# Vetiver grass model for long term carbon sequestration and development of designer genotypes for implementation

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# Vetiver grass model for long term carbon sequestration and development of designer genotypes for implementation

**To reduce atmospheric carbon it is desirable that carbon is blocked into subsoil horizon to minimize its recycling back to atmosphere**

## This can be best achieved either by

(a) Locking of carbon in basalt rocks as carbonate
(b) Photosynthetic capture converted into bio-char
(c) Photosynthetic capture locked deep into the soil

- Deep rooted forest trees are most suitable to realize this objective
- In the absence of above Vetiver is the suitable alternative option

# Vetiver grass model for long term carbon sequestration and development of designer genotypes for implementation

## Vetiver is the most suitable alternative option

- It has deep penetrating high biomass producing root system
- Its roots offer a growth potential of 3 cms. per day reaching upto 2.5 meters just in six months
- Root degradation is minimal in deep sub-soil horizon facilitating long-term carbon sequestration
- Degradation could be further minimized by selecting casparian strip thick genotypes

# Vetiver is the most suitable alternative option

**India being the native home of Vetiver offers repertoire of genetic diversity to select / develop designer genotype**

**However, the seed forming genotypes that offer such an opportunity are not suitable for ecological plantations**

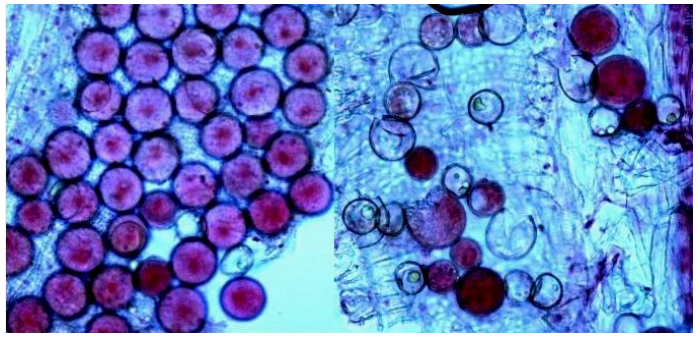
# Vetiver is the most suitable alternative option

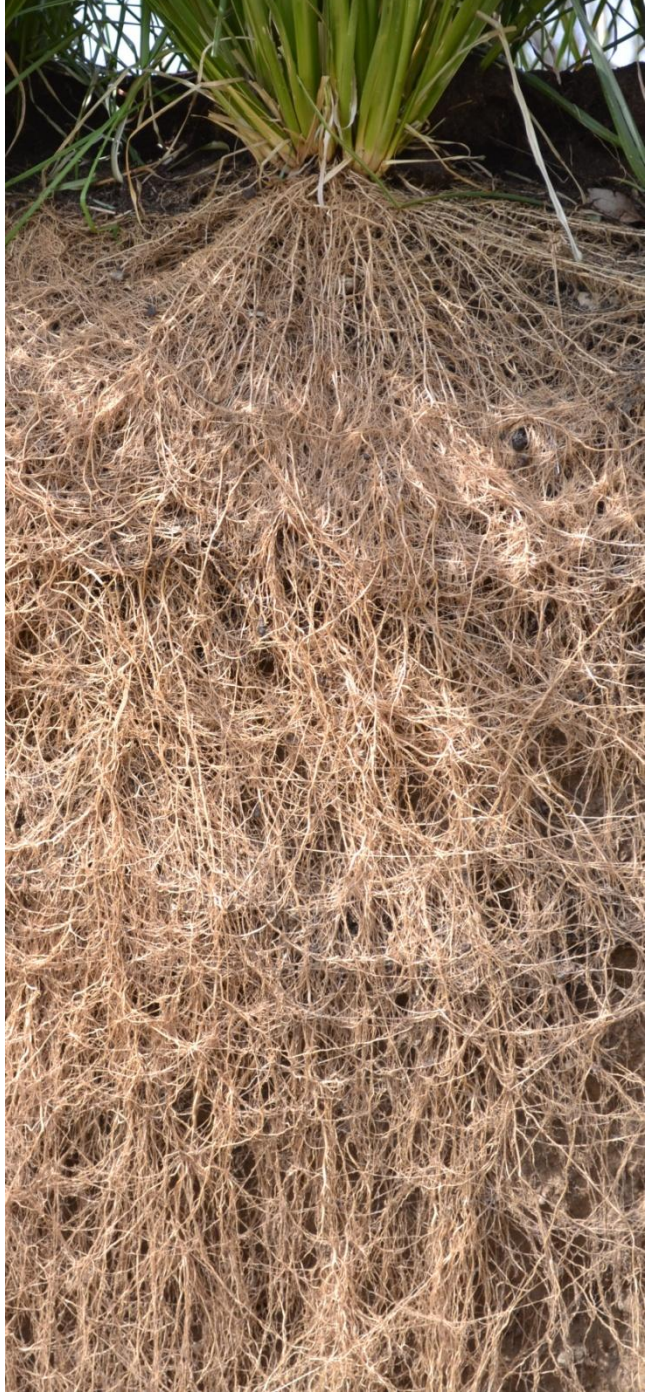
**Development of designer genotypes for implementation**

