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PERSPECTIVE

Designer Vetiver genotypes for environmental and industrial applications

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Abstract

Background and Aims: The Vetiver grass [*Vetveria zizaniodes* (L) Nash. syn. *Chrysopogon zizaniodes* (L.) Roberty], traditionally valued in aroma industry for its essential oil extracted from roots is now extensively used as a green technology for its multifarious environmental applications. With rising global interest in Vetiver Grass Technology, it becomes desirable to identify / develop designer genotype/s of vetiver for specific application/s to realize its efficient implementation. Whereas the penetrating roots reaching deep into subsoil horizon promises vetiver as a suitable candidate for carbon sequestration likened to trees, its web forming tufted roots supported with lateral roots make this grass a model soil binder. Further, owing to its efficient phyto-absorption potential for toxic chemicals and metalloids that are primarily stabilized in root zone, the above ground shoot mass of this grass could also be utilized as a forage when combined with nutritional qualities required in a fodder. On the other side for its utilization as an essential oil crop it is desirable to develop short duration genotypes that could yield maximum productivity of high-quality essential oil keeping in fitness with the requirements of aroma industry. Therefore, it was endeavored to develop designer genotype/s to aim the following specifications: (a) Efficient soil binder and soil ameliorator combined with fodder qualities, (b) Root based sequestration of atmospheric carbon deep into subsoil horizon likened to trees, (c) Short duration crop for high essential oil productivity.

Methods: Keeping in fitness with the concept of 'root ideotype' and 'root phenomics' suggested for vetiver grass for specific applications, extensive efforts were made to isolate genotypes from the range of diversity prevalent across the length and breadth of India. Further selection pressure and genomic manipulation was applied to identify genotypes that meet the 'best fit' to realize the requirement of environmentalists in a global perspective, including non-invasiveness. The genotypes were tested for their efficiency as a soil ameliorator by growing them in iron mine spoil dumps, nutritional and palatability qualities of leaves desirable as a fodder, growth efficiency and deep penetrability of roots for carbon sequestration potential, and concentration and quality of essential oil in the roots harvested after six months.

Key results: The following three kinds of designer genotypes are developed for environment and industry specific applications: (i) A non-seeding ideal plant type that sports profuse rooting with high secondary roots (with least oil) for enhanced soil binding properties, coupled with profuse tiller and shoots rich in high fiber content and nutritional qualities suitable for fodder. This genotype is suitable for ecological plantations in degraded soil / iron mine spoil dump; (ii) A seed infertile plant type that has thick deep penetrating roots with least secondary roots and high biomass. This plant type is ideal for sequestration of atmospheric carbon deep into subsoil region likened to trees as well as suitable for improving soil fertility through enriching the soil carbon pool; (iii) A short duration clone that could yield high amount of essential oil of desirable quality from its roots harvested just after six months of plantation, otherwise obtainable after 18 months in the standard cultivars.

Conclusions: Keeping in view the two diverse requirements i.e. for environmentalists on one side and industrialists on the other, intensive efforts were exercised that led to development of three designer genotypes : (i) Genotype 'CIMAP-FORAGIKA' : It is suitable as an efficient soil binder on account of its designer root architecture that sports intense root-web, as well as soil ameliorator on account of its high iron / heavy metal absorption potential in the root zone, and at the same time possessing shoot characteristics that make it suitable for usage as fodder under ecological plantations, (ii) The clone 'CIMAP-KH40' : This clone sports deep penetrating fast growing smooth roots with efficient carbon sink, and is thus ideal for carbon sequestration deep into the subsoil likened to trees. Further, this clone was made seed infertile through genomic manipulation to realize non-invasiveness for its suitability in ecological plantations, (iii) The genotype 'CIMAP-KHUSINOLIKA': This is a short duration clone that could yield essential oil of desirable quality just after 6 months crop cycle against the existing varieties that require 18 months crop cycle.

Keywords: *Chrysopogon zizanioides*, Designer genotype, Root architecture, Root-web, Vetiver fodder, Soil binder, non-seeding vetiver, short duration vetiver, soil iron mitigation, autopolyploidy, seed infertility, carbon sequestration, ecological plantation, climate change, carbon credit, DNA finger prints, DNA markers

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Conflict of interest: (i) In order to complete the story, necessary data from earlier published work by the authors has been used/ reproduced with due citation to the original source. (ii) This manuscript is based on the presentation made in the Plenary Session- Part 2.2, p 44--; during the ICV- i.e. 7 th International Conference on Vetiver: Vetiver for Soil and Water Conservation, organized by the Chaipattana Foundation & the Office of the Royal Development Projects Board (ORDPB), at Chiang Mai, Thailand from 29 May– 1 June 2023; Proceedings: https://drive.google.com/file/d/1ZSTYj1BNf0zs3qpeG5uo1dvA3Au_yLwH/view (iii) This article is being published here with necessary edits and permission of the Conference Organizers through the President of The Vetiver Network International -TVNI (Jim Smyle) and the Editor (Paul Truong).

Introduction

Vetiver [*Vetveria zizaniodes* (L) Nash. syn. *Chrysopogon zizaniodes* (L.) Roberty] is a perennial densely tufted C4 grass native to India (Lavania 2002, 2008). This grass traditionally used for extraction of essential oil, has attracted world attention as a natural inexpensive and practical means for its multifarious environmental applications, including conservation and amelioration of degraded / mine tailing soil loaded with excess Fe, Cu and other heavy metals (Truong 1999, Roongtanakiat *et al.* 2008), control of soil erosion, sedimentation, flood and landslide disaster mitigation (www.vetiver.org, National Research Council monograph 1993). There are indications that cattle can feed on vetiver leaves (Lukiwati 2015) having digestibility and protein content comparable to other pasture grasses such as Rhodes and Kikuyu (Paul Troung, personal communication). Therefore, this grass could be targeted to develop as a prospective fodder. With the initiatives undertaken by the World Bank in 1980s followed by proactive support and dissemination from the Royal Project Development Board of Thailand over the last three decades, the vetiver grass has since emerged as an important candidate to address host of environmental concerns and human well-being across the globe. Adding value to vetiver grass applications in global environmental perspective in view of looming threats of climate change, Lavania and Lavania (2009) proposed a "vetiver grass model" for sequestration of atmospheric carbon into subsoil horizon likened to trees. Further, Lavania S (2003, 2019) outlined the 'root ideotype' and 'root phenomics' based criteria to develop designer genotypes for environment and industry specific applications to realize optimum utilization of this grass under agri- and eco-plantations.

