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# Propagation GAC Fruit Plants in Vitro and Study Their Stability by ISSR Markers

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**Abstract:** Genetic Stability analysis of *in vitro* cultivated GAC fruit *(Momordica cochinchinensis Spreng)* from the Horticulture Department station using ISSR markers. These are difficult to apply for breeding, resulting in limited availability. Resulting in a lack of seed availability. However, tissue culture allows the development of micropropagate variations, especially in plantlets undergoing several subculture stages. This study aimed to identify somaclonal variations in GAC fruit seedlings generated from in vitro culture using Inter Simple Sequence Repeat (ISSR) markers. Two accessions were used in this study consisting of GAC fruit grown in its original environment and in vitro cultured. Total of five primers, of which four primers detected polymorphisms among the population. This study resulted in a somewhat similar genetic similarity among the GAC fruit. The genetic diversity of GAC fruit plants propagated by *in vitro* culture had a similarity level of 92%, showing somaclonal variations *in vitro* culture.

**Keywords**: genetic stability, *Momordica cochinchinensis* Spreng. *in vitro* plants, micropropagation, ISSR marker.

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## Introduction

*Momordica cochinchinensis* (Lour.) Spreng. Cucurbitaceae, is a dioecious plant that develops all through Southeast Asia and few Places of Northern Australi(1) *M. cochinchinensis* natural products are known as Baby jackfruit or "gac" in Vietnam. External shell of the organic product is not viewed as eatable, yet the seeds and the delicate mash are palatable and they utilized in Conventional Chinese Medication. *M. cochinchinensis* seeds utilized for the vast majority restorative purposes including easing muscle fits, rheumatic torment, hemorrhoids, and injuries (2). Ready GAC organic products contain high measures of lycopene and -carotene and are related with different medical advantages including stroke decrease and lack of vitamin A counteraction (3). Past synthetic examinations on this natural product have exhibited the presence of significant supplementary metabolites including triterpenoids and saponins(4,5). New review uncovered those saponins from M. *cochinchinensis* seed have renoprotective impacts against cisplatininduced harm to LLC-PK1 kidney cells (6). Plant cell and tissue culture turned into a flexible tool for fast spread and biomass creation of important

species (7,8). *In vitro* culture of plant cell, tissue and organ is related with contrasts in physiological, epigenetic and hereditary quality, to be specific, nonappearance or absence of organogenic potential (obstinacy), hyperhydricity (vitrification) and somaclonal variety. These characteristic are reliant of genotype and culture conditions and influence the useful utilization of tissue culture in plant proliferation and hereditary control(9,10). As a rule, by *in vitro* culture a large number of somaclone could be got in short period and space. Increase of first class genotypes follow the protection of the important genotype, consequently the somaclonal variety is certainly not a typical relation. Notwithstanding of this, in certain case, somaclonal variety could be an important wellspring of changeability to choose new genotypes. (11,12). hereditary changes do occur in undifferentiated cells, Disengaged protoplasts or callus continual study have uncovered that cell or tissue association go through continuous hereditary changes (polyploidy, aneuploidy, chromosomal breakage, erasure, movement, quality intensifications and transformations) and these are additionally communicated at biochemical or molecular levels (13,14) Somaclonal variation in identical plants arises from genetic and epigenetic changes during tissue culture, not necessarily during dedifferentiation. While it can be a source of new traits, many somaclonal variations are random, unstable, and non-heritable. This means they are not consistently passed down through generations and may not be useful for plant improvement. The event of reserved hereditary deficiency emerging by means of somaclonal variety in the regenerates can actually limit the more large utility of micropropagation frameworks. Clonal spread and safeguarding of tiptop genotypes, chose by their prevalent qualities, require serious level of hereditary correspondence among the recovered plants. The event of somaclonal variety is a detriment for both *in vitro* cloning as well as germplasm preserve strategy, thusly, the examination of hereditary changeability/ stability of *in vitro* plants is critical. There are a few methodologies to discover the hereditary changeability or security, every one of them having benefits and constraints (15). Procedures in view of morphophysiological, biochemical and cytological methodologies are principally founded on characters, which can be affected by the *in vitro* control, climate, and kinds of plant tissue, accordingly the isolation of somaclonal variety is hard to accomplish. Notwithstanding of this, DNA-based Molecular markers are a flexible device in different areas of science (16). The important advantage presented by DNA molecular markers is objective examination, in this way the outcomes could be handily rehashed and divided among research centers. Molecular markers are utilized to screen somaclonal variety, check the hereditary constancy of micropropagated plants and to distinguish genotypes with the ideal reaction to *in vitro* culture conditions. The most involved DNA-based Molecular markers for evaluation of security of *in vitro* plants. Examination of hereditary dependability of *in vitro* establishes Different markers strategies are utilized to really take a look at hereditary security and the absence of somaclonal variety in tissue determined plants as Restriction Fragment Length Polymorphism (RFLP), Amplified Fragment Length Polymorphism

