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Bud sports in the popular mulberry cultivar, Victory-1 and their characteristics

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Abstract

Bud sport (bud mutation) is a valuable source for existing new genotypes in mulberry (*Morus* spp.) as well as critical materials for studying the molecular mechanisms underlying essential traits. Thus, identification, collection, characterization, and conservation of such natural variants are prerequisites for enhancing the mulberry genetic resource in the germplasm. In this context, we identified and characterized three bud sports (VBS-1, VBS-2, and VBS-3) of a popular mulberry cultivar, Victory-1 (V-1). These bud sports are morphologically, anatomically, and genetically more distinct from their mother plant, Victory-1. Moreover, these bud sports display lower growth and yield potential. Furthermore, these showed remarkably lower 2C DNA contents of 0.74 pg (VBS-1), 0.78 pg (VBS-2), and 0.76 pg (VBS-3), when compared to their mother plant V-1 (2C = 0.81 pg). On the other hand, molecular characterization between the bud sports and their mother plant revealed the existence of genetic variation due to the natural bud mutation that occurred in the mulberry cultivar Victory-1.

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Mulberry,
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Victory-1

Introduction

The *Morus* species are woody perennials with a long juvenile period and generation time and are highly heterozygous in nature, which means valuable qualities may be lost through sexual reproduction. Hence, mulberries are vegetatively propagated by cutting, grafting, and budding, which can preserve desirable genotypes over long periods of time. A bud sport is a phenotypically distinct part of a plant that frequently occurs in woody perennials with vegetative propagation (Foster and Aranzana, 2018). Bud sports usually harbor a limited number of mutations based on the original plants and retain most of the original traits, making them an excellent resource for breeding new cultivars (Wu *et al.,* 2021). Many bud sports have been introduced as new cultivars in horticulturally important crops, such as mango (Young and Leidin, 1954), citrus (Usman and Fatima, 2018), apple (Li *et al.,* 2018), grape (Xu *et al.,* 2019), peach, and nectarine (Okie, 1998). In sericulture, many genotypes/varieties of mulberry were identified through clonal selection (Yamanouchi, 2019; Kumara *et al.,* 2021; Kumara *et al.,* 2022), which are the result of natural bud mutations (Dzhafarov, 1966; Dandin *et al.,* 1996). Therefore, somatic mutation represents a mechanism to generate new genetic variability in mulberry, as presently observed in the cultivar, Victory-1 (*Morus indica*).

The Victory-1 (2n=2x=28) mulberry cultivar, also known as V-1, is a popular mulberry cultivar in southern India. It is a hybrid developed in the late 1990s from controlled-pollinated

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hybrids of S-30 and Ber. C-776. The variety is distinguished by its erect branches and greyish stem color. Leaves are thick, smooth, succulent, large, entire, and ovate with a truncate base. It has good agronomic characteristics like high rooting ability, fast growth, and high yield. Under irrigated conditions, with the recommended package of practices, it yields about 60 MT/ha/year. Bioassay and chemoassay tests indicated the superiority of this variety for silkworm rearing (Saratchandra *et al.,* 2011). Survey, exploration, collection, and introduction of mulberry germplasm resources are the prerequisites for the conservation and exploitation of mulberry genetic resources for their further use. Realizing the importance of impending global climatic changes and the threatened sustainability of biodiversity wealth in India at a faster rate, systematic survey and exploration for the collection of mulberry biodiversity have gained greater momentum in the recent past (Nagoo *et al.,* 2019). Hence, the present study was undertaken for the identification, collection, characterization, and conservation of bud sports/variants of the mulberry cultivar, Victory-1 through field surveys.

