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EVALUATION OF GENETIC FIDEBILITY IN REGENERATED PLANTLETS AND NATURAL PLANT USING MOLECULAR MARKER RAPD

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ABSTRACT
Analysis of genetic diversity could be an important parameter in crop improvement and plant breeding. There observation through 5 random RAPD primers namely OPA-3, OPC-6, OPC-12, OPD-3 and OPD-20 among the given tissue cultured plant samples *Gymnemasylvestre* and their source or mother plant. It was observed that banding pattern of DNA samples of tissue cultured plants samples of *Gymnemasylvestre.*

KEYWORDS: *Gymnemasylvestre*, OPA-3, OPC-6, OPC-12, OPD-3 and OPD-20.

INTRODUCTION

Biological diversity may be defined as the variation present in all species of plants and animals, their genetic material and the ecosystems in which they occur. The importance of biodiversity for humankind has been well recognised in the recent decades and many would argue that diversity is essential for allowing sustainable development of various human activities. Biological diversity can enable social and economic systems to flourish in ways that allow the poorest to meet their food and nutritional needs and retain the cultural diversity of countries throughout the world (Shiva, 1994).

METHODS AND MATERIALS

The methodology involved the collection of leave of *G.sylvestre* their washing and cleaning, surface sterilization with alcohol cutting into small pieces. Genomic DNA was extracted from leaf samples by CTAB solvent extract ion method with some modification. Qualitative assessment of extracted genomic DNA from leaf samples was done on agarose gel Polymerase chain reaction was performed with extracted genomic DNA from *G. Sylvestre* samples using selected primers as RAPD markers. PCR was performed on Microprocessed Peltier based thermocycler "Prima96" from Himedia. The amplified PCR products were electrophoresed on 1to3% Agarosegel All the images of Agarose gel were taken on gel documentation system "E-gel Imager" from ABIInvitrogen. The number of amplicons generated neachlane were counted and detection of any unique band in tissue cultured plant clones would be considered a sindication of genetic variation compared to mother plants in present investigation.

Table 1: List of RAPD Primers used in PCR Amplification of *Gymnema.sylvestre*DNA Samples.

S. No.	Primer name	PRIMER SEQUENCE (5'-3')
1	OPA-3	AGTCAGCCAC
2	OPC-6	GAACGGACTC
3	OPC-12	TGTCATCCCC
4	OPD-3	AACCCGGTCA
5	OPD-20	AACCCGGTCA

Result and discussion- There were total 10 tissue cultures *Gymnemasylvestre* plant samples were randomly collected to check out their genetic homogeneity compared to their mother plant. The mother plants is represented with code GSM while their supposed decedent micro propagated plants were designated with codes GS-1, GS-2, GS-3,.. . . . .up to GS-10.

RAPD Profile of G. sylvestre DNA samples with Primer OPA-3

The PCR amplification when performed with DNA samples using short RAPD oligomer "prime OPA-3" yielded the banding pattern with DNA samples of *G. sylvestre* was electrophoresed on 3% Agarose gel yielded

a banding pattern. A 50 bp DNA ladder (MBT084 from HiMedia) was also used for comparison.