**Therefore,**
**We looked for strategic solutions that minimizes seed fertility**

**Selected a suitable genotype with fast growth potential and minimum fertility**

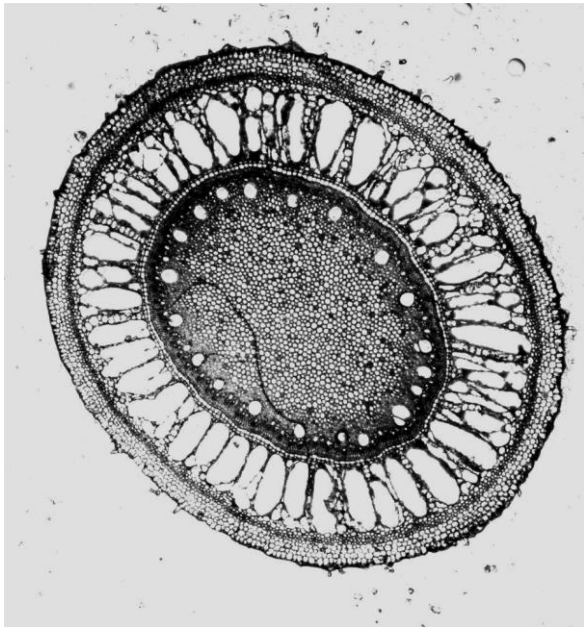
**Changed to polyploid to realize seed infertile designer genotype**