Materials and Methods

Plant materials and plot establishment

The three natural bud variants were identified from the Victory-1 mulberry garden in the Ramanagara district of Karnataka (India) through surveys conducted in 2016-2017. The identified variants were allowed in the field for four months for seed cutting preparations. Further, the saplings were raised from the selected stock of bud variants. The six-month-old saplings were selected for the plantation. The variants were named VBS-1 (Victory-1 Bud Sport-1), VBS-2 (Victory-1 Bud Sport-2), and VBS-3 (Victory-1 Bud Sport-3). The experiment was laid out in RCBD (Randomized Complete Block Design) with 10 replications at the spacing of 90 × 90 cm. The soil was red sandy loam with an average pH of 7.65. The recommended doses of farm yard manure (20 MT/ha/yr) and chemical fertilizer (NPK 350:140:140 kg/ha/yr), irrigation, and weeding were done as per the requirement. Plant protection measures were ensured throughout the experiment. After one year of establishment, eight successive harvests were taken with an interval of 65-70 days, and all the harvests were made by shoot pruning at 45 cm height.

Morpho-physio-anatomical and Molecular studies

The data on plant morphology, growth, and yield characteristics were recorded from the second year onwards of the plantation with the adoption of standard procedures, as described by Sahay *et al.* (2016). Leaf anatomy and stomata studies were carried out following the method described by Mallikarjunappa *et al.* (2007). The leaf moisture content and retention capacity were estimated using medium (5^{th} order to 10^{th} order from the top position) leaves, following respective standard methods (Thangavelu *et al.,* 2000). The relative leaf chlorophyll content was recorded in the fully expanded leaf (5^{th} order from the top position) near the middle of the lamina, avoiding the midrib, using the SPAD (Soil Plant Analysis Development)-502 Chlorophyll Meter. Three randomly selected plants with six readings per plant from each genotype were measured in the field.

All the genotypes were subjected to molecular characterization using Inter Simple Sequence Repeat (UBC ISSR Primers) markers by adopting standard protocols for DNA extraction (Murray and Thompson, 1980), PCR amplification (Williams *et al.,* 1990), and gel electrophoresis separations (Agnese *et al.,* 2004). The nuclear DNA (2C) amount was estimated by flow cytometry from the relative fluorescence strengths of sample peaks and internal standards as previously described by Yamanouchi *et al*. (2008). Data were analyzed adopting the method of one-way analysis of variance using the Statistical Tool for Agricultural Research (STAR). Tukey's Honest Significant Difference (HSD) was used for further testing of significance

Results and Discussion

Origin of Bud Sports

Despite the fact that vegetative propagation is used in mulberry to multiply plants that are identical to the original type, spontaneous phenotypic variation occasionally appears on some shoots (known as "bud sports") as a result of somatic mutations (Dzafarov, 1966; Tojyo, 1979; Dandin *et al.,* 1996). Identifying such bud sports in the mulberry garden, on the other hand, is a difficult task; initially, the bud mutation originates in a single bud, and as a result, the branch appears distinct from the rest of the branches of the same plant (Fig. 1a). Subsequently, due to repeated punning of such a single branch, it later appears busy (Fig. 1b). At this stage, such variants can be identified. Bud

