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# Molecular Characterization and Validation of Micro-Satellite Markers Linked to Powdery Mildew Disease Resistance in Mini Core Germplasm of Urdbean [*Vigna mungo* (L.) Hepper]

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## Abstract

Twenty-nine Urdbean [*Vigna mungo* (L.) Hepper] genotypes, including four check varieties 'Prasad,' 'Ujala,' 'PU-31' and 'OBG-31' were evaluated in the experimental farm of Odisha University of Agriculture and Technology, Bhubaneswar, India, for two seasons to assess these genotypes against powdery mildew disease incidence and to estimate the extent of genetic divergence and character association employing molecular markers. For molecular diversity studies five molecular markers *i.e.*, MB-SSR238, VrCsSTS1, CEDG191, VrCsSSR1, CEDG166 were evaluated on the Urdbean genotypes. The results showed the association of VrCsSTS1 marker with the powdery mildew resistance gene in different genetic backgrounds. The genotypes 'OBG-31', 'Ujala,' 'Prasad,' and 'Nayagarh-I local' were resistant to powdery mildew disease, which can be further used for breeding programs.

**Keywords:** Molecular marker, Powdery mildew, Resistance, Urdbean

## Introduction

Urdbean or Black gram (*Vigna mungo*) belongs to the family Fabaceae. It is a staple crop in central and South East Asia; however, it is extensively used only in India and is now grown in the Southern United States, West Indies, Japan, and other tropics and subtropics (Mader *et al*., 2011). Urdbean is consumed as dal because of its body-building properties due to various amino acids and medicinal properties. Powdery mildew disease caused by *Erysiphe polygoni* is one of the serious constraints that affects the cultivation of Urdbean in India and other countries. Yield loss is much high when the pathogen infects the crop before flowering. However, it results in complete crop loss if the disease occurs at the seedling stage. Abbaiah (1993) reported that the powdery mildew in Urdbean was generally noticed in 45 days old crops upon attack by *Erysiphe polygoni* D.C. This fungus affects Urdbean in high humid conditions and is easily transmitted through sporulation. They affect all the parts of the plants. However, leaves are affected initially, and the morphology of the plant begins to change as the progress of the disease (Jayasekhar and Ebenezar, 2016; Sahoo *et al.*, 2020a). It is especially devastating in the cool-dry growing season. Although urdbean has a short growth duration, progress in breeding cultivars with Powdery mildew disease resistance has been slow (Sahoo *et al.*, 2020b). A factor limiting breeding progress is selection for powdery mildew disease resistance, which is is confined to the cool-dry season (Chankaew *et al*., 2013).

Screening of breeding population for Powdery mildew disease is difficult, especially when weather conditions do not favour fungal growth, and hot spots of natural Powdery mildew disease epidemics are not always available (Sahoo *et al.*, 2020c). To ensure error-free identification of resistant plants in segregating populations, developing a molecular marker linked to the resistant gene could be helpful. Among several classes of DNA markers developed (Singh *et al.*, 2018), microsatellite simple sequence repeat (SSR) markers have proven highly versatile and useful for genetic studies (Sahoo *et al.*, 2018; Sahoo *et al.*, 2019). The present investigation was planned for field screening for powdery mildew resistance in a set of Urdbean germplasm, agro-economic assessment of elite powdery mildew resistance genotypes,

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molecular characterization for powdery mildew resistance in selected germplasm, and validation of molecular markers associated with powdery mildew resistance.

## Materials and Methods

### *Plant Materials and Screening the Accessions against Powdery Mildew Incidence*

Twenty-nine accessions of Urdbean were sown in January 2017 and 2018 at the experimental farms of Odisha University of Agriculture and Technology Bhubaneswar, Odisha, India. The seeds of twenty-nine Urdbean genotypes were sown in the field in a Randomised Block Design. The first experiment was planted during *rabi* 2017, and the second experiment was planted during *rabi* 2018 for the characterization of genotypes. Recommended package of agronomic practices and plant protection measures were adopted as and when required. The field experiments were carried out under conditions favouring normal growth and expression of all the characteristics (Prathyusha *et al*., 2017). The disease intensity of powdery mildew disease was recorded using a 0 to 9-point scale given by Mayee and Datar (1986) on five marked plants of each entry at the pod maturity stage of the crop growth.

