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Genetic Potentiality of Pumpkin Genotypes Against Pumpkin Yellow Vein Mosaic Virus

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Abstract

Pumpkin (*Cucurbita moschata* Duch. ex Poir.), a nutritious vegetable, is susceptible to several diseases, with Pumpkin Yellow Vein Mosaic Virus (PYVMV) causing severe impact by affecting plants at all growth stages, leading to substantial yield losses, vegetable shortages, and malnutrition in Bangladesh. This study evaluated thirty pumpkin genotypes for PYVMV resistance, genetic potential, and yield characteristics, sourced from Advanced Chemical Industries (ACI) Agribusiness and various districts in Bangladesh. For agronomic and molecular assays, thirty pumpkin genotypes along with the standard check were planted following RCBD with three replications at BAU-GPB farm. Whitefly-mediated inoculation in a net house assay determined the Area Under Disease Progress Curve (AUDPC) under controlled conditions. Tissue Blot Immunoassay (TBIA), using rabbit antiserum specific to PYVMV, showed no cross-reactivity with non-target viruses, with consistent results across TBIA, AUDPC, and molecular assays. Infected younger leaves were collected for virus-specific molecular analyses. Three sets of SSR markers were used to identify genes responsible for PYVMV resistance. PYABFP & PYABRP and PYBHFP & PYBHRP primer sets were capable of producing expected fragment.ToLCV1100F & ToLCV1650R primer sets were used for final confirmation. Genotypes PK2, PK5, and PK17 showed superior performance with higher individual fruit weights, more fruits per vine, higher overall yields, and minimal viral infection compared to the check variety (Baromashi). In contrast, PK1, PK6, PK25, and PK27 exhibited poor yield and related traits. Marker analysis revealed the absence of resistance genes in PK1, PK3, PK4, PK6, PK7, PK8, PK9, PK10, PK11, PK12, PK13, PK14, PK15, PK16, PK18, PK19, PK20, PK21, PK22, PK23, PK24, PK25, PK26, PK27, PK28, PK29, and Baromashi (control), but confirmed the presence of resistance genes in PK2, PK5, and PK17. Consequently, PK2, PK5, and PK17 were recommended as PYVMV-resistant genotypes with high yielding genetic potentiality.

Keywords: Genetic Potentiality, Pumpkin,*PYVMV,* Resistance, TBIA, AUDPC

Abbreviations
PYVMV- Pumkin Yellow Vien Mosaic Virus,
ACI- Advanced Chemical Industries,
BAU-GPB Farm– Bangladesh Agriculture University- Genetic and Plant Breeding Farm
RCBD- Randomize Complete Block Design
AUDPC- Area Under Disease Progress Curve
TBIA- Tissue Blot Immunoassay

1. Introduction

Pumpkin or sweet gourd (*Cucurbita moschata*) is a vital vine crop in Bangladesh, grown both commercially and domestically (Hoque et al., 2015). It is also significant in subtropical regions (Tadmore et al., 2005). In 2021-2022 cropping season 145205 metric ton pumpkin was cultivated in 12207.045 ha with a productivity of 11.895 ton/ha (BBS, 22) was recorded in Bangladesh. Pumpkin's fruits, vines, flowers, seeds, and rinds are extensively consumed. Its fruits can be stored for 3-4 months, fetching higher prices off-season. Nutritious and rich in β-carotene, minerals, and vitamins, pumpkins help combat malnutrition (Hoque et al., 2015; Yadav et al., 2010). They also have therapeutic properties and bioactive compounds (Saavedra et al., 2013). Pumpkins are used in confectionery, beverages, and alcohol (Yadav et al., 2010). Disease-tolerant varieties are essential to meet rising demand. Among all diseases, Pumpkin Yellow Vein Mosaic Disease is particularly significant.Yellow vein mosaic disease in pumpkin crops was first reported in northern India in the early 1940s (Vasudeva & Lal, 1943). Diseased plants are infected by a begomovirus, designated *Pumpkin yellow vein mosaic virus* (PYVMV).The disease manifested initially as a yellowing of veins on the younger leaves, which progressively evolved into mosaic-like patches in the advanced stages of infection. Infected plants exhibited stunted growth and premature flower abscission. Diseased plants exhibit veinal chlorosis, which can sometimes coalesce to form extensive chlorotic patches, significantly diminishing yields. An epidemic of PYVMD was recorded for the first time in South India in 2004 with disease incidences of up to 100% and significant yield losses(Maruthi et al., 2007). According to Akhter *et al.* vegetable production in Bangladesh also faces a significant yield loss every year due to virus infection. The virus is transmitted readily and in persistent manner by the whitefly, (*Bemisiatabaci*). Transmission of PYVMV requires minimum acquisition and inoculation access periods of 30 min and 10 min, respectively. The minimum latent period in the insect is 6 h and the virus persists in the vector for at least 8 days (MUNIYAPPA., et al 2003). A begomovirus causing PYVMD in South India was characterized recently but the nature of virus causing the disease in North India was not known. Samples of PYVMD were obtained from North India and two putative begomoviruses were PCR-amplified and sequenced. Comparison of complete DNA-A sequences indicated that PYVMD in North and South India were caused by two distinct begomoviruses and shared only approximately 88% DNA-A nucleotide identity (Maruthi et al., 2007).