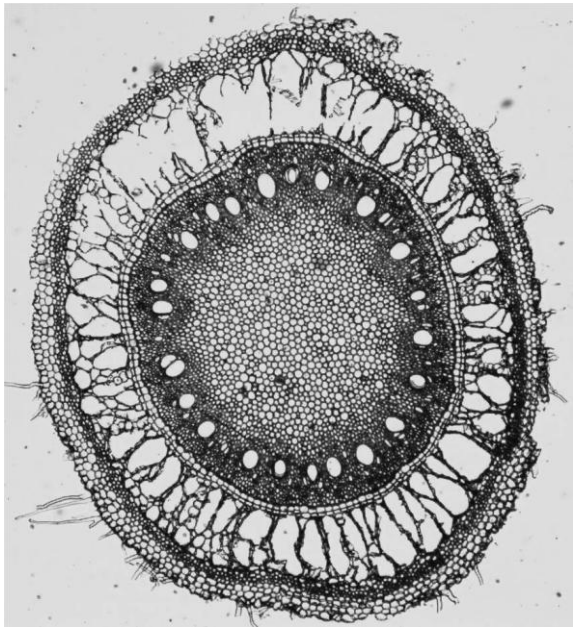


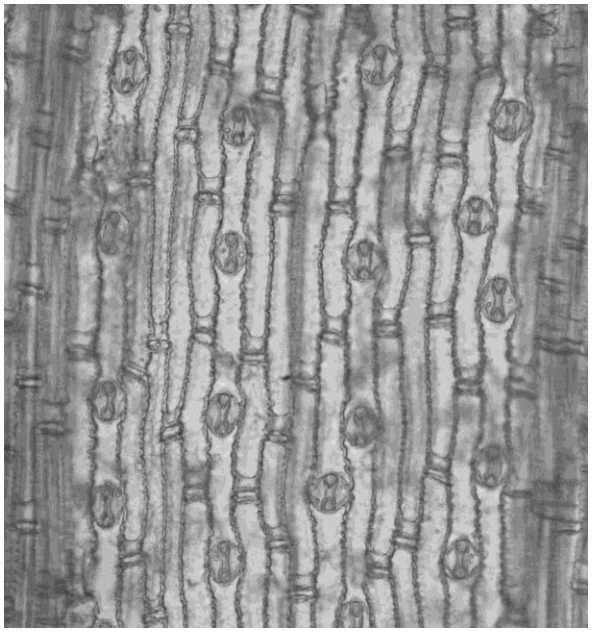


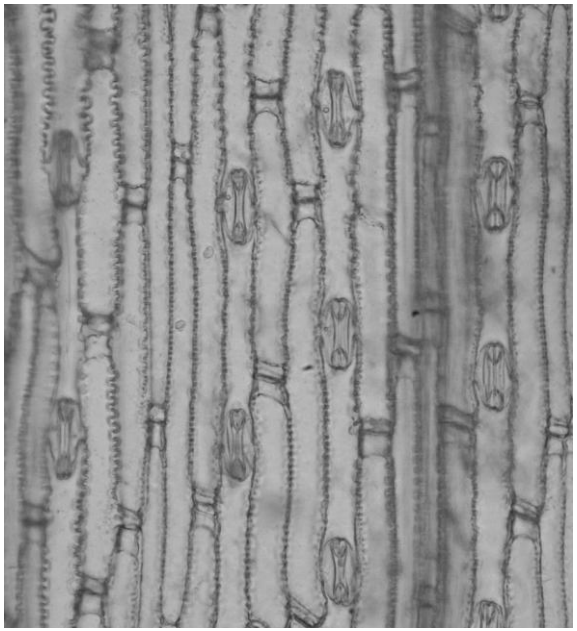
**Root anatomy and Stomatal features in the diploid vs tetraploid clone of Vetiver**