India being the native home of vetiver evince its natural presence across the length and breadth of the country in the tropical and subtropical regions, and is enriched with repertoire of genetic diversity (Lavania 1985, 2008). The latter offers opportunity to score / develop designer plant types to realize specific applications. Whereas, both profuse to low seed forming genotypes occur in India respectively in the northern and southern regions of the country (Lavania 2008), they distinctly differ for their transcriptome as well (Chakrabarty *et al.* 2015), having value in identification of reference genes (Chauhan *et al.* 2023). Nevertheless, the low seed forming types from south do set seed when grown under north Indian conditions. Therefore, north Indian conditions are ideally suited to tap the inherent genetic diversity from the segregating progenies to score the desired plant type. It was therefore planned to score the attendant native genetic diversity occurring in India after growing the diverse collections under agroclimatic conditions of Lucknow located at 26°55′ N and 80°59′ E at an elevation of 123 M, in order to further select / develop designer genotypes for specific environmental and industrial applications as detailed in this communication. Due emphasis was laid to score a non-seeding / seed infertile genotype for environmental applications to realize its global acceptability as conceptualized by Lavania and Kumar (1996) and Lavania *et al.* (2006). For industrial application, the major emphasis was for productivity of essential oil of high quality with minimum crop gestation. The three designer genotypes thus developed are presented here.

Experimentation and Demonstration

Choice of the Material and Method of development of designer genotypes

Material

A large number of plants and seeds of vetiver were collected from diverse natural habitats along the agricultural plains, river banks, terai regions, road sides from the northern, central and southern regions of India, and grown at the experimental farm of the CSIR-Central Institute of Medicinal and aromatic plants, Lucknow, India. Such plants were screened for their growth and reproductive behavior and root architecture and root histology (Figures 1-3). Based on initial screening plants were classified into three diverse categories targeting the diverse applications. (i) profuse rooting supported with high secondary and tertiary roots with low number of essential oil secretory cells, nutritionally rich leaves and low / no seed formation, (ii) fast growing thick smooth roots with low

Figure 1: Exo-morpholoigcal diversity in vetiver. Clone FORAGIKA - extreme right with profuse tillers

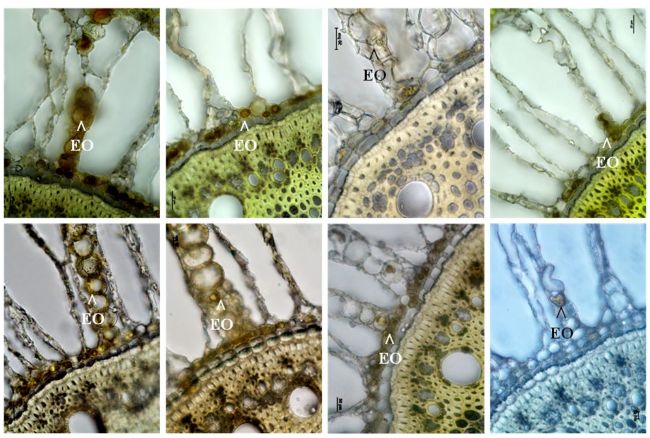

Figure 2: TS of the different vetiver roots showing diversity in essential oil secretory cell (EO)

seed formation, (iii) fast growing roots with large number of essential oil secretory cells. The selected clones were further processed for identification of desired plant type for specific applications as per the procedure and details given below:

Method of development / identification of designer clones for specific applications

- *Development of clone suitable as soil binder, soil ameliorator, and as cattle feed, i.e. clone 'CIMAP-FORAGIKA'*

Out of the diverse collections twelve clones were initially screened out for fast growth and high tillerring, and were further screened out for root growth pattern with a focus on presence of secondary / tertiary roots, broader leaves with thin lamina and low seed set under field conditions. Side by side these clones were histologically examined for frequency of occurrence of essential oils secretary cells in the roots in six-month aged roots, cortex / vascular cylinder ratio, leaf lamina for thickness of leaf sclerenchyma, seed set and seed fertility. Out of these six morphologically diverse

Figure 3: Fully grown-up plant, leaf width and root-pattern of the Check - Clone CIM-Vriddhi (left) vs Clone CIMAP-FORAGIKA (right). Note high tiller, and drooping and broad leaves, and profuse secondary roots in the clone CIMAP-FORAGIKA – source Lavania et al. 2021

clones that promise high shoot biomass, broad and soft textured leaves and fast-growing high number of roots were evaluated for root and shoot number per tiller and growth behavior under field conditions to identify a clone of choice. The clone 'CIMAP-FORAGIKA' thus identified was further tested for its biological attributes and performance vis-à-vis other standard clones earlier released for industrial and environmental applications under field conditions to record empirical data on various morphological, reproductive, anatomical, physiological and nutrition quality parameters. Further, in order to elucidate the tolerance and metal extraction potential of vetiver in rehabilitating iron spoil soil-dump sites, selected vetiver clones were evaluated by growing them on the soil from an iron ore spoil dump under bench scale as well as in field conditions on the mining site. The details of the data recorded are given below in Tables 1-3, and also depicted by way of figures (Figures 3-8).

Figure 4: Root pattern of Clone CIMAP-FORAGIKA.Left – under field conditions, Right – under experimental conditions grown in microcosm. Note profuse primary, secondary and tertiary roots. -source Lavania et al. 2021

- *Development of clone suitable for sequestration of atmospheric carbon in subsoil horizon likened to trees i.e. clone 'CIMAP-KH40'*

Diverse accessions were screened to select a clone that sports thick and smooth (with least secondary roots) fast growing roots and at the same time having low seed fertility as referred to above under the Section-Material (ii). A desirable clone thus selected was further targeted for the induction of polyploidy to make it fully seed infertile suitable for ecological plantations. The lateral shoot buds near the leaf base from the fast-growing tillers were exposed by removing the cover leaf. 100 numbers of such tillers were immersed in 0.1% aqueous solution of Colchicine prepared

Table 1: Nutritional characteristics of various vetiver clones based on biochemical analysis of leaves at two / three months of growth after planting*

