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STABILITY STUDIES FOR HIGHER ROOT BIOMASS AND ESSENTIAL OIL CONTENT IN *INULA RACEMOSA* HOOK. F. PUSHKARMOOL SELECTIONS UNDER WESTERN HIMALAYAN CONDITIONS

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ABSTRACT

Inula racemosa is an important medicinal herb lacking in superior and stable varieties for cultivation in the varied climates of the Western Himalayas. In the present experiment, genotypic effects and environment interaction among ten newly developed selections were explored to identify superior and stable genotypes under different environments of Himachal Pradesh. The multi location trials were laid out during the years 2021 and 2022 in a completely randomized block design with three replications. The pooled analysis of variance has shown significant ($p \geq 0.05$) variations for genotype, environment and G × E interactions for all the traits under study. The maximum mean performance for most of the traits suggests that Env-1 followed by Env-2 has the most suitable environments for its cultivation. The genotype CSIR- IHBT-IR-09 has been identified as best performer for root biomass (553.39 g) and essential oil content (3393.21 mg /Kg) over the environments. The results of Eberhard and Russell's regression-based model confirm that genotype CSIR-IHBT-IR-09 is stable and superior genotype. Further, the gas-chromatography mass spectrophotometry analysis of essential oil revealed that the genotype CSIR-IHBT-IR-09 was unique in terms of having the maximum percentage of marker compounds *i.e.*, sesquiterpene lactones eudesmanolides namely alantolactone (51.10 -54.60%) and isoalantolactone (21.50 -25.80%) across all the test environments. The GGE biplot analysis confirmed that all the test environments form a single mega-environment. The study laid a strong base for the screening of stable genotypes and develop a selection strategy for future genetic improvement programs in *Inula racemosa*.

Key words : Stability, G × E interaction, Selection, Eberhart and Russel Model, Essential oil.

Introduction

Inula racemosa Hook. f., commonly known as "Pushkarmool" is a critically endangered perennial herb belonging to genus *Inula* of Asteraceae family (Shabir *et al*., 2013). The plant species is found abundantly throughout the Himalayas (Europe, China and India). It grows well in the temperate regions ranging from Afghanistan to Central Nepal and Kashmir to Kumaon with an altitudinal range of 1500 – 4200 m above mean sea level (Firdous *et al*., 2018). The cold, arid, and alpine regions of the Western Himalayas at an altitudinal range of 2700–3500 mamsl are the natural habitat of the species (Amin *et al*., 2013; Seth *et al*., 2022).

The plant grows up to a height of 1.5 m with large basal leaves arranged in racemose manner (Arora *et al*., 1980; Wangchuk and Jamtsho, 2023). The leaves areelliptic-lanceolate in shape, usually large thick, rough and leathery with dense hairs on the abaxial surface.

The flower heads (bisexual florets) are usually terminally borne and yellow forming a capitulum (Fig. 1). The species is entomophilous having 10-20 capitula per plant aggregated to form racemes. The disc florets are protandrous and are often assumed to be selected for avoiding self-fertilization. The chromosome number of *Inula racemosa* is diploid with 2n = 20 (Shabir *et al*., 2015).

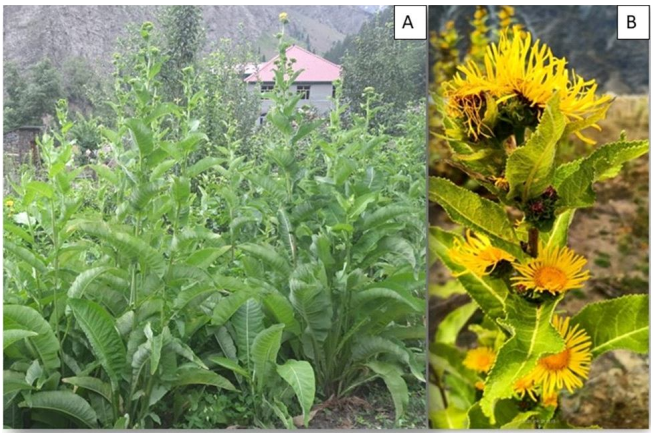

Fig. 1 : Plant growth **(A)** at vegetative and **(B)** reproductive stage.

The plant is known to be used in traditional medicine throughout the world, especially in East Asia and Europe (Rathore *et al*., 2022). Root powder is consumed with lukewarm water as diuretic, rejuvenating and anti-aging agent. Besides, it is also used for the treatment of high cholesterol, diabetes and tooth diseases (Jaiswal *et al*., 2022). The extract of the plant is used against abdominal pain, acute enteritis, and bacillary dysentery (Firdous *et al*., 2018). The extracts of the plants are reported to have anti-inflammatory, analgesic, hepatoprotective, antiasthmatic, cytotoxic, cardioprotective antispasmodic, antiseptic, hypoglycaemic, antimicrobial and antioxidant activities (Seth *et al*., 2022; Jaiswal *et al*., 2022). The fresh roots of the plant are having a camphor-like aroma. The major active phytochemical constituents in the roots with medicinal properties are alantolactone, isoalantolactone, dihydroisoalantolactone, neoalantalactone, sesquiterpene lactone, germacranolide β-sitosterol and alantodiene (Kapoor, 2018; Ketai, 2000). The roots and rhizome of the plant contains essential oil that can be extracted through hydro distillation. The essential oil of *Inula racemosa* is reported to have two marker compounds (sesquiterpene lactones eudesmanolides) namely alantolactone and isoalantolactone (4:6 ratio), which are being commercially used as an active pharmaceutical ingredient for the antiulcer drug "Alanton" (Agnihotri *et al*., 2017; Rathore *et al*., 2022; Zhang *et al*., 2010). Sesquiterpene lactones are the sesquiterpenoids that contain a lactone ring with broad range of biological activities such as anti-inflammatory, antibacterial and anticancer (Kour *et al*., 2020).

