Bangladesh J. Environ. Sci., Vol. 48, 25-30, 2025 @ BAED ISSN 1561-9206

SCREENING AND GENETIC ANALYSIS OF PUMPKIN (*Cucurbita moschata*) GENOTYPES FOR RESISTANCE TO PUMPKIN YELLOW VEIN MOSAIC VIRUS (PYVMV)

M. J. H. Khan[1*], B. Saha[2], B. Roy[3], M. F. A. Siddique[3] and M. A. Haque[4]
[1]Soil Conservation and Watershed Management Centre, SRDI, Bandarban
[2]Bangladesh Rice Research Institute (BRRI), Gazipur 1701, Bangladesh
[3]Department of Agricultural Extension (DAE), Ministry of Agriculture, Bangladesh
[4]Department of Genetics and Plant Breeding, BAU, Mymensingh-2202, Bangladesh
*Corresponding author's e-mail: jonayed71@gmail.com

ABSTRACT

Two separate experiments were done with eighteen locally available pumpkin genotypes. First was done under field condition where there was no attempt taken to control the infestation by white fly which is the key vector for transmitting pumpkin yellow vein mosaic virus at Field laboratory. Second experiment was done net at Net House of Department of Genetics and Plant Breeding. About seven genotypes were selected for next year for second experiment which was mechanically inoculated with virus by rubbing of virus sap. The performance of 18 pumpkin genotypes for yield and different yield contributing traits were evaluated and observed that there were significant variations for all the traits studied among the genotypes. Genetic variability analysis revealed that there is significant amount of variability present among genotypes of pumpkin which can be exploited for qualified and high yield pumpkin production. Fruit weight of pumpkin is positively correlated with the yield of pumpkin. Genotypes PK2, PK5, PK6, PK13, PK15, PK16 and PK17 showed resistance against PYVMV under field condition. Genotype PK13 showed resistance against PYVMV both under field condition and artificial rubbing.

Key words: Screening, genetic analysis, pumpkin, yellow vein mosaic virus

Introduction

Yellow vein disease was first reported in northern India in the early 1940s affecting pumpkin (Vasudeva and Lal, 1943). Symptoms of the disease include yellowing of veins on younger leaves which ultimately produce mosaic patches in the later stages of infection. The presence and distribution of Pumpkin yellow vein mosaic disease (PYMV) was reported throughout India. There are two species of Gemini viruses which cause PYVMD have been reported in India. One from northern India Tomato leaf Curl New Delhi virus-India and the other from southern India Squash leaf curl China virus-India and both are bipartite (Singh *et al*. 2009). Finally, pumpkin yellow vein mosaic disease (PYVMD) causes significant damage to pumpkin production, which belonging to the Cucuarbitacae family and one of the most important vegetables in Bangladesh as well as throughout the world. It is an important vegetable for its high yields, good storage, and longer period of consumption, high nutritive values, substantial medicinal properties and fitness in transport. Therefore it is an urgent need to develop yellow vein mosaic virus free pumpkin lines. Phenotypic screening is a foundational step in identifying resistant genotypes. Researchers evaluate symptoms such as yellowing, vein clearing, and mosaic patterns, often using a severity scale. For example, a 0-5 scale has been used to assess symptom severity in response to PYVMV infection, with lower scores indicating higher resistance (Seda-Martinez *et al*., 2021; Kavalappara *et al.*, 2024). Both greenhouse and field trials are essential for validating resistance under different environmental conditions. Greenhouse trials provide controlled conditions for precise inoculation and monitoring, while field trials assess performance under natural infection pressures. For instance, trials conducted in Puerto Rico evaluated tropical pumpkin genotypes for resistance to PYVMV and other potyviruses, revealing significant differences in symptom severity and yield impact (Seda-Martinez *et al*., 2021). Molecular markers, such as SNPs and SCAR markers have been developed to facilitate marker-assisted breeding for PYVMV resistance. For example, RAPD and SCAR markers related to WMV and ZYMV resistance were developed