CLONE SAMPLE	*N*	*Proteins*	*Carbo-hydrates*	*P*	*Crude fibre*	*Fe*	*Cu*	*Zn*	*Ca*	*K*	*Na*
at 2 months											
THAILAND	14.11	30.29	59.58	21.85	0.218	216.03	0.024	5.346	21	85	12
CIMAP-FORAGIKA	6.6	36.03	32.13	29.78	0.205	262.1	0.031	6.048	17	197	7
CIM-VRIDDHI	7.37	29.64	143.8	48.64	0.154	262.82	0.033	7.112	21	191	12
CIMAP-KH40	5.9	29.54	159.17	35.19	0.172	334.25	0.03	4.751	18	111	7
CIMAP-KHUSINOLIKA	2.92	47.38	91.61	54.39	0.19	157.46	0.022	5.559	22	181	15
at 3 months											
THAILAND	12.14	38.18	31.47	55.89	0.163	218.00	0.026	5.349	23	115	45
CIMAP-FORAGIKA	3.77	41.17	31.15	26.22	0.244	263.00	0.032	6.049	22	45	21
CIM-VRIDDHI	3.99	27.19	138.53	41.74	0.206	262.98	0.032	7.114	42	161	22
CIMAP-KH40	4.26	29.64	198.11	40.59	0.229	336.30	0.031	4.750	19	103	19
CIMAP-KHUSINOLIKA	3.51	48.46	100.86	41.74	0.197	156.00	0.022	5.561	21	58	34

* Estimated values measures as : N, Carbohydrate, P and Crude fibre as mg/gm dry weight, Protein taken as mg/gm fresh weight, K, Na, Fe, Cu, Zn as ppm/25 mg dry weight (source - Lavania et al 2021)

Table 2: Estimation of metal contents (mg kg^{-1} DW) in shoot and root of vetiver varieties (CIM-Vriddhi, CIMAP-KH40, Thaialand-Surathani, and CIMAP-FORAGIKA) grown on iron mine overburden soil. (values represent three replicates)*

Months	*Vetiver Clone*	*Fe*		*Cu*		*Zn*		*Cr*		*Mn*	
		Shoot	*Root*	*Shoot*	*Root*	*Shoot*	*Root*	*Shoot*	*Root*	*Shoot*	*Root*
0	CIM-Vriddhi	6.31	9.12	2.10	1.83	2.79	7.82	2.78	4.85	1.56	8.50
	CIMAP-KH40	5.13	8.45	1.92	1.97	1.73	6.99	2.52	3.28	1.70	8.22
	Surathani	6.12	8.13	2.37	2.54	1.35	5.99	2.85	3.17	1.23	7.96
	CIMAP-FORAGIKA	5.97	8.82	2.68	1.89	2.47	6.27	3.07	4.36	1.34	8.27
6	CIM-Vriddhi	478.40	1498.81	47.42	36.83	5.91	14.12	8.37	15.94	16.37	21.40
	CIMAP-KH40	486.30	1614.12	61.27	45.57	6.47	10.70	8.40	12.38	10.61	18.23
	Surathani	544.55	1642.01	37.72	44.85	7.31	13.50	9.36	14.36	16.96	21.25
	CIMAP-FORAGIKA	556.70	1661.37	46.67	56.35	6.93	11.90	7.39	9.95	12.57	27.49
12	CIM-Vriddhi	1276.8	2992.86	128.03	84.71	10.64	36.71	16.74	39.85	29.47	51.36
	CIMAP-KH40	1041.6	3684.72	165.43	104.81	11.65	27.82	16.80	30.95	19.10	43.75
	Surathani	916.1	3852.06	101.84	103.16	13.16	35.10	18.72	35.90	30.53	51.00
	CIMAP-FORAGIKA	1189.3	3968.22	126.01	129.61	12.47	30.94	14.78	24.88	22.63	65.98

*Data obtained from the collaborative experiment conducted at Kolkata (Banerjee, Goswami, Lavania S, Mukherjee & Lavania UC, 2019. Ecological Engineering, **132**, 120-136).

Table 3: Comparison of Description of morphometric, histological and physiological features of clone 'CIMAP-FORAGIKA' vis-à-vis other developed varieties (source – Lavania et al. 2021)

Characteristics	*CIMAP-FORAGIKA*	*CIM Vriddhi*	*CIMAP-KH40*	*Thailand*	*Khusinolika*
General morphology	*Very broad and soft leaf, high tillerring and profuse secondary roots*	*Profuse flowering, low seed set, smooth and roots*	*Low and late flowering, broad leaf, non-seeding, thick and smooth roots*	*Semi-Spreading, low flowering, smooth and thin roots*	*Dwarf, spreading type canopy, late and low flowering, smooth and thin roots*
Growth Habit	Fast growing, profuse tillerring	Fast growing	Slow growing	Medium growing	Fast growing
Plant height in cms (taken as leaf length)	164	168	142	145	141
Shoot yield (culm / leaf dry matter) after 5 months	120 g	85 g	95g	65 g	75
Carbon content in shoots (%)	37	36	38	37	36
Number of leaves / tiller	7-9	6-9	7-9	8-11	7-9
Number of slip/ tillers after 5 months/12 months	25-28 / 47-50	20-23/ 28-35	18-20/ 32-38	25-28/ 40-44	22-24/ 30-34
Inflorescence stalk length in cms (culm and inflorescence combined)	230	235	190	245	197
Culm length (cms)	136	140	95	128	127
Leaf color	RHS 143B Green	RHS 137A Green	RHS 138B Green	RHS 137 B Green	RHS 137 B Green
Leaf Texture / No. of air chambers	Smooth / 24-26	Stiff/ 20-22	Stiff/20	Stiff/24-26	Stiff/20-22
Leaf cuticle thickness (mm) Adaxial/abaxial	0.0037/0.0037	0.0037/0.0055	0.0038/0.0050	0.0041/0.0048	0.0048/0.0048
Leaf Thickness at midrib (mm)	0.418	0.45	0.66	0.35	0.54
Vascular tissue thickness at mid rib (mm)	0.138	0.18	0.25	0.125	0.115
Leaf Width (mm)	12.0	8.0	6.5	9.0	8.0
Sclerenchyma thickness (mm)	0.055	0.07	0.057	0.060	0.062
Leaf Sclerenchyma (%)	12.43	13.62	16.42	15.05	11.4
Leaf blade stomatal index	7.57	7.62	6.47	7.39	9.04
Leaf base stomatal index	2.25	2.93	1.52	1.62	2.5
Average size of guard cell	116 μm^2	75 μm^2	119 μm^2	103 μm^2	81 μm^2
Stomata / mm^2	197	181	77	199	255
Seed set and germination	00 %	2-3%	0.8-1.0 %	8-10%	8-11%
Number of primary roots after 5 months	223	193	208	198	195
Root Length (cm) after 5 months	135 - 160	150 - 155	153 - 185	130 - 140	125-135
No. of Primary roots per tiller	12-25	10-18	8-18	12-20	10-23
No. of Second. roots / 2.5 Cms	25-55	12-22	8-18	12-18	12-20
No. of Tertiary roots / 2.5 Cms	27-45	11-17	10-18	12-18	15-24
Average root diameter / stele diameter (at the base of main root) cms.	1.84/0.86	2.1 / 1.11	0.249 / 0.153	0.19/0.14	1.75/0.9