**Note: Higher Vascular cylinder vs. cortex ratio in the terraploid**

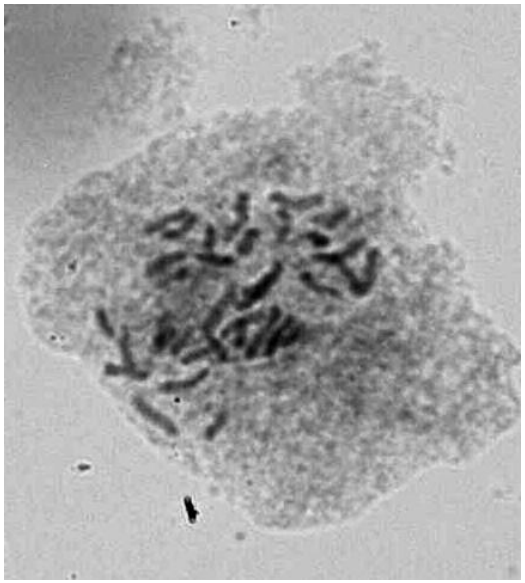




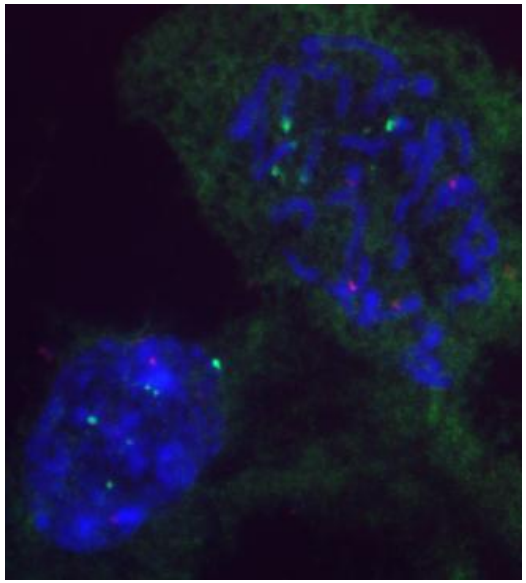




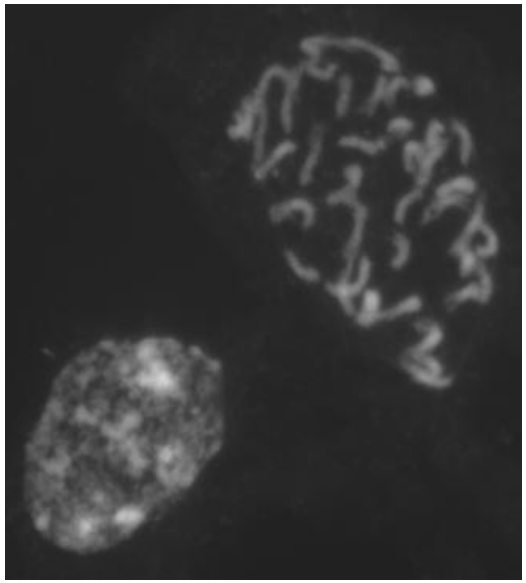
# Cytological characterization of seed infertile autotetraploid clone of Vetiver



