, BARI mistikumra 2, Pk101, Pk102 andPk105**)** showed positive reaction against at least any one of four antisera namely PRSV-W, ZYMV, CMV and WMV2which were used for detection of viruses. Among the test lines, five (Pk55-2-2, Pk05-1-2, BARI mistikumra1, BARI mistikumra 2 and Pk101) showed positive reaction to PRSV-W, five (Pk05-4-1, Pk75-1, Pk05-8-2, Pk07-4-7 and Pk102) ZYMV, two (Pk34-4-3 and Pk67-1-9) to CMV and one (Pk105) WMV2. Samples of the rest of the lines did not show any reaction in DAS-ELISA against any of these four antisera. Same type of viruses has already been reported in pumpkin in previous work in Bangladesh (Masud *et al*. 2009). Few lines showed virus like symptoms in the field i.e, symptomatic, but negative reaction in DAS-ELISA, which might be due to abiotic agents (Bos 1969, Noordam 1973, Roy 1993) or infection of other viruses for which antiserum was not used (Yuki *et al.* 2000) or infection with an unidentified virus as yet not characterized or suffering from physiological or nutritional disorder (Bos 1983). Similar anomalous results were reported by Vincelli and Seebold 2009.

***Table 2.*** *Response of pumpkin breeding lines against four viruses by DAS-ELISA.*

| Lines | *PRSV-W* | *ZYMV* | *CMV* | *WMV2* |
|---|---|---|---|---|
| Pk05-4-1 | - | + | - | - |
| Pk31-2-4 | - | - | - | - |
| Pk05-8-2 | - | + | - | - |
| Pk34-4-3 | - | - | + | - |
| Pk75-1 | - | + | - | - |
| Pk67-1-9 | - | - | + | - |
| Pk37-1-4 | - | - | - | - |
| Pk07-4-7 | - | + | - | - |
| Pk13-1-1 | - | - | - | - |
| Pk20-2-1 | - | - | - | - |
| Pk02-2-1 | - | - | - | - |
| Pk19-4-1 | - | - | - | - |
| Pk55-2-2 | + | - | - | - |
| Pk61-1-1 | - | - | - | - |
| Pk54-4-12 | - | - | - | - |
| Pk05-1-2 | + | - | - | - |
| Pk04-7-12-3 | - | - | - | - |
| Pk05-7-11-8 | - | - | - | - |
| Pk01-10-9-4 | - | - | - | - |
| BARI mistikumra1 | + | - | - | - |
| BARI mistikumra 2 | + | - | - | - |
| Pk101 | + | - | - | - |
| Pk102 | - | + | - | - |
| Pk105 | - | - | - | + |
| Pk106 | - | - | - | - |
| Pk107 | - | - | - | - |

In Bangladesh, based on the serological detection of four types of viruses PRSV-W, ZYMV, CMV and WMV2 have been identified in pumpkin. Besides, virus-like symptoms in few lines in the field but negative reaction of those lines to any of these four viruses suggested there might have other viruses in pumpkin which are not yet to be identified.

### 3.3. Variability in Pumpkin Breeding Lines for Horticultural Qualities

Variability in pumpkin breeding lines for the characters measured is presented in Table 3. All the lines varied significantly for their response to quantitative characters like fruits per plant, average fruit weight (kg), yield/plant (kg), flesh thickness (cm) and % Total Soluble Solid; and qualitative characters like flesh color, fruit skin color and fruit shape. Significant variation with respect to quantitative and qualitative traits provides good scope for selection of desired lines. Maximum number of fruits per plant (4) was recorded in two lines (Pk106 and Pk20-2-1) while minimum (1) in four (Pk05-8-2, BARI mistikumra1, BARI mistikumra2 and Pk101). Maximum average fruit weight (3.9 kg) was recorded in Pk101 whereas minimum (1.0-1.5 kg) in 11 lines. Minimum weight of fruit may be considered as family size. Yield per plant ranged from 1.4-8.1 kg which is unexpectedly low. This low yield might be due to genetic reason or adverse plant environment or interaction of both. Yield is a quantitative trait which is often greatly influenced by the plant environment than by the underlying genes themselves (Bernardo 2002). Maximum flesh thickness was recorded 6.5cm in Pk31-2-4 and minimum 3.1cm in Pk07-4-7.Total soluble solid, which has positive correlation with β-carotene content, precursor of vitamin A ranged from 12.0% in Pk05-7-11-8 to 5.5% in Pk20-2-1 and Pk105. Wide variation was found in the flesh color, fruit shape and fruit skin color different lines for qualitative traits. Flesh color varied from yellow (Y) to orange (O). Similarly fruit skin color at maturity ranged from light green (LG) to orange (O). Fruit shape also varied from flat (F) to dumble (D). Rahman *et al*. (1990) found wide variations in sweet gourd for fruits per plant and fruit weight. Earlier reports indicated remarkable variations in fruits/plant and yield per plant in pumpkin (Rana *et al*. 1986, Masud *et al.* 2009, Masud 1995). Respective CV percent of these characters were also appreciable. Wide variation among the tested lines for different traits suggested giving priority to these characters as selection parameter as also opined by Vijay (1987).Considering such a wide variations for these characters 15 lines (Pk13-1-1, Pk20-2-1, Pk02-2-1, Pk19-4-1, Pk54-4-12, Pk01-10-9-4, Pk31-2-4, Pk04-7-12-3, Pk07-4-7**,** Pk55-2-2, BARI mistikumra1, BARI mistikumra 2, Pk101, Pk106 and Pk107) may be selected for their improvement.