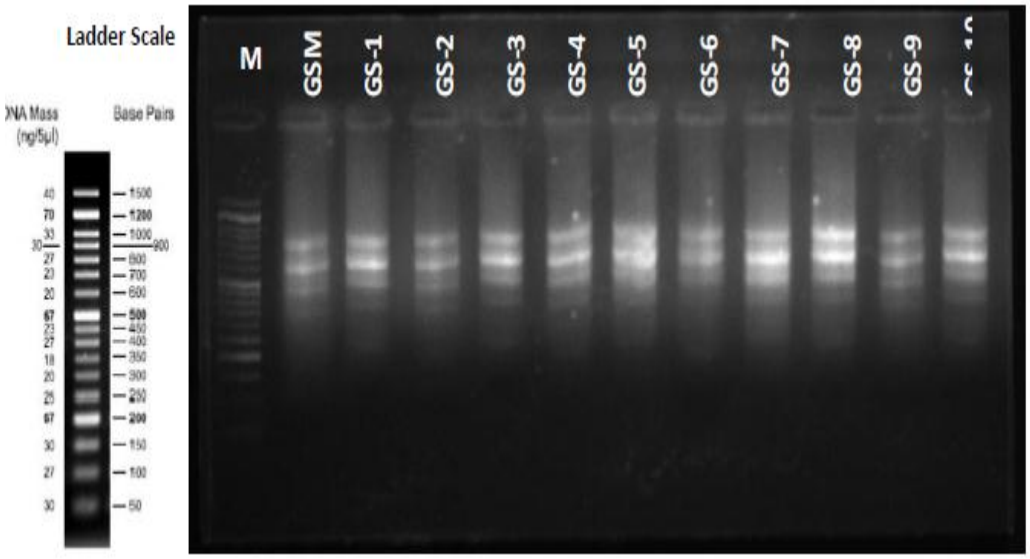

Fig- 1 Bands pattern of DNA samples amplified with Primer OPA-3 extracted from leaves of 10 tissue cultured *G. sylvestre*plants samples, compared to their mother plant.

Table 2: Total number of bands generated due to RAPD primer OPA-3 on *G. sylvestre*DNA samples from mother & tissue cultured plants.

S.NO	Sample code	Primer used	Total number of bands generated	Number of unique bands generated
1	GSM	OPA-3 Primer Sequence 5”- AGTCAGCCAC-3”	4	0
2	GS-1		4	0
3	GS-2		4	0
4	GS-3		4	0
5	GS-4		4	0
6	GS-5		4	0
7	GS-6		4	0
8	GS-7		4	0
9	GS-8		4	0
10	GS-9		4	0
11	GS-10		4	0

RAPD Profile of *G. sylvestre* DNA samples with Primer OPC-6

Table 3: Total number of bands generated due to RAPD primer OPC-6 on *Gymnemasylvestre* DNA samples from mother & tissue cultured plants.

S.NO.	Sample code	Primer used	Total number of bands generated	Number of unique bands generated
1	GSM	**OPC-6 Primer Sequence 5”- GAACGGACTC -3”**	6	0
2	GS-1		6	0
3	GS-2		6	0
4	GS-3		6	0
5	GS-4		6	0
6	GS-5		6	0
7	GS-6		6	0
8	GS-7		6	0
9	GS-8		6	0
10	GS-9		6	0
11	GS-10		6	0

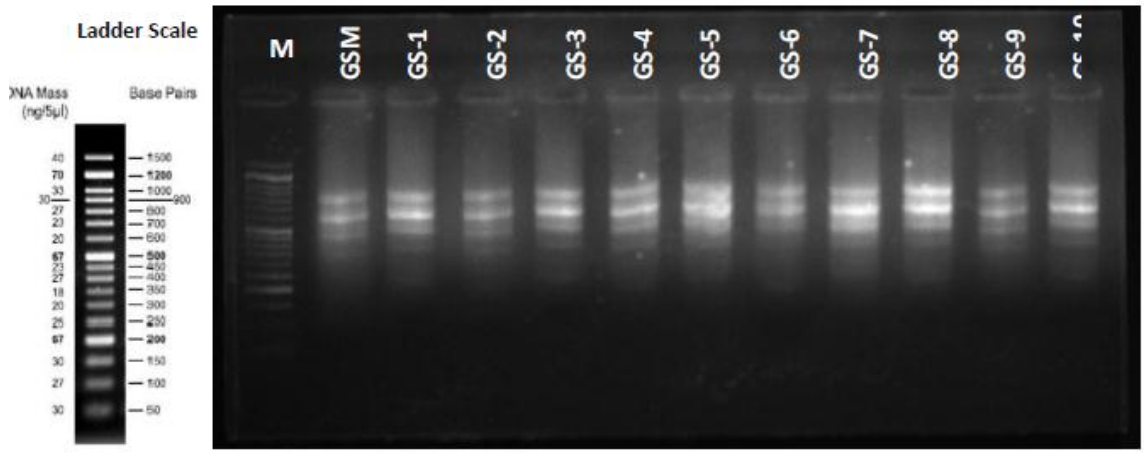

Fig. 2: Bands pattern of DNA samples amplified with Primer OPC-6 extracted from leaves of 10 tissue cultured *G. sylvestre*plants samples, compared to their mother plant.