(AFLP), Random Amplified Polymorphic DNA (RAPD), Simple Sequence Repeat (SSR), Inter Simple Sequence Repeat (ISSR) and Sequence-related Amplified Polymorphism (SRAP) (17). Molecular markers as RAPD and ISSR are not difficult to utilize, modest and requiring no past grouping data, in this manner the vast majority of the examinations showing the hereditary soundness of in vitro establishes utilized these markers (18). Among the various sub- molecular markers accessible, somewhat recently the most involved molecular markers for various applications incorporating appraisal of *in vitro* establishes devotion are RAPD, SSR and ISSR markers since they are more efficient and require low measures of DNA (19). These kind of markers were appropriate for laying out hereditary strength of a few micropropagated plants in yields, for example, gac natural product (20). Molecular markers are likewise significant apparatuses for portrayal of *in vitro* plants of organic product trees. Thus, RAPD and ISSR markers were used to prove the genetic fidelity of clonally propagated apple from adventitious buds (21). RAPD markers used to prove the genetic stability of somatic embryos of peach (22). The paternity of incipient organism culture derived cherry plants affirmed with RAPD markers (23).

## Materials and Methods

Plant materials GAC fruit plant leaf tests from Cultivation Division station utilized as parental plants, which were replanted at the College of Horticultural Designing Sciences, College of Baghdad Tissue Culture Lab. For examination, plants were refined in various conditions; alongside the adjusted plants and plantlets from *in vitro* culture, the reaction of Gac organic product explants for disinfection was analyzed. The tests were conveyed to choose the most proper strategies for proliferation relying upon the technique (24) after modification. Methods DNA extraction and intensification DNA tests from GAC fruit plants removed from frozen youthful leaves at - 20°C utilizing the Geneaid Genomic DNA Mini Kit Optimization and the primary selection stage used in this study directly from the DNA isolation stage (25).

**Table (1): List of 5 ISSR primers to analyze genetic diversity (26).**

| Primer | Primer sequence (5'-3') | Annealing temp. (°C) |
|---|---|---|
| **1** | ACACACACACACACACT | 46.8 |
| **2** | GAGAGAGAGAGAGAGAC | 52.6 |
| **3** | CTCTCTCTCTCTCTCTA | 49.4 |
| **4** | AGAGAGAGAGAGAGAGG | 50.2 |
| **5** | TCTCTCTCTCTCTCTCRT | 51.1 |

