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On carbon sequestration efficient clones/genotypes selection for high essential oil yield over environments in Khus (*Chrysopogon zizanioides* (L.) Roberty)

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ABSTRACT

Khus/vetiver (*Chrysopogon zizanioides* (L.) Roberty), family Poaceae, commonly known as khus grass, is important for essential oil used in the perfumery industry as well as in aromatherapy in India, and so is its value world over. The genetic diversity and genotype × environment studies using agronomical traits study in this crop are available. Still, the genotype × environment study using the physiological economic traits is very meager. Therefore, the objectives in the present investigation were to estimate the khus genotypes response over multi-years interaction and the selection of stable high essential oil yielding and carbon sequestration efficient genotypes in the khus. In this study, sixty-five diverse genotypes of khus collected from twenty-five states/places of India and abroad were studied for the seven physiological economic traits in the three consecutive years. The genotypes, environments/years, genotypes × environments/years, years + (genotypes × environments/years), genotype × environments/years (Linn.) related pooled analysis of variance and deviations for the seven characters were found highly significant. This indicates that these genotypes had diverse linear interactions in change environmental conditions. The pooled deviation significances showed that deviation in linear regression contributes towards the genotype's stability. The predictable/linear or unpredictable/non-linear components significantly contributed to the genotype's stability. Both Eberhart and Russell and GGE biplot analyses identified six promising and stable genotypes for essential oil yield and the three highly stable genotypes for the trait photosynthesis rate. The essential oils of the selected genotypes were also found rich for the major compounds: khusilal, preziza-7(15)-en-12-ol, khusol, and khusimol, along with other minor compounds. The preziza-7(15)-en-12-ol, a prezizaen class of compound, is being reported in high proportions for the first time in *Chrysopogon zizanioides* (L.) Roberty).

1. Introduction

Khus (*Chrysopogon zizanioides* (L.) Roberty), family- 'Gramineae' is an important aromatic grass cultivated for roots and its much valuable khus essential oil. Essential oil is used extensively in a diverse range of consumer products, such as after-shave creams, room fresheners, and perfumes (Virmani and Datta, 1975; Lal et al., 1998). It is also used in flavoring and is an excellent preservative agent for culinary products, cordials, and toilet articles (Virmani and Datta, 1975; Lal, 2013). Stability analysis is an essential tool for stable genotype selection after tests in different growing environments. The exclusivity of khus oil lies in its typical base note characteristics; it is much precious oil as it lacks any synthetic substitute (Lawrence, 1997; Gupta et al., 2015).

The essential oil of khus roots oil has marked influence on the perfumery and essential oil industries as well as on aromatherapy in India, and so is its value world over. Besides, khus roots oil is highly

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Table 1
Origin of sixty five accessions of khus maintained at CSIR-CIMAP, Lucknow (India).

S,No.	Accession's code	Origin	S,No.	Accession's code	Origin
1.	Vc-1	Bihar, India	34.	Vc-34	U.P., India
2.	Vc-2	Rajasthan, India	35.	Vc-35	U.P., India
3.	Vc-3	Jammu and Kashmir, India	36.	Vc-36/Vridhi	U.P., India
4.	Vc-4	Punjab, India	37.	Vc-37	M.P., India
5.	Vc-5	Odisha, India	38.	Vc-38	Kerala, India
6.	Vc-6	Odisha, India	39.	Vc-39	Gujarat, India
7.	Vc-7	Thailand	40.	Vc-40	U.P., India
8.	Vc-8	Haiti	41.	Vc-41	U.P., India
9.	Vc-9	M.P., India	42.	Vc-42/Selection-1	U.P., India
10.	Vc-10	Chhattisgarh, India	43.	Vc -43	U.P., India
11.	Vc-11	Jharkhand., India	44.	Vc-44	M.P., India
12.	Vc-12/ Samriddhi	U.P., India	45.	Vc-45	Kerala, India
13.	Vc-13	U.P., India	46.	Vc-46	U.P., India
14.	Vc-14	U.P., India	47.	Vc-47	Kerala, India
15.	Vc-15	Uttarakhand, India	48.	Vc-48/Keshari	U.P., India
16.	Vc-16	Bihar, India	49.	Vc-49	U.P., India
17.	Vc-17	Rajasthan, India	50.	Vc-50	U.P., India
18.	Vc-18	Jammu and Kashmir, India	51.	Vc-51	Reunion, Island
19.	Vc-19	Punjab, India	52.	Vc-52	Andhra Pradesh, India
20.	Vc-20/Dharini	U.P., India	53.	Vc-53	Mizoram, India
21.	Vc -21	Odisha, India	54.	Vc -54	U.P., India
22.	Vc-22	U.P., India	55.	Vc-55	Indonesia
23.	Vc-23	U.P., India	56.	Vc-56	U.P., India
24.	Vc-24	Haiti	57.	Vc-57	Rajasthan, India
25.	Vc-25	Maharashtra, India	58.	Vc-58	Arunachal Pradesh, India
26.	Vc-26	U.P., India	59.	Vc-59	U.P., India
27.	Vc-27	U.P., India	60.	Vc-60/Gulabi	U.P., India
28.	Vc-28	New Delhi, (India)	61.	Vc-61	Kerala, India
29.	Vc-29	U.P., India	62.	Vc-62	Karnataka, India
30.	Vc-30/ DH 1	U.P., India	63.	Vc-63	W.B., India
31.	Vc-31	Meghalaya	64.	Vc-64	Gujarat, India
32.	Vc -32/KS 1	U.P., India	65.	Vc -65	Kerala, India
33.	Vc-33	U.P., India			

Where, Vc = Khus clone; U.P. = Uttar Pradesh; M.P. = Madhya Pradesh; W.B. = West Bengal.

Table 2
Pooled analysis of variance and deviation of the seven traits of khus.