The *Inula racemosa* have high demand in the pharmaceutical industry. The global market of herbal drugs is approximately ` 300,000 crores in which the contribution of India is about ` 3000 crores. Currently, the market of medicinal plants in India is increasing at a compound annual growth rate (CAGR) of 38.5% to reach US$ 188.6 million in 2026. The domesticated forms of the cultigens are cultivated in Kashmir and Lahaul valley of Himachal Pradesh (Singh *et al*., 1959). Presently, the cultivation of *Inula racemosa*is restricted to a few locations in the Lahaul Valley of Himachal Pradesh. Thus, a large-scale commercial cultivation is required to fulfill the demand of the pharmaceutical industry. Further, there is a lack of superior and stable varieties of the species in the Western Himalayan regions as the farmers are growing the existing landraces which are heterogenous in produce and maturity. The genetic improvement of landraces is necessary to develop new varieties with uniformity of produce (Dillon *et al*., 2007).

Wild relatives under natural habitats are the major source of genetic variations in *Inula racemosa* but unsystematic, illegal and unscientific harvesting practices have posed a high pressure on this taxon (Vashisht *et al*., 2016). The genetic variation may respond differently over different environments. Few earlier attempts have been made concerning germplasm evaluation and interaction studies in *Inula racemosa* based on morphological traits and quantitative characteristics (Singh *et al*., 2018). Further, Literature studies have reported significant variations for the morphological traits and active phytochemical markers in the roots of *Inula racemosa* (Mudassir Jeelani *et al*., 2022; Rathore *et al*., 2022; Seth *et al*., 2022; Singh *et al*., 2018). The genetic makeup of the plants influences the phytochemical content (Batubara et al., 2020)and the varying environmental conditions have been reported to cause significant variations in the marker compounds (Isoalantolactone and Alantolactone) among different populations of *Inula racemosa* (Mudassir Jeelani *et al*., 2022).

The Indian Himalayan Region harbours rich biodiversity of medicinal and aromatic plants. The mountain range of the North-Western Himalayas at an altitude of 2700-3500 m is the natural habitat of *Inula racemosa*. Thus, there is sufficient scope to explore the existing variability to identify superior and stable genotypes for future genetic improvement programs of *Inula racemosa*. The multi location evaluation helps identify

plant traits that contribute to adaptability and productivity across various geographical locations. The exploration of the available variability will help to screen superior genotypes and establish a selection strategy for future genetic improvement programs of *Inula racemosa*. Currently, there is a need to evaluate the available variability of *Inula racemosa* to enhance its cultivationin the Western Himalayan region. Therefore, the present investigations were carried out at four diverse locations of Himachal Pradeshin search of superior and stable genotypes, development of a selection strategy for higher root biomass, and essential oil contentby analyzing the effects of genotypes, environments, and their interaction.

Materials and methods

Experimental material and layout

The present studywas carried over ten superior diploid selections (2n=20) of *Inula racemosa* (Table 1). These selections (for higher root biomass) were made through a single plant selection approach from the base population (IR B) of *Inula racemosa* being maintained at the Centre for High Altitude Biology (CeHAB) farm in Ribling, Himachal Pradesh. Selections were subsequently maintained in isolation at CeHAB as separate plots and sib-mating was allowed within the progenies (half-sib progenies). The experiment to assess performance for morphological traits among the genotypes was laid out at four diverse locations of Himachal Pradesh in a randomized block design (RBD) during the years 2021 and 2022. Twenty-four plants per selection were planted in an experimental plot of size 3 × 2 m and spacing of 50 × 50 cmat each location. Manual weeding was performed during the entire cropping period to ensure weed-free plots at all four locations. Proper irrigation facility to every plot was ensured for good crop growth. The well-rotten farmyard manure was applied in a dose of 15 tons per hectare during field preparation before transplanting. All the recommended agronomical practices for raising a healthy crop stand were applied at all four locations (Rathore *et al*., 2022).

Table 1 : Detail of genotypes used in the study.

S. no.	Genotype (progeny line)	Pedigree (Selection)
1	CSIR-IHBT-IR-01	IR B (Sel-01)
2	CSIR-IHBT-IR-02	IR B (Sel-02)
3	CSIR-IHBT-IR-03	IR B (Sel-03)
4	CSIR-IHBT-IR-04	IR B (Sel-04)
5	CSIR-IHBT-IR-05	IR B (Sel-05)
6	CSIR-IHBT-IR-06	IR B (Sel-06)
7	CSIR-IHBT-IR-07	IR B (Sel-07)
8	CSIR-IHBT-IR-08	IR B (Sel-08)
9	CSIR-IHBT-IR-09	IR B (Sel-09)
10	CSIR-IHBT-IR-10	IR B (Sel-10)

Experimental locations

The trials were laid out in four different locations representing three districts of Himachal Pradesh in the Western Himalaya. Detailed information on all four experimental sites with their environmental conditions is given in Fig. 2. The experimental locations lie in the range from 1219 to 3400 m amsl. The studied locations fall under mid-hills (sub-humid mid-hill zone) to high hills (wet and dry temperate high hills zones).

Data recording and essential oil extraction

Ten plants from each plot were recorded for morphological traits *viz*., Plant height (cm), number of branches, basal leaf length (cm), basal leaf width (cm), Number of basal leaves, leaf length upper leaf (cm), leaf width upper leaf (cm), root biomass (g) and essential oil content (mg/Kg).

Dried root powder of *Inula racemosa* was used for essential oil extraction from all the locations. The essential oil extraction was performed with 500 g of sample to estimate the essential oil yield. The extraction process follows hydro-distillation using Clevenger apparatus under optimal operating conditions for 5 hours. After the extraction process, the moisture content in the essential oil is removed by adding a pinch of sodium sulphate (anhydrous). The amount of essential oil extracted is expressed in terms of milligram per kilo gram of the root sample on a dry matter basis. The oil was stored in amber glass bottles at a temperature of 4-5^{0}C in refrigerator till further characterization of essential oil compounds through gas- chromatography mass spectrophotometry.