### *Genotyping of Urdbean Varieties*

Genomic DNA was isolated from 15 days old Urdbean seedlings following the modified protocol of Agbagwa *et al*. (2012). The quality and quantity of DNA were checked by agarose gel electrophoresis and nanodrop spectrophotometer reading. The final concentration to do PCR was adjusted to 100 ng $\mu l^{-1}$. DNA templates from twenty-nine Urdbean genotypes were amplified using five SSR primer pairs (Table 1) selected from those previously identified for powdery mildew resistance (Zhang *et al*., 2008; Kasettranan *et al*., 2010). The amplification was performed in a reaction volume of 25 µl containing 10 mM Tris-HCl, pH 9.0, 1.5 mM $MgCl_2$, 50 mM KCl, 0.01% gelatine, 100 µM each of dNTPs, 10 ng of single random primer, 10 ng of genomic DNA and 1 unit of Taq polymerase (Genei, Bangalore). Amplifications were performed in a Thermocycler (Bio-Rad T-100M), programmed for 3 min at 94 °C, 35 cycles of 1 min at 94 °C, 1 minute at annealing temperature (varied with primer), and 2 minutes at 72 °C, and final extension for 7 min at 72 °C followed by storing at 4 °C till loading to the agarose gel.

Table 1: List of SSR markers used in the present study

| Sl. No. | Marker Name | Forward Sequence (5' to 3') | Reverse Sequence (5' to 3') | Annealing Temp. |
|---|---|---|---|---|
| 1 | CEDG166 | GGTACAACATTCTTCTATTTG | GGCTTATGAGTTTATCTTATC | 48 °C |
| 2 | MB-SSR238 | AGCTATTGGTGCATAGGTTC | GATATGATGAGTATGGTGTAG | 54 °C |
| 3 | CEDG191 | CAATAAGCAATCTGTGGAGAG | CTGCAGGAAACTTGGAATTGC | 60 °C |
| 4 | CEDG0282 | CAGCAACAAGACATGGAGTG | GGTGACCACTTAGACAGAC | 52.5 °C |
| 5 | VrCSSTS1 | ATTACTTGAGGTGGGGATAAT | AATAGACCACTTTTTCCGT | 56 °C |

The amplified products were loaded in respective wells alongside a 100 bp molecular marker in 2.4% agarose gel containing 0.5 mg $ml^{-1}$ of ethidium bromide and electrophoresed at a constant voltage of 50 V. The amplifications were checked twice for their reproducibility. The gel was visualized by the gel doc system (Uvtec, Cambridge, UK). The size of the amplicon(s) was determined by comparing it with the DNA ladder (100 bp) with known fragment sizes. Scoring of the amplification product was done by '1' if the allele is present and '0' when the allele is absent (Figure 1). Homology of alleles was based on the distance migration of amplified DNA fragments according to their molecular weights in the gel. Jaccard's similarity coefficient estimated pair-wise genetic similarities between genotypes. Clustering was done using the symmetric similarity coefficient matrix, and the clusters were obtained based on unweighted pair group arithmetic mean (UPGMA) using the software program NTSYS pc Ver 2.1 (Rohlf, 1998).

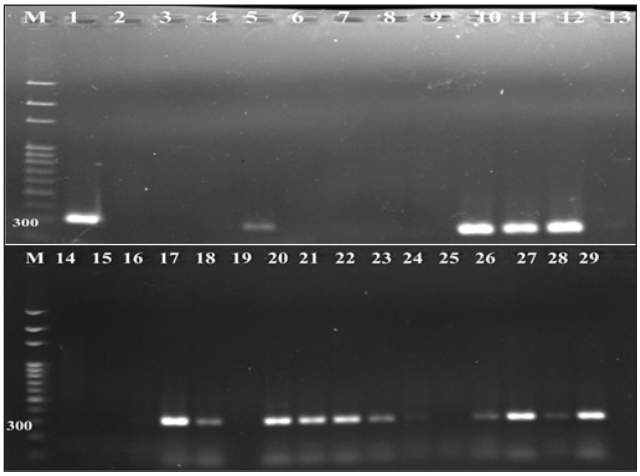


Figure 1: SSR genotyping profile of urdbean genotypes by the primer VrCsSTS1 on agarose gel