using resistant and susceptible *C. moschata* lines. These markers enable early selection of resistant genotypes in breeding programs (Kim *et al*., 2016).The current study has been attempted to identify yellowing virus resistant lines or landraces among locally available pumpkin lines. Generally, viral diseases are controlled by removal of virus reservoirs and killing of vector but this cause reduction in biodiversity. Again, chemical control of vectors is not possible due to non-persistent mode of virus transmission (Adams *et al*. 2005; Ingvardsen *et al*., 2010). So, cultivation of resistant Pumpkin varieties is the most effective way to control virus infections. In Bangladesh, no line or landrace has been identified yet as a resistant one. Resulting screening of locally available pumpkin lines or landraces as a source of virus resistance is a current need. The specific objectives of current research program are as: i) to study genetic diversity of locally available pumpkin lines using morphological data for better utilization of pumpkin germplasm and ii) to identify locally available pumpkin lines containing pumpkin yellow vein mosaic virus disease resistant gene.

Materials and Methods

The field experiment was designed and conducted in the field lab and net house of the Department of Genetics and Plant Breeding, Bangladesh Agricultural University (BAU), Mymensingh. Molecular work for screening was carried out at the molecular laboratory of Department of Genetics and Plant Breeding, BAU, Mymensingh and the Biotechnology Laboratory of Biotechnology Division, Bangladesh Institute of Nuclear Agriculture (BINA), Mymensingh. Eighteen locally available pumpkin lines were selected as experimental materials; those were designated as PK 1 to PK 18 (Table 1).

Table 1. List of pumpkin genotypes used in the experiment

Genotypes	Description/source	Genotypes	Description/source
PK1	Jessore-1	PK10	Saver-3
PK2	Jessore-2	PK11	Thakurgaon-1
PK3	Jessore-3	PK12	Thakurgaon-2
PK4	Jessore-4	PK13	Thakurgaon-3
PK5	Commilla-1	PK14	Thakurgaon-4
PK6	Commilla-2	PK15	Meherpur-1
PK7	Commilla-3	PK16	Meherpur-2
PK8	Saver-1	PK17	Meherpur-3
PK9	Saver-2	PK18	Meherpur-4

Field experiment: Eighteen genotypes with three replications following RCBD design was performed at the field of Genetics and Plant Breeding department. Appropriate measurement was taken for the growth and development of plant as necessary.

Pot experiment and design: For the proper selection of genotypes, another experiment was conducted on earthen pot following RCBD also. It is done with the superior genotypes derived from field experiment data for the observation of PYVMV resistant genotypes. The pots were labeled with tag. Recommended production packages were followed as and when necessary to ensure the normal plant growth and development.

Virus inoculation and scoring: Pumpkin plants are frequently attacked by pumpkin yellow vein mosaic virus. White fly is the vector for pumpkin yellow vein mosaic virus. Two separate experiments were done for virus inoculation .One is pumpkin plants were kept naturally and white flies were allowed to attack pumpkin plants. Other was artificial inoculation. Newly grown plants were inoculated with virus by rubbing inoculation sap. The inoculation sap was made by homogenizing young leaves with typical mosaic symptoms of virus infected plants. Mortar and pestles were used for grinding virus infected leaves.

Statistical analysis on morphological data: The morphological data were collected at field maturity of red pumpkin. The recorded data were analyzed statistically according to the design used in the experiment.

Analysis of variance: Analysis of variance (ANOVA) was done on the sample for all the 7 character mentioned using MStat4c statistical program. The total variance of each character was partitioned into replication, genotype and error. The differences within the classes of effects were tested by F-test.

Regarding genetic analysis, estimation of genotypic and phenotypic variances; heritability; genotypic co-efficient of variation (GCV) and phenotypic co-efficient of variation (PCV); genetic advance (GA) and correlation coefficient (r) were done following the wide circulated and established formulae.

Analysis on scoring of virus symptoms: Scoring of virus symptoms had been used to calculate % infection and AUDPC (area under disease progress curve). AUDPC was calculated from means of disease ratings for each line in each replicate using trapezoid method from the time of first disease scoring. The trapezoid method is the most common way to calculate AUDPC. The genotype with 100% infection would possess 14 score in AUDPC.