*Pumpkin yellow vein mosaic virus*is not transmitted to healthy pumpkin through sap inoculation but only by the whitefly, (*Bemisiatabaci*). A single viruliferous whitefly was able to cause 21.67 per cent infection(Jayashree et al., 1999). PYVMV infected five of 67 species tested when inoculated by viruliferous *B. tabaci*. The host range consisted of the viruses are presumed natural host, pumpkin and three other cucurbit species (summer and winter squash, and bottle gourd) and a solanaceous species, (*N. tabacum*). Of the plant species tested, many were host plants of other begomoviruses and also vectored by *B. tabaci*. This host range difference between PYVMV and other begomoviruses is, therefore, not

due to the inability of the vector to feed on other species, several of which are hosts of ToLCNDV and/ or begomoviruses of the cotton leaf curl disease complex, which were transmitted using the same whitefly colony (Muniyappa *et al*., 1991; Nateshan*et al*., 1996). However, it should be emphasized that the host range assessment was based on symptom appearance alone and plant species supporting a symptomatic infection would not have been identified. Controlling viral diseases is highly complex in field conditions i.e. vector control, making the use of resistant cultivars one of the three major strategies for managing cucurbit viruses (Lecoq and Desbiez, 2012). Given the aforementioned facts, this paper aims to provide a comprehensive approach to identifying pumpkin genotypes free from Pumpkin Yellow Vein Mosaic Virus (PYVMV) by evaluating their field performance, AUDPC (Area Under Disease Progress Curve) data, and molecular assay results. The objectives of this study are to collect underutilized pumpkin genotypes from various locations across the country and to select and conserve PYVMV-free genotypes based on rigorous field performance assessments and molecular analyses.

2. Methodology

2.1. Plant material

Thirty pumpkin genotypes including check variety were collected from Advanced Chemical Industries (ACI) and from different district of Bangladesdh and were planted following RCBD with three replications in Bangladesh Agricultural University Genetic and Plant Breeding Farm for this investigation. In field condition all thirty pumkin genotypes along with the standard were left for natural pumkin yellow vien mosaic virus infestation. However, Virus transmission was done in net house where a insect free condition was made, other than whiteflies (Bemisiatabaci) as whiteflies are proved to the vector for PYVMV. For virus transmission we followed the protocols that were mentioned in (Muniyappa et al., 2003). Intercultural operations were done maintain standard procedure. For molecular analysis, infected leaf samples were collected from these genotypes at earlier stage of infection.

2.2. Data collection

Morphological data such as Days to first male flowering, Days to first female flowering, Node number to first female flowering, Number of primary branches per vine, Fruit length, Fruit diameter, Flesh thickness, Flesh cavity, Number of fruits per vine, and Yield per vine of all the genotypes were recorded to access the field performance of the genotypes. To visually score viral symptoms we followed a simplified rating system outlined by Haque and Mou. (2015), Haque *et al*.(2014) where plants with no symptoms or asymptomatic plants were rank 0, plant with partial symptoms 1 and plants with mosaic symptoms 2. Individual plants were scored three times for the appearance and type of symptoms between 7 to 14 days of post-inoculation (dpi).The viral symptoms scored by visual analyse were further confirmed by Tissue blot immunoassay (TBIA). In TBIA, PYVMVA specific rabbit antiserum was used for detection and for thisrepresentative plant from each genotype in two of the three replicates was used in TBIA and when virus was detected by TBIA having no symptoms were scored. The TBIA data were used to adjust percent infection calculations (Stewart *et al*.,2013).Area under the disease-progress curve (AUDPC) were determined for each plant in each replicate using the mean plot disease score at each rating data (Shaner and Finney 1977). Analysis of variance (ANOVA) and means separations followed by lsd test were calculated using SAS PROC GLM (SAS Version 9.1; SAS Institute, Cary, NC).

2.3. Isolation of genomic DNA for PCR analyses

Genomic DNA of all genotypes were isolated separately along with the standard check from infected leaf smaples DNA were extracted by using the CTAB method as described by Brown et al., 1998 with some modification. Twenty days old seedlings were collected for each sample and leaves were grinded using a GENO grinder in a 96 well titer plate. Extraction buffer (660 ul) and 20% SDS were added and vortexed. Incubation for 10 min at 65 degree C in addition to 100 ul 5M NaCl and100 ul 10X CTAB solution were performed, respectively. About 400 ul samples were spun at 12000 rpm for 3-5 mins by adding 900 ul chloroform (chloroform: isoamyl alcohol = 24:1) solution. The top supernatant phase was again spun at 14000 rpm for 5-7 minutes with 600 ul isopropanol. The DNA pellets were air dried and rinsed with 70% ethanol and again air-dried. The DNA were re-suspend in 50 ul of 1X TE buffer and stored at -20 degree C. DNA concentration was determined by electrophoresis of 5 ul of sample along with serial dilutions of Lambda DNA in 0.8% agarose(Navas et al., 1999).

2.3.1. PCR amplification: For DNA amplification reactions for each of the PCR primers were performed in a 20 µl reaction volume containing 25 ng of genomic DNA, 4µl of green and white Go Taq PCR buffer, 0.2 mM of each dNTP, 0.1 µM of each forward and reverse primer and 0.5 U Taq polymerase (Promega, USA).