## Results and Discussion

### *Screening of Urdbean Germplasm against Powdery Mildew*

The *kharif* season was favourable for powdery mildew disease incidence, and most genotypes showed moderate resistance and moderate susceptible reaction. Seven ('OBG-31', 'Prasad,' 'Kendrapada local,' 'Nayagarh local.' 'Denkhanal local,' 'Jagatsinghpur local' and 'Golapali local') genotypes were found to be resistant, and seven varieties were found moderately resistance, 13 moderately susceptible and one highly susceptible among these genotypes. In other studies, scientists used excised leaves to study powdery mildew and found that the RUM lines resisted powdery mildew (Reddy

Table 2: Powdery mildew disease reaction of urdbean genotypes based on phenotypic observations

| Disease reaction | No. of Genotypes | Name of the Genotypes |
|---|---|---|
| MR | 8 | OBG-31, PU-31, Ujala local, Charipalli local, Nayagarh-2 local, Bhawanipatna local, Badamba local-2, Dharakote local |
| R | 7 | Aska local, Badamba local-1, Nayagarh-1 local, Denkhanal local, Jagatsinghpur local, Prasad local, Golapali local |
| MS | 13 | Puri-2 local, Kendrapada local, Suvrnagiri local, Kendrapada local, Bolangiri local, Rourkela local, Nayagarh-3 local, Kantapada local-1, Kotthagarha local, Mohana local, Keonjhar-1 local, Keonjhar-3 local, Seimiliguda local |
| HS | 1 | Boudha-1 local |

MR: Moderately resistance; R: Resistance; MS: Moderately susceptible; HS: Highly susceptible

*et al*., 1994). Subsequently, some scientists found fewer accessions resistant to powdery mildew (Sivaprakasam *et al*., 1976; Consonni *et al*., 2006; Sahoo *et al*., 2021a). Scientists also reported resistance varieties in urdben, *i.e.*, LBG17 (Agarwal *et al*., 1989); P115 (Kaushal and Singh, 1989), LBG-623, and LBG-648 (Prashanthi *et al*., 2010), BS 2-3, IPU 02-43 and B 3-8-8 (Akthar *et al*., 2014). However, in the present study, the absence of resistance lines from the test germplasm population highlights the need for extensive work in exploring new sources (Sahoo *et al.*, 2021b).

*Genetic Diversity Analysis*

The UPGMA dendrogram (Figure 2) was constructed using Jaccard's similarity coefficient of SSR markers on 29 genotypes by scoring the bands in the agarose gel of employed markers and using the NTSYS program (Table 3). In the present investigation, the minimum dissimilarity coefficient of 0.44 was recorded between 'Nayagarh local' and 'Ujala' along with 'Dharakote local' and 'Charipalli local,' indicates that these four genotypes were closely related to each other and were most similar pairs among all the genotypes under study. The dendrogram (Figure 2) was divided into two major clusters at a dissimilarity coefficient of 7.0. Cluster-I has 17 genotypes, and cluster-II includes 12 genotypes. Clusters I and II are subdivided at a dissimilarity coefficient of 6.1. Sub-Cluster-I is further divided into two sub-clusters, IA and IB. Cluster IA included 7 genotypes *viz*., 'OBG- 31', 'Nayagarh-3 local', 'Seimiliguda local', 'Bhawanipatna local', 'Boudha local', 'kendrapada local', 'Mohanalocal'. Cluster IB Included the genotypes 'Aska local,' 'Suvrnagiri local,' 'Keonjhar local,' 'Rourkela local,' 'Denkhanal local,' 'Nayagarh local,' 'Prasad Badamba local,' and 'Kendrapada local' among them 'Suvrnagiri local' and 'Keonjhar local,' were in pair showing distinct showing wider divergence, other genotypes are closely related, and also 'Keonjhar local,' and 'Denkhanal local' were in pair, were in pair showing distinct showing wider divergence and remaining one genotype 'Kendrapada local' was separated and showing wider divergence. Sub-cluster II is divided into three sub-clusters IIA, IIB, and IIC. Cluster IIA included five genotypes 'PU-31, Ujala', 'Nayagarh local,' 'Charipalli local,' and 'Dharakote local; IIB included the genotypes 'Puri local,' 'Bolangiri local,' 'Badamba local,' 'Jagatsinghpur local,' 'Kantapada local' and 'Golapali local and IIIC included 'Kotthagarha local' which was separated and showing wider divergence.