Source of variation	d.f.	Traits (mean sum of squares)						
		Plant height (m)	Photosynthesis rate/net CO_2 assimilation rate (u mol m^{-2} s^{-1})	Transpiration rate (m mol m^{-2} s^{-1})	Stomatal conductance (m mol m^{-2} s^{-1})	Root yield/plot (g)	Essential oil content (%)	Essential oil yield/ plot (ml)
Genotypes	64	0.02**	10.90**	1.72**	25084.78**	8086.31**	0.10**	1.98**
Years	2	0.002	1.811	2.92**	143164.00**++	7760.00**	0.01	4.87**++
Year × genotypes	128	0.02**	13.22**+	2.81**	52396.85**++	7249.94**	0.14**	3.27**+
Years + (genotypes × years)	130	0.02**	13.05**+	2.81**	34100.95**++	7257.78**	0.13**	3.29**+
Years (lin.)	1	0.004	3.62	5.83**++	286328.20**++	15516.85**++	0.01	9.75**++
Genotypes × years (lin.)	64	0.02**	15.73**++	2.71**	43112.96**++	6176.31**	0.13**	3.70**
Pooled deviation	65	0.02	10.55	2.87	21347.12	8195.67	0.13	2.80
Pooled error	390	0.01	5.54	0.30	4871.13	1775.96	0.0319	0.31
Total	194							

**-$p < 0.01$ and +, ++- $P < 0.05$ and 0.01 significant level against pooled error and pooled deviation, respectively.

useful for general health due to its many medicinal properties like anti-inflammatory, anti-fungal, antimicrobial, and antimycobacterial. India is importing khus essential oil of about 350 metric tons/year (Virmani and Datta, 1975; Lawrence, 1997; Lal et al., 1998a). The essential oil production of khus is lowering down in India due to limited availability of short duration (twelve months or six months) maturing stable essential oil-producing varieties. The available long-duration varieties of khus are ready for the extraction of essential oil in more than 18–24 months. In India, due to smallholding/fields, the farmers do not want to engage his cultivated area for a long time. They like to cultivate short-duration varieties of khus without affecting his conventional crops.

Recently, some varieties of khus developed and released for the commercial cultivation by CSIR-CIMAP, Lucknow, India, for example, KS-1, KS-2, and CIMAP KH-40 (autotetraploid, US Patent number - PP26474, Lavania et al., 2012) with khus note are ready in 18–24 months for oil extraction/distillation. Similarly, the five varieties Dharini (khus note), Gulabi (rosy note), Kesari (saffron note), CIM-Vridhi (earthy note), and CIM-Samriddhi (spicy/fruity note) were developed and released as short duration crop ready in twelve months only for oil extraction/distillation possessing different oil yield and quality for commercial cultivation. Notably, one another unique variety of khus also developed and released as CIM-Khusinolika gets ready for the digging of roots and oil extraction in the only six months (US Patent No. PP28388). This variety is available for cultivation in India (Chauhan et al., 2017; Kumar et al., 2018; Lal et al., 1998, 2017a; Lal et al., 2017b, c). The essential oil of these varieties is also well accepted

Fig. 1. Morphological variations in plants (a–c), leaves (d–f), inflorescences (g) and roots (h–i) in khus.

Table 3
Environmental indices for the seven economic characters of khus.

S. No.	Characters	Khus crop growing environments		
		Year 1	Year 2	Year 3
1.	Plant height (m)	0.003	0.003	−0.01
2.	Photosynthesis rate/net CO_2 assimilation rate (u mol m^{-2} s^{-1})	−0.08	0.19	−0.12
3.	Transpiration rate (m mol m^{-2} s^{-1})	−0.23	0.04	0.19
4.	Stomatal conductance (m mol m^{-2} s^{-1})	−33.86	−19.71	53.57
5.	Root yield/plot (g)	−9.30	−2.71	12.02
6.	Essential oil content (%)	−0.01	0.01	−0.003
7.	Essential oil yield/plot (ml)	−0.13	0.32	−0.18

by the essential oil industries in India and abroad. Many workers studied the genetic diversity and variability using different agronomical traits and plant sets/materials in the Khus (Srifah et al., 1996; Lal, 2013; Lal et al., 2018a, b; Singh et al., 2019; etc.) and the genotype × environment (g × e) interactions studies (Gupta et al., 2015; Lal et al., 2017a, b; Lal et al., 2018b).

The genotype × environment studies using these physiological economic traits in khus are very meager or absent. Therefore, this communication deals with objectives, 1) to estimate the khus genotypes response and interaction over multi-years, 2) selection of high essential oil yielding genotypes, and 3) identification of carbon sequestration efficient stable genotypes in khus crop.

2. Materials and methods

Sixty-five genotypes of khus (*Chrysopogon zizanioides* (L.) Roberty), were collected from twenty-five states/places of India and abroad were multiplied and maintained at the National Gene Bank of CSIR-Central Institute of Medicinal and Aromatic Plants, P.O. CIMAP-Lucknow, U.P. (India) with botanical authentication, voucher specimen/accession's code numbers with their botanical origin are presented in (Table 1). All sixty-five genotypes were evaluated using a randomized block design in three replications at the distance of 60 × 60 cm plant to plant and row to row (plot size = 1.8 m^2) at the experimental farm of CSIR-CIMAP, Lucknow, India. The institute's farm located at 26°5′ N latitude, 80°5′ E longitude and 120 m above the mean sea level.

The climate of the experimental site is classified as semi-arid subtropical with severe hot summer and relatively cold winter. In this region, monsoon starts in the last week of June and continues till the end of September, with an average annual rainfall of 960 mm. The average maximum and minimum temperatures are fluctuated from 23 to 45 °C in the summer and 5.6–26.5 °C in the winter seasons. The soil of the experimental site was sandy-loam in texture having pH 7.8 with low in organic carbon (OC) 0.25 % and available nitrogen (N) 128 kg/ha, medium in available phosphorus (P)11.8 kg/ha and potassium (K) 223 kg/ha, respectively. All sixty-five genotypes were evaluated in the three consecutive years: 2016–2017, 2017–2018, and 2018–2019, respectively. The crop was planted in each year between dates 1–5 February and uprooted for their roots between dates 1–10 January after 12 months of transplanting.

The fertilizers were applied as 80 N: $60P_2O_5$: 60 K_2O kg/ha. In whole crops, four weeding and irrigations were given for better crop growths. The data were taken on all genotypes for the seven characters: Plant height (m), photosynthesis rate/net CO_2 assimilation rate (u mol m^{-2} s^{-1}), transpiration rate (m mol m^{-2} s^{-1}), stomata conductance (m mol m^{-2} s^{-1}), root yield/plot (g), oil content (%) and oil yield/plot (ml), respectively.

2.1. Measurement of photosynthesis parameters

Photosynthesis parameters were measured on photosynthesis rate or net CO_2 assimilation rate (μ mol m^{-2} s^{-1}), transpiration rate (m mol m^{-2} s^{-1}), and stomatal conductance (m mol m^{-2} s^{-1}) in the attached khus leaf using a portable photosynthesis system (CIRAS-3, PP Systems, USA). For photosynthesis measurement, khus leaves were kept in leaf cuvette having 400 μmol photons m^{-2} s^{-2} light, 400 ppm CO_2, and

Fig. 2. Variations in roots and roots hairs architecture in the different genotypes of khus.