Results and Discussion

The performance of 18 pumpkin genotypes for yield and different yield contributing traits (morpho-agronomic traits) were evaluated and observed that there were significant variations for all the traits studied among the genotypes (Tables 2-3). From the morphological data, each trait indicated separate genotype as best one, such as early male flowering was observed in genotype 9 (45 days), early female flowering was observed in genotype in genotypes 1 and genotypes 17 (66 days). The highest value for vine length was observed in genotype 12 (5.267 m), the highest value for no. of primary branches per plant was observed in genotype 10 and genotype 12 (14.33), the highest value for average fruit weight (kg) was observed in genotype 9 and genotype 12 (4.567 kg), the highest value for fruits per plant was observed in genotype 10 (3.5) and the highest value for yield was observed in genotype 12 (10.05 kg).

Table 2. Mean and range variation of 18 pumpkin genotypes for seven morpho-agronomic traits

Character	Mean ± Standard Error(mean)	Range	F-test	CV%
1st male flower opening(Days)	47.889 ± 2.52	45-54	**	2.03%
1st female flower opening (Days)	69.204 ± 2.40	65-77	**	1.93%
Vine length (m)	4.680 ± 0.34	4-5.3	**	2.52%
No. of primary branches per plant	11.426 ± 2.16	7-15	**	7.34%
Average fruit weight (kg)	2.441 ± 1.03	1-4.6	**	5.02%
Fruits per plant	2.707 ± 0.50	2-3.5	**	4.55%
Yield/plant (kg)	6.240 ± 1.83	4-10.58	**	8.43%

Table 3. Analysis of variance (mean squares) for different characters of 18 genotypes of pumpkin

Source of Variation	Degrees of freedom (df)	1st male flower opening (Days)	1st female flower opening (Days)	Vine length (m)	No. of primary branches per plant	Average fruit weight (kg)	Fruits Per plant	Yield/ plant (kg)
Replication	2	5.056	6.241	0.019	2.296	0.006	0.004	0.028
Genotype	17	17.843**	14.162**	0.333**	13.051**	3.256**	0.756**	9.848**
Error	34	0.944	1.778	0.014	0.704	0.015	0.015	0.277

Phenotypic variance was greater than the genotypic variances for all the traits which indicated the influences of environmental factor on these traits. Almost all traits studied here showed high heritability (Table 4) except vine length. Genetic advance was highest for 1st male flower opening followed by no. of primary branches per plant, yield/plant, 1st female flower opening, average fruit weight, fruits per plant and vine length. The mean percentage of genetic advance was highest in case of average fruit weight (87.05%) followed by yield/plant and lowest for days to 1st female flower opening**.** Genetic variation, heritability and genetic advance provides the opportunity to predict the amount of genetic gain that could be obtained in later generations, if proper selection is made for improving the particular trait under study.

Character exhibiting high heritability may not necessarily give high genetic advance. Johnson *et al*., (1955) showed high heritability should be accompanied by high genetic advance to arrive at more reliable conclusion. In general, it is the additive gene action where high heritability is controlled by high genetic advance (Panse and Sukhatm, 1957) and can be improved through simple or progeny selection methods. Selection for the traits having high heritability coupled with high genetic advance is likely to accumulate more additive genes leading to further improvement of their performance. In this experiment, five associations showed positive significant correlation: yield/plant with average fruit weight, 1st male flower opening with 1st female flower opening, No. of primary branches per plant with 1st male flower opening, 1st female flower opening and vine length. Two associations showed a significant negative correlation: fruits per plant with yield/plant and average fruit weight (Table 5).