2.3.2. Electrophoresis: The PCR products were electrophoresed in 1.5% Agarose Gel in TAE buffer stained with 40 ul ethidium bromide and photographed under UV light.

2.4. PYMV detection by specific marker: To detect PYMV detection we used three sets of markers(Table 5.1) as applied by Chakraborty *et al*., 2009; Muniyappa *et al*., 2003. For each marker we simply tested the PCR product of genomic DNA with three sets of selected primers, whether capable of producing erxpected band in electrophoresis or not and results were presented using +/- sgin.

Table 2.1. List of PCR primers for *Pumpkin yellow vein mosaic virus* detection

PRIMERS NAME	DNA FRAGMENTS	SEQUENCE (5'-3')
PYABFP-Forward	DNA-A (full length)	GTGGGGGATCCATTATTGCACGGG
PYABRP--Reverse	DNA-A (full length)	CCGGATCCCACATGTTTGTAGA
PYBHFP-Forward	DNA-B (Partial)	GAAAGCTTACTGGTCTTACCATGTCC
PYBHRP--Reverse	DNA-B (Partial)	TGAAGCTTGATATATGAACGAACCCTG
ToLCV 1100F	DNA-B (Partial)	TGGRYWACGTTCAAGGAYSMWG
ToLCV 1650R	DNA-B (Partial)	YTKGAYTTYTGGTCTGTKG

Source of primers:Chakraborty *et al*., 2009; Muniyappa *et al*., 2003

3. Results

The experiment results are presented in three different subheads:

3.1.Field performance of pumpkin genotypes

The outcome of the field assay showed highly significant (0.1% level) variation among different genotypes for all the traits *viz.* Days to first male flowering, Days to first female flowering, Node number to first female flowering, Number of primary branch per vine, Fruit length, Fruit diameter, Flesh thickness, Flesh cavity, Number of fruits per vine, Yield per vine and showed moderately significant (1% level) variation for the trait of Vine length (Table 6.1).

3.1.1 Days to first male flowering [DFMF]

Days to first male flowering showed highly significant at 0.1% level of probability in the analysis of variance (Table 6.1). The significant differences indicated a wide range of variation with the mean value of 49.914 days. Highest days for first male flowering were required by PK13 (54.08 days) and minimum days were required by PK10 (46.83 days) (Table 6.2). The phenotypic variance (4.78) is comparatively higher than the genotypic variance (4.29) for this trait indicating presence of some sort of environmental influences. The phenotypic and genotypic coefficients of variations were considerably low which were 4.38% and 4.15%, respectively. The character also showed high heritability (89.81%), low genetic advance (4.05) and genetic advance in percentage of mean 8.105% (Table 6.3).

3.1.2 Days to first female flowering [DFFF]

Days to first female flowering showed significant differences among the genotypes studied (Table 6.1). The genotype PK27 required maximum number of days (69.42) to first female flowering and genotype PK29 required minimum number of days (56.17) to first female flowering. The average value of days to first female flowering was 61.50 (Table 6.2).The phenotypic variance (10.06) is comparatively higher than the genotypic variance (8.96) for this trait showing some sort of environmental influences on the expression of the character. For first female flowering, heritability (89.06%) was high with low genetic advance (5.82) and genetic advance in percentage of mean (9.46%) (Table 6.3).

3.1.3 Node number to first female flowering [NFF]

The different genotypes showed significant differences in node number to first female flowering. The average value of this character was 21.62 (Table 6.2). Maximum node number to first female flowering was required by PK19 (26.92) and minimum node number was required by PK4 (18.42). The phenotypic variance (3.79) was relatively higher than the genotypic variance (3.05) and they were slight difference in between phenotypic coefficient of variations (9.01%) and genotypic coefficient of variations (8.07%) (Table 6.3).The genetic advance (3.22) and genetic advance in percentage of mean (14.90%) were low for this character. But the heritability was relatively higher (80.30%) (Table 6.3).

3.1.4 Vine length [VL]

The analysis of variance showed moderately significant (1% level) variation among the genotypes for the trait of vine length (Table 6.1). Among the genotypes, PK11 (6.77 m) gave highest vine length and PK13 (2.6 m) gave the lowest vine length. The mean value of this character was 4.38 (Table 6.2).The genotypic variance (0.49) was relatively lower than the phenotypic variance (1.59) for this character among the genotypes. The genotypic coefficient of variations was 16.01 and the phenotypic coefficient of variations was 28.75. The heritability (31.02) as well as the genetic advance (0.81) and genetic advance in percentage of mean (18.37%) were low for this trait (Table 6.3).

3.1.5 Number of primary branches per vine [NPB]

Number of primary branches per vine showed significant differences among the genotypes studied (Table 6.1). The genotype PK28 occupied maximum number of primary branch (11) and genotype PK29 occupied minimum number of primary branch (3.58). The average value of Number of primary branches per vine was 5.99 (Table 6.2).

The genotypic variance and the phenotypic variance for this trait were 4.82 and 5.52 respectively. Of them phenotypic variance was relatively higher than the genotypic variance. Genotypic coefficient of variations was 36.65 and phenotypic coefficient of variations was 39.21 (Table 6.3). The genetic advance (4.23) was low but the heritability (87.35) and the genetic advance in percentage of mean (70.56) were significantly higher for this trait (Table 6.3).