25 °C temp.

2.2. Extraction of essential oil

The roots of khus extracted for the roots oil by hydro-distillation (Clevenger, 1928) for 24 h. The extracted essential oils were kept at 4 °C prior to analysis. The essential oil was measured directly from Clevenger, and essential oil content (%) was determined as volume (ml) of essential oil per 100 g of roots. The oil quality was measured after dehydrating the oil over anhydrous Na_2 SO_4.

2.3. Gas chromatography (GC) and GC Mass spectrometry analysis

GC and GC–MS analyses were performed as per our reported method (Pragadheesh et al., 2015). The relative retention index was calculated by injecting a homologous series of *n*-alkanes (C_6-C_{28} hydrocarbons, Polyscience Corp. Niles IL). Compound identification was achieved by recording NMR experiments, comparison of MS libraries (TurboMass NIST 2011 version 2.3.0 and Wiley registry of mass spectral data 9th edition,) and reference guide on mass spectral data (Adams, 2007).

2.4. Isolation of marker compounds from khus essential oils

The essential oil was fractionated by column chromatography using silica gel (mesh size 230–400; Merck) with hexane and diethyl ether as a mobile phase solvent. Four marker compounds viz., khusilal (1), preziza-7(15)-en-12-ol (2), and khusimol (4) were eluted in 3–6 % diethyl ether in hexane whereas khusol (3) was eluted in 15–20 % diethyl ether in hexane. The isolation and purification of the column chromatography fractions were based on their thin layer chromatography (TLC) pattern in hexane and diethyl ether as a mobile phase solvent in TLC.

2.5. Nuclear magnetic resonance spectroscopy

NMR measurements were recorded on a Bruker AVANCE III HD spectrometer (Bruker BioSpin GmbH, Germany; 500 MHz (*B0* = 14 T). Besides, 2D-NMR experiments were also carried out for oil samples and standards. The structures were confirmed using Heteronuclear Single Quantum Coherence Spectroscopy and Heteronuclear Multiple Bond Correlation experiments. All spectra were recorded using $CDCl_3$.

Each isolated compound (30 mg each) was dissolved in $CDCl_3$ in 5 mm NMR tube. Chemical shifts were reported in ppm units.NMR solvent ($CDCl_3$) set to 7.26 (^{1}HNMR) and 77.0 (^{13}C NMR). Signal multiplicities were denoted as s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet, etc.). Compound identity was established by comparison of spectral data. The structures were determined through ^{13}C NMR spectral data of compound 1–4 (125 MHz for, $CDCl_3$, TMS as internal standard).

2.6. Statistical analysis

Three years mean of all seven characters were subjected to statistical analyses for the stability analyses by Eberhart and Russell (1996) model and GGE biplot model by using Sigma plot 13.0 and Institutes software as described by (Yan, 2002; Yen and Tinker, 2006; Yan et al., 2007 and Singh and Chaudhury, 2014). Using GGE biplot model, the GGE biplot and genotype by environment-traits interactions figures were also generated by principal components: PC1 = 72.1 % and PC2 = 20.2 % with transform = 0, scaling = 0, centering = 0, singular value partitioning (SVP) = 1 and PC1 = 93.6 % and PC2 = 3.0 % with transform = 0, scaling = 1, centering = 1 and SVP = 2 are presented in the Fig. 4. The GGE stands for genotype main effect (G) plus genotype by environment interaction as (GE), which is the only source of variation that is relevant to genotype evaluation. Mathematically, GGE is the genotype by environment data matrix after the environment means are subtracted (Yen and Tinker, 2006).