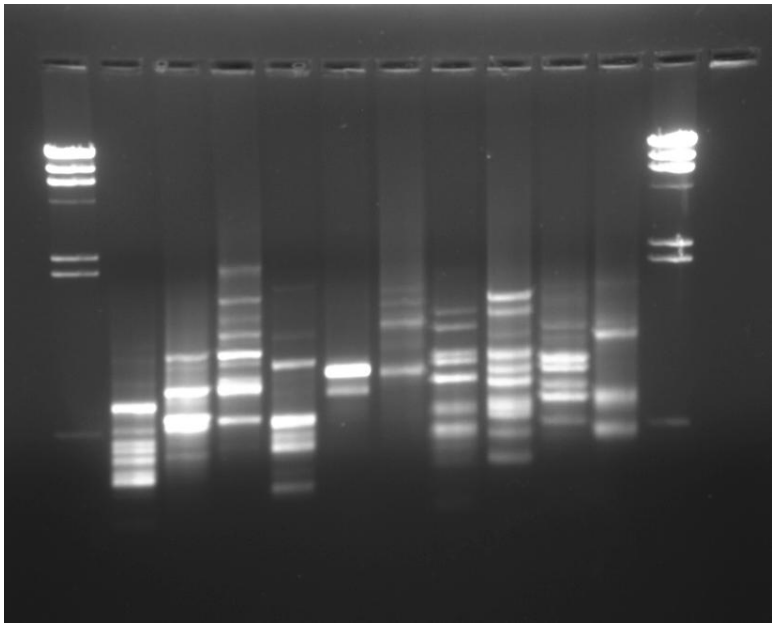
Feulgen

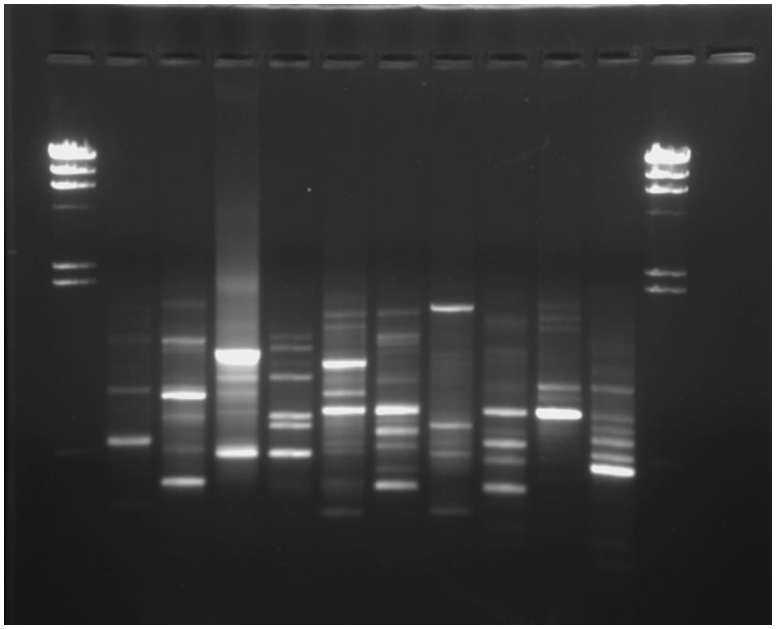


rDNA FISH



DAPI





## DNA fingerprints of the autopolyploid clone of Vetiver

### ISSR Markers :

Lane M : λ DNA / Hind III digest
Lane 1 : UBC807- AGAGAGAGAGAGAGAGT
Lane 2 : UBC810- GAGAGAGAGAGAGAGAT
Lane 3 : UBC811- GAGAGAGAGAGAGAGAC
Lane 4 : UBC812- GAGAGAGAGAGAGAGAA
Lane 5 : UBC814- CTCTCTCTCTCTCTCTA
Lane 6 : UBC818- CACACACACACACACAG
Lane 7 : UBC823- TCTCTCTCTCTCTCTCC
Lane 8 : UBC825- ACACACACACACACACT
Lane 9 : UBC826- ACACACACACACACACC
Lane10 : UBC828 -TGTGTGTGTGTGTGTGA

### RAPD Markers :

Lane M : λ DNA / Hind III digest
Lane 1 : OPJ 6- TCGTTCCGCA
Lane 2 :OPJ 9 - TGAGCCTCAC
Lane 3 : OPJ10- AAGCCCGAGG
Lane 4 :OPJ12 - GTCCCGTGGT
Lane 5 :OPJ13 - CCACACTACC
Lane 6 : OPJ14- CACCCGGATG
Lane 7 :OPJ15 - TGTAGCAGGG
Lane 8 : OPJ16- CTGCTTAGGG
Lane 9 :OPJ17- ACGCCAGTTC
Lane10 :OPJ18- TGGTCGCAGA