Table 4. Genetic parameters of seven different characters of 18 pumpkin genotypes

	Phenotypic variance	Genotypic variance	PCV (%)	GCV (%)	Heritability (%)	GA	GA (%)
1st male flower opening (Days)	6.577	5.633	5.355	4.956	85.646	4.522	9.44
1st female flower opening (Days)	5.906	4.128	3.511	2.935	69.895	3.494	5.04
Vine length (m)	0.81	0.106	19.23	4.956	13.086	0.241	5.14
No. of primary branches per plant	4.819	4.115	19.212	17.753	85.391	3.857	33.76
Average fruit weight (kg)	1.095	1.080	42.86	42.573	98.630	2.125	87.05
Fruits per plant	0.262	0.247	18.908	18.359	94.274	0.993	36.68
Yield/plant (kg)	3.467	3.190	29.839	28.622	92.01	3.528	56.55

Table 5. Correlation co-efficient between yield and other yield related characters

	1st male flower opening (Days)	1st female flower opening (Days)	Vine length (m)	No. of Primary branches per plant	Average fruit weight (kg)	Fruits per plant
1st female flower opening (Days)	0.752**					
Vine length (m)	0.243	0.25				
No. of Primary branches per plant	0.559**	0.365**	0.547**			
Average fruit weight (kg)	-0.142	0.052	0.159	0.004		
Fruits per plant	0.145	0.119	-0.008	-0.083	-0.697**	
Yield/plant (kg)	-0.156	0.065	0.147	0.060	0.909**	-.357**

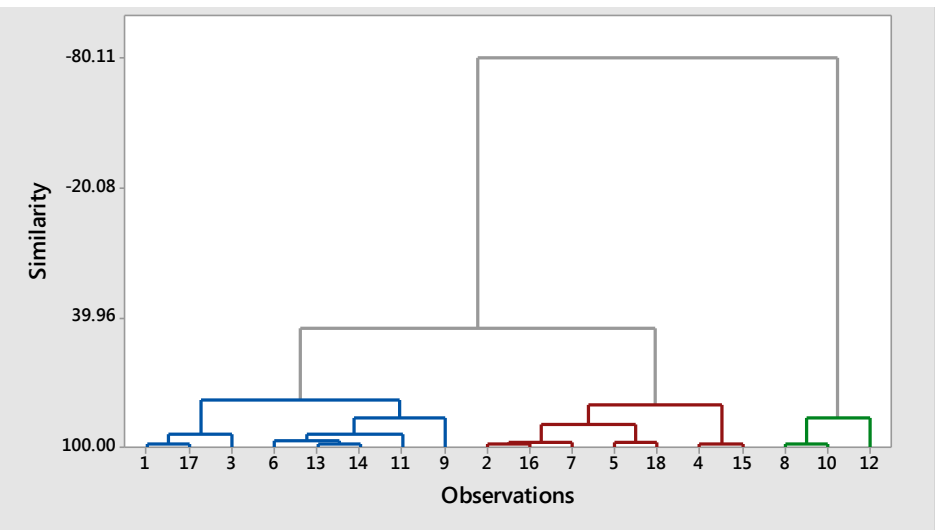

Fig. 1. Dendrogram showing distribution of eighteen genotypes among three clusters

Table 6. Clustering of 18 pumpkin genotypes

Cluster	No. of Genotypes	Genotypes
I	8	1,17,3,6,13,14,11 and 9
II	7	2,16,7,5,18,4 and 15
III	3	8,10 and 12

Maximum numbers of genotype were under cluster I which representing early flowering genotypes and having relatively high yield potentiality. Seven genotypes were classified in second cluster accounting for 38.88 per cent of total genotypes representing lower yields. Third cluster exhibits late flowering and lower yields (Fig. 1 and Table 6). Rogers (1972) described that the cluster analysis is an important tool for assessing family relationships.

From the observation of infection of pumpkin yellow vein mosaic virus, seven genotypes (such as 2, 5, 6, 13, 15, 16 and 17) showed resistance (Table 7). These seven genotypes considered superior genotypes considering the aim of the study and in the net house these genotypes were planted and inoculated with virus through mechanical rubbing with virus sap and scored for symptoms development at 7, 10 and 14 dpi (days post inoculation). From the value of % infection and AUDPC it was seemed that genotypes, PK 13 might be considered as highly resistant, PK17 and PK2 might be considered as moderately resistant, PK5 and PK15 might be considered as moderately susceptible and PK6 and PK16 might be considered as susceptible (Table 8).