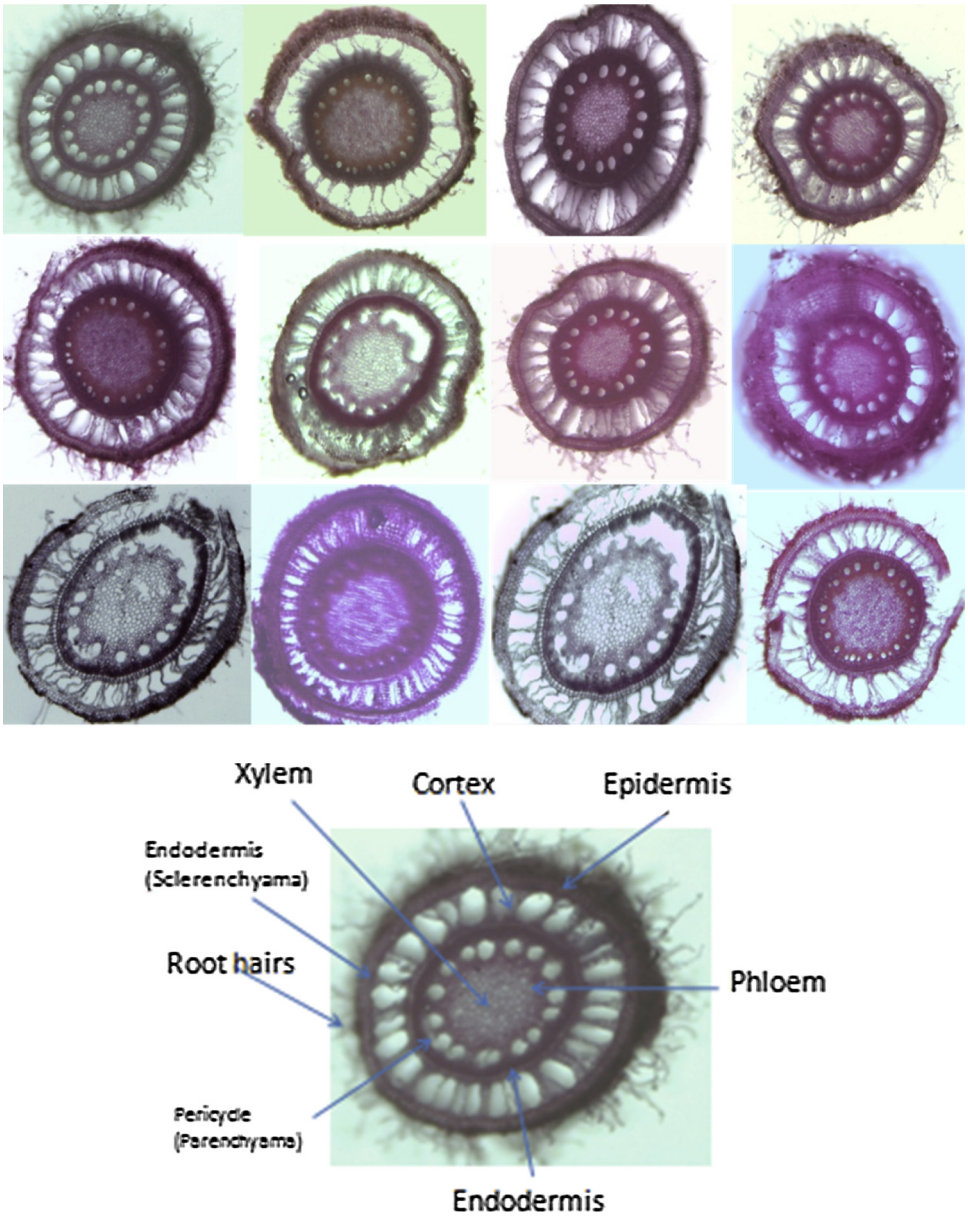

Fig. 3. Variations in root architecture and anatomy in the different genotypes of khus.

3. Results

The pooled analysis of variances (ANOVA) showed highly significant differences among genotypes and years/environments for all seven traits except two characters, namely plant height (m) and photosynthesis rate/net CO_2 assimilation rate (u mol m^{-2} s^{-1}) in years/environments (Table 2). Therefore, the above results indicated the presence of significant differences/variability among genotypes over the years/environments. The mean sum of squares due to genotypes environments (linear), tested against pooled deviation, was significant for all the traits, indicating that substantial genetic differences were present among the genotypes for linear regression and environmental index (Table 2, Fig. 1). The source of the variation environment (linear) was highly significant for only four characters, namely, transpiration rate (m mol m^{-2} s^{-1}), stomatal conductance (m mol m^{-2} s^{-1}), root yield/plot (g) and essential oil yield/plot (ml). The other three characters, namely plant height (m), photosynthesis rate/net CO_2 assimilation rate (u mol m^{-2} s^{-1}), and oil content (%), were found not significant, which were exhibited differences in years to years/environments.

The values of the environmental index indicated the favorable and unfavorable situations over the years, related to all the seven characters (Table 3). Stability parameters such as mean performance ($\bar{x}$), regression coefficient (bi) and mean square deviations from regression (S^2d) for the oil yield/plot (ml), root yield/plot (g), oil content (%) and plant height (m) and photosynthesis parameters: net CO_2 assimilation, transpiration rate and stomatal conductance in the best stable genotypes are also analyzed. The variations in the root's thickness, size, root's hairs (thin or thick hairs) architecture, and anatomy small to big pith size were also recorded in the selected stables genotypes (Fig. 2–3). Using GGE biplot model, the GGE biplot and genotype by environment-traits interactions figures were also generated, and the results are presented in Fig. 4. The essential oil qualities of some selected stable genotypes are also presented in (Table 4–5; Fig. 5–8).

4. Discussion

Presently, the changes in climate (variations in rainfall, winds, and

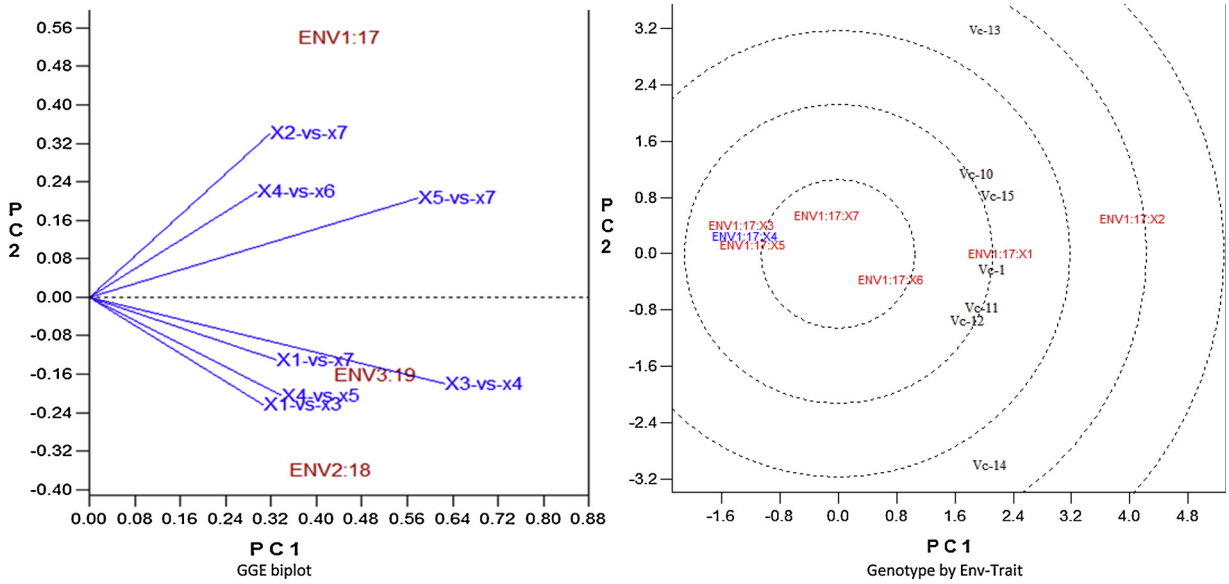

Fig. 4. GGE biplot and genotype by environment-traits interactions in the sixty-five genotypes in three environments of khus.

Table 4
Chemical composition of some promising stable khus clones.

S. No.	Entries RI*	khusilal (1) 1651	preziza-7(15)-en-12-ol (2) 1752	khusol (3) 1809	khusimol (4) 1747
1.	VC-42	–	–	8.82	21.96
2.	VC-59	33.04		22.89	–
3.	VC-22	–	–	–	24.21
4.	VC-30		26.49	8.94	–
5.	VC-12	33.23	–	25.47	–
6.	VC-60	–	–	–	23.98

* Retention Index (RI): a) on DB-5 capillary column using a homologous series of *n*-alkanes (C_6-C_{28}) hydrocarbons, Polyscience Corp. Niles IL.

Table 5
^{13}C NMR spectral data of compounds 1–4 (125 MHz, in $CDCl_3$ with TMS as internal standard).