3.1.6 Fruit length [FL]

The analysis of variance was highly significant (0.1% level) for the trait of fruit length i.e. the genotypes showed significant variation for fruit length (Table 6.1). The mean value of this variable was 9.50. Maximum fruit length (11.3 cm) and minimum fruit length (7.67 cm) were occupied by the genotype Baromashi and PK13 respectively (Table 6.2).The variance of genotype was 0.98 and that of for phenotype was 1.005. The heritability (97.72) was very high for this trait. The genotypic coefficient of variations and the phenotypic coefficient of variations were 10.43 and 10.55 respectively. The genetic advance (2.02) and the genetic advance in percentage of mean (21.23%) were low for the variable fruit length (Table 6.3)

Table 3.1. Analysis of variance for different morphological plant characters of pumpkin genotypes

Characters	df	DFMF	DFFF	NFF	VL	NPB	FL	FD	FT	FC	NFPV	YPV
Replication	2	0.9528	0.9674	0.0021	0.637	0.0812	0.0344	0.1254	0.0028	0.1117	0.0111	0.09
Genotypes	29	13.369***	27.989***	9.886***	2.57**	15.164***	2.968***	5.551***	0.633***	5.743***	0.213***	0.825***
Error	58	0.487	1.1	0.747	1.094	0.698	0.0229	0.0448	0.005	0.06	0.065	0.076

*** and ** indicate significant at 0.1% and 1% probability level respectively.

Legend:

DFMF= Days to first male flowering, **DFFF**= Days to first female flowering, **NFF**= Node number to first female flowering, **VL**= Vine length, **NPB**= Number of primary branch per vine, **FL**= Fruit length, **FD**= Fruit diameter, **FT**= Flesh thickness, **FC**= Flesh cavity, **NFPV**= Number of fruits per vine, **YPV**= Yield per vine

Table 3.2. Mean performance of pumpkin genotypes on different morphological traits related to yield

Genotypes	DFMF	DFFF	NFF	VL	NPB	FL	FD	FT	FC	NFPV	YPV
PK1	47.33nop	58.17lm	20.92fgh	4.63bcdef	9.17bc	9.63e	13.47m	2.7i	10.05ij	1.67defg	2.41klmno
PK2	47.67mnop	59.25kl	23.25cd	5.03bc	8.83bc	11.17ab	18.27abc	3.88a	10.51h	2.65a	4.226ab
PK3	51.42efg	63.67cde	19.58hijk	4.57bcdefg	5.5fgh	8.6k	16.8fg	3.71ab	11.38def	2.17bc	3.05efgh
PK4	47.67mnop	58.33lm	18.42k	4.97bc	7.17de	9.1hij	16.67g	3.27de	10.12hij	1.83cdef	3.19defg
PK5	49.42jk	62.42efgh	20.17ghij	4.9bcd	8.83bc	9.31fgh	18.41ab	3.79ab	9.18lm	2.27ab	4.05ab
PK6	48.17lmn	59.33kl	23cd	5.37abc	10.67a	10.27d	14.33l	3.18defgh	11.11fg	2bcd	2.59ijklmno
PK7	49jkl	60.67ijk	21.5efg	4.87bcde	3.67kl	9.77e	16.33g	3.16efgh	8.01n	2.17bc	3.61abcd
PK8	51.67defg	63.58de	20.83fghi	4.53bcdefg	4ijkl	11.1ab	17.6d	3.6bc	10.4hi	1.75def	2.91ghij
PK9	50hij	61.5fghi	20.58ghij	4.57bcdefg	5.33fghi	10.33d	15.17k	3.1h	8.97m	1.83cdef	2.65hijklmn
PK10	46.83p	56.67mn	21.5efg	4.17bcdefgh	5.75fg	10.6c	18.27abc	3.29d	11.68cd	2bcd	3.74abc
PK11	48lmno	58lm	20.83fghi	6.77a	3.67kl	8.33l	15.97h	2.49j	10.99fg	2.25ab	2.22no
PK12	53abc	66b	22.67cde	5.33abc	4.67fghijkl	8.57kl	14.13l	3.12gh	7.9n	2.25ab	2.66hijklmn
PK13	54.08a	65bcd	23.42cd	2.6h	5fghijk	7.67m	15.17k	3.53bc	8.11n	1.92bcde	3.41cdef
PK14	47op	57.92lm	22.08def	4.23bcdefgh	4.5ghijkl	7.77m	16.53g	3.22defg	10.09ij	1.33g	2.38lmno
PK15	50hij	61.08hij	20.42ghij	5.67ab	3.92jkl	8.53kl	18c	3.24def	11.51cde	1.83cdef	3.58abcd
PK16	51.5efg	63.58de	20.42ghij	3.17efgh	5.92ef	9.07hij	13.47m	2.17k	9.13lm	2bcd	3.51bcd
PK17	48.67klm	59.42jkl	21.25fg	5.13abc	7.33d	9.37fg	18.07bc	3.21defgh	11.65cd	2.58a	4.01ab
PK18	51.08fgh	63.08ef	19.5ijk	5.23abc	8.42cd	10.3d	18.4ab	3.26def	11.87bc	2bcd	3.49cde
PK19	48lmno	58.67l	26.92a	4.97bc	5.5fgh	11.3a	16.57g	3.55bc	9.47kl	2.58a	3.02 fghi
PK20	49jkl	62.33efghi	20.17ghij	3.87cdefgh	3.67kl	8.63k	18.47a	2.21k	14.22a	2.17bc	3.01fghi