***Table 3.*** *Variability in pumpkin breeding lines for horticultural qualities.*

| Lines | Fruits per plant | Average Fruit wt.(kg) | Yield per plant (kg) | Flesh thickness (cm) | %TSS | Flesh color | Fruit skin color at maturity | Fruit shape |
|---|---|---|---|---|---|---|---|---|
| Pk05-4-1 | 2 | 3.0 | 5.2 | 5.6 | 7.3 | Y | YG | HFR |
| Pk31-2-4 | 3 | 1.4 | 4.4 | 6.5 | 8.9 | Y | YG | R |
| Pk05-8-2 | 1 | 2.3 | 2.8 | 5.5 | 7.8 | Y | DG | E |
| Pk34-4-3 | 3 | 1.3 | 3.3 | 4.4 | 7.4 | Y | YG | R |

| Lines | Fruits per plant | Average Fruit wt.(kg) | Yield per plant (kg) | Flesh thickness (cm) | %TSS | Flesh color | Fruit skin color at maturity | Fruit shape |
|---|---|---|---|---|---|---|---|---|
| Pk75-1 | 3 | 1.4 | 4.6 | 4.7 | 10.7 | Y | O | F |
| Pk67-1-9 | 3 | 1.3 | 4.2 | 3.4 | 11.7 | Y | OG | HR |
| Pk37-1-4 | 2 | 1.8 | 2.6 | 4.0 | 7.5 | O | Y | FR |
| Pk07-4-7 | 3 | 1.8 | 4.4 | 3.1 | 10.0 | O | O | FR |
| Pk13-1-1 | 3 | 1.3 | 4.2 | 3.5 | 8.5 | O | O | FR |
| Pk20-2-1 | 4 | 1.9 | 8.1 | 3.6 | 5.5 | O | O | D |
| Pk02-2-1 | 2 | 2.4 | 5.0 | 5.0 | 8.0 | Y | O | FR |
| Pk19-4-1 | 3 | 2.5 | 7.8 | 4.5 | 9.5 | Y | O | R |
| Pk55-2-2 | 2 | 3.1 | 5.9 | 4.8 | 9.6 | O | O | D |
| Pk61-1-1 | 2 | 2.7 | 5.9 | 4.5 | 9.0 | O | O | FR |
| Pk54-4-12 | 3 | 1.9 | 4.8 | 3.4 | 11.0 | Y | O | D |
| Pk05-1-2 | 3 | 2.5 | 5.9 | 4.6 | 11.0 | O | OG | HR |
| Pk04-7-12-3 | 3 | 2.3 | 5.9 | 4.0 | 8.6 | O | O | O |
| Pk05-7-11-8 | 3 | 2.5 | 6.3 | 4.9 | 12.0 | O | O | FR |
| Pk01-10-9-4 | 2 | 1.5 | 2.9 | 5.5 | 7. 0 | O | OG | FR |
| BARI mistikumra1 | 1 | 2.7 | 3.2 | 5.0 | 9.5 | Y | OG | G |
| BARI mistikumra 2 | 1 | 1.2 | 1.4 | 3.8 | 7.0 | O | O | FR |
| Pk101 | 1 | 3.9 | 4.9 | 4.0 | 6.5 | Y | OG | FR |
| Pk102 | 3 | 1.0 | 3.0 | 4.8 | 7.0 | O | O | HR |
| Pk105 | 3 | 1.1 | 3.4 | 6.0 | 5.5 | Y | LG | F |
| Pk106 | 4 | 1.1 | 4.0 | 4.5 | 8.0 | O | YG | F |
| Pk107 | 3 | 1.1 | 3.6 | 4.0 | 7.0 | Y | G | FR |
| LSD | 0.5 | 0.5 | 1.4 | 1.2 | 2.2 | | | |
| CV (%) | 8.3 | 10.9 | 10.9 | 13.2 | 12.7 | | | |
| Range | 1.2-4.1 | 1.0-3.9 | 1.4-8.1 | 3.1-6.5 | 5.5-12.0 | | | |

*Y=Yellow, O= Orange, **O=Orange, G=Green, YG=Yellow Green, OG=Orange Green; ***R=Round, FR= Flat Round, HR= High Round, HFR=High Flat Round, F=Flat, G=Glubose, D= Dumble, O= Oval

## 4. Conclusion

Results of this study revealed that sources of natural resistance to PRSV-W, ZYMV, CMV and WMV2 in pumpkin are very limited. However, some promising resistance responses were found in a number of breeding lines which can be potential sources of resistance for pumpkin breeding program.

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