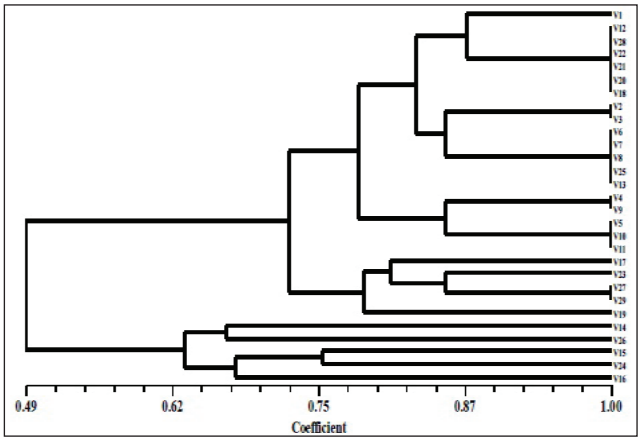


Figure 2: Dendrogram depicting the classification of 29 urdbean genotypes; in which and investigated scale at the bottom is Jaccard's similarity coefficient

Similar results were also obtained from previous studies where little divergence in Urdbean was found, with the inter-cluster distance ranging from 1.94 to 6.9 (Chauhan *et al*., 2008). This revealed a significant amount of variety in the germplasm collections that were investigated for this research. This is also in accordance with findings that previously suggested a large variability in urdbean materials (Chauhan *et al*., 2008). According to the research findings, the cultivars of urdbean have a high degree of genetic resemblance with one another. This may be because their ancestry has a significant amount of shared ancestry. In this investigation, we could determine that the urdbean cultivars come from a small genetic background. The findings of the analysis highlight how important it is for cultivar development initiatives to use huge germplasm pools, including a wide variety of morpho-agronomic features (Basak *et al*., 2005; Thirumalaiandi *et al.*, 2008; Sahoo and Sharma, 2018). Previous investigators did the research (Lakhanpaul *et al*., 2004; Chattopadhyay *et al*., 2007; Souframanien and Gopalakrishna, 2004; Priyadarshini *et al.*, 2020) and investigated the diversity in *Vigna* species using

RAPD and SSR primers which has also shown similar findings. Even they have observed that the genetic foundation of *Vigna* species is limited. On the other hand, the diversity obtained in this study can be utilized to develop mapping populations.

*Validation of Molecular Marker for Powdery Mildew in Urdbean Genotypes*

The present investigation showed molecular diversity studies with five molecular markers *viz*., MB-SSR238, VRCSSTS1, CEDG191, VrCsSSR1, and CEDG166 employing upon 29 urdbean genotypes. Out of five molecular markers, two MB-SSR238 and VrCs STS1 amplified the all powdery mildew resistance genotypes and generated bands at 300 bp (Table 4). Similarly, CEDG191, CEDG282, and CEDG166 markers are amplified all other genotypes linked to powdery mildew resistance at 150 bp, 200 bp, and 100 bp, respectively. For marker MB-SSR238 and VrCs STS1, the results obtained from PCR amplification were found to the deviation (41.37% and 6.89% respecively) from the result obtained from field screening. Similarly, for markers (CEDG191, CEDG282, and CEDG166), the result obtained from PCR amplified DNA banding pattern and result obtained from field screening were found to have higher deviation (41.37%, 34.48% and 31.08%).

These results showed that the association of this VrCsSTS1 marker closely with the powdery mildew resistance gene in different genetic backgrounds (a small deviation might be due to an error in phenotypic data). However, microsatellite markers have been used for genome mapping and genetic diversity studies in many crop plants (Ford *et al*., 2002; Kasettranan *et al.*, 2010; Sangiri *et al*., 2007; Gupta *et al*., 2013; Singh *et al*., 2014). Identification of a linked marker