Genotypes	DFMF	DFFF	NFF	VL	NPB	FL	FD	FT	FC	NFPV	YPV
PK21	48.08lmno	59.17kl	21.5efg	2.87gh	5.67fgh	9.53ef	17.13ef	2.5j	12.13b	2bcd	2.75ghijklm
PK22	51fgh	65bcd	23.33cd	3.67cdefgh	5fghijk	11b	18.13abc	3.15fgh	11.83bc	1.5fg	2.24no
PK23	52.25cde	62.83efg	22.92cd	4.4bcdefg	9.92ab	9.17ghi	15.37jk	2.54j	10.29hi	1.83cdef	2.83ghijk
PK24	51.83def	65.33bc	25.17b	4.27bcdefgh	3.67kl	9ij	16.03h	3.11gh	9.81jk	1.5fg	2.35mno
PK25	50.67ghi	61.33ghi	20.42ghij	4.2bcdefgh	4.33hijkl	8.9j	13.47m	2.57j	10.93g	1.58efg	2.19o
PK26	50.92fgh	61.67fghi	21.17fg	4.03bcdefgh	4.42ghijkl	9.53ef	16.1h	2.53j	11.05fg	1.92bcde	2.49jklmno
PK27	53.67ab	69.42a	23.92bc	3.23defgh	5.58fgh	9.63e	15.87hi	3.18defgh	9.5kl	2bcd	2.97fghi
PK28	52.67bcd	62.67efgh	21.17fg	3.17efgh	11a	8.57kl	16.47g	3.14fgh	10.18hij	1.92bcde	2.8ghijkl
PK29	47.17nop	56.17n	19.42jk	3.87cdefgh	3.58l	10.73c	15.97h	2.18k	11.71cd	1.83cdef	2.51jklmno
BAROMASHI	49.67ijk	62.67efgh	22.08def	3.13fgh	5.08fghij	9.63e	18.17abc	3.51c	11.15efg	2.17bc	3.96ab
$LSD_{0.05}$	1.140874	1.71437	1.412646	1.709585	1.36581	0.2475	0.345	0.120	0.400	0.416	0.450
Mean	49.914	61.50	21.62	4.380	5.992	9.504	16.58	3.048	10.50	1.919	2.962
SE (±)	0.40	0.57	0.35	0.23	0.42	0.18	0.25	0.08	0.25	0.06	0.10
Standard Deviation	2.17	3.14	1 .93	1.25	2.32	0.99	1.36	0.46	1.38	0.33	0.57
Minimum	46.83	56.17	18.42	2.60	3.58	7.67	13.47	2.17	7.90	1.33	2.19
Maximum	54.08	69.42	26.92	6.77	11.00	11.30	18.47	3.88	14.22	2.58	4.02
CV%	1.39849	1.70566	3.998	23.881	13.9472	1.594	1.275	2.4105	2.332	13.281	9.335

Table 3.3. Estimation of genetic parameters for 11 morphological characters related to yield in 30 genotypes of pumpkin

Characters	GV	PV	Heritability	GCV	PCV	GA	GAM
DFMF	4.29	4.781	89.814	4.151	4.381	4.045	8.105
DFFF	8.96	10.06	89.069	4.868	5.158	5.82	9.464
NFF	3.05	3.793	80.308	8.074	9.009	3.222	14.905
VL	0.49	1.586	31.021	16.011	28.746	0.805	18.37
NPB	4.82	5.52	87.355	36.647	39.21	4.228	70.559
FL	0.98	1.005	97.72	10.425	10.546	2.018	21.23
FD	1.84	1.88	97.617	8.17	8.269	2.757	16.629
FT	0.21	0.214	97.667	15.011	15.189	0.931	30.559
FC	1.89	1.954	96.93	13.111	13.317	2.791	26.59
NFPV	0.05	0.114	43.149	11.574	17.62	0.301	15.662
YPV	0.25	0.326	76.663	16.921	19.325	0.901	30.52

Legend:

GV= Genotypic variance, **PV** = Phenotypic variance, **GCV** = Genotypic coefficient of variation, **PCV** = Phenotypic coefficient of variation, **GA** = Genetic advance, **GAM** = genetic advance in percentage of mean

3.1.7 Fruit diameter [FD]

Fruit diameter of 30 pumpkin genotypes showed significant variation (Table 4.1). In the mean performance, FD varied between 13.47 cm and 18.47 cm. Genotype PK20 gave the highest FD which was 18.47 cm and PK16 gave the lowest FD which was 13.47 cm. Beside this,PK2 and PK5 having FD very closure to PK20 (Table 6.2). The genotypic variance (1.84) was relatively lower than the phenotypic variance (1.88) for this character among the genotypes. The genotypic coefficient of variations and the phenotypic coefficient of variations were 8.17 and 8.27 respectively (Table 6.3). The

character was highly heritable (97.62) but the genetic advance for fruit diameter was very low (2.76). The genetic advance in percentage of mean was 16.63 (Table 6.3).