RAPD Profile of G. sylvestre DNA samples with Primer OPC-12-

Table 4: Total number of bands generated due to RAPD primer OPC-12 on G. *sylvestre* DNA samples from mother & tissue cultured plants.

S.NO.	Sample code		Total numbers of band generated	Number of unique bands generated
1	GSM	OPC-12 Primer sequence	7	0
2	GS-1		7	0
3	GS-2		7	0
4	GS-3		7	0
5	GS-4		7	0
6	GS-5		7	0
7	GS-6		7	0
8			7	0
9	GS-8		7	0
10	GS-9		7	0
11	GS-10		7	0

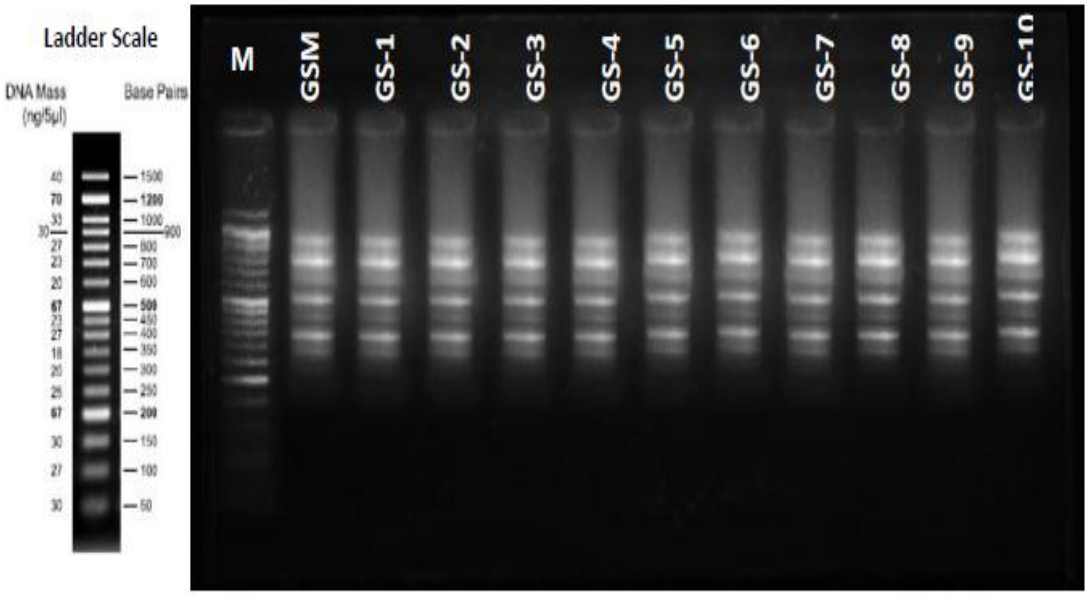

Fig. 2: Bands pattern of DNA samples amplified with Primer OPC-12 extracted from leaves of 10 tissue cultured *G. sylvestre*plants samples, compared to their mother plant.

RAPD Profile of G. sylvestre DNA samples with Primer OPD-3

Table 5: Total number of bands generated due to RAPD primer OPD-3 on G. *Sylvester* DNA samples from mother & tissue cultured plants.