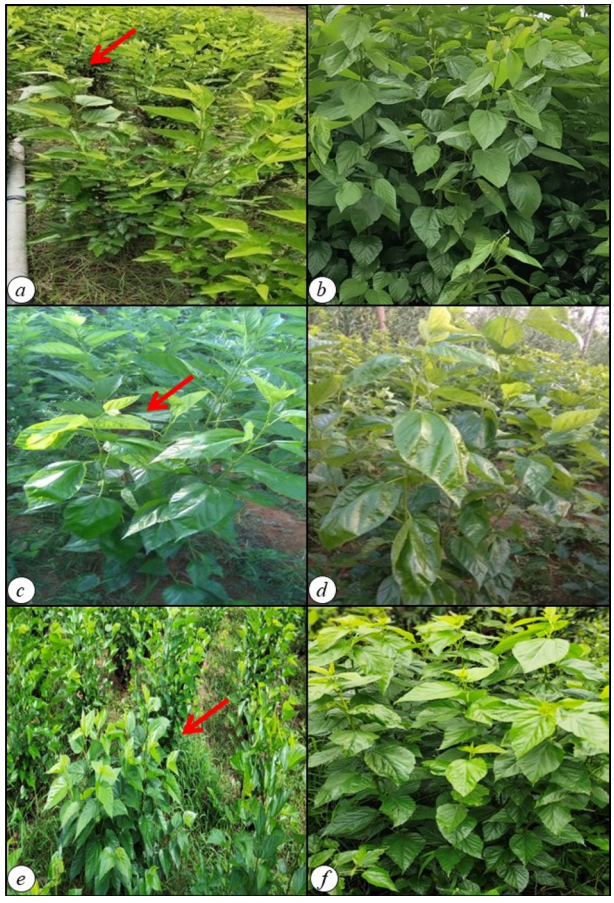

Fig. 1. Occurrence of bud sports in the Victory-1 mulberry garden: a) Initial stage of VBS-1; b) Later stage of VBS-1, c) Initial stage of VBS-2; d) Later stage of VBS-2, e) Initial stage of VBS-3; f) Later stage of VBS-3. (*Arrow shows the bud sport branch/plant)

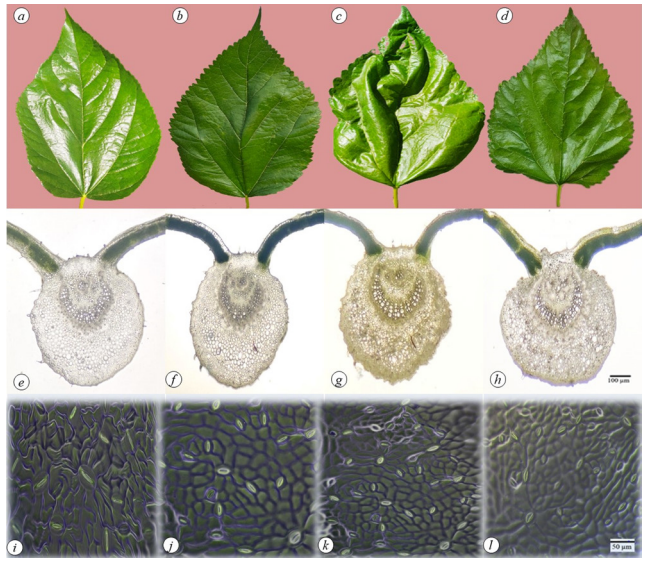

Fig. 2. Comparative account of leaf morphological and anatomical view of Victory-1 and its bud sports: a) Leaf of V-1, b) Leaf of VBS-1, c) Leaf of VBS-2, d) Leaf of VBS-3, e)Transfer section of V-1 leaf blade, f) Transfer section of VBS-1 leaf blade, g) Transfer section of VBS-2 leaf blade, h) Transfer section of VBS-3 leaf blade, i) Stomatal view of V-1, j) Stomatal view of VBS-1, k) Stomatal view of VBS-2, l) Stomatal view of VBS-3.

sports can display any type of phenotypic variation in any organ: leaf, stem, bunch, berries, seeds, etc. (Carbonell-Bejerano *et al.,* 2019).

Leaf morphology

In mulberry plants, the leaf morphological traits are the prime markers for distinguishing the variants. The leaf morphological characters have been utilized to examine genetic diversity, variation, phylogeny, and grouping. The mother plant V-1 has dark green leaves, while the variants have light green (VBS-1) and green (VBS-3) colored-leaves. The VBS-1 (Fig. 2b) and VBS-3 have a non-glossy, slightly rough surface compared to the smooth, strongly glossy leaf of their mother plant. Contrastingly, VBS-2 exhibited wrinkled leaves with impressed veins (Fig. 2c). The dentate (VBS-1) and crenate (VBS-2) leaf margins of the bud sports contrasted with the serrate margins of the mother plant. The leaf is ovate-shaped in VBS-2 and cordate-shaped in VBS-3, (Fig. 2d) as compared to the narrow ovate of V-1(Fig. 2a). VBS-2's leaf has a horizontal angle in comparison to its mother plant's acute angle. In terms of leaf morphological traits, these are the distinguishing characteristics of the bud variants from their mother plant, V-1. The remaining leaf morphological traits, such as leaf apex, leaf base, leaf texture, leaf nature, and leaf lobation, are unchanged in the variants.