## Internal transcribed sequence based variability in diploid (2V) and tetraplooid (4V) plants of Vetivar

**(Red colour- variation (4.63%)*, green colour - Conserved region(5.8S) and Black with red colour - ITS-1 & ITS-2 region of 45S rDNA)**

```
2V                GATTTTGGGTATCCCGACT-GAC-TGGGGTCGCGGTCCGAGCGGCGTGCGCTGCGGTGCT 58
4V                AAAAGTTGTTGTTCAAACTTGACCTGGGGTCGCGGTCCGAGCGGCGTGCGCTGCGGTGCT 60
                   *   * * * * *  *** *** ************************************
2V                CGATGGGTCGTAAGGGCCGATACGCCGGCCGCGTGCCGGGGCGCTGCACCGAGAACAACT 118
4V                CGATGGGTCGTAAGGGCCGATACGCCGGCCGCGTGCCGGGGCGCTGCACCGAGAACAACT 120
                  ************************************************************
2V                AGGTGTCGCCCACCACGTGCTGTGCCCGGCACGATCCGCCGGCAGCCCCAACTTGGGCCC 178
4V                AGGTGTCGCCCACCACGTGCTGTGCCCGGCACGATCCGCCGGCAGCCCCAACTTGGGCCC 180
                  ************************************************************
2V                ACCGCGCCCTGCGGCGCGGGGAGCCAAACGCCACGTCCCTCCCCACGGGTGGGTTGGGAA 238
4V                ACCGCGCCCTGCGGCGCGGGGAGCCAAACGCCACGTCCCTCCCCACGGGTGGGTTGGGAG 240
                  ************************************************************
2V                TGTCTTTTGGCGTGACGCCCAGGCAGACGTGCCCTCGACCAGAAGGCCTCGGGCGCAACT 298
4V                TGTCTTTTGGCGTGACGCCCAGGCAGACGTGCCCTCGACCAGAAGGCCTCGGGCGCAACT 300
                  ************************************************************
2V                TGCGTTCAAAAACTCGATGGTTCCGGGATTCTGCAATTCACACCAGGTATCGCATTTTG 358
4V                TGCGTTCAAAAACTCGATGGTTCGCGGGATTCTGCAATTCACACCAGGTATCGCATTTTG 360
                  ************************ ************************************
2V                CTACGTTCTTCATCGATGCCAAAACCGAAATATCCGTTGCCGAAAGTCGTGTGGGTTAAG 418
4V                CTACGTTCTTCATCGATGCGAGAGCCGAGATATCCGTTGCCGAGAGTCGTGTGGGTTAAG 420