3.1.8 Flesh thickness [FT]

The character flesh thickness which was manually tested showed significant variation among the genotypes (Table 6.1). The highest (3.88 cm) and lowest (2.18 cm) value for FT were occupied by the genotype PK2 and PK29 respectively (Table 6.2).The genotypic variance (0.21) and the phenotypic variance (0.214) were around similar to each other. The genotypic coefficient of variations was 15.01 and the phenotypic coefficient of variations was 15.19 for this variable (Table 4.3). The high heritability (97.67) was estimated for the variable flesh thickness with low genetic advance (0.93) and genetic advance in percentage of mean (30.56) (Table 6.3).

3.1.9 Flesh cavity [FC]

Flesh cavity showed significant differences among the genotypes studied (Table 6.1).The mean value of the genotypes for this trait was 10.50. The maximum flesh cavity (14.22 cm) and the minimum flesh cavity (7.9 cm) were occupied by the genotype PK20 and PK12 respectively (Table 6.2). The phenotypic variance (1.95) was slightly higher than the genotypic variance (1.89). The genotypic coefficient of variations and the phenotypic coefficient of variations were 13.11 and 13.31 respectively. The genetic advance (2.79) and the genetic advance in percentage of mean (26.59%) for this variable were low but the heritability was very high (96.93) (Table 6.3).

3.1.10 Number of fruits per vine [NFPV]

The different genotypes showed significant differences in number of fruits per vine (Table 6.1). The maximum number of fruits per vine (2.65) was calculated for the genotype PK2 and the minimum number of fruits per vine (1.33) was estimated for the genotype PK14. The average value was 1.93 for this trait. Since the genotypic variance (0.05) was lower than the phenotypic variance (0.114), it's indicating some sort of environmental influence in the expression of this character. The genotypic coefficient of variations was 11.58 and phenotypic coefficient of variations was 17.62 for this variable.
The heritability (43.15), the genetic advance (0.30), the genetic advance in percentage of mean (15.66%) was low for the trait number of fruit per vine (Table 6.3).

3.1.11 Yield per vine [YPV]

All the characters studied showed significant variations in mean square. Yield showed wide range of variation in the analysis of variance (Table 6.1). In the mean performance, the value varied between 4.22Kg (PK2) and 2.19 Kg (PK25) per vine. Comparatively higher yield per vine was noticed by the genotypes PK2, PK5 and PK17 which were 4.22 Kg, 4.05 Kg and 4.01 Kg respectively. On the other hand, the lowest yield was estimated for the genotype PK25 which was 2.19 Kg (Table 6.2). The phenotypic variance (0.35) was slightly higher than the genotypic variance (0.25). The phenotypic coefficient of variations and genotypic coefficient of variations were 19.33 and 16.92 respectively. The yield per vine showed moderate heritability (76.66) with low genetic advance (0.90) and moderate genetic advance in percentage of mean (30.52%) (Table 6.3).

3.2. Detection of PYVMV infection and AUDPC

The genotypes PK2, PK5, PK17 showed no virus infection symptoms in TBIA whereas other 27 genotypes including the check showed viral symptoms. Disease severity, as measured by the AUDPC scores, indicated that symptoms for PYMV correlate with % infection. Genotypes PK2, PK4, PK5, PK6, PK17 and the control genotypes resulted lower infection rate along with lower AUDPC (Table 6.4 and

Fig.6.1 & Fig.6.2) and the rest of the genotypes produced higher infection rate as comparable with the check.

3.3. Molecular detection using SSR primer

Three sets of primer were used to detect desired gene in the thirty pumpkin genotypes. Detection of PYVMV gene in the genotypes was carried out using PYABFP-Forward / PYABRP-Reverse, PYBHFP-Forward PYBHRP-Reverse & ToLCV1100F and ToLCV1650R primers (Table 5.1) based on simple PCR detection system. The viral resistance system is a complex mechanism provides resistance in a semi- dominant manner, so a certain degree of resistance can be expected if the major gene is present in a genotype. Our results indicated that a fragment of 550bp was absent in the genotypes PK2, Pk5 and in Pk17 that are resistant to *Pumpkin yellow vein mosaic virus* (Table 6.4, Fig.6.3 & Fig.6.4).The rest of the genotypes showed presence of a clear fragment of 550 bp indicated that these 27 genotypes including check were susceptible to PYVMV. A number of other bands at different positions were identified in most of the genotypes which might be due to presence of other gene(s) capable of amplifying those primer sets.

Table 3.4. Resistance pattern of thirty pumpkin genotypes for Pumpkin yellow vein mosaic disease resistance

Sl. No.	Genotypes	Presence/ Absence of gene(s) against *pyvmv*	AUDPC value	TBIA response for PYVMV	% Infection(Adjusted with TBIA)
1.	PK1	-	11.3cd	+	78.66d
2.	PK2	+	2.99i	-	27.66i
3.	PK3	-	12.13b	+	88.66b
4.	PK4	-	4.81h	+	33.16h
5.	PK5	+	5.81g	-	44.33g
6.	PK6	-	2.97i	+	22.01j
7.	PK7	-	11.3cd	+	78.66d
8.	PK8	-	8.63f	+	55.33f
9.	PK9	-	12.14b	+	88.66b
10.	PK10	-	14a	+	100a
11.	PK11	-	10.83de	+	72.01e
12.	PK12	-	11.3c	+	83.6c
13.	PK13	-	10.77e	+	72e
14.	PK14	-	12.13b	+	88.66b
15.	PK15	-	14.02a	+	100.04a
16.	PK16	-	10.83e	+	72.01e
17.	PK17	+	2.99i	-	27.66i
18.	PK18	-	12.13b	+	88.66b
19	PK19	-	14a	+	100a
20.	PK20	-	10.85cde	+	72.01e