Gas-chromatography mass spectrophotometry (GC-MS) and gas chromatography (GC) analysis

The qualitative analyses of the isolated essential oils were carried out using GC-MS on Shimadzu QP 2010 (Kyoto, Japan) with a ZB-5MS capillary column (30 m 0.25 mm 0.25 m film thickness) inserted with an AOC-5000 auto-injector. Helium was employed as carrier gas, with a flow rate of 1.05 mL/min. The oven temperature was set to 70°C for 5 minutes and then gradually raised to 220°C at a rate of 4°C per minute, holding for 5.0 minutes. The temperature of the injector and the interface were set to 240°C and 250°C, respectively and the mass range of MS data was 40-800 m/z at 70 eV. Dichloromethane (5 mg/ 2 mL) was used to thin down the isolated essential oils samples, and 2 L volumes of the entire solution were added to the system. An inoculation was carried out using a split ratio of (1:10). Similar circumstances were used to administer a standard

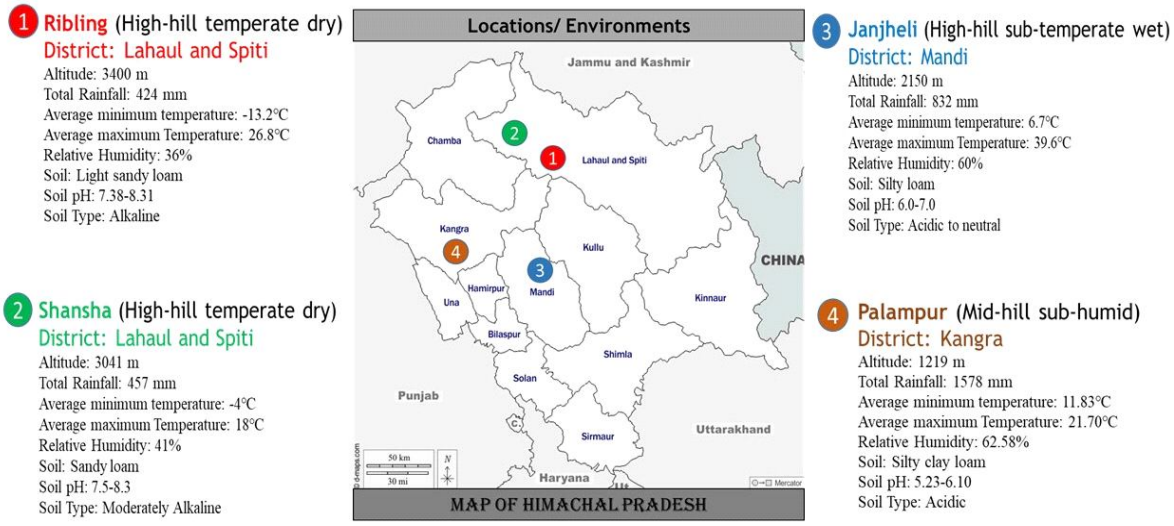

Fig. 2 : Detail of meteorological and soil conditions at the studied locations.

mixture of *n*-alkane chains (C9-C24) to the GC-MS to assess the Arithmetic Index (AI). Further, quantitative analysis was carried out on a Shimadzu GC 2010 (Kyoto, Japan) equipped with a FID detector using a GC coupled with an AOC-20i auto-injector and a ZB-5MS (30 m x 0.25 mm i.d., 0.25 m film thickness) capillary column from Phenomenex, USA. Nitrogen was the carrier gas, and column flow rate was set at 1.05 mL/min (87.4 kPa). The temperature of the oven was programmed at 70°C, kept for 5 minutes, extended to 220°C at a rate of 4 °C per minute, and hold at this temperature for 5 minutes. The injector and detector were adjusted at 240°C and 250 °C, respectively.

Identification and confirmation of isolated essential oil components was done by comparing mass spectra with spectral library database (NIST) and Arithmetic Index (AI) of a series of *n*-alkane chains (C_9-C_{24}) w.r.t literature values (Adams, 1995; Stein, 2005). AI calculated as reported earlier in the literature (Adams, 1995).

Statistical analysis

The two-factor (genotypes × environment) combined analysis of variance (ANOVA) and stability analysis using the "Eberhart and Russel Model" (Eberhart and Russell, 1966) were performedusing OPSTAT (Sheoran *et al.*, 1998). The multiple comparisons for mean performance were performed using Fisher's LSD test. The heat maps and cluster analysis was generated to study the mean performance of genotypes at different locations using the "heatmap" function in Rstudio. The "which-won–where and Descriminitiveness vs. Representativeness' plots for G×E interaction were performed using the "gge_biplot" function in Rstudio (RStudio Team, 2022). Pearson's correlation matrix and genetic parameters were performed using associated functions "corr_coef" and "gen.var" in Rstudio.

Results and Discussion

Analysis of variance

Pooled data of two years 2021 and 2022 was used to perform the combined analysis of variance (ANOVA) to identify significant variations. The results of two-factor combined analysis of variance are presented in Table 2. The genotype (G), environments (E) and genotype × environment interaction (G×E) were found significant for all the traits. The significant results for G×E interaction in the present study indicates that the performance of the superior genotypes are influenced not only by their inherent genetic makeup but also by the specific environment conditions, where the genotypes are being tested. Our findings are in consistence with Singh *et al.* (2018), where they have reported significant variations for several morphological and quantitative traits (*e.g.*, plant height, leaf length, leaf breadth, number of stems) including root biomass and essential oil content in the germplasm collected from eight different sites (6 sites of Himachal Pradesh and 2 sites from Jammu and Kashmir) suggesting that there is a significant effect of location on the growth and performance of *Inula racemosa*.

The variations in performance of different genotypes have utility in breeding programs of *Inula racemosa*. Significant variations for the quantitative morphological traits in the present study suggest that there is no influence of the environments on these traits. These traits can be directly utilized in the genetic improvement program of *Inula racemosa* to develop a selection strategy for higher root biomass and essential oil content. The multi-environment evaluation of *Inula racemosa* genotypes will contribute to strengthening the process of selection. Root biomass is the economically important trait due to

Table 2 : Analysis of variance for all the traits studied at four different environments pooled over the years 2021 and 2022.