δ (ppm)				
Carbon No.	khusilal	preziza-7(15)-en-12-ol	khusol	khusimol
1	43.19	52.08	44.13	53.22
2	24.07	46.96	25.70	48.38
3	21.80	21.62	30.48	26.49
4	140.98	21.93	135.16	25.12
5	152.42	59.41	121.96	48.68
6	47.01	37.70	44.50	156.48
7	47.58	162.76	41.95	40.29
8	35.22	46.69	26.98	49.32
9	35.55	26.30	36.06	25.40
10	150.75	29.90	152.88	35.76
11	140.84	44.39	34.77	33.29
12	116.58	64.94	10.19	66.36
13	194.49	31.95	67.15	105.29
14	105.12	27.02	23.88	25.99
15	–	105.67	103.47	28.46

temperature, etc.) profoundly influences eco-system, respiration, nutrients cycle, growth, and essential oil yield of the khus crops. Although the khus plant is a C_4 plant but might be prone to change climate conditions, including the carbon sequestration efficiency that is directly or indirectly affects the growth and synthesis of essential oil in khus plant. Not all genotypes may always express positive responses for the desired traits in the over the years/environmental conditions (Yen and Tinker, 2006; Yizhao et al., 2016; Gupta et al., 2015; Lal et al., 2018a, b). Therefore, it will always be better than the highly stable genotypes should be selected based on $\bar{x}$ (high mean value), bi (regression coefficient) around unity and s^2d_i (mean square deviations from the regression) near-zero and by GGE biplot model (Eberhart and Russell, 1996; Yen and Tinker, 2006; Gupta et al., 2015).

Genotypes with high mean value, regression coefficient approaching one, and low deviation mean square were considered to be a stable genotype and expected to perform uniformly over the years whereas, high mean value, regression coefficient less than unity means a khus genotype related to above medium or average stability. These type genotypes should be better adapted for low yielding years/environments, and a coefficient greater than unity indicates khus genotype expressing below average stability. Such types of genotypes should be suitable for high yielding years/environments.

Based on the stability parameters like mean ($\bar{x}$), regression coefficient (bi), and mean square deviation from regression (s^2d_i), the high essential oil yield was expressed by the six out of sixty-five genotypes. These genotypes exhibited high oil yield along with the regression coefficient approaching one and low deviation mean square considered to be a stable genotype. The two genotypes were expressed the high oil yield, regression coefficient < than unity showed the average stability or adapted explicitly to low yielding years. The other three genotypes had coefficient values > unity indicated a genotype with stability below than average means such type of genotypes were suitable for high yielding years/environments could be selected for essential oil yield trait (Table 2–3).

Ostensibly, for the root yield, out of sixty-five genotypes evaluated, the only four genotypes were found high stable. In which only two genotypes were with high mean, bi approaching one and low s^2d_i were selected as moderate stable genotype. The two genotypes of the khus were also expressed the good mean, bi < unity indicated genotype as above-average stability. Only one genotype was greater coefficient than unity indicated a genotype as below average stability. It is imperative to note that out of seven average stable genotypes, and two genotypes were also expressed, the regression coefficient value > unity indicated genotypes as below average stability for root yield/plot (g). The six genotypes were with high mean, bi approaching one, and low s^2d_i considered to be the medium stable genotype for the root yield/plot, which expected to be performed uniformly over variable environments. The two genotypes were with high mean, bi < unity would perform

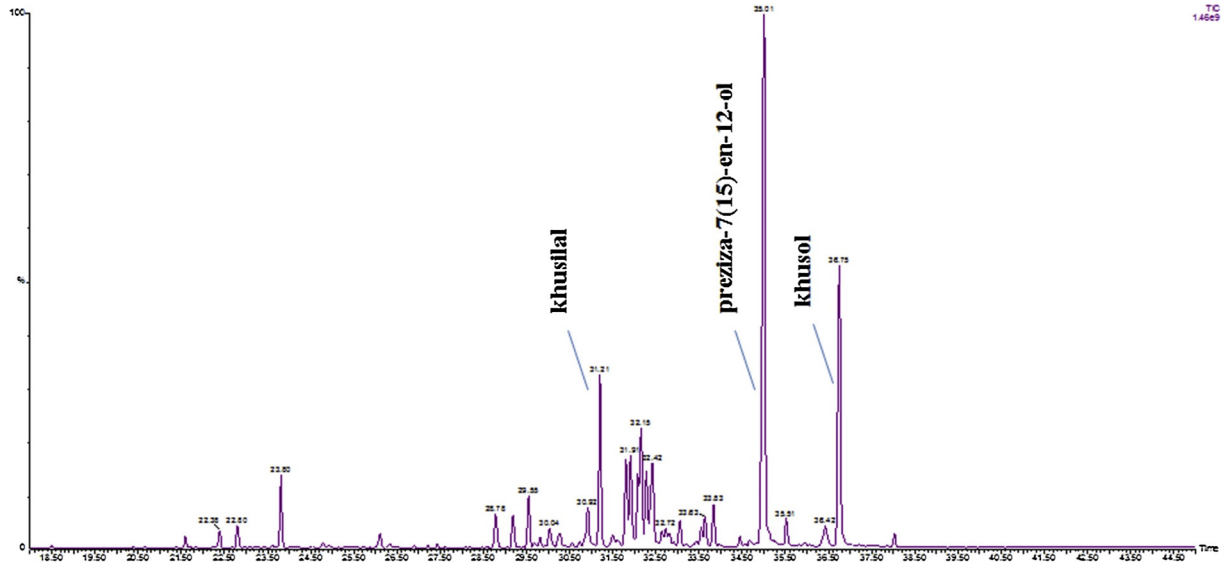

Fig. 5. Total ion chromatogram of the unique and best stable khus genotype Vc-30.