Total root dry weight (g/ plant) after 5 months	40 g	38 g	43 g	31 g	37g
Carbon content in Roots (%)	44	42	43	42	44
Oil Content (%) in fresh roots) at one year	0.3	1.0	0.7	0.8	1.0
Photosynthesis efficiency i.e. CO_2 exchange rate at 10-15 µmol / m^2 / sec photosynthetic active radiation	5.46/ m^2 / sec	4.44/ m^2 / sec	7.634 µmol / m^2 / sec	5.49/ m^2 / sec	5.12/ m^2 / sec
Chlorophyll content	2.186 µg / g fresh wt.	2.336 µg / g fresh wt.	2.386 µg / g fresh wt.	2.376 µg / g fresh wt.	2.343 µg / g fresh wt.
Cytological differentiation	2n = 20	2n = 20	2n = 20	2n = 20	2n=20
Range in Chromosome size	2.0 to 4.0 µm	2.1 to 4.0 µm	2.1 to 4.4 µm	2.1 to 4.2 µm	1.9 to 4.0µm
Haploid chromatin length	28 µm	28 µm	29 µm	29 µm	28 µm

in 2% DMSO (Dimethyl Sulphoxide) for 07 hours at ~ 25^0 C followed by thorough washing in running water. Only the lower 8 cm portion of the tillers that had axillary buds was kept immersed in colchicine solution. After washing the treated tillers were given overnight recovery period from colchicine shock in 1X Hoagland's solution, and then planted in the experimental field followed by optimum cultural care. The leaves from lateral tillers emerging from the colchicine treated tillers were examined for the size of stomata in the epidermal peel from the adaxial side of the leaf. Only one leaf-shoot emerging from one of the treated tillers showed linear streaks of larger stomata in the central region of the leaves. This tiller was scored out, and its vegetative progenies were screened cytologically for polyploid chromosome number and uniform occurrence of larger stomata in the entire leaf. Cytologically stable polyploid clone was multiplied vegetatively for further evaluation, for root growth and biomass. Further details are given under section 3.1.2, and also depicted by way of figures (Figures 9 and 10).

- *Development of clone that produces khusinol rich essential oil under short duration cultivation i.e. clone 'CIMAP-KHUSINOLIKA'*

Intensive recurrent selection breeding was exercised at the experimental farm of the Central Institute of Medicinal and Aromatic Plants, Lucknow, India to obtain a clone suitable for commercial cultivation as a short duration crop out of the poly-crossed population generated from the bulk of wild collection. The clone 'CIMAP-KHUSINOLIKA' initially identified as strain "G-12" in the breeding population is a descendant from population improvement programme. The parent source material is the wild population of 4000 plants raised from open pollinated wild population of vetiver occurring across the 09 districts located in the state of Uttar Pradesh, India. Standard breeding parameters of initial and large-scale evaluations were exercised to isolate a clone of choice. Further details are given under the section 3.1.3 in Table 4, and also depicted by way of figures (Figure 11).

Botanical description, evaluation and utilization of the developed clones

Clone 'CIMAP-FORAGIKA' suitable as soil binder, soil ameliorator, and as cattle feed

General

This clone is distinct and unique in its exo-morphological appearance on account of its markedly broad leaves and drooping appearance of its thin leaves, and profuse tiller formation. The clone sports high rooting coupled with secondary and tertiary roots; and its leaves are palatable and nutritionally rich meeting fodder qualities. Also, this clone could be efficiently grown on iron mine spoil dumps thus ameliorating and rehabilitating metal infested mining over burden soil and at the same time making its above ground biomass available for grazing and other uses since the phyto- absorbed metalloids are largely confined to root zone with least translocation to the shoot zone.

As such this clone is suitable for ecological plantations as hedge rows for soil / slope stabilization along pastures, mudslide prone foothills; contour protection of river banks, ponds / bunds, on account of its massive web-forming roots that facilitate enhanced soil binding / stabilization of degraded soil, as well as for amelioration of mine over burden soil owing to its high phyto-absorption for iron and other metalloids. Further, this clone based ecological plantations could be effectively sustained when allowed to be grazed by cattle enabling their continued rejuvenation. Moreover, since this clone is non-seeding therefore its plantation would not pose any threat of getting invasive / weedy.

Table 4: Performance of 5 elite lines + 4 checks of vetiver in respect to fresh root and shoot weight (g)/plant and oil content (%) in PST after 06 months of plantation*

Strain	*Fresh root weight g/plant*	*Fresh shoot weight g/plant*	*Root: Shoot*	*Oil content (%)*
G3	70	543	0.1289	0.86
G12	65	440	0.1478	1.08
G15	74	699	0.1059	0.95
G16	75	1000	0.0750	1.06
G21	68	566	0.1201	0.88
KS-1	76	1340	0.0567	0.79
Dharini	70	1150	0.0609	1.00
Kesari	100	1280	0.0781	0.80
Gulabi	81	1480`	0.0547	0.98
MEAN	75.44			0.93
SE ±	3.457			0.0356
SD	9.830			0.400
Variance	96.647			0.160

Of these selections, G12 was observed to be having highest Root: Shoot ratio (0.1478) and oil content (1.08% v/w). * based on data recorded in Chauhan et al. 2017

Description and Characterization

- *Growth behavior and root pattern for enhanced soil binding potential*

The plant is quite distinct in its growth behavior, sporting profuse tiller production i.e. >1.5 times compared to others, and its roots bear huge secondary and tertiary roots far more compared to all other clones occurring in the natural habitats. Its leaves are quite broad but thin (less lignified) compared to others.

- *Uniformity, stability and noninvasiveness*

The given plant is a seed sterile clone that could propagate only vegetatively promising uniformity, and as such ideal in ecological plantation without posing any threat of becoming weedy. Its stability has been tested and ensured through several multiplication cycles over five years. The clone could be easily propagated through vegetative slips to generate planting material.

- *Flowering behavior*

Although, this clone flowers as usual, but the pollen borne are sterile and the seeds formed are empty / sterile and does not germinate at all.

- *Essential oil secretary cells*

The number and volume of oil secretary cells is quite low, and so does the essential oil content (0.3 %) far below than the other cultivated varieties. Therefore, this plant is quite ideal for ecological plantations since this will not attract the root diggers that wish to uproot the plant for its aromatic roots.