DNA amplification responses were performed with 25 µL solution consisting of 2.5 µL ISSR primer, 2.5 µL sample DNA, 7.5 µL aquabides, and 12.5 µL PCR Kit. DNA amplification was performed on a PCR gradient tool, Labcycler, programmed to start at 95°C for 4 minutes. Furthermore, each of the 37 consecutive cycles was denatured at 95°C for 45 seconds, annealing at each primary temperature for 50 seconds, and extension at 72°C for 1 minute, followed by post-extension at 72°C for 10 minutes and cooling at 4°C for 10 minutes. DNA electrophoresis DNA amplification was followed by separating the DNA band by electrophoresis on 1% agarose gel. In each agarose, gel was inserted 1.5 µL of

loading dye with 8.5 µL of amplified DNA. The gel was dissolve in 0.2x TBE solution for 75 minutes at 70 volts. Electrophoresis results were observed with the gel documentation (Syngene InGenius3). The standard DNA size used was 100 bp of the DNA ladder to determine the size of the DNA band. Data analysis. The appearance of DNA bands was read and scored using the Gen Pro Analyzer 3.1 software. The scores were one (present) and zero (absent or doubtful existence). Genetic similarity and diversity were analyzed with Similarity Qualitative (SIMQUAL) (27).

**Results AND Discussion**

Best shoots increase accomplished on MS medium enhanced with 2 mgL-1 of BA as the most elevated normal of primary and optional informal breakfasts (3.0 and 23.2 individually), shoots length (7.3 mm), leaves number (28.6), new and dry weight (0.570 and 0.054 gm separately) thatwas recorded. Recovered shoots were moved to MS media containing (0, 0.5, 1 and 1.5) mgL-1 Indole-3 butyric corrosive (IBA). The most noteworthy normal of root numbers (14.10) and length (7.5 cm) was seen on medium improved with 1.5 mgL-1 of IBA. Recovered plantlets made due and filled vivaciously in the field when refined on peat greenery with endurance rate (90%). (24) In view of the consequences of present paper, a decent multiplication which was gotten utilizing direct pathway by means of organogenesis, that ought to be valuable for the quick proliferation of this significant plant and prompted produce plantlets that have capacity to in any case alive in the field. (Figure 1)

**Figure (1): A) Plantlets via hardening stage in tap water for one week. B) Plantlets in pots covered with glasses jar to keep humidity. C) Gac plant which grown in green house after four weeks of planting.**

The all out genomic DNA of two gac increases were effectively separated from the fresh leaves through a changed CTAB extraction technique. The typical grouping of genomic DNA 189.8 ng/µL. DNA evaluation was led through spectrometry changed at 260/280 nm and agarose gel electrophoresis to confirm the purity of DNA. with an concentration of 1.34. Agarose Gel Electrophoresis Figure (2) (left to right) represents results of gel electrophoresis of (L) 1kp - 100 bp DNA Ladder marker, (P) genomic DNA extracted from mother plant leaves and (L) plantlets from tissue propagation, on agarose gel (1%) after electrophoresis at 76 volt for 80 min

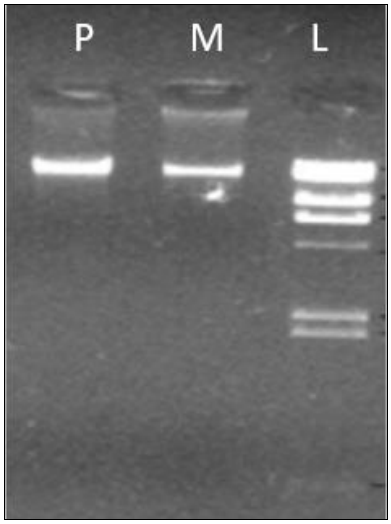


**Figure (2): Gel electrophoresis represent genomic DNA extracted from mother plant leaves(M) and from plantlets of tissue propagation from *Momordica cochinchinensis* (P).**

DNA-based molecular markers are ideal contrasted with morphological markers as they can give a superior gauge of hereditary variety and are not impacted by the climate (28). The ISSR strategy was taken on in this review. Aside from its ability to evaluate hereditary variety among firmly related species, ISSR offers a few critical benefits, including its speed, cost-viability, effortlessness and constancy(29). DNA polymorphisms the electrophoresis results and band size of each primer are displayed in Figure 3 and Table (2).