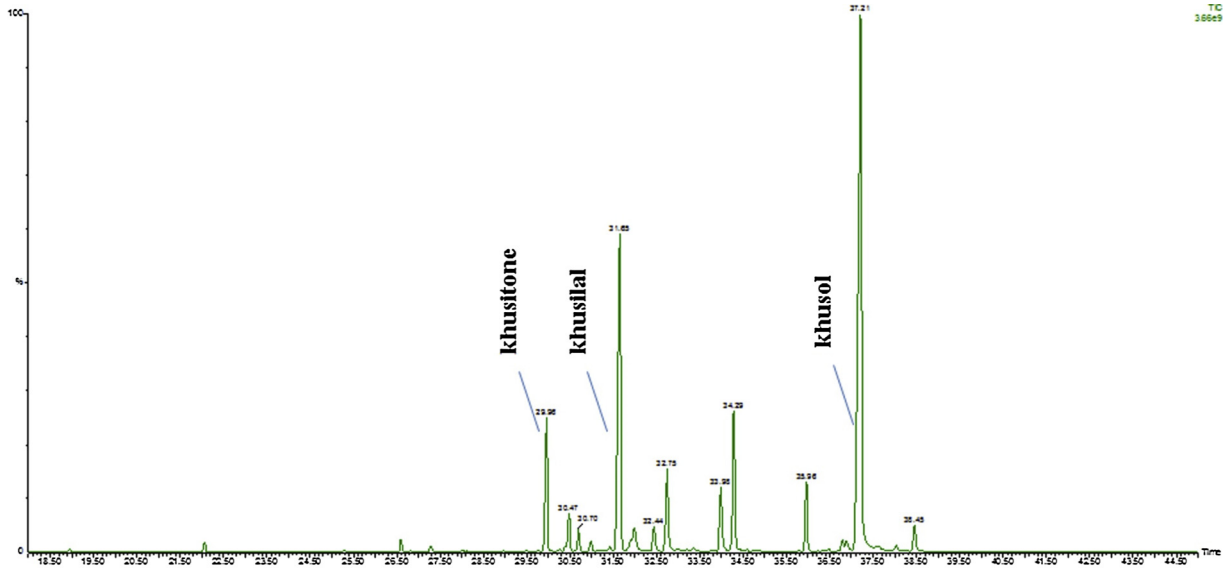

Fig. 6. Total ion chromatogram of the unique and best stable khus genotype Vc- 12.

above average stability. The one genotype was showed the regression coefficient > unity would be a genotype below average stability. The seven genotypes were mediocre stable genotypes for the essential oil content (%).

The meticulous study of results indicated that out of sixty-five, only four genotypes came under the category of stable genotypes for the plant height (m). The genotypes with high mean, bi approaching one, and low s^2d_i were indicated to be an average stable genotype. Therefore, the two genotypes expressed the high mean, bi < unity would be genotype above-average stability. The other two genotypes with bi > unity, would indicate genotype below average stability; for the character photosynthesis rate/net CO_2 assimilation rate (u mol m^{-2} s^{-1}), the one highly stable genotype was with high mean, bi approaching one and low s^2d_i were considered to as medium stable genotype. The one genotype was with high with mean, bi < unity, genotype above-average stability. The one khus genotype had bi > unity to be a genotype with below-average stability. These findings are also in agreement with the results of Yizhao et al., (1916).

Notwithstanding that for the character transpiration rate (m mol m^{-2} s^{-1}), the three genotypes were high mean, bi approaching one, and low s^2d_i expressed as moderate stable genotypes. However, one genotype revealed the high mean, bi < unity would be a genotype to have above-average stability. The other one genotype expressed bi > unity, would show a genotype related to below-average stability. Likewise, out often khus genotype, three genotypes were highly stable than others. For the character stomatal conductance (m mol m^{-2} s^{-1}), one genotype with high mean bi approaching one, and low s^2d_i was a medium stable genotype. The one genotype with high mean, bi < unity may be considered as above average stability and one another genotype which showed bi > unity found as average stability type. It is evidenced from results that all the studied physiological traits directly or indirectly affect the stability of genotypes (Table 2 –5; Fig. 4–8). Our results are also in agreement with a number of research workers (Kempton, 1984; Gupta et al., 2015; Lal et al., 2017a; Lal et al., 2018a,b; Sarkar and Lal, 2018; etc.).

A critical perusal of the results showed that the mechanisms for stability accomplishment within different khus genotypes were unique and character-specific. The above findings are also supported by the GGE biplot analysis results (Fig. 4). The GGE biplot and genotype, genotype by environment-traits interactions, is also the very

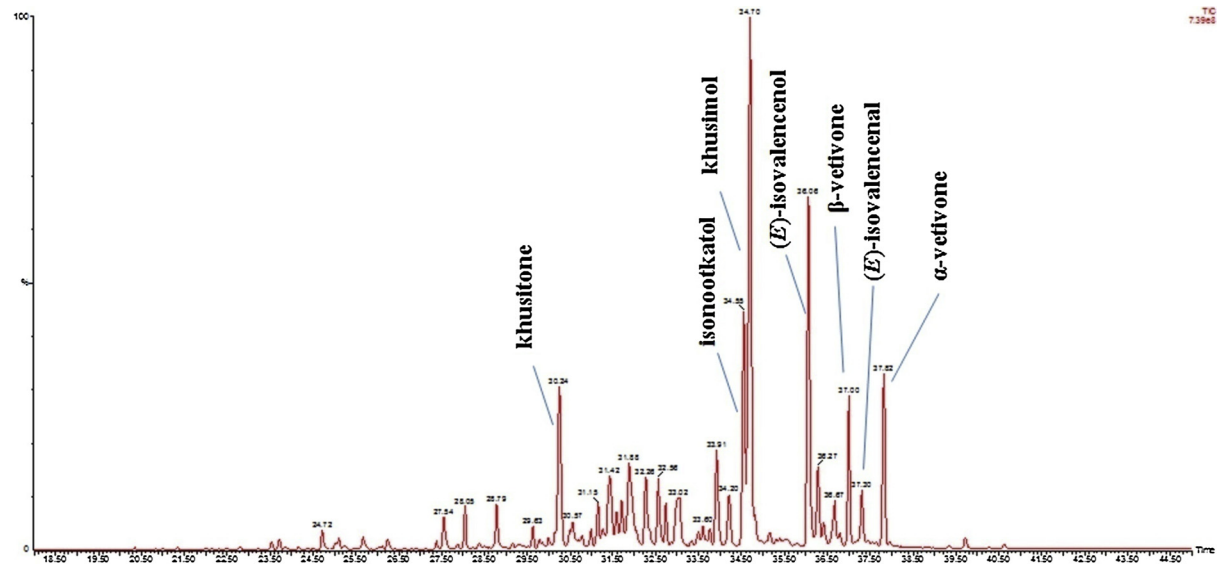

Fig. 7. Total ion chromatogram of the unique and best stable khus genotype Vc-2.