Source of variation	Degree of freedom	Mean sum of squares							
		Plant height	Number of branches	Basal leaf length	Basal leaf width	Number of leaves	Upper leaf length	Upper leaf width	Root Biomass	Essential oil
Replication	2	64.864	0.454	17.157	2.28	0.162	2.454	0.007	1.242	19.28
Genotypes (G)	9	649.229*	6.63*	608.656*	18.053*	11.195*	70.795*	5.75*	1,17,364.30*	50,71,098.94*
Environments (Env)	3	6,802.05*	8.735*	10,067.53*	208.048*	149.133*	80.638*	59.592*	8,71,545.97*	1,47,041.23*
G × Env	27	714.807*	0.961*	84.779*	8.742*	6.931*	13.387*	2.427*	21,216.64*	21,890.47*
Error	78	18.778	0.255	2.07	1.235	0.37	1.087	0.481	2.84	4.062

*, significant at $p \leq 0.05$ and $p \leq 0.01$, respectively.

the presence of marker compounds such as flavanol glycosides, sesquiterpenoids and sesquiterpene lactones (Seth *et al*., 2022). The two sesquiterpene lactones namely Isoalantolactone and Alantolactone have recently been identified in the essential oil of *Inula racemosa* (Agnihotri *et al*., 2017). These marker compounds have enormous pharmacological importance. The results of significant variations for root biomass and essential oil content based on combined analysis of variance (ANOVA) further allow to perform stability analysis.

Mean performance for genotype and environment

The mean performance of genotypes under multi-environment evaluation helps assess the overall adaptability and stability of genotypes across various environmental conditions providing valuable in sights for plant breeding and selection processes. The overall mean performance of the genotypes for studied traits is presented in Table 3. The results revealed that Env-1 has the maximum performance for most of the traits including economically important traits e.g., root biomass (562.99 g) and essential oil content (1939.24 mg/Kg). However, leaf width of upper leaf has maximum performance under the Env-2. The mean performance of the genotypes under tested environments follows the order as; Env-1> Env-2 > Env-3 > Env-4. This finding suggest that Env-1 (Ribling, Lahaul and Spiti) is most favorable environments for the cultivation of *Inula racemosa*. Whereas, the Env-4 (Palampur, Kangra) was observed with minimum performance compared with other environments studied. Lowest mean performance of root biomass (191.63 g) and essential oil content (1676.45 mg/Kg) under Env-4 indicates that this environment is not favorable for *Inula racemosa* cultivation. Such type of variations in morphological traits under multi-environment conditions can be attributed to a combination of genetic factors, environmental influence and their interaction. Genetic factors determine the inherent potential of a genotype for specific traits, while the environmental conditions such as temperature, rainfall pattern, humidity, soil type and management practices can influence how those traits are expressed (Ji *et al*., 2016; Pacheco-Hernández *et al*., 2021; Singh *et al*., 2015).

Overall, the genotype, CSIR-IHBT-IR-03 has the maximum plant height (166.02 cm). Whereas, the genotype CSIR-IHBT-IR-02 has the maximum leaf length of upper leaf (11.29 cm). The genotype CSIR-IHBT-IR-09 has the maximum performance for all other traits studied *viz*, number of branches (4.21), basal leaf length (74.88 cm), basal leaf width (15.83 cm), number of basal leaves (7.25) and leaf length of upper leaf (24.98 cm). In addition, the genotype CSIR-IHBT-IR-09 was recorded with maximum mean performance across test environments for economically most important quantitative traits *i.e*., root biomass (553.39 g) and essential oil content (3393.21 mg/Kg). The genotype with maximum expression for desirable traits are important aiming to develop novel improved varieties with enhanced performance. Although, the higher expression of a trait depends upon the genetic makeup of the plant but it can also be influenced by different environmental factors (Shakya *et al*., 2023).

The root biomass and essential oil contentare the most important economical traits in *Inula racemosa*. For root biomass, significant variations have been observed for genotypes CSIR-IHBT-IR-02, CSIR-IHBT-IR-03, CSIR-IHBT-IR-07, CSIR-IHBT-IR-09, and CSIR-IHBT-IR-10. Whereas, the essential oil content was significantly higher in genotypes CSIR-IHBT-IR- 02, CSIR-

Table 3 : Means comparison of genotypes and tested environments for all the traits.