21.	PK21	-	14.01a	+	100a
22.	PK22	-	12.13b	+	88.66b
23.	PK23	-	8.63f	+	55.33f
24.	PK24	-	8.63f	+	55.33f
25.	PK25	-	12.13b	+	88.66b
26.	PK26	-	8.63f	+	55.33f
27.	PK27	-	10.79e	+	72.01e
28.	PK28	-	8.63f	+	55.33f
29.	PK29	-	14a	+	100a
30.	Baromashi (BADC)	-	2.99i	+	27.66i

Note: **Resistant gene for PYVMV**: "+" indicated genes for PYVMV resistance was present; "-" indicated absence of genes for PYVMV resistance. **TBIA response for PYVMV:** "+" indicated infection caused by PYVMV and "-" indicated there was no infection of **PYVMV**. TBIA data was adjusted with % Infection using 2-3 plants whenever essential% Infection is the mean for three independent replications of 5 plants. Values within a column followed by the same letter were not different (LSD **0.6872443**; $p<0.05$)AUDPC is the mean for three independent replications of 2-3 plants. Values within a column followed by the same letter were not different (LSD **0.475624**; $p<0.05$)

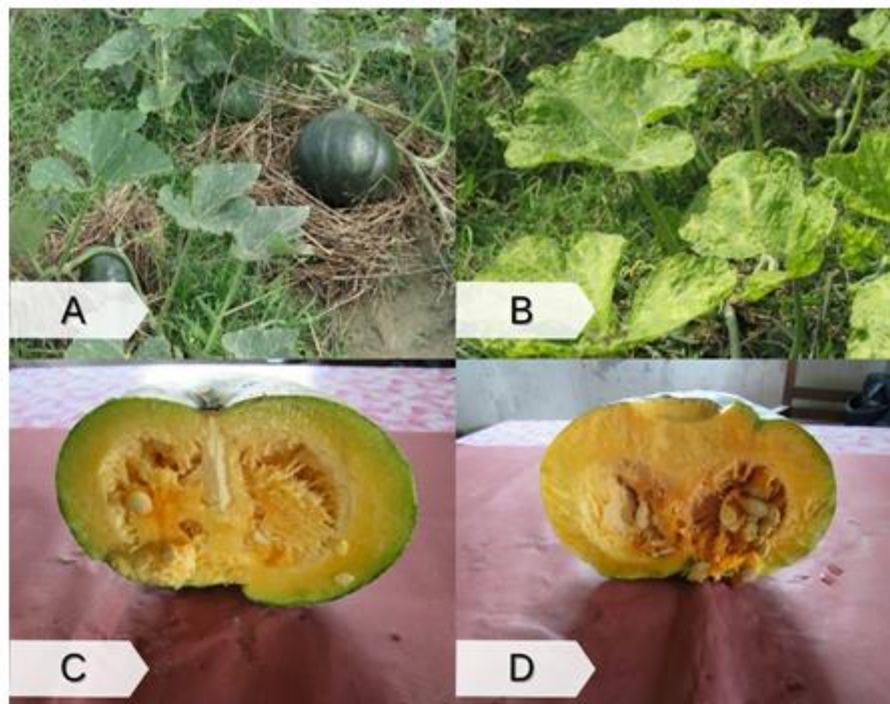

Fig.3.1. Field view of a representative healthy Pumpkin plant (A) PK17 and a *Pumpkin yellow vein mosaic* virus affected plant (B) Pk15. Dissected pumpkin fruit of a healthy plant of PK17 (C) and virus affected fruit of PK15 (D).