**Table (2): ISSR primer amplification of polymorphic marker selection in GAC fruit plants.**

| Primer identification | Number of amplification bands | Amplicon size (bp) |
|---|---|---|
| **1** | 16 | 800, 700, 600,400 |
| **2** | 14 | 700, 600, 400 |
| **3** | 11 | 800, 600, 500 |
| **4** | 15 | 600, 550, 500, 400 |
| **5** | 13 | 800.500,400 |

Note: The absolute number of obviously recognized DNA groups was 69. The quantity of polymorphic loci of all preliminaries utilized was 6 groups. The amplicon size in every preliminary went from 500 to 800 bp. The quantity of DNA groups on each ISSR preliminary went from 11 (ISSR 3) to 16 (ISSR 1) the total number of clearly identified DNA bands was 69. The quantity of polymorphic loci of all preliminary utilized was 12 groups. The amplicon size in every preliminary went from 1000 to 100 bp. The quantity of DNA groups on each ISSR preliminary went from 11 (ISSR 3) to 16 (ISSR 1) (Table 2). The electrophoresis aftereffects of the preliminaries showed a few distinctions in enhanced groups in the parental plant test with the examination test (Figure 3). This showed that the hereditary qualities in each plant was temperamental. The perception of DNA intensification on ISSR3 was muddled. This can be brought about by the low number of duplicates of DNA sections that can influence the loci to be dissected (30).

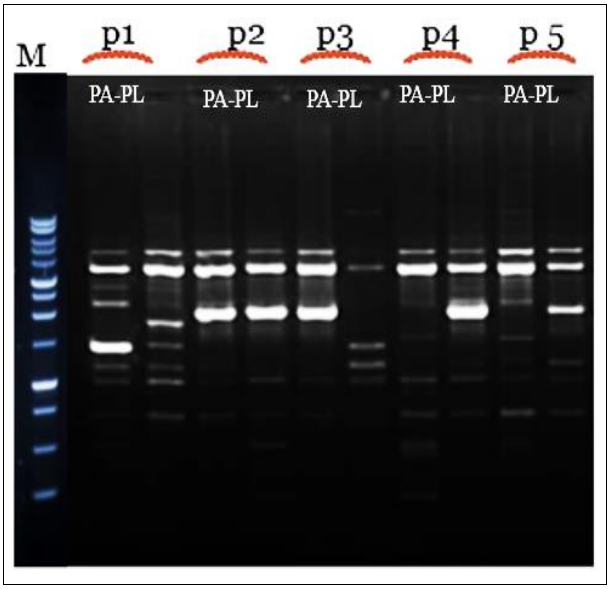


**Figure (3): The ISSR profile of the *in vitro* cultured GAC fruit using primer: p1. ISSR 1; p2. ISSR 2; p3. ISSR 3; p4. ISSR4; p5. ISSR5; M: Marker ladder 100 bp; PA-: parental plants; PL.**

Gac fruit plant is a dioecious plant that regenerative techniques frequently bring about a populace with a serious level of hereditary heterogeneity because of quality material traded by means of outcrossing rearing frameworks (31, 32). Relating with this, dioecious Momordica species show a huger extent of hereditary variety than monoecious species, most likely inferable from their fertilization technique, which incorporates wind and bugs (33, 34). Nonetheless, this procedure's regenerative achievement might be undermined by self-inconsistency (35). Geological imperatives and pollinators' short flight distance. The human component has recently been shown to be liable for the shortfall of relationship among hereditary and geological distance in certain condition.

**Analysis of genetic stability of GAC fruit plants**

Different markers procedures are utilized to really look at hereditary security and the absence of somaclonal variety in tissue determined plants including ISSR Examination. the hereditary variety of GAC fruit plants (Figure 2), which had a likeness level of 92%. The hereditary comparability between in vitro refined plants and accustomed parental plants was better than expected (36). This was impacted by the wellspring of acclimatization plant separates from plant tests filled in various conditions and a similar ecological development factors in the Tissue Culture Research facility. Hereditary contrasts in parental plants beginning from the Cultivation Division station, in plants filled in various conditions, and in adjusted plants refined in vitro could happen in light of elevation, development region, and natural circumstances (37). Hereditary likeness testing is one of the prerequisites in the creation of plants by involving in vitro culture procedures to guarantee that seed creation is consistent with type. The early identification of hereditary variety in plants from in vitro culture is critical to try not to foster plants that might have changed hereditarily. This in vitro culture as the spread technique brings about a high comparability of the refined plant to the parent plant and is powerful and