Growth nature

The growth vigor is low in the VBS-2, and medium in the VBS-1 and VBS-3 (Fig. 1e&f) variants, compared to the high vigor of their mother plant. The VBS-2 grew semi-erect with slightly curved branches (Fig. 1c&d), in contrast to its mother plant's erect growth and straight branching nature (Table 1). The shoot height, shoot thickness, number of branches per plant, leaf area, leaf weight, and leaf yield per plant were recorded lower in the bud variants compared to their mother plant (Table 2). Moreover, these are major contributory traits for mulberry leaf yield (Tikader and Roy, 2001). Hence, these bud variants are not suitable for commercial exploitation for silkworm rearing. It was known that mulberry tree bud mutations are induced

Table 1. Comparative account of morphological traits between the mother plant, V-1 and its bud sports

Characteristics	Victory -1	VBS-1	VBS-2	VBS-3
Leaf color	Dark green	Light green	Dark green	Green
Leaf glossiness	Strongly-glossy	Non-glossy	Glossy	Non-glossy
Leaf wrinkles	Non-wrinkle	Non-wrinkle	Wrinkle with impressed veins	Non-wrinkle
Leaf apex	Acuminate	Acuminate	Acuminate	Acuminate
Leaf base	Ttruncate	Ttruncate	Truncate	Truncate
Leaf margin	Serrate	Dentate	Crenate	Serrate
Leaf surface	Smooth	Slightly rough	Smooth	Slightly rough
Leaf texture	Charataceous	Charataceous	Charataceous	Charataceous
Leaf shape	Narrow ovate	Narrow ovate	Ovate	Cordate
Leaf angle	Acute	Acute	Horizontal	Acute
Leaf nature	Homophyllous	Homophyllous	Homophyllous	Homophyllous
Leaf lobation	Unlobed	Unlobed	Unlobed	Unlobed
Leaf hairiness	Glabrous	Sparsely hairy	Glabrous	Sparsely hairy
Plant vigor	High	Medium	Low	Medium
Growth nature	Erect	Erect	Semi-erect	Erect
Branching nature	Straight	Straight	Slightly curved	Straight
Shoot color (mature)	Brown	Brown	Brown	Brown
Sex expression	Androecious	Androecious	Androecious	Androecious

Table 2. Comparative analysis of growth and yield parameters between the mother plant, V-1, and its bud sports

Genotypes	Shoot thickness (cm)	No. of branches/ plant	Average shoot length (cm)	Inter-nodal distance (cm)	Petiole length (cm)	Leaf area (cm^2)	25 leaf weight (g)	Leaf yield / plant (g/harvest)
Victory -1	1.24^{a}	24.33^{a}	164.28	4.28	5.34	377.52^{a}	151.36^{a}	754.31^{a}
VBS-1	0.87^{b}	18.45^{b}	154.81	4.11	5.16	311.32^{ab}	93.27^{b}	415.22^{c}
VBS-2	0.61^{c}	7. 34^{c}	147.31	3.36	4.28	204.87^{c}	47.98^{b}	312.55^{d}
VBS-3	0.92^{c}	16.87^{c}	157.23	4.16	5.37	294.35^{c}	82.34^{c}	498.47^{b}
CV %	11.81	18.28	10.16	30.61	32.72	18.34	24.09	13.19

Means within a column with the same letter are not significantly different at 5 % level (HSD)

spontaneously, but most of them are malformations that have no practical value (Hazama, 1968; Dandin *et al.,*1996).