Table 3: Pair wise similarity matrix based on Jaccard's similarity coefficient of 29 urdbean genotypes by SSR marker analysis

| | $V_1$ | $V_2$ | $V_3$ | $V_4$ | $V_5$ | $V_6$ | $V_7$ | $V_8$ | $V_9$ | $V_{10}$ | $V_{11}$ | $V_{12}$ | $V_{13}$ | $V_{14}$ | $V_{15}$ | $V_{16}$ | $V_{17}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $V_1$ | 1.00 | | | | | | | | | | | | | | | | |
| $V_2$ | 0.86 | 1.00 | | | | | | | | | | | | | | | |
| $V_3$ | 0.86 | 1.00 | 1.00 | | | | | | | | | | | | | | |
| $V_4$ | 0.63 | 0.71 | 0.71 | 1.00 | | | | | | | | | | | | | |
| $V_5$ | 0.75 | 0.63 | 0.63 | 0.86 | 1.00 | | | | | | | | | | | | |
| $V_6$ | 0.75 | 0.86 | 0.86 | 0.86 | 0.75 | 1.00 | | | | | | | | | | | |
| $V_7$ | 0.75 | 0.86 | 0.86 | 0.86 | 0.75 | 1.00 | 1.00 | | | | | | | | | | |
| $V_8$ | 0.75 | 0.86 | 0.86 | 0.86 | 0.75 | 1.00 | 1.00 | 1.00 | | | | | | | | | |
| $V_9$ | 0.63 | 0.71 | 0.71 | 1.00 | 0.86 | 0.86 | 0.86 | 0.86 | 1.00 | | | | | | | | |
| $V_{10}$ | 0.75 | 0.63 | 0.63 | 0.86 | 1.00 | 0.75 | 0.75 | 0.75 | 0.86 | 1.00 | | | | | | | |
| $V_{11}$ | 0.75 | 0.63 | 0.63 | 0.86 | 1.00 | 0.75 | 0.75 | 0.75 | 0.86 | 1.00 | 1.00 | | | | | | |
| $V_{12}$ | 0.88 | 0.75 | 0.75 | 0.75 | 0.88 | 0.88 | 0.88 | 0.88 | 0.75 | 0.88 | 0.88 | 1.00 | | | | | |
| $V_{13}$ | 0.75 | 0.86 | 0.86 | 0.86 | 0.75 | 1.00 | 1.00 | 1.00 | 0.86 | 0.75 | 0.75 | 0.88 | 1.00 | | | | |
| $V_{14}$ | 0.38 | 0.43 | 0.43 | 0.67 | 0.57 | 0.57 | 0.57 | 0.57 | 0.67 | 0.57 | 0.57 | 0.50 | 0.57 | 1.00 | | | |
| $V_{15}$ | 0.25 | 0.29 | 0.29 | 0.50 | 0.43 | 0.43 | 0.43 | 0.43 | 0.50 | 0.43 | 0.43 | 0.38 | 0.43 | 0.75 | 1.00 | | |
| $V_{16}$ | 0.38 | 0.43 | 0.43 | 0.43 | 0.38 | 0.57 | 0.57 | 0.57 | 0.43 | 0.38 | 0.38 | 0.50 | 0.57 | 0.60 | 0.75 | 1.00 | |
| $V_{17}$ | 0.63 | 0.50 | 0.50 | 0.71 | 0.86 | 0.63 | 0.63 | 0.63 | 0.71 | 0.86 | 0.86 | 0.75 | 0.63 | 0.43 | 0.50 | 0.43 | 1.00 |
| $V_{18}$ | 0.88 | 0.75 | 0.75 | 0.75 | 0.88 | 0.88 | 0.88 | 0.88 | 0.75 | 0.88 | 0.88 | 1.00 | 0.88 | 0.50 | 0.38 | 0.50 | 0.75 |
| $V_{19}$ | 0.63 | 0.71 | 0.71 | 0.71 | 0.63 | 0.86 | 0.86 | 0.86 | 0.71 | 0.63 | 0.63 | 0.75 | 0.86 | 0.43 | 0.50 | 0.67 | 0.71 |
| $V_{20}$ | 0.88 | 0.75 | 0.75 | 0.75 | 0.88 | 0.88 | 0.88 | 0.88 | 0.75 | 0.88 | 0.88 | 1.00 | 0.88 | 0.50 | 0.38 | 0.50 | 0.75 |
| $V_{21}$ | 0.88 | 0.75 | 0.75 | 0.75 | 0.88 | 0.88 | 0.88 | 0.88 | 0.75 | 0.88 | 0.88 | 1.00 | 0.88 | 0.50 | 0.38 | 0.50 | 0.75 |
| $V_{22}$ | 0.88 | 0.75 | 0.75 | 0.75 | 0.88 | 0.88 | 0.88 | 0.88 | 0.75 | 0.88 | 0.88 | 1.00 | 0.88 | 0.50 | 0.38 | 0.50 | 0.75 |
| $V_{23}$ | 0.63 | 0.50 | 0.50 | 0.50 | 0.63 | 0.63 | 0.63 | 0.63 | 0.50 | 0.63 | 0.63 | 0.75 | 0.63 | 0.25 | 0.29 | 0.43 | 0.71 |
| $V_{24}$ | 0.38 | 0.25 | 0.25 | 0.43 | 0.57 | 0.38 | 0.38 | 0.38 | 0.43 | 0.57 | 0.57 | 0.50 | 0.38 | 0.60 | 0.75 | 0.60 | 0.67 |
| $V_{25}$ | 0.75 | 0.86 | 0.86 | 0.86 | 0.75 | 1.00 | 1.00 | 1.00 | 0.86 | 0.75 | 0.75 | 0.88 | 1.00 | 0.57 | 0.43 | 0.57 | 0.63 |
| $V_{26}$ | 0.63 | 0.50 | 0.50 | 0.50 | 0.63 | 0.63 | 0.63 | 0.63 | 0.50 | 0.63 | 0.63 | 0.75 | 0.63 | 0.67 | 0.50 | 0.67 | 0.50 |
| $V_{27}$ | 0.75 | 0.63 | 0.63 | 0.63 | 0.75 | 0.75 | 0.75 | 0.75 | 0.63 | 0.75 | 0.75 | 0.88 | 0.75 | 0.38 | 0.43 | 0.57 | 0.86 |
| $V_{28}$ | 0.88 | 0.75 | 0.75 | 0.75 | 0.88 | 0.88 | 0.88 | 0.88 | 0.75 | 0.88 | 0.88 | 1.00 | 0.88 | 0.50 | 0.38 | 0.50 | 0.75 |
| $V_{29}$ | 0.75 | 0.63 | 0.63 | 0.63 | 0.75 | 0.75 | 0.75 | 0.75 | 0.63 | 0.75 | 0.75 | 0.88 | 0.75 | 0.38 | 0.43 | 0.57 | 0.86 |