productive. Molecular markers used to notice the hereditary similitude of gac fruit plants from in vitro culture were RAPD (38), AFLP (39), and ISSR (40). The principal motivation behind why picked ISSR markers in this study was they have a higher reproducibility rate than the RAPD markers, which are less expensive than the AFLP markers (41). The hereditary contrasts between in vitro refined plants, field plants, and other examination plants can be brought about by different variables, for example, the wellspring of explants, focus and kind of development controllers, length of culture, recurrence of subculture, and responsiveness of the DNA markers utilized (41). The wellspring of explants from tissues still effectively isolating, like the cambium, for the most part does not change. Interestingly, separated tissues, like roots, leaves, delusion, and callus tissues, for the most part have enormous varieties (42). In this study, the plantlets had a removed source from the callus. The expansion of development controllers (the two auxins and cytokinins) could cause varieties. little BA fixations (2 mgL-1) could cause somaclonal focuses in gac organic product by diminishing the quantity of chromosomes and animating morphogenesis in the cell cycle (43). BA grouping of 2 mgL-1 causes modest quantities of somaclonal varieties in gac fruit plant (24) and 3 mgL-1 BA in media could cause somaclonal varieties of GAC plants refined in vitro contrasted and parental plants. In this review, hereditary variety in plants refined in vitro might measure up to parent plants due to the media that included BA, a development controller, at a high convergence of 2 ppm. The way of life period's term and subcultures' recurrence likewise influence the hereditary closeness of plants refined in vitro. This is on the grounds that the higher subsuquent of sub culturing, the more drawn out the length of the phones being presented to different elements that can cause transformations and hereditary variety when refined (44). For instance, hereditary variety was seen in pineapple plants developed from in vitro culture for a considerable length of time without. Hereditary variety of pineapple plants coming about because of in vitro culture for 8, 12, and two years with five subculture medicines with every subculture for 45 days on MS medium with no expansion in Plant Development Controller was low (9). Another review detailed a hereditary variety level of 85%-89% in grapes subcultures multiple times, though grapes that subcultured multiple times had a hereditary variety level of 66% (45). The plantlets were sub cultured at regular intervals. In this review, the time of plantlet culture was 4 months, with two subcultures on MS with 2 mgL-1 of BA. Generally, ISSR markers are helpful and effective for distinguishing crossovers in controlled crosses from the different hereditary foundations (46). but also can be used extensively for fingerprinting, phylogenetic analysis, population structure analysis, varietal/line identification, genetic mapping and marker-assisted selection (47).

**Conclusion**

Hereditary constancy of in vitro plants is critical to assess to foster legitimate protection programs. The most important devices for examination of in vitro plants are DNA-based molecular markers. Among these, RAPD, SSR and ISSR markers are modest and simple to utilize. ISSR creators don't require past data about the designated grouping, the groundworks could be utilized in various plants species. Regardless of these. For

hereditary fluctuation and loyalty appraisal utilizing various sorts of molecular markers is firmly suggested the hereditary closeness of the GAC fruit plant was better than expected. The hereditary variety of GAC plants from Agriculture Division station has a closeness level of 92%. The hereditary disparity between parental plants, plants are filled in various conditions, and the different development conditions caused accustomed plants. The parent plant was taken straightforwardly from its unique cultivation region. While GAC fruit plants filled in various conditions and accustomed plants were both filled in the Tissue Culture Research center. The hereditary disparity of parent plants and spread gac fruit plants was the consequence of in vitro culture, which could be brought about by the plantlet establishing medium containing a convergence of 2 ppm of BA, a year of the way of life period, and the recurrence of subculture (twice) utilizing a similar medium.

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