Foliar characteristics

Mulberry leaves with higher moisture content have a direct effect on silkworm growth and development by promoting nutrient ingestion, digestion, and assimilation. The leaf moisture content may serve as one of the criteria for estimating the leaf quality (Parpiev, 1968). Leaf anatomical parameters like stomatal size, stomatal frequency, mesophyll tissue, cuticle thickness, and leaf thickness also influence the moisture content of the leaf and its retention capacity (Ninge Gowda and Sudhakar, 2002). The thickness of the leaf, its cuticle, and stomata size are decreased, while stomatal frequency is increased in all bud sports compared

Table 3. Comparative analysis of leaf anatomical characteristics between the mother plant, V-1, and its bud sports

Genotypes	Leaf thickness (µm)	Upper cuticle thickness (µm)	Lower cuticle thickness (µm)	Upper epidermal thickness (µm)	Lower epidermal thickness (µm)	Palisade thickness (µm)	Spongy thickness (µm)	Chloroplast No./stomata	Stomatal size (µm)	Stomata frequency (no./mm^2)
Victory -1	153.77^{a}	7.48	4.30	26.08^{a}	12.33	47.23^{a}	56.35^{a}	10.22	36.28	724.53^{c}
VBS-1	120.69^{b}	5.12	3.14	18.25^{bc}	10.04	38.87^{b}	45.27^{bc}	8.13	31.38	816.37^{b}
VBS-2	116.93^{b}	6.14	4.01	16.27^{c}	10.49	37.14^{b}	42.88^{c}	9.14	27.19	977.41^{a}
VBS-3	139.16^{ab}	5.88	4.12	24.67^{ab}	11.06	42.16^{ab}	51.27^{ab}	8.77	29.22	974.96^{a}
CV %	14.63	53.33	38.15	25.11	18.09	12.05	13.52	15.3	22.50	8.51

Means within a column with the same letter are not significantly different at 1 % level (HSD)

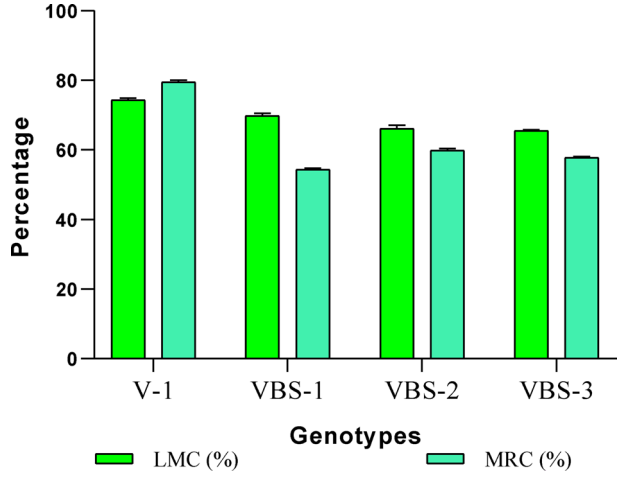

Fig. 3. Comparative analysis of Leaf moisture content (LMC %) and leaf moisture retention (LMR %) capacity between the mother plant, V-1 and its bud sports

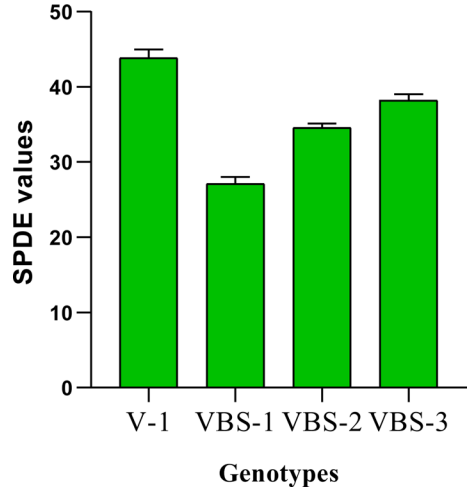

Fig. 4. Comparative analysis of leaf chlorophyll content between the mother plant, V-1 and its bud sports