- *DNA finger printing*

The clone has a characteristic DNA finger print identifying it as a distinct clone

- *Nutritional Quality vis-à-vis suitability as a fodder*

The leaves of the clone 'CIMAP-FORAGIKA' are enriched with protein (41 g / kg fresh weight), crude fiber content (25 g/kg dry weight), but low in carbohydrates (31 g/ kg dry weight), alongwith desirable amount of essential minerals. The presence of given amount of fiber content makes it digestible by the cattle, and the protein content adds to its nutritional quality thus making it suitable as a forage. Thus it is inferred that the Clone 'CIMAP-FORAGIKA' besides being suitable for soil conservation, is high in crude fibre content and contains reasonable amount of protein. It has low Ca and Na levels in the leaves but high level of potassium to tone up metabolism. July to October and Feb to May are favorable for growth and useful for consumption. Presence of moderate Fe, Cu and Zn levels keep major synthetic pathways to function harmoniously.

- *Iron mine soil dump amelioration potential*

Results obtained after growing this clone vis-à-vis other clones on the iron overburden (OB) soils on the iron mining site show that whereas vetiver grass *per se* could be suitably

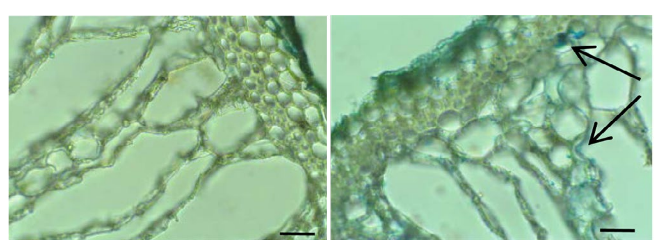

Figure 5: Transverse section of root, Left- grown on garden soil, Right - grown at Iron ore mine soil. Note uptake and localization of iron particles in mine soil (marked in blue as localized by Perls blue test). – source- Banerjee et al. 2019

Figure 6: Plantation of clone CIMAP-FORAGIKA at the iron ore overburden spoil dump site – source Vimala et al. 2022

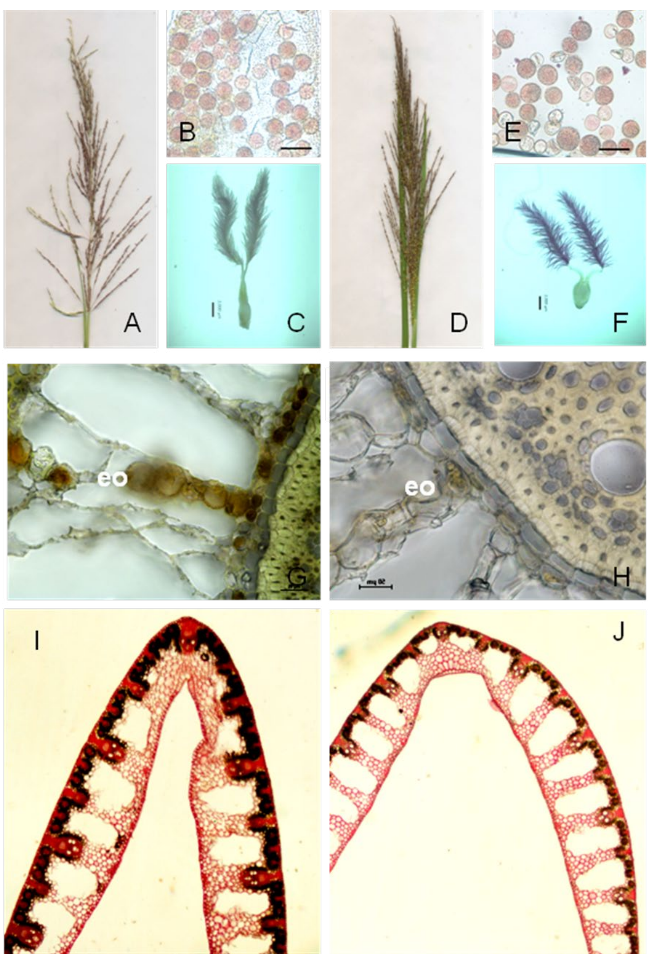

Figure 7: Depiction of micromorphological features (inflorescence, pollen grains, pistil, essential oil secretory cell (eo) and TS of leaf showing comparison between Clone CIM-VRIDDHI (A,B,C, G,I) and Clone CIMAP-FORAGIKA (D,E,F, H, J). Note low essential oil cells, reduced leaf – source Lavania et al. 2021

grown on iron mine OB soil to help rehabilitate soil without any sign of phytotoxicity, but the instant clone 'CIMAP-FORAGIKA' is most efficient for rehabilitation of Fe infested spoil soil-dump from iron mining site (Figure 6). Curiously the accumulation of Fe was higher in roots than in shoots thus having least toxic effect on leaves that could be usable as a fodder. Presence of Fe in plant tissue, in particular, was confirmed by histochemical localization by Perls blue staining (Figure 5). SEM images of the transverse sections of leaves and roots of vetiver growing on mine soil revealed no noticeable structural abnormalities.

It is inferred from the data above that vetiver grass *per se* can tolerate high concentrations of Fe along with other heavy metals in the soil as well in its tissues. Such potential varies across the four ecotypes and can be effectively used for phytoextraction on sites contaminated with high levels of heavy metals, particularly Fe, Mn, Zn and Cr.

Clone 'CIMAP-KH40' suitable for sequestration of atmospheric carbon in subsoil horizon likened to trees

General

Large scale deforestation has necessitated the search for suitable alternatives for locking of the atmospheric

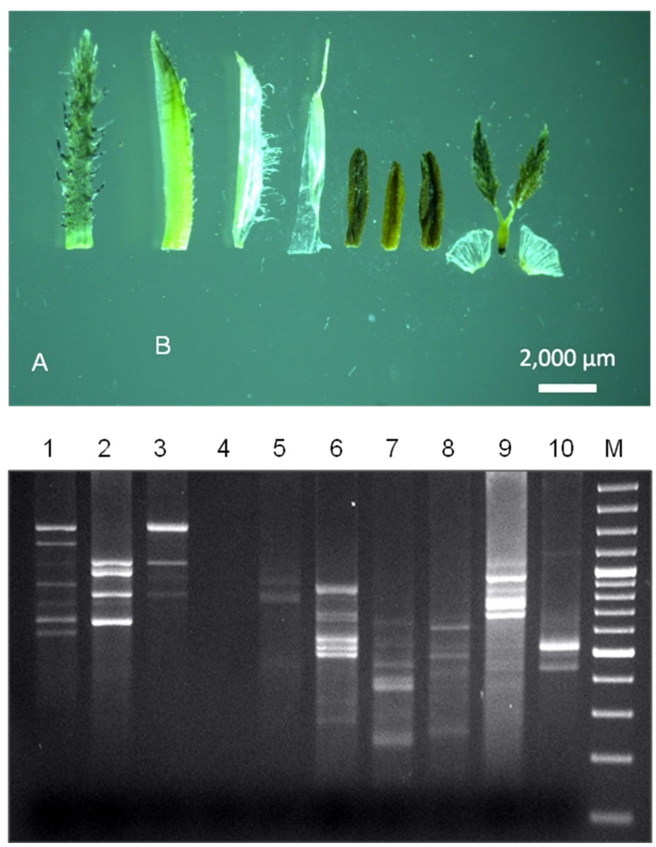

Figure 8: Characterization of clone 'CIMAP-FORAGIKA'. A . Floral organs, B. ISSR DNA finger print (M- Marker- 100 bp; and 1-10 ISSR primers- UBC 807,810,811,812,814,818,823,825,826,828) – source - Lavania et al. 2021