Table 7. Screening pumpkin genotypes under field condition against PYVMV

Genotypes	Total PYVMV affected plant	% of PYVMV affected plant
PK1	3	30
PK2	0	0
PK3	2	20
PK4	3	30
PK5	0	0
PK6	0	0
PK7	2	20
PK8	5	50
PK9	6	60
PK10	6	60
PK11	3	30
PK12	1	10
PK13	0	0
PK14	1	10
PK15	0	0
PK16	0	0
PK17	0	0
PK18	3	30

Table 8. The mean ordinal score and AUDPC for each line

Sl	Genotype name	Mean ordinal score	% Infection	AUDPC (area under disease progress curve)
1	PK2	1.11	37.03	7.99
2	PK5	1.22	40.73	8.99
3	PK6	1.66	55.55	12.74
4	PK13	00	00	00
5	PK15	1.44	48.13	10.99
6	PK16	1.55	51.83	11.49
7	PK17	1	33.33	7.5

Covering all aspects genotype PK13 may be declared as the best genotypes among all the 18 locally available pumpkin genotypes due to its resistance against pumpkin yellow vein mosaic virus and considerable yield.

Conclusion

This study evaluated the resistance of locally available pumpkin genotypes to Pumpkin Yellow Vein Mosaic Virus (PYVMV) through field and net house experiments. The field trial, under natural whitefly infestation, assessed 18 genotypes for disease resistance and yield traits under real-world conditions. Significant genetic variability was observed, indicating strong potential for selecting resistant, high-yielding genotypes. Parameters like infection percentage and AUDPC identified genotype PK13 as the most promising, showing complete resistance and strong yield performance. Statistical analyses confirmed high heritability and genetic advance for key traits such as fruit weight and yield, supporting the potential for successful selective breeding. The study concludes that local pumpkin germplasm holds valuable traits for developing PYVMV-resistant cultivars. Further multi-season trials are recommended to validate the stability of these findings. Overall, the research offers a solid foundation for breeding resilient pumpkin varieties, emphasizing the value of integrating field data, controlled testing, and genetic analysis in crop improvement programs.

References

Adams, M., et al. 2005. Molecular criteria for genus and species discrimination within the family Potyviridae. Archives of virology 150(3): 459-479.

Ingvardsen, C. R., et al. (2010). "Genetic and physical fine mapping of Scmv2, a potyvirus resistance gene in maize. Theoretical and Applied Genetics, 120(8): 1621-1634.

Johnson, H. W., Robinson, H. F. and Comstock, R. E. 1955. Estimates of genetic and environmental variability in soybean. *Agron. J.*,(473):14-318.

Kavalappara, S. R., Bag, S., Luckew, A. S., McGregor, C. E., Culbreath, A., & Simmons, A. M. (2024). Evaluation of Squash (Cucurbita pepo L.) Genotypes for Resistance to Cucurbit Chlorotic Yellows Virus. *Horticulturae*. https://doi.org/10.3390/horticulturae10030264

Kim, D. K., Seo, S.-G., Kwon, S. B., & Park, Y.-D. (2016). Development of RAPD and SCAR markers related to watermelon mosaic virus and zucchini yellow mosaic virus resistance in Cucurbita moschata. *Horticulture Environment and Biotechnology*, *57*(1), 61–68. https://doi.org/10.1007/S13580-016-0090-0

Rogers, J. S. 1972. Measures of genetic similarity and genetic distance. *Studies Genetics*, 7(7213): 145-153.

Seda-Martinez, W., Wessel-Beaver, L., Linares-Ramírez, A., & Verle Rodrigues, J. C. (2021). Virus Quantification, Flowering, Yield, and Fruit Quality in Tropical Pumpkin (Cucurbita moschata Duchesne) Genotypes Susceptible or Resistant to Two Potyviruses. *Hortscience*, *56*(2), 193–203. https://doi.org/10.21273/HORTSCI15525-20

Singh, A., et al. 2009. Biological and molecular characterization of a begomovirus associated with yellow mosaic vein mosaic disease of pumpkin from Northern India. Virus genes, 39(3): 359-370.

Vasudeva, R. and T. Lal, 1943. A mosaic disease of bottle gourd. *Indian J. Agric. Sci.*, 13: 182-198.