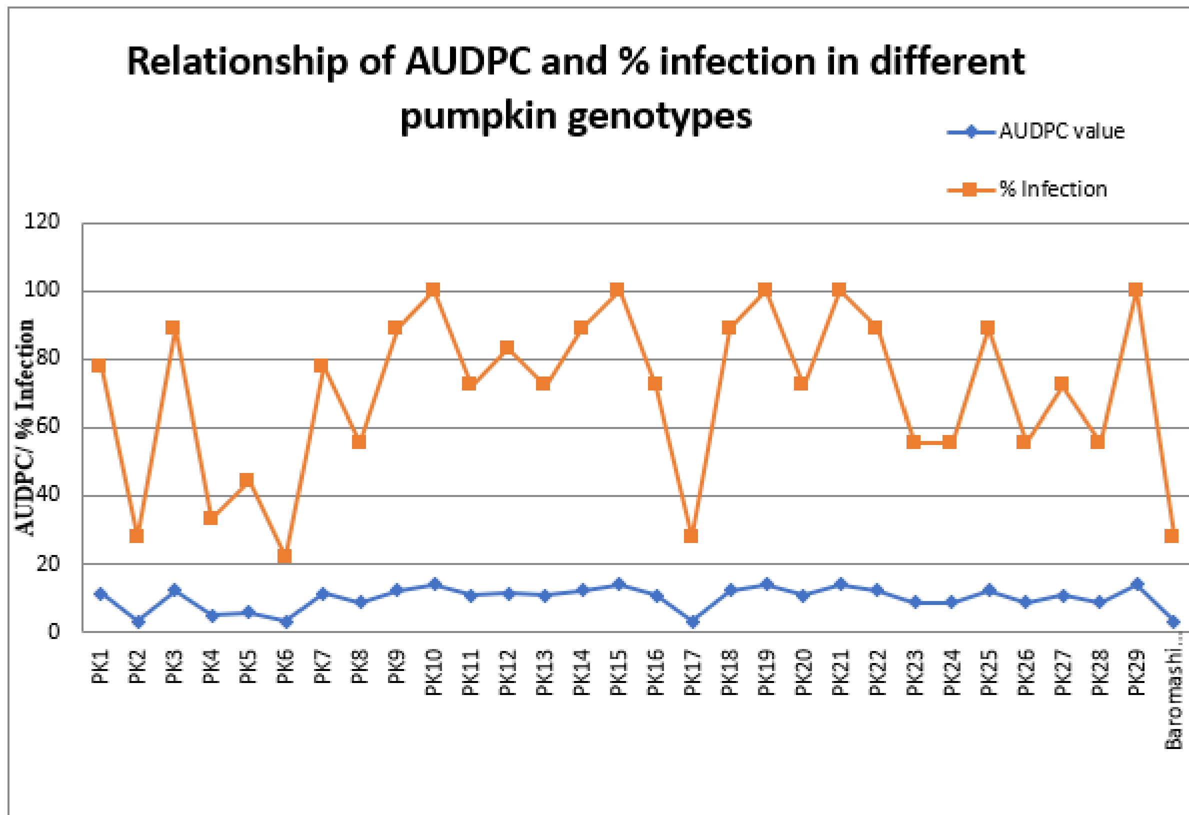

Fig.3.2. The nature and extend of relationship between AUDPC and % Infection in the thirty pumpkin genotypes. This graph also reflects the resistance pattern of different pumpkin genotypes under controlled condition where vector transmission was allowed.

4. Discussion

Yellow vein mosaic disease in *Cucurbita moschata*, Duch. ex Poir. caused by a begomovirus, has become increasingly severe in recent years across India, Bangladesh, and even the Middle East, leading to a notable fraction of yield loss. This disease is transmitted by whiteflies (*Bemisia tabaci* biotype B). In this study, we attempted to characterize pumpkin genotypes based on field performance, AUDPC, percentage infection, TBIA, and molecular assays. The field assay results indicated that most genotypes had lower yields, except for PK2, PK5, and PK27. This reduced yield is likely due to viral infection, as even the control genotype, Baromashi, was unable to produce higher yields. The crop has an indeterminate bearing habit, producing a very small number of unhealthy fruits until death; thus, data were recorded until fruit maturity. Additionally, our study indicated that the genotype PK6 was less infected by PYVMV (Table 6.4), but the gene responsible for PYVMV was absent (Fig. 6.3 & Fig. 6.4), despite initial assumptions of resistance. It is possible that another virus-resistant gene or allele, other than the PYVMV gene, may have contributed to PK6's resistance mechanism. Further detailed and extensive studies are essential for a definitive conclusion.

Both the movement protein (MP) and coat protein (CP), rather than any elicitor, are implicated in begomovirus infection, as stated by Muniyappa et al., 2002. Previous research indicated that DNA-A and DNA-B specific primers amplify products from PYVMV-affected tissues, suggesting that the associated virus is probably bipartite. However, in our PCR assay, we only amplified DNA-B (550 bp), an MP amplicon. We did not achieve the expected 850 bp CP amplification from infected samples. The

absence of a 550 bp amplification in healthy samples indicated the high specificity of our primers. Therefore, these primers could be used in developing a molecular diagnostic kit for rapid PYVMV pathogen detection. As mentioned previously, the ability of MP-specific primers to amplify products from PYVMV-affected samples suggests that the virus associated with this disease is probably monopartite, as MP genes are encoded by DNA-B (Varma, 1955; Saikia & Muniyappa, 1989; Nateshan et al., 1996).
Moreover, earlier reports on geminivirus genome organization suggested that monopartite viruses do not possess DNA-B or MP, with both encapsidation and movement functions controlled by CP (Harrison et al., 2002; Fauquet and Stanley, 2003). We did not perform any phylogenetic analysis, but both the CP and MP gene sequences showed maximum similarity to Squash leaf curl China virus [Pumpkin: Coimbatore database survey], aligning with observations by Singh et al., (2009). Muniyappa et al., (2003) reported that PYVM disease in Karnataka state is caused by a strain of tomato leaf curl New Delhi virus. In our laboratory, we could not sequence the infected DNA samples; hence, PCR-based detection remains the only method to infer the tested genotypes. However, comprehensive database information combined with our results may aid in rapidly screening PYVMV-free underutilized pumpkin genotypes.

5. Conclusion

A combination of different approaches facilitated our rapid virus detection technology that may be sustainable in pumpkin virus resistance system without hampering environment. Three genotypes were identified to have PYVMV resistant gene while most of the genotypes were susceptible against yellow vein mosaic virus. It is also suggested that this kind of research could be highly beneficial for both scientists and farmers in Bangladesh, offering valuable insights into the advancement of rapid virus detection technologies.

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