carbon deep into the subsoil horizon so as to minimize its recycling back into the atmosphere. Lavania and Lavania (2009) proposed a Vetiver grass model suggesting that deep penetrating roots of vetiver could be a suitable alternative likened to trees for long-term sequestration of photosynthetic carbon deep into the soil in the climate change regime. However, for effective implementation of vetiver grass model there is need to identify a clone that has thick, smooth and fast growing roots with least degradation. The clone 'CIMAP-KH40' meets the designer characteristics on account of its fast-growing deep penetrating tufted roots that are smooth and thick producing high root mass rich in organic carbon, high stele-pith/ cortex ratio and thick casparian strips enabling minimum degradation. This clone is capable of sequestering 860 g of carbon / square meter / year. Further, for its global acceptance this clone was made seed infertile by changing it to polyploid state. Besides, this clone is equally effective for conservation of degraded soil on account of its formidable root-web.

Description and Characterization

- *Carbon sequestration potential of roots in sub- soil horizon*

When grown in sandy soil with optimum cultural care the instant autopolyploid clone is capable of producing 1.02 kg. of dry root biomass (of which 43% is the organic carbon content) from one square meter area with single slips planted at 40 cms x 40 cms plant – to- row distance after six months. Owing to the exponential root growth, this root biomass yield is likely to double after one year, with an estimated carbon sequestration potential of 860 g / square meter / year. Organic carbon content in the dry roots was estimated by standard chromic acid oxidation method according to Walkley and Black (1934).

- *Uniformity, genetic stability and seed infertility*

The progenitor diploid clone pre-selected for the desirable characteristics was changed to autopolyploid state to realize seed infertility. The clone CIMAP-KH 40 thus developed was field evaluated for growth and seed fertility. The polyploid clone is genetically pure and cytologically stable for its autotetraploid nature (4X=40). Sufficient quantity of planting material could be conveniently generated by vegetative propagation for plantation purpose.

Compared to all other fast-growing clones, the present autotetraploid clone exhibits delayed flowering (a delay of over one month) and seed fertility almost zero (i.e. no seed germination under laboratory conditions) compared to its source diploid clone that show 3% germination, and other

Figure 9: Exomorphology and root-growth pattern of clone CIMAP-KH40. A. plant grown from single slip at 40 days showing shoot-root ratio, B. Root-growth and root-web as seen in situ under field conditions at six months, C. Late lowering and fast growth in the clone CIMAP-KH40 (left) compared to south Indian clone (right), D-E root transverse section showing cortex/ vascular cylinder ratio : D. progenitor diploid and E. CIMAP-KH40. Note higher vascular cylinder ratio in CIMAP-KH40 - source Lavania et al. 2016

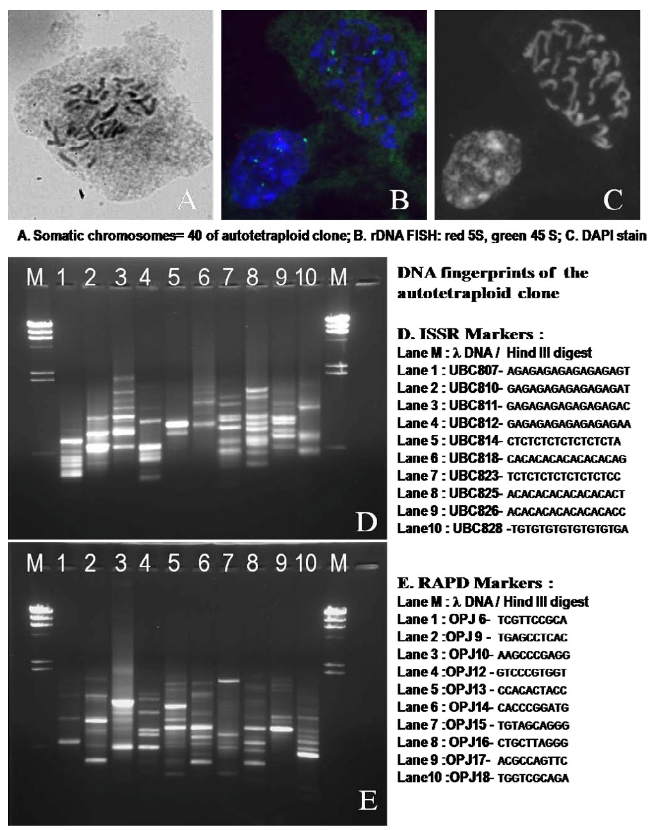

Figure 10: Chromosomal and DNA finger-based characterization of clone CIMAP-KH40. A-C. Chromosomal characterization for autotetraploid nature, D-E. Molecular characterization based on DNA finger prints, D-ISSR primer, E-RAPD markers. Source- Lavania et al. 2016

clones showing seed germination ranging from 10 – 50 %. It may be mentioned that Lucknow climate is otherwise most suitable climate for fertile seed formation in Vetiver since even the low seed forming vetiver from south India, and the clone from Thailand does form fertile seeds under Lucknow conditions. Therefore, it is believed that the instant autotetraploid clone does not form fertile seeds when grown in other parts of the globe.

- *Growth potential*

Under optimum field conditions with sandy soil the initial root emerging from the single tiller reaches up to the length of 90 cms in one month, and a 130 cms. long tuft of 160 nos. of roots and a clump of 40 nos. of tillers after three months. In six months this tufted root system grows up-to 250 cms and shoot: root length ratio of 1:2. The roots could grow through the soil hardpan crust as well.