to their mother plant (Table 3). Hence, the leaf moisture content and its retention capacity in the bud sports are very low (Fig. 3). Due to changes in the mulberry leaf quality parameters, there will be a great impact on the rearing performance of the silkworm and the quality of the silk. Different genotypes are said to influence the leaf moisture content and its retention in harvested leaves. Kumara *et al.* (2021) and Kumara *et al.* (2022) analyzed the variability in leaf anatomical and leaf quality parameters in the clonally selected mulberry genotypes. The cultivar, Victory-1 is superior due to the association of favorable physiological and anatomical characteristics: high photosynthetic rates, leaf photoreceptor chlorophyll pigments, and a higher ratio of chlorophyllous palisade tissue, leaf thickness, and stomatal frequency, all of which are directly correlated with high leaf yield in mulberry (Kumar *et al.,* 2012).

The SPAD (Soil Plant Analysis Development) chlorophyll value can be used as a reference index for evaluating mulberry leaf quality and breeding improved varieties. The SPAD chlorophyll content of mulberry leaves is significantly positively correlated with the commercial characteristics of the silkworm cocoons (Yu *et al.,* 2015). The genetic variability in SPAD chlorophyll content in mulberries is well studied, and its association with photosynthesis has been established (Rukmangada *et al.,* 2016). The chlorophyll content of the bud sports varied significantly from that of their mother plant, V-1 (Fig. 4). The light green color of the leaves in VBS-1 is correlated with their lower content of chlorophyll.

Genetic analysis

One approach is the analysis of genome size by flow cytometry in these variants as a proxy to study their genetic variability. The

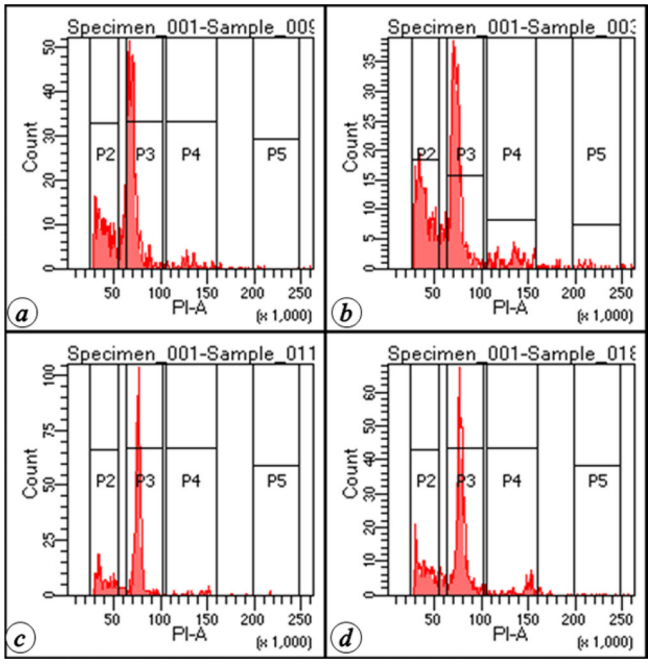

Fig. 5. Representative histogram of the flow cytometry analysis: a) VBS-1; b) VBS-2; c) VBS-3; d) V-1.

bud sports showed remarkably lower 2C DNA contents of 0.74 pg, 0.78 pg, and 0.76 pg in VBS-1(Fig. 5a), VBS-2(Fig. 5b), and VBS-3(Fig. 5c), respectively, compared to their mother plant V-1 (2C=0.81 pg) (Fig. 5d). The cultivars resulting from spontaneous mutations have a greater or lower DNA content than the cultivars from which they originated (Ortega-Ortega *et al.,* 2019). On the other hand, the molecular marker technique is an efficient tool for genetic variation evaluation in plants. Inter-simple sequence repeat (ISSR) markers are found to be useful in analyses of genetic variation below the species level, mainly in studying population structure and differentiation (Mohammad *et al.,* 2018). The ISSR markers are used for mulberry genetic diversity analysis and germplasm characterization (Awasthi *et al.,* 2004). The molecular characterization of somatic variants provides basic information that aids in understanding gene biological function (Carbonell-Bejerano *et al.,* 2019). The number of amplified fragments in the total of 10 ISSR primers varied from one to five, with fragment sizes ranging from 300 to 700 kb. All ten primers (Table 4) showed differential DNA banding patterns between