                  ********************* * * **** ************** ****************
2V                AAATCATCGCTGCTGCGGGGAACCGAAGGCAGGCCGACCGCTCCGCCGAACAAGGCAATA 478
4V                ATATCATCGCTGCTGCGGGGAGCCGAAGGCAGGCCGACCGCTCCGCCGAGCAAGGCAATA 480
                  * ******************* ************************** **********
2V                TAAGTGTTCCTTGACGCCTAAGGCGCCGTGGGTTCTGTTGCGGCCCCTCCGCCGAAGCTC 538
4V                TAAGTGTTCCTTGACGCCTAAGGCGCCGTGGGTTCTGTTGCGGCCCCTCCGCCGAAGCTC 540
                  ************************************************************
2V                GGGGGCCTTGGCCGAACCGAAGCCCGGCGGCACGAGAGACTCGTTCGCGGTCGGTTTTGT 598
4V                GGGGGCCTTGGCCGAGCCGAAGCCCGGCGGCACGAGAGACTCGTTCGCGGTCGGTTTTGT 600
                  ************** ******************************************
2V                TTAAGGGTCACGACAATGATCCTTCC-GCAGGTTCACCTACGGAAACCTTGTTACGATTT 657
4V                TTAAGGGTCACGACAATGATCCTTCCCGCATGT-CACCTACGGGGA-------------- 645
                  ************************** *** ** *********  *
2V                TTACTTCCCA 667
4V                ---------- 
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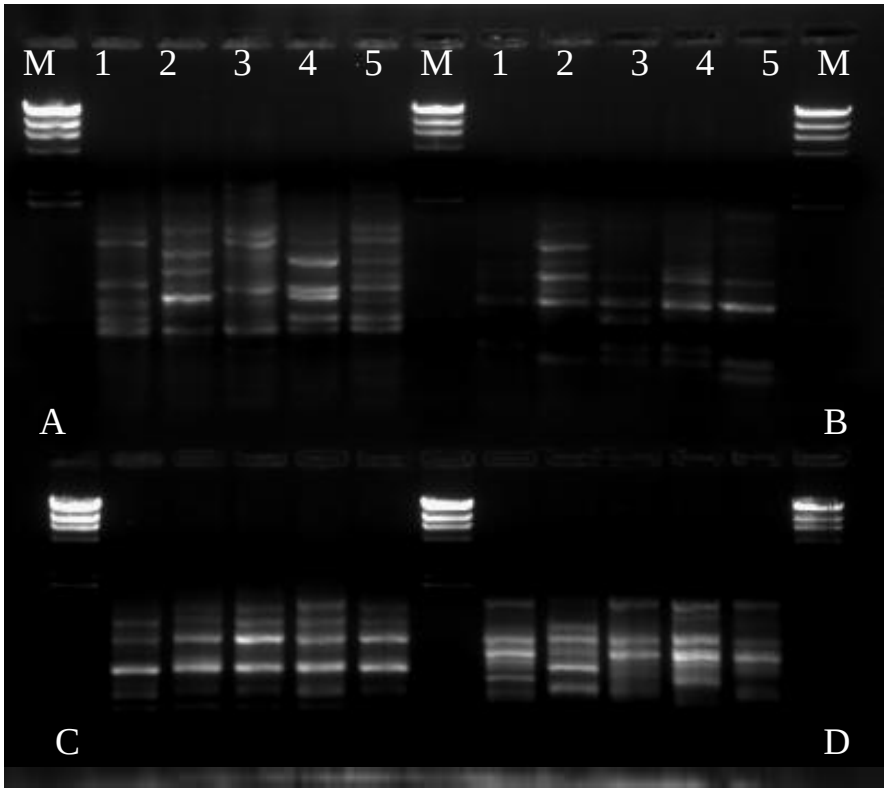
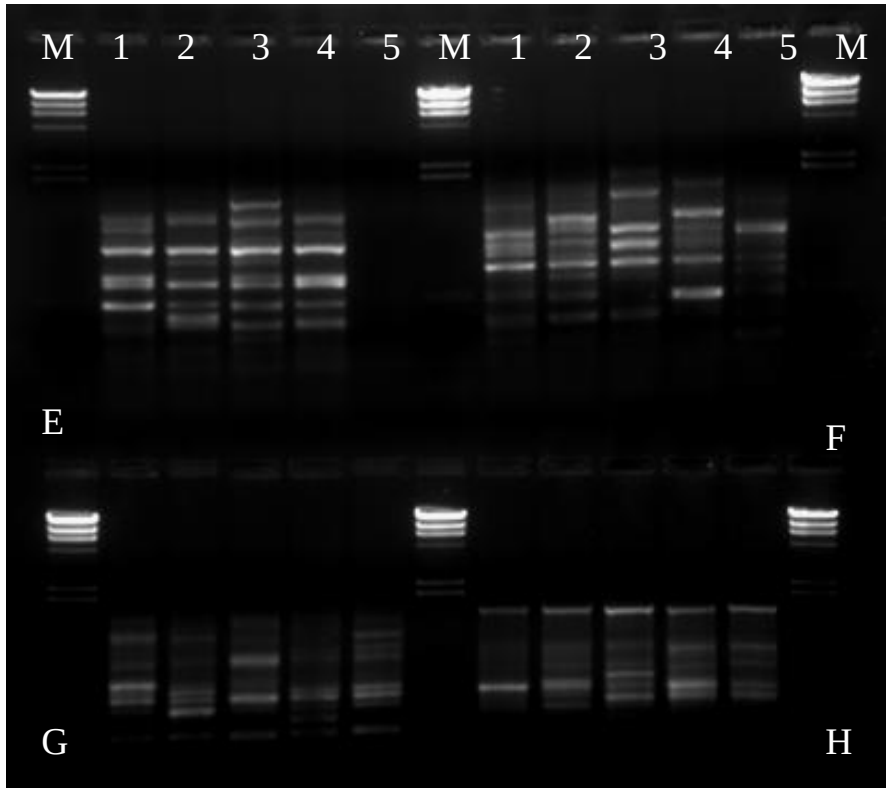
* estimated by Mega 4

**DNA Finger prints of five diverse clones of Vetiver as amplified with 8nos. of UBC-ISSR Primers**

(1- Autotetraploid, 2. South Indian, 3. Dharani, 4. KS-1, 5. Thailand)




**Lane M : λ DNA / Hind III digest**

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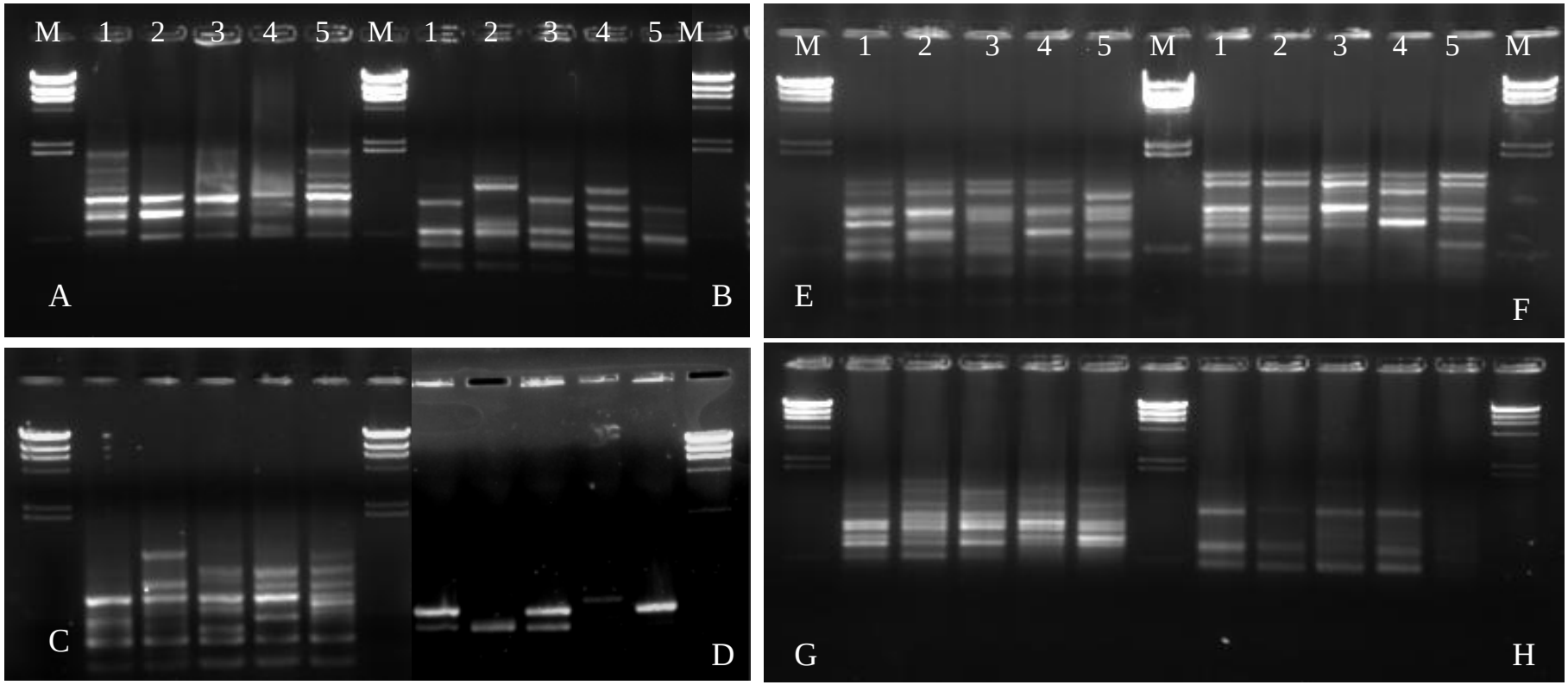
**E : UBC814-** CTCTCTCTCTCTCTCTA; **F : UBC818-** CACACACACACACACAG; **G: UBC823-** TCTCTCTCTCTCTCTCC; **H : UBC825-** ACACACACACACACACT

**DNA Finger prints of five diverse clones of Vetiver as amplified with 8nos. : 04 OPO + 04 OPJ of RAPD Primers**

(1- Autotetraploid, 2. South Indian, 3. Dharani, 4. KS-1, 5. Thailand)