Table 3: Continue...

| | $V_{18}$ | $V_{19}$ | $V_{20}$ | $V_{21}$ | $V_{22}$ | $V_{23}$ | $V_{24}$ | $V_{25}$ | $V_{26}$ | $V_{27}$ | $V_{28}$ | $V_{29}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $V_{18}$ | 1.00 | | | | | | | | | | | |
| $V_{19}$ | 0.75 | 1.00 | | | | | | | | | | |
| $V_{20}$ | 1.00 | 0.75 | 1.00 | | | | | | | | | |
| $V_{21}$ | 1.00 | 0.75 | 1.00 | 1.00 | | | | | | | | |
| $V_{22}$ | 1.00 | 0.75 | 1.00 | 1.00 | 1.00 | | | | | | | |
| $V_{23}$ | 0.75 | 0.71 | 0.75 | 0.75 | 0.75 | 1.00 | | | | | | |
| $V_{24}$ | 0.50 | 0.43 | 0.50 | 0.50 | 0.50 | 0.43 | 1.00 | | | | | |
| $V_{25}$ | 0.88 | 0.86 | 0.88 | 0.88 | 0.88 | 0.63 | 0.38 | 1.00 | | | | |
| $V_{26}$ | 0.75 | 0.50 | 0.75 | 0.75 | 0.75 | 0.50 | 0.67 | 0.63 | 1.00 | | | |
| $V_{27}$ | 0.88 | 0.86 | 0.88 | 0.88 | 0.88 | 0.86 | 0.57 | 0.75 | 0.63 | 1.00 | | |
| $V_{28}$ | 1.00 | 0.75 | 1.00 | 1.00 | 1.00 | 0.75 | 0.50 | 0.88 | 0.75 | 0.88 | 1.00 | |
| $V_{29}$ | 0.88 | 0.86 | 0.88 | 0.88 | 0.88 | 0.86 | 0.57 | 0.75 | 0.63 | 1.00 | 0.88 | 1.00 |