Khusilal (1) **Preziza-7(15)-en-12-ol (2)** **Khusol (3)** **Khusimol (4)**

Fig. 8. Marker terpenoids 1 and 3 in Vc-12; 2 in Vc-30 and 4 in Vc-2 identified in stable khus genotypes.

appropriate statistical model for the comparing multi-characters in the sixty-five genotypes of khus. Based on it, the interpretations are the overall dissimilarity between them present in ample amount. The distances between the two genotypes were found very high, low, or quite similar in the environments one; however, they showed different attitudes in the other two environments (Fig. 4). The dissimilarity can be due to the difference in means of the genotypes (G), environments, and genotypes × environments (GE) interaction. An average value of genotypes in each of the environments assumes the origin of biplot denotes as "virtual" genotypes. These "average" genotypes have nearly zero contributions to the genotype and genotype × environment interactions. The distance of the vector genotypes is the distance of genotypes. The estimate variations of genotypes from the biplot are the real estimation of stability from high to medium performance. It is a contribution to either genotype or genotypes × environments or all of them. The genotypes found near the biplot origin have a low contribution to genotypes and genotype × environment interactions (Fig. 4).

The genotype with having the longer vector has a high contribution to either genotypes and genotype × environment interactions or all of them. Thus, the genotype having the most top vector is best, most impoverished, or most unstable. The angles of the vector of genotypes and partitions are the vector length into genotypes and genotype × environment interaction components. The right angle represents the roll of the genotype × environment interactions only. The mean's obtuse angle contribution is due to genotype, which is lower-than-average mean performance. The acute angle denotes contribution is primarily due to the genotype, which means > average mean performance (Fig. 4). The ideal genotype near the center of the concentric circles to a point on absolutely stable in the plus directions have vector length equal towards the most extended vector of the genotype on the direction of the positive sides of the better mean value. The genotypes found the nearest to the ideal genotype is highly desirable than the other one. Hence, three genotypes of the khus were more desirable and had a higher mean performance than others (Fig. 4). The one genotype was the poorest because it was consistently showed low mean performance for the essential oil yield. The three genotypes were highly stable for the photosynthesis rate/net CO_2 assimilation rate (u mol m^{-2} s^{-1}) because the performance of these genotypes was always found consistent. The other genotypes were more deficient than the least stable genotype because it performed well in some years. Therefore, it is clear cut findings that stable genotypes are desirable only when they have high yield performances along with consistency over environments/years.

Nevertheless, it was a clear indication that the GGE biplot and mean ($\bar{x}$), b_i, and s^2d_i stability parameters should be considered for the selection of stable genotypes for the essential oil yield along with high carbon sequestration efficient genotypes in the khus. The three genotypes were found highly stable for the character photosynthesis rate/net CO_2 assimilation rate (u mol m^{-2} s^{-1}). It is also interesting to note that the primary roots, along with thin secondary roots of khus are expressed association with high essential oil yield directly. In contrast, primary long thick roots are useful for the control of soil/water conservation from erosion (Figs. 2). The variations in the thickness, sizes, length of the primary and secondary roots, their architecture, and in the anatomy of the structure of the root along with pith size found directly correlated to stability of the genotypes for the high essential oil yield (Figs. 2 and 3). Out of the sixty-five, only six genotypes were proved high stability for the high essential oil yield. These stable genotypes also found rich in major 1–4 (khusilal, preziza-7(15)-en-12-ol, khusol, and khusimol) along with other minor compounds (Figs. 5–8).

The preziza-7(15)-en-12-ol compound, which traced in the one genotype 30, is the world first report in *Chrysopogon zizanioides* (L.) Roberty (Tables 4–5; Figs. 5–8). This compound reported in another species of khus *Vetiveria njgritana* (Benth) Stapf belongs to Mali. This Mali khus species contains the fifty-four constituents 79.70 % of the

whole essential oil including preziza-7(15)-en-12-ol (9.5 %), prezizanoic acid (15.0 %), cedren-8-en-15-ol (6.2 %), preziza-7(15)-en-3α-ol (6.0 %) and zizanoic acid (5.9 %) as major components (Champagnat et al., 2006).

Finally, out of sixty-five, only six genotypes with high mean, bi approaching one, and low s^2d_i, was identified as highly stable for the essential oil yield. The other two genotypes with a high mean ($\bar{x}$), bi < unity would show as above-average stability, and one genotype (bi > unity) would indicate below-average stability for the essential oil yield. The three genotypes for photosynthesis rate/net CO_2 assimilation rate identified as highly stable from both Eberhart and Russell and GGE biplot analyses. Therefore, these khus genotypes may be exploited for further commercial cultivation.

5. Conclusions

The sixty-five diverse genotypes of khus were collected from twenty-five different states/places of India and abroad and studied on the seven physiological and quantitative characters in the three consecutive years. The genotypes, environments, genotypes × environments, environments/years + (genotypes × environments), genotype × environments (Linn.) related pooled analysis of variance (ANOVA), and deviations were found highly significant. The predictable and unpredictable components significantly contributed to stability. Both Eberhart and Russell and GGE biplot analyses identified six promising and stable genotypes for essential oil yield and the three highly stable genotypes for the trait photosynthesis rate/net CO_2 assimilation rate. The essential oil of the three genotypes found rich for the major compounds: khusilal, preziza-7(15)-en-12-ol, and khusol and khusimol along with minor. These stable genotypes of khus may be exploited for further commercial cultivation.

Author contributions

RKL was involved in planning, actual experimentation, statistical analyses, manuscript preparation; PG, AM, SS, SHM in data collection; RM, SUS, YP in distillation, chemical analysis; CS, AY in chemical analysis GC, GC–MS including NMR, identification of compound; SSP in Physiological data collection.

Declaration of Competing Interest

The authors have no conflict of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.indcrop.2020.112139.