Genotypes/ Environment	Plant height (cm)	Number of branches	Basal leaf length (cm)	Basal leaf width (cm)	Number of basal leaves	Leaf length upper leaf (cm)	Leaf width upper leaf (cm)	Root biomass (g)	Essential oil (mg/ Kg)
CSIR-IHBT-IR-01	154.52 ^{c}	2.50 ^{b}	60.75 ^{c}	14.08 ^{c}	4.75 ^{b}	20.83 ^{c}	9.85 ^{bc}	338.50 ^{d}	1566.91 ^{e}
CSIR-IHBT-IR-02	161.58 ^{d*}	3.25 ^{c*}	74.67 ^{g*}	15.29 ^{d*}	4.79 ^{b}	22.94 ^{d*}	11.29 ^{d*}	475.52 ^{h*}	2101.62 ^{f*}
CSIR-IHBT-IR-03	166.02 ^{e*}	2.83 ^{b}	70.42 ^{e*}	14.13 ^{c}	4.96 ^{b}	21.31 ^{c}	10.50 ^{c}	433.96 ^{f*}	1685.10 ^{f}
CSIR-IHBT-IR-04	142.46 ^{a}	2.08 ^{a}	62.96 ^{d}	13.19 ^{bc}	4.04 ^{a}	18.65 ^{a}	10.13 ^{bc}	340.17 ^{e}	1487.17 ^{d}
CSIR-IHBT-IR-05	150.73 ^{bc}	2.13 ^{ab}	59.69 ^{c}	12.90 ^{b}	4.63 ^{b}	18.15 ^{a}	8.94 ^{a}	307.10 ^{c}	1417.03 ^{c}
CSIR-IHBT-IR-06	151.00 ^{bc}	2.00 ^{a}	58.35 ^{b}	12.56 ^{ab}	4.00 ^{a}	18.02 ^{a}	9.56 ^{b}	287.10 ^{b}	1343.90 ^{b}
CSIR-IHBT-IR-07	159.48 ^{d*}	2.71 ^{b}	72.33 ^{f*}	13.94 ^{c}	5.88 ^{c*}	23.04 ^{d*}	10.75 ^{cd*}	447.46 ^{g*}	1906.22 ^{h*}
CSIR-IHBT-IR-08	145.83 ^{a}	1.88 ^{a}	56.81 ^{a}	11.73 ^{a}	5.00 ^{b}	19.65 ^{b}	9.60 ^{b}	257.96 ^{a}	991.84 ^{a}
CSIR-IHBT-IR-09	160.17 ^{d*}	4.21 ^{d*}	74.88 ^{g*}	15.83 ^{d*}	7.25 ^{d*}	24.98 ^{e*}	10.71 ^{c}	553.39 ^{j*}	3393.21 ^{j*}
CSIR-IHBT-IR-10	153.75 ^{bc}	3.38 ^{c*}	70.88 ^{e*}	13.73 ^{bc}	5.71 ^{c*}	23.21 ^{d*}	10.27 ^{c}	483.88 ^{i*}	1764.11 ^{g}
Env-1	173.94 ^{4*}	2.88 ^{2*}	89.37 ^{4*}	16.04 ^{3*}	6.80 ^{3*}	20.63 ^{2}	10.62 ^{3*}	562.99 ^{4*}	1839.24 ^{4*}
Env-2	158.22 ^{3*}	3.37 ^{3*}	69.43 ^{3*}	15.69 ^{3*}	7.23 ^{4*}	22.30 ^{3*}	11.79 ^{4*}	523.35 ^{3*}	1798.14 ^{3*}
Env-3	146.58 ^{2}	2.37 ^{1}	60.32 ^{2}	12.75 ^{2}	3.43 ^{2}	22.43 ^{3*}	9.79 ^{2}	288.93 ^{2}	1749.01 ^{2}
Env-4	139.48 ^{1}	2.17 ^{1}	45.58 ^{1}	10.47 ^{1}	2.93 ^{1}	18.95 ^{1}	8.44 ^{1}	191.63 ^{1}	1676.45 ^{1}
Overall Mean	154.55	2.70	66.17	13.74	5.10	21.08	10.16	392.28	1765.71
CD (Genotypes)	3.53	0.41	1.17	0.91	0.50	0.85	0.57	1.37	1.64
CD (Environments)	2.23	0.26	0.74	0.57	0.31	0.54	0.36	0.86	1.04
CD (G × E)	7.06	0.82	2.34	1.81	0.99	1.70	1.13	2.74	3.28

Different superscript letters and numbers show significant ($p \leq 0.05$) difference between the means within the column for genotypes and environments, respectively (Fisher's LSD test); *, Significantly ($p \leq 0.05$) higher than overall population mean; *CD*- critical difference.

IHBT-IR-07 and CSIR-IHBT-IR-09. The genetic variations with maximum expression serve as principal criteria for breeding superior genotypes (Vanavermaete *et al*., 2020). Such genotypes have utility in future genetic improvement programs of *Inula racemosa* to generate variations stable over different locations.

The heat map visuals were created to understand the comparative mean performance of each genotype in the tested environments (Fig. 3). The dark yellow color is depicting the maximum scaled value and the dark blue color is depicting the lowest scaled value. The genotype CSIR-IHBT-IR-06 has the maximum plant height (190.2 cm) under Env-1. The maximum number of branches (5.5), basal leaf width (19.5 cm), number of basal leaves (11.2) and upper leaf length (27.7 cm) has been recorded for genotype CSIR-IHBT-IR-09 under Env-2. Whereas, the basal leaf length (103.6 cm), root biomass (744.7 g) and essential oil content (3487.7 mg/Kg) were maximum in the genotype CSIR-IHBT-IR-09 under Env-1. Additionally, the genotype CSIR-IHBT-IR-10 was recorded with maximum root biomass (753.8 g) under Env-2. The genotypes with maximum expression can be used in future genetic improvement programs of *Inula racemosa* for location-specific target traits. Further, the findings suggest that the genotype and environmental interaction have a significant influence on phenotypic and quantitative traits.

The present investigations align with the previous report that remarks significant variation in morphological and quantitative traits (plant height, leaf length, leaf breadth, number of stems and fresh root weight) during germplasm evaluation and interaction studies in *Inula racemosa* (Singh *et al*., 2018) from eight high altitude sites (2146 -3550 m amsl) of Himachal Pradesh and Jammu & Kashmir. They