Table 4. List of ISSR primers and their sequences

Sl. No.	Sample code	Primer	Nucleotide Sequence (5'- 3')	Size (bp)
1	1a,1b,1c,1d	UBC-812	GAGAGAGAGAGAGAGAA	300–1500
2	2a,2b,2c,2d	UBC-813	CTCTCTCTCTCTCTCTT	300–1500
3	3a,3b,3c,3d	UBC-815	CTCTCTCTCTCTCTCTG	300–1500
4	4a,4b,4c,4d	UBC-824	TCTCTCTCTCTCTCTCG	300–2000
5	5a,5b,5c,5d	UBC-834	AGAGAGAGAGAGAGAGYT	200–1500
6	6a,6b,6c,6d	UBC-840	GAGAGAGAGAGAGAGAYT	300–1000
7	7a,7b,7c,7d	UBC-843	CTCTCTCTCTCTCTCTRA	300–1500
8	8a,8b,8c,8d	UBC-844	CTCTCTCTCTCTCTCTRC	300–2000
9	9a,9b,9c,9d	UBC-845	CTCTCTCTCTCTCTCTRG	100–2000
10	10a,10b,10c,10d	UBC-853	TCTCTCTCTCTCTCTCRT	300–1500
*Where 1a to10a =V-1;1b to10b =VBS-1;1c to10c = VBS-2;1d to10d = VBS-3.			*Where Y = C and T & R= A/G	

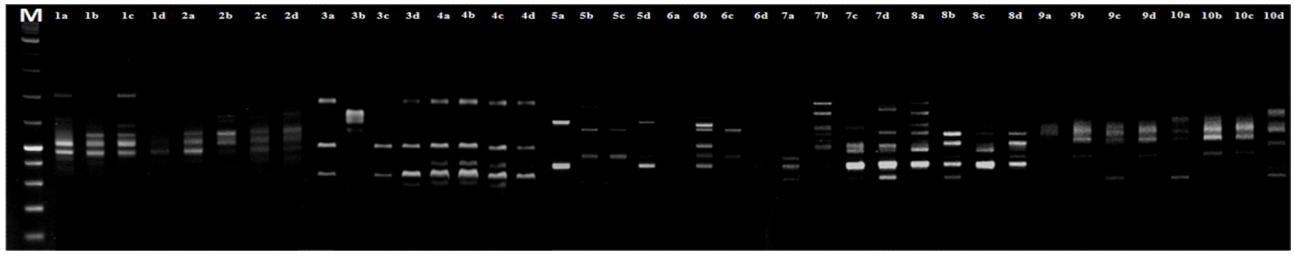

Fig. 6. ISSR banding patterns between the mother plant, V-1 and its bud sports

the bud variants and their mother plant (Fig. 6). The banding pattern polymorphism is commonly attributed to either mutation affecting a restriction enzyme target site (three-band difference) or DNA rearrangements involving deletions or insertions (two-band difference) (Hall, 1994; Tenover *et al.,* 1995). Hence, the bud sports evolved due to the natural bud mutation in the mulberry cultivar, Victory-1, that generated the novel phenotype at a distinguishable level with their mother plants.

Conclusion

Genetic variation/mutation is essential and vitally important for genetic improvement and the development of new cultivars of mulberry. From bud sports, the new variant phenotype can be established as a whole plant and, eventually, as a new variety, using the same propagation strategy. The present bud sports are not suitable for commercial exploitation but rather are maintained as genetic stocks in the mulberry germplasm bank.

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