References

Adams, R.P., 2007. Identification of Essential Oil of Components by Gas chromatography/mass Spectrometry. Allured Publishing Corporation, Carol Stream, Illinois, USA.
Champagnat, P., Figueredo, G., Chalchat, J.C., Bessière, J.M., 2006. Essential oil composition of *Vetiveria nigritana*(Benth.) Stapf from Mali. J. Ess. Oil. Res. 18 (6), 647–649. https://doi.org/10.1080/10412905.2006.9699193.
Chauhan, H.S., Singh, H.P., Chanotiya, C.S., Shasany, A.K., Lavania, U.C., Tomar, V.K.S., Kalra, A., Singh, A.K., 2017. Vetiver plant named 'CIMAP-Khusinolika' US Patent number, PP28388.
Clevenger, J.F., 1928. Apparatus for the determinations of volatile oils. J. Am. Pharm. Assoc. 17 (4), 345–349. https://doi.org/10.1002/jps.3080170407.
Eberhart, S.A., Russell, W.L., 1996. Stability parameters for comparing varieties. Crop Sci. 6, 36–40. https://doi.org/10.2135/cropsci1966.0011183X000600010011x.
Gupta, P., Lal, R.K., Banerjee, N., 2015. Stable genotype selection in Khus (*Veteveria zizanioides* L. Nash). J. Herb. Spice. Med. Plant. 21 (4), 392–409. https://doi.org/10.1080/10496475.2014.995329.
Kempton, R.A., 1984. The use of biplots in interpreting variety by environment interactions. J. Agric. Sci. 103 (1), 123–135. https://doi.org/10.1017/S0021859600043392.
Kumar, Y., Prakash, O., Tripathi, H., Tandon, S., Gupta, M.M., Rahman, L.U., Lal, R.K., Semwal, M., Darokar, M.P., Khan, F., 2018. AromaDb: a database of medicinal and aromatic plant's aroma molecules with phytochemistry and therapeutic potentials. Front. Plant Sci. 9 (1081), 1–11. https://doi.org/10.3389/fpls.2018.01081.
Lal, R.K., 2013. On genetic diversity in germplasm of vetiver *Vetiveria zizanioides* (L.) Nash. Ind. Crop Prod. 43, 93–98. https://doi.org/10.1016/j.indcrop.2012.07.005.
Lal, R.K., Chanotiya, C.S., Dhawan, S.S., Gupta, P., Sarkar, S., 2018a. Genotypic and morphological appearance of the traits in relation to genetic diversity of essential oil yield in khus grass (*Chrysopogon zizanioides* Roberty). Acta. Sci. Agri. 2 (8), 62–72.
Lal, R.K., Chanotiya, C.S., Gupta, P., Sarkar, S., Singh, S., Maurya, R., Srivastava, S., Chaudhary, P.K., 2017a. Phenotypic stability, genotype × environmental interactions, and cultivar recommendations for essential oil yield in khus aromatic grass (*Chrysopogon zizanioides* Roberty). Ind. Crops Prod. 111, 871–877. https://doi.org/10.1016/j.indcrop.2017.11.003.
Lal, R.K., Chanotiya, C.S., Shasany, A.K., Gupta, A.K., Singh, V.R., Sarkar, S., Singh, S., Dhawan, S.S., Gupta, P., Dhawan, O.P., Kalra, A., Singh, H.P., Kumar, B., Tomar, V.K.S., Kumar, S., Singh, S., Singh, S.K., Chandra, R., Yadav, A., Maurya, R., 2017b. Registration of a high-yielding khusilal [nor-sesquiterpene (C_{14}) aldehydes] rich variety CIM-Samriddhi of vetiver (*Chrysopogon zizanioides* (L.) Nash. J. Med. Arom. Pl. Sci. 39 (2-4), 139–144.
Lal, R.K., Gupta, P., Sarkar, S., 2018b. Phylogenetic relationships, path, and principal component analysis for genetic variability and high oil yielding clone selection in khus (*Vetiveria zizanioides* L.) Nash. J. Pl. Genet. Breed. 2 (1), 105–113.
Lal, R.K., Sarkar, S., Zaim, M., 2017c. Genotype × environment interaction, rhizome yield stability and selection for region-specific stable genotypes in turmeric (*Curcuma longa* L.). Trends. Phyto. chem. Res. 1, 93–102. http://tpr.iau-shahrood.ac.ir.
Lal, R.K., Sharma, J.R., Naqvi, A.A., Misra, H.O., 1998. Development of new varieties – dharni, Gulabi, Kesari of khus (*Vetivaria zizanioides* (L.) Nash. J. Med. Arom. Plant. Sci. 20, 1067–1070.
Lavania, U.C., Rai, S.K., Lavania, S., Basu, S., Dubey, B.K., Ujagir, Ram., 2012. Autotetraploid plant useful for carbon sequestration and soil conservation named 'CIMAP-KH 40'US Patent number, PP26474.
Lawrence, B.M., 1997. Progress in essential oils. Perfumer Flavorist. 22 (5), 71–83.
Pragadheesh, V.S., Yadav, A., Chanotiya, C.S., 2015. Role of substituents in cyclodextrin derivatives for enantio selective gas chromatographic separation of chiral terpenoids in the essential oils of *Mentha spicata*. J. Chromatogr. B. 1002, 30–41. https://doi.org/10.1016/j.jchromb.2015.07.034.
Sarkar, S., Lal, R.K., 2018. Genetics, inheritance pattern and genotype selection in isabgol (*Plantago ovata* Forsk). SCIOL. Genet. Sci. 1, 68–79.
Singh, R.K., Chaudhury, B.D., 2014. Biometrical Methods of Quantitative Genetic Analysis. Chapter 13. Kalyani Pub. Ludhiana, New Delhi, pp. 253–280.
Singh, N., Singh, V.R., Lal, R.K., Verma, R.S., Mishra, A., Yadav, R., 2019. Quantification of genotypic and chemotypic diversity for elite clone selection with high-quality essential oil traits in germplasm of vetiver {*Chrysopogon zizanioides* (Roberty)}. J.E.O. B. Pl. 22 (4), 1150–1162. https://doi.org/10.1080/0972060X.2019.1661795.
Srifah, P., Ruanjaichon, V., Sangduen, N., 1996. The use of random amplified polymorphic DNA for classification and identification of *vetiveria* species in Thailand. In: Chomchalow, Narong, Henle, Hans V., Rai, Chiang (Eds.), Proceeding of the First International Conference on Vetiver: A Miracle Grass. Thailand. pp. 141–146.
Virmani, O.P., Datta, S.C., 1975. *Vetiveria zizanioides* (L.) Nash. Indian Perfum. 19, 73–95.
Yan, W., 2002. Singular-value partitioning in biplot analysis of multi-environment trial data. Agron. J. 94, 990–996.
Yan, W., Kang, M.S., Baoluo, M., Woods, S., Cornelius, P.L., 2007. GGE Biplot vs. AMMI analysis of genotype-by-environment data. Crop Sci. 47 (2), 643–655. https://doi.org/10.2135/cropsci2006.06.0374.
Yen, W., Tinker, A.N., 2006. Biplot analysis of multi-environment trial data: principals and applications. Can. J. Plant Sci. 86 (3), 623–645.
Yizhao, C., Shaojie, M., Zhengguo, S., Chengcheng, G., Jianlong, L., José, P., Pavel, G., Jingming, C., Siwei, L., 2016. Grassland carbon sequestration ability in China: a new perspective from terrestrial aridity zones. Rangel. Ecol. Manag. 69 (1), 84–94. https://doi.org/10.1016/j.rama.2015.09.003.