Morphometric description of the clone 'CIMAP—KH40'

General morphology

Clumpy with long erect leaves, capable of rattooning; Growth habit: Profuse synchronous whorly tiller pattern. Under Lucknow conditions the instant autopolyploid clone flowers after eight months of planting done in the month of March, i.e. a delay of 30-45 days compared to other genetic stocks; Plant Height: After 180 days of planting: 1.50 meter, Inflorescence stalk length (i.e. 240 days after planting); 2.0 meters Culm and leaf: Tiny shoot (culm) hidden in the leaf sheath with 6-8 leaves per tiller sprouting from the bottom of the clump; Leaf: Dark green in color, smooth and waxy texture, Leaf blade is broad, width at leaf base = 2.6 cms. (compared to 2.2 cms. in diploid source), the adaxial surface of the two halves of the leaf blade are inwardly folded at the base but gradually open towards apical side, leaf apex acute, petiole length (outer three leaves) = 120 – 150 cms., with their average leaf area 180 cms^2 (compared to 160 cms^2 in source diploid).The average size of stomata (both guard cells) = 935 μm^2 with a stomatal frequency of 77 stomata / mm^2 of leaf surface compared to 491 μm^2 with a stomatal frequency of 144 stomata / mm^2 in the diploid clone, respectively; Inflorescence: Panicle type, purplish red, stalk and panicle together reaching upto two meter long; Seed set: Infertile seeds virtually with no seed germination; Oil content: 1.5 % in fresh roots (with 45% moisture) after 15 hrs of hydrodistillation at 10 months of growth; Root diameter (at the base of main root): 2.3 mm compared to 2.0mm in the diploid. The vascular cylinder is distinctly larger with reduced cortex; Root yield (dry matter) / sq. meter at 180 days = 1.02 kg.; Shoot yield (culm / leaf dry matter) / sq. meter at 180 days = 1.54 kg.; Carbon content: in the roots: 43%, in shoots: 37%. Further details could be found in Lavania et al (2016)

Estimated carbon sequestration potential into subsoil/ year/square meter: 860 g.

Clone 'CIMAP-KHUSINOLIKA' that produces khusinol rich essential oil under short duration cultivation

General

The new and distinct clone of *Chrysopogon zizanioides* `CIMAP - KHUSINOLIKA' is suitable for cultivation as a short duration crop for its essential oil rich in 'Khusinol'. The latter has value in perfumery, as well as, is a potential substitute / alternative to the other essential oil component 'Khusimol'. The clone holds commercial advantage since the plant roots of desirable quality can be harvested after 6 months that contain high essential oil content (1% v/w), rich in Khusinol (45 % v/v), not reported in any other variety and genotype. The said clone is developed through recurrent selection in polycrossed progenies and possesses the following combination of characters: Fresh Root Yield:18-20q/ha (at an average age of 6 months); Oil Yield: 18-20 kg/ha. This clone could be distinguished based on characteristic DNA finger print.

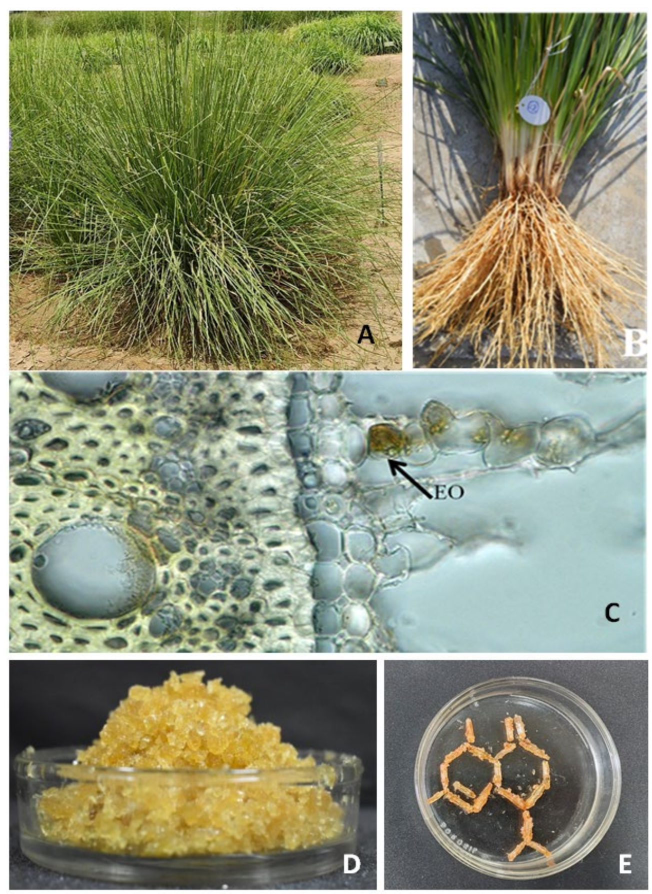

Figure 11: Characterization of clone CIMAP-KHUSINOLIKA. A. Exomorphology showing spreading plant canopy, B. Root pattern, C. Root TS showing localization of Essential oil secretory cells (EO), D. Khusinol crystals, E. Molecular structure of (bicyclic Cadinane) made from Khusinol crystals. Source: B & C - Chauhan et al. 2017 – D&E - Courtesy CS Chanotia

Description and Characterization

- *Morphological Characterization*

It is a fast growing diploid (2n=20), late and low flowering clone, with spreading plant canopy initially, smaller stomata, lax inflorescence, smaller spikelet and white feathery stigma, capable of producing >1 % essential oil rich in Khusinol (>45%) obtained from fresh roots after six months of plantation.

- *Uniformity and stability*

The instant plant type is a clone, tested for growth behavior, essential oil yield and essential oil quality that show its uniformity and stability examined over vegetative multiplication cycles. Sufficient quantity of planting material could be easily generated by asexual propagation through slips.

- *Flowering behavior*

This clone is late flowering and low seed setter. Under Lucknow conditions it flowers in the months of August-September, compared to others that start flowering from April / May onward.

- *Growth behavior*

In its initial growth stage at 2-3 months, the plant shows prostrate-spreading type of plant canopy. Under Lucknow conditions, a six-month-old plant planted at plant to row distance of 60 x 60 cms in the month of July and harvested at the end of December gives an average root yield of 65g / plant. However, the flowering occurs only in the second year after initial planting.

- *Essential oil productivity and composition*

The fresh roots harvested from six month old plant on hydro-distillation for 18 hours yield 1% essential oil that contains 45-50 % khusinol v/v. Performance data is provided in Table 4.

- *Physicochemical parameter of essential oil*

Odor: woody/ earthy note, Colour: Light yellow in appearance, Refractive Index n_D^{20}: 1.5193-1.5209, Optical rotation [α]: -68.680° at 23.8°C temperature; Chemical composition: Capillary GC-FID and GC/MS analysis of the essential oil obtained from roots harvested after 6 months of planting showed oxygenated sesquiterpenes (approx. 88%); out of which khusinol contribute approx. 47%.