**Lane M : λ DNA / Hind III digest**

**A: OPJ 9 - TGAGCCTCAC ; B : OPJ10- AAGCCCGAGG; C: OPJ12 – GTCCCGTGGT; D: OPJ13 – CCACACTACC ;**

**E: OPO-4- AAGTCCGCTC; F: OPO-5 – CCCAGTCACT; G: OPO-6- CCACGGGAAG; H: OPO-7- CAGCACTGAC**

# Vetiver grass model for long term carbon sequestration and development of designer genotypes for implementation

**The Vetiver autopolyploid clone christianed as "CIMAP-40" is characterized by:**

- **Somatic chromosome number of 4x=40 (compared to 2n=20 found in progenitor / natural source)**
- **Presence of one site each of 45S and 5S ribosomal DNA represented four times in its somatic cell as delineated by FISH**
- **larger stomata, larger root vascular cylinder, genetically distinguished by unique ISSR and RAPD profiles that serve as DNA fingerprints**

## Vetiver grass model for long term carbon sequestration and development of designer genotypes for implementation

**The autopolyploid clone :**

- **Owing to its fast growing deep penetrating root system could potentially sequester 900 g of atmospheric carbon per year from one meter square surface area deep into sub-soil horizons likened to forest trees, with least risk of carbon being recycled into atmosphere.**

- **Seed infertile suitable for ecological plantations that does not pose any problem of becoming weedy**

- **Sports dense mesh forming root system that can withstand landslide disaster and facilitate soil conservation**

- **Strategic plantation of this autotetraploid clone in crop fields tree lines, river, road and rail-line embankments as hedgerows could potentially contribute to carbon sequestration vis-à-vis eco-technological management of land and soil, and as a resource for biomass and bioenergy with no threat of becoming weedy in unattended plantations**