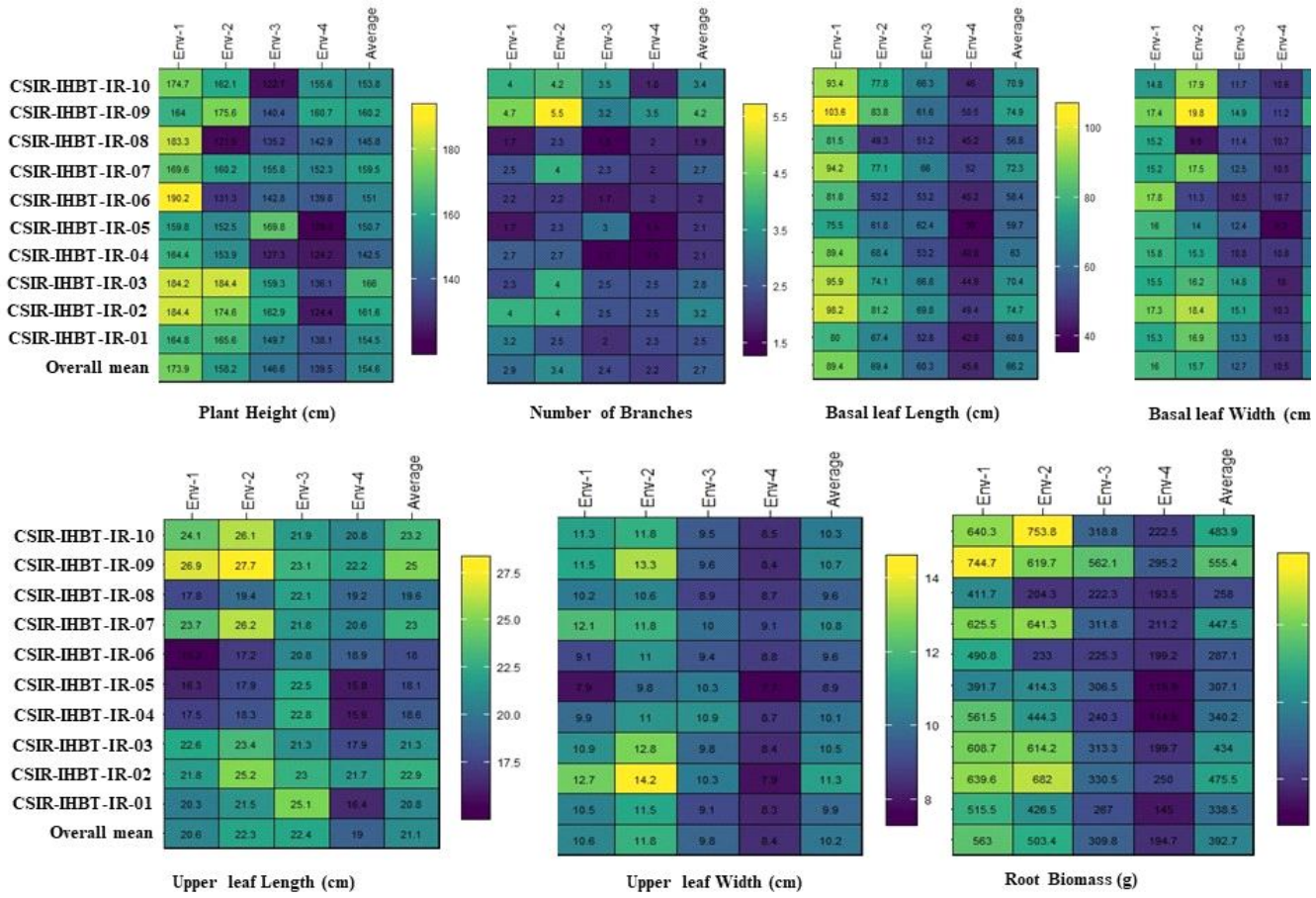

Fig. 3 : Heat map visuals for the mean performances of genotypes at different environments for all the traits. The scale on the side indicates magnitude of the trait.

have reported the maximum root weight (659.30 and 636.50 g) from high altitude regions at 3417 and 3116 m amsl, respectively.

However, in the present study, genotype CSIR-IHBT-IR-09 produced maximum root biomass (753.80 g/ plant) under Env-2 followed by Env-1 (744.70 g/ plant). The finding suggests that the Env-1 (3400 m amsl) and Env-2 (3040 m amsl) were found most suitable for *Inula racemosa* cultivation in the Western Himalayas indicating that the high altitude temperate regions with alkaline (pH 7.38-8.3), sandy loam soil, comparatively lower temperature and rainfall conditions favours better root growth. Roots (especially root hairs) are the most important part of the plants that face the heterogonous nature of the soil in terms of penetration, resistance, and availability of nutrients (Lippold *et al*., 2022). Sandy loam soil (a mixture of sand, silt and clay) is considered best for plant growth as it provides proper aeration and space for air and water to flow and roots to penetrate soil, thereby enhancing plant growth and development.

Cluster analysis and genetic relationship

Cluster analysis has wide use in predicting kinship that groups individuals based on genetic similarities. The genetic relationship among the genotypes and the quantitative traits were assessed using cluster analysis (Fig. 4). The dark red color depicts the maximum value and the dark blue color depicts the minimum value for a trait. Clustering grouped ten genotypes in to two clusters (G-1 and G-II). Cluster G-1 consists of 5 genotypes *i.e.*, CSIR-IHBT-IR-02, CSIR-IHBT-IR-03, CSIR-IHBT-IR-07, CSIR-IHBT-IR-09 and CSIR-IHBT-IR-10. The findings revealed that the genotype CSIR-IHBT-IR-09 from this cluster was recorded with the maximum value for all the quantitative traits (including root biomass and essential oil content). Additionally, the genotype CSIR-IHBT-IR-02 from this cluster group was recorded with maximum leaf width of upper leaf. Cluster G-II also grouped 5 genotypes *i.e.*, CSIR-IHBT-IR-01, CSIR-IHBT-IR-04, CSIR-IHBT-IR-05, CSIR-IHBT-IR-06 and CSIR-IHBT-IR-08. All these genotypes have comparatively lower performance for all the traits studied.