Table 4: Amplified DNA banding pattern and disease reaction pattern

| Sl. No. | Name of genotype | Disease reaction | MBSSR238 | CEDG166 | CEDG191 | VrCsSTS1 | CEDG282 | |
|---|---|---|---|---|---|---|---|---|
| 1 | OBG-31 | MR | 0 | 0 | 1 | 1 | 1 | 1 |
| 2 | Aska local | R | 1 | 1 | 1 | 1 | 1 | 1 |
| 3 | Kendrapada local | MS | 1 | 1 | 1 | 0 | 1 | 1 |
| 4 | Suvrnagiri local | MS | 1 | 0 | 1 | 0 | 1 | 1 |
| 5 | PU-31 | MR | 1 | 0 | 1 | 1 | 1 | 1 |
| 6 | Rourkela local | MS | 1 | 1 | 1 | 0 | 1 | 1 |
| 7 | Nayagarh local-3 | MS | 1 | 1 | 1 | 0 | 1 | 1 |
| 8 | Ujala | MR | 1 | 1 | 1 | 0 | 1 | 1 |
| 9 | Keonjhar local | MS | 1 | 0 | 1 | 0 | 1 | 1 |
| 10 | Charipalli local | MR | 1 | 0 | 1 | 1 | 1 | 1 |
| 11 | Prasad | R | 1 | 0 | 1 | 1 | 1 | 1 |
| 12 | Badamba local | MR | 1 | 1 | 1 | 1 | 1 | 1 |
| 13 | Keonjhar local | MS | 1 | 1 | 1 | 0 | 1 | 1 |
| 14 | Seimiliguda local | MS | 1 | 0 | 1 | 0 | 0 | 0 |
| 15 | Boudha local | HS | 0 | 0 | 1 | 0 | 0 | 0 |
| 16 | Puri local | MS | 0 | 1 | 1 | 0 | 0 | 0 |
| 17 | Badamba local | R | 1 | 1 | 1 | 1 | 1 | 1 |
| 18 | Kendrapada local | MS | 1 | 1 | 1 | 0 | 1 | 1 |
| 19 | Bolangiri local | MS | 0 | 1 | 1 | 0 | 1 | 1 |
| 20 | Nayagarh local | R | 1 | 1 | 1 | 1 | 1 | 1 |
| 21 | Denkhanal local | R | 1 | 1 | 1 | 1 | 1 | 1 |
| 22 | Jagatsinghpur Local | R | 1 | 1 | 1 | 1 | 1 | 1 |
| 23 | Kantapada local | MS | 0 | 1 | 0 | 1 | 1 | 1 |
| 24 | Kotthagarha local | MS | 0 | 0 | 1 | 1 | 0 | 0 |
| 25 | Mohana local | MS | 1 | 1 | 1 | 0 | 1 | 1 |
| 26 | Nayagarh local | MR | 1 | 1 | 1 | 1 | 0 | 0 |
| 27 | Bhawanipatna local | MR | 0 | 1 | 1 | 1 | 1 | 1 |
| 28 | Golapali local | R | 1 | 1 | 1 | 1 | 1 | 1 |
| 29 | Dharakote local | MR | 0 | 1 | 1 | 1 | 1 | 1 |

with the desired trait is an essential requirement for marker assisted selection (MAS) in an advanced breeding program (Sahoo *et al.*, 2022; Samal *et al.*, 2021). Validation of marker by efficient screening is the process of unambiguous association of marker with the trait of interest (Maiti *et al.*, 2011); however biochemical analysis could also be employed to assess the biochemical parameters (Dash *et al.,* 2022).

**Conclusion**

Both morphological traits and SSR markers were highly effective in estimating genetic diversity and distinguishing genotypes in urdbean. Direct selection for yield improvement through traits such as number of branches plant$^{-1}$, number of pods plant$^{-1}$, number of seeds pod$^{-1}$, days to 50% flowering, and 100 seed weight would be rewarding. In the present study, out of the 29 genotypes, seven were found resistant, eight were moderately resistant, 13 were found moderately susceptible and only one was highly susceptible to the disease. The genotypes 'OBG-31', 'Ujala,' 'Prasad,' and 'Nayagarh-I local' were good yielders with resistance to powdery mildew disease, which can be used for future breeding programs. Molecular diversity assessment results showed that the association of VrCsSTS1 marker closely with powdery mildew resistance gene in different genetic backgrounds of urdbean, which can be further validated in a large population panel and used in future breeding programs for the development of powdery mildew resistance genotypes.

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