Morphometric description of the clone 'CIMAP - KHUSINOLIKA'

General morphology

It is a fast growing diploid (2n=20), plant height 1.0 to 1.7 m, late and low flowering clone, with spreading plant canopy initially with tufted roots, smaller stomata, lax inflorescence, smaller spikelet and white feathery stigma, capable of producing >1 % essential oil rich in Khusinol (>45%) obtained from fresh roots after six months of plantation, late and low flowering; delay in flowering by over 45 -60 days compared to other north Indian genotypes, and lax inflorescence with white feathery stigma Flowering occurs in the second year after initial planting i.e. in the months of August – November, compared to other varieties that flower in June – September.

Growth Habit

Spreading plant type initially nearly prostrate but becomes erect at later stage, flowering is initiated in August (Figure 9), Culms compressed in early stages cylindrical in later stages, well defined solid nodes and internodes, number of tillers 25 to 42 after six months (cf. 30-45 in standard check).

Stomata

Stomatal Index 19.5; size of stomatal complex and stomatal guard cell 498 μm^2 and 72.5 μm^2 respectively (compared to 870 μm^2 and 115 μm^2 in standard check); Seed: Oblong and oblique at the top; Root: Diameter near the root base 1.7 mm (compared 2.0 + mm in others), colour pale whitish, average root length 15-20 cms.

Essential oil Content and composition

Medium (1.0%) in freshly harvested roots having moisture content 45%.; Physicochemical parameter of essential oil: Odor: woody/ earthy note, Colour: Light yellow in appearance, Refractive Index n_D^{20}: 1.5193-1.5209, Optical rotation [α] : -68.680° at 23.8°C temperature; Chemical composition: Capillary GC-FID and GC/MS analysis of the essential oil obtained from roots harvested after 6 months of planting showed oxygenated sesquiterpenes (approx. 88%); out of which khusinol contribute approx. 47%. Further details could be found in Chauhan et al. (2017).

Distinctiveness of the plant through IISR Fingerprints

The clone could be characterized through DNA Fingerprints (Figure 11)

Discussion

Vetiver grass technology, ICVs and beyond

Tracing back its usage as vetiver hedge rows for contour protection and root essential oil in aroma industry in its native home in India (Lavania 2002, 2008), vetiver grass technology has made significant strides and international impact leading to its multifarious applications and take-home message for practical implementation through several regional, national and thematic international conferences. The 7th International conference planned in Thailand is another milestone focused to dwell upon vetiver for soil and water. In this context concerns about the soil health vis-à-vis environmental conservation are equally important necessitating the development of specific genotypes to address the outstanding practical issues in a global perspective. Whereas, Lavania S (2003, 2019), and Lavania UC (2008) underscored the need to develop designer genotypes, they also proposed a vetiver grass model (Lavania and Lavania 2009) suggesting that vetiver grass could be a suitable alternative for long-term sequestration of atmospheric carbon deep into the soil likened to tree plantations. Another equally important area that needs to be addressed is to strengthen the traditional usage of vetiver for its root oil, since vetiver oil is one of the three essential oils that is perfume in its own right and is an important fixative highly valued in aroma industry (Lavania UC 2003, a, b); the other two are from Sandalwood oil and Pandanus oil. In view of above the three designer genotypes are developed that are presented here.

Novelty, Importance, and advantages of the developed designer clones

The two clones, 'CIMAP-FORAGIKA' and 'CIMAP-KH40' are non-seeding types and therefore globally acceptable in ecological plantation without posing any threat of becoming weedy,

Clone 'CIMAP-FORAGIKA' could be considered as an efficient soil binder on account of its profuse rooting. Presence of secondary and tertiary roots makes this clone even more efficient soil binder on account of intense root-web thus formed,

In addition, the clone 'CIMAP-FORAGIKA' could be suitably grown in iron mine over burden soil, and help ameliorate soil for its iron and other metalloid infestation, thereby making such soil cultivable in the long run. Moreover, the toxic metalloids phyto-absorbed by this clone do not / least translocate to the shoot zone, therefore no adverse affect/ contamination of the shoot biomass. The latter could be used for fodder and other standard applications.

The clone 'CIMAP-FORAGIKA' is unique in its own right having significantly broad leaves that are nutritionally rich and palatable suitable as a cattle feed. Profuse tiller production further adds value to this clone for its usage for grazing animals. Latter shall help sustain such ecological plantations.

The clone 'CIMAP-KH40' is unique in its own way being an experimentally developed autotetraploid, thus adding thickness, strength and biomass to the otherwise fast growing roots,

The clone 'CIMAP-KH40' is a genetically superior clone for its fast growing deep penetrating and interwoven mesh forming roots having utility in ecological plantations for its carbon sequestration in sub-soil horizon and soil conservation.

Clone 'CIMAP-KHUSINOLIKA' is a high essential oil yielding clone suitable as a short duration crop that can be harvested just after six months (*c.f.* other existing clones that require 18 month crop duration).

Concluding remarks regarding implementation and utility of the developed clone

The two clones 'CIMAP-FORAGIKA' and 'CIMAP-KH40' are suitable for ecologically sustainable environmental plantations, since both these clones are non-seeding with no threat of becoming weedy, their plantations could be terminated at will when no more required simply by excising the shoot crown, enabling release of such land for other applications,

Whereas, clone 'CIMAP-FORAGIKA' has value as a soil binder in stabilization of soil along the slopes to check erosion, and foothills to check the mudslides on account enhanced soil binding potential of its roots, this clone is equally effective in ameliorating and stabilization of mine over burden soil. The above ground biomass of clone 'CIMAP-FORAGIKA' could be used as a fodder under ecological plantations at the erosion and mudslide prone sites,

The autotetraploid clone 'CIMAP-KH40' offers enhanced opportunities for its utilization in mitigating global warming through photosynthetic capture of atmospheric carbon dioxide and its long-term sequestration in sub-soil horizons through its fast-growing deep penetrating roots, and as such offers tremendous utility in ecological plantations for (a) mitigation of global warming, (b) earning carbon credit, (c) environmental conservation.

The clone 'CIMAP-KHUSINOLIKA' is intended for its utility in industrial plantation for optimum harvest of its quality essential oil as a short duration crop. This Clone is a best fit as a short duration crop to realize commercial harvest for its essential oil highly valued in aroma industry.

Acknowledgements

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Declarations

In order to complete the story, necessary data from earlier published work by the authors has been used/ reproduced with due citation to the original source.

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