Overall, cluster G-1 consists of best performing and stable genotype. Thus the genotype CSIR-IHBT-IR-09 from this group can be promoted as superior variety in Western Himalayan regions. The genotype CSIR-IHBT-

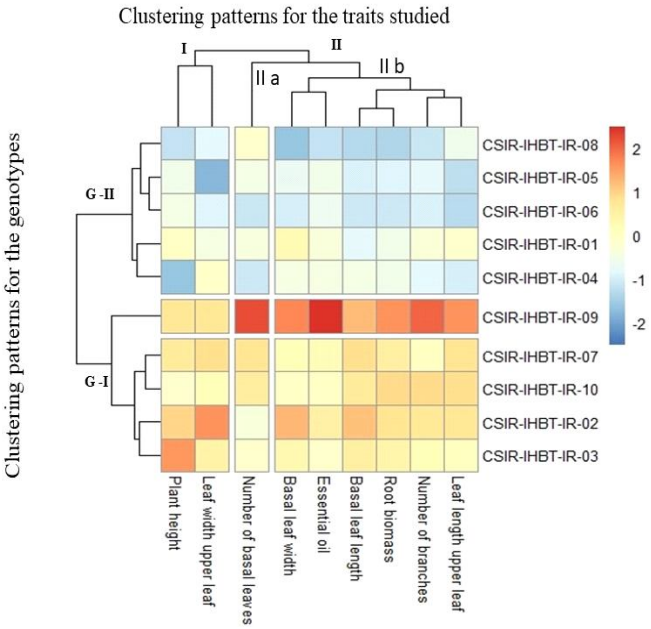

Fig. 4 : Heat map visuals and clustering patterns for the comparative performance of phenotypic traits and genotypes across all test environments.

IR-09 was significantly higher for root biomass and essential oil content compared with the population mean at all the test environments. This genotype can be used in hybridization programs of *Inula racemosa* to generate variations for higher root biomass and essential oil content in future breeding programs. The results of cluster analysis are quite promising providing in sights in to genetic relationship and predicting kinship.

The grouping patterns based on morphological traits differentiate nine traits into two clusters (I and II). Cluster -1 consists of plant height and leaf width of upper leaf where, the genotype CSIR-IHBT-IR-03 and CSIR-IHBT-IR-02 were recorded with maximum expression for these traits, respectively. Cluster –II consists of the economically important traits (Root biomass and essential oil content) along with other agro-morphological traits and further sub-clustered in to IIa and IIb. The sub cluster-II a consists of number of basal leaves for which the highest expression was recorded for genotype CSIR-IHBT-IR-09. The sub cluster II b grouped basal leaf width, essential oil content, basal leaf width, root biomass, number of branches and leaf length of upper leaf. The sub group II b was unique in terms of grouping the economically most important traits of *Inula racemosa i.e.*, root biomass and essential oil content. The genotype CSIR-IHBT-IR-09 consistently performed well for all the traits (including root biomass and essential oil content) in this sub group across all the test environments. However, the mean performance of the genotype was observed maximum under Env-1. A similar type of cluster analysis to predict kinship among genotypes has earlier been successfully utilized in german chamomile (*Matricaria chamomile*) and lavender (*Lavandula angustifolia*) for identification of highest-yielding genotypes in terms of essential oil content (Gupta *et al.*, 2023; Shakya *et al.*, 2023).

Variations for essential oil composition

Essential oil analysed through gas-chromatography mass spectrophotometry (GC-MS) have shown significant variations for essential oil compounds based on a t-test across all the environments (Table 4). The GC-MS analysis led to the identification of total ten compounds. Out of which six major compounds (β-Pinene, β-Elemene, β-Elemene enantiomer, β-Selinene, alantolactone and isoalantolactone) were identified that contributes 87.5 – 95.8% of total volume of the essential oil. The representative chromatograph for the presence of marker compounds is given in Fig. 5. Earlier literature hasalso reported the presence of two major sesquiterpene lactones eudesmanolides *i.e.*, alantolactone and isoalantolactone in a ratio of 4:6 in the essential oil of *Inula racemosa* (Firdous *et al.*, 2018; Rathore *et al.*, 2022).

In the present study, alantolactone (51.10 - 54.60%) and isoalantolactone (21.50 -25.80%) were found maximum in genotype CSIR-IHBT-IR-09 across all the test environments. Additionally, the variation in essential oil composition across different environments suggest that the Env-1 and Env-2 were most appropriate for obtaining maximum percentage of marker compounds (sesquiterpene lactones) in the essential oil. Sesquiterpene lactones are rapidly evolving group of natural products known for therapeutic properties and often possess anti-inflammatory, antimicrobial and antifungal properties (Kour *et al.*, 2020).

Overall, the genotype CSIR-IHBT-IR-09 was unique in terms of highest proportion of marker compounds in the essential oil. The variations for alantolactone content across all the test environments follows the order as; Env-1 (54.60%) > Env-2 (54.20%) > Env-3 (52.70%) > Env-4 (51.10%). Whereas, for isolantolactone content the order is as: Env-2 (25.80%) > Env-4 (24.40%) > Env-3 (22.70%) > Env-1 (21.50%). Thus, the genotype CSIR-IHBT-IR-09 can be recommended for varietal development programs of *Inula racemosa*.

Stability analysis

Stability analysis is an analytical approach used to assess the performance of genotypes across different environments. Eberhart and Russell's model is a widely used univariate parametric method for regression-based

Table 4 : Variation among major chemical constituents in the essential oil of clary sage for all the genotypes tested at four different locations.