S.NO.	Sample code	Primer used	Total numbers of bands generated	Total number of unique bands generated
1	GSM	OPD-3 Primer Sequence 5"- GTCGCCGTCA -3"	8	0
2	GS-1		8	0
3	GS-2		8	0
4	GS-3		8	0
5	GS-4		8	0
6	GS-5		8	0
7	GS-6		8	0
8	GS-7		8	0
9	GS-8		8	0
10	GS-9		8	0
11	GS-10		8	0

Fig. 3: Bands pattern of DNA samples amplified with Primer OPD-3 extracted from leaves of 10 tissue cultured *G. sylvestre*plants samples, compared to their mother plant.

RAPD Profile of *G. sylvestre* DNA samples with Primer OPD-20-

Table 6: Total number of bands generated due to RAPD primer OPD-20 on *G. sylvestre*DNA samples from mother & tissue cultured plants.

S.N0.	Sample code	Primer Used	Total number of bands generated	Number of unique bands generated
1	GSM	OPD-20 Primer Sequence 5"- AACCCGGTCA -3"	5	0
2	GS-1		5	0
3	GS-2		5	0
4	GS-3		5	0
5	GS-4		5	0
6	GS-5		5	0
7	GS-6		5	0
8	GS-7		5	0
9	GS-8		5	0
10	GS-9		5	0
11	GS-10		5	0

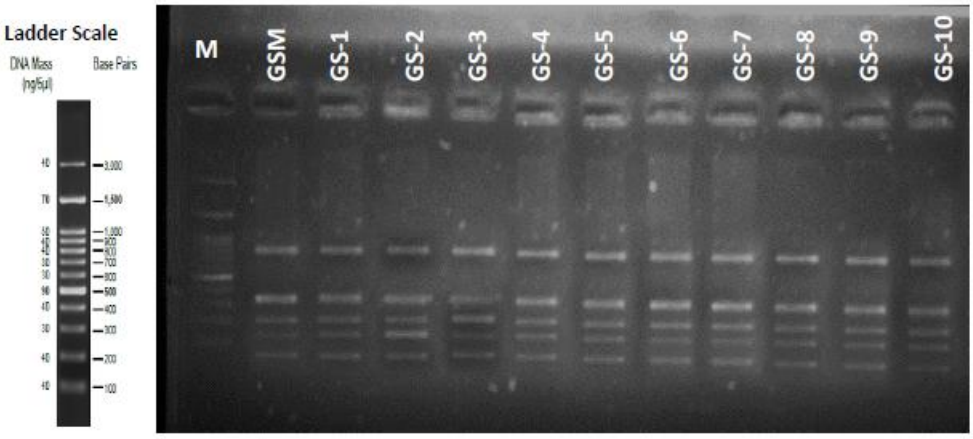

Fig. 4: Bands pattern of DNA samples amplified with Primer OPD-20 extracted from leaves of 10 tissue cultured *G. sylvestre*plants samples, compared to their mother plant.

The 5 random RAPD primers namely OPA-3, OPC-6, OPC-12, OPD-3 and OPD-20 when used for PCR amplification of DNA samples of test plant materials yielded variable number of banding pattern which were used to assess the genetic homogeneity of tissue cultured plants compared to their mother plant.Generation of at least 2 to 5 bands are suitable for RAPD genomic profile of any sample group and evaluation of genetic variation or similarity. In present investigation the primers used were successfully yielded the 4 to 8 bands upon PCR amplification.There observe at genetic homogeneity based on the use of 5 random RAPD primers namely OPA-3, OPC-6, OPC-12, OPD-3 and OPD-20 among the given tissue cultured plant samples *Gymnemasylvestre* and their source or mother plant.

CONCLUSION

PCR amplification with the 5 different random oligomers or primers were similar to the banding pattern of their mother or sources plant of *Gymnemasylvestre* which indicates that the locus for the sequence of random oligomers/ primers are present the DNA of sample plants and also the position and sized of amplicons formed using sample DNA is also similar in each plant samples used. Also the results may vary depending on the methods, techniques of experiments by individual and laboratory/place of experimentation.

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