Environments	**Genotypes**	**α-Pinene**	**β-Pinene**	**β-Elemene**	**β-Elemene enantiomer**	**β-Selinene**	**α-Selinene**	**Alantol-actone**	**Isoalanto-lactone**
AI^{exp}		934	979	1389	1469	1489	1497	1906	1947
AI^{lit}		932	974	1389	-	1489	1498	-	-
Identification method		AI, MS	AI, MS	AI, MS	NIST-MS	AI, MS	AI, MS	NIST-MS	NIST-MS
Env-1	CSIR-IHBT-IR-01	2.10	18.10*	12.70	8.90*	*nd*	*nd*	37.90	13.90
Env-1	CSIR-IHBT-IR-02	*nd*	4.30	10.10	6.70	3.40	*nd*	42.90	25.90*
Env-1	CSIR-IHBT-IR-03	2.30	17.20*	13.90	4.70	3.00	*nd*	33.90	17.40
Env-1	CSIR-IHBT-IR-04	1.60	13.00	13.00	9.90*	*nd*	*nd*	37.60	16.20
Env-1	CSIR-IHBT-IR-05	*nd*	3.70	17.00*	7.30	3.40	*nd*	41.50	16.00
Env-1	CSIR-IHBT-IR-06	*nd*	7.90	6.60	4.60	3.80	1.90	47.50*	23.40*
Env-1	CSIR-IHBT-IR-07	1.70	16.50*	17.20*	7.40	1.90	*nd*	35.50	14.00
Env-1	CSIR-IHBT-IR-08	1.70	18.30*	19.20*	8.50*	*nd*	*nd*	31.30	13.60
Env-1	CSIR-IHBT-IR-09	*nd*	4.10	4.10	6.20	4.30	2.10	54.60*	21.50*
Env-1	CSIR-IHBT-IR-10	1.30	12.10	9.30	4.40	4.10	1.90	44.90	18.40
Mean (Env-1)		1.78	11.52	12.31	6.86	3.41	1.97	40.76	18.03
LSD (P=0.05)		0.26	4.33	3.46	1.37	0.57	0.08	5.01	3.05
Env-2	CSIR-IHBT-IR-01	*nd*	15.10	10.50	6.50	6.10*	2.50*	37.90	16.00
Env-2	CSIR-IHBT-IR-02	2.10	14.20	8.40	5.40	6.20*	2.00	42.50	15.40
Env-2	CSIR-IHBT-IR-03	2.20	18.50*	17.50*	5.60	*nd*	*nd*	33.30	12.60
Env-2	CSIR-IHBT-IR-04	1.60	14.20	12.70*	9.20*	3.30	1.30	36.90	16.50
Env-2	CSIR-IHBT-IR-05	*nd*	11.30	10.70*	4.90	3.60	1.20	41.40	21.90*
Env-2	CSIR-IHBT-IR-06	*nd*	6.80	8.40*	7.40	5.10	2.20	46.20*	18.90
Env-2	CSIR-IHBT-IR-07	1.30	13.50	14.90*	9.50*	2.50	*nd*	35.50	15.60
Env-2	CSIR-IHBT-IR-08	3.60	25.80*	12.30	3.60	3.00	*nd*	29.10	15.30
Env-2	CSIR-IHBT-IR-09	*nd*	4.50	4.10	2.90	3.80	1.70	54.20*	25.80*
Env-2	CSIR-IHBT-IR-10	*nd*	6.60	11.90	6.30	0.60	2.50*	44.50	22.80*
Mean (Env-2)		2.16	13.05	11.14	6.13	3.80	1.91	40.15	18.08
LSD (P=0.05)		0.63	4.50	2.65	1.54	1.28	0.38	5.14	2.97
Env-3	CSIR-IHBT-IR-01	2.60*	19.90*	7.70	8.40	3.50	1.30	37.50	15.70
Env-3	CSIR-IHBT-IR-02	*nd*	7.70	11.10	5.70	5.70*	2.90*	42.10	15.50
Env-3	CSIR-IHBT-IR-03	1.90	15.90	13.70	8.90*	2.20	nd	32.80	17.50
Env-3	CSIR-IHBT-IR-04	*nd*	3.20	17.40*	12.00	6.60*	2.60*	36.90	14.70
Env-3	CSIR-IHBT-IR-05	1.60	14.00	10.60	5.30	4.10	nd	39.70	18.70
Env-3	CSIR-IHBT-IR-06	*nd*	5.40	9.90	5.80	5.40*	2.40	46.90*	18.70*
Env-3	CSIR-IHBT-IR-07	2.30	18.10*	13.10	5.90	*nd*	nd	34.70	18.10
Env-3	CSIR-IHBT-IR-08	2.20	19.40*	19.30*	6.50	4.00	nd	28.70	11.70
Env-3	CSIR-IHBT-IR-09	*nd*	3.90	4.60	9.60*	2.30	0.90	52.70*	22.70*
Env-3	CSIR-IHBT-IR-10	*nd*	6.50	8.80	5.90	5.00	2.10	44.20	21.80*
Mean (Env-3)		2.12	11.40	11.62	7.40	4.31	2.03	39.62	17.51
LSD (P=0.05)		0.27	4.80	3.16	1.59	1.08	0.56	5.07	2.35
Env-4	CSIR-IHBT-IR-01	*nd*	2.50	15.50*	12.40*	7.90*	2.90*	37.40	15.10
Env-4	CSIR-IHBT-IR-02	*nd*	9.40	10.20	4.80	3.70	1.40	41.90	23.30*
Env-4	CSIR-IHBT-IR-03	*nd*	20.30*	13.30	9.30*	4.50	1.30	31.70	14.50
Env-4	CSIR-IHBT-IR-04	1.10	18.40*	17.30*	4.80	2.80	*nd*	36.10	13.80

Table 4 continued...

Table 4 continued...

Env-4	CSIR-IHBT-IR-05	1.60*	13.50	4.90	4.80	5.80	2.90	41.10	22.10*
Env-4	CSIR-IHBT-IR-06	*nd*	6.90	9.10	8.90	4.20	1.60	45.90*	17.80
Env-4	CSIR-IHBT-IR-07	*nd*	13.50	8.80	7.80	7.30*	3.30	34.50	18.40
Env-4	CSIR-IHBT-IR-08	1.50	25.60*	16.00*	8.30	2.90	*nd*	26.70	12.30
Env-4	CSIR-IHBT-IR-09	*nd*	6.90	4.30	5.10	3.40	1.60	51.10*	24.40*
Env-4	CSIR-IHBT-IR-10	1.40	12.10	7.90	5.90	2.80	*nd*	43.90	21.60*
Mean (Env-4)		1.40	12.91	10.73*	7.21*	4.53*	2.14	39.03	18.33
LSD (P=0.05)		0.15	5.00	3.29	1.83	1.33	0.61	5.15	3.09

AI^{exp}: Arithmetic index calculated against n-alkanes (C9 – C24) on the ZB-5MS column, **AI^{lit}:** Arithmetic index from literature, IM: Identification method. **MS:** identified on the basis of mass of computer matching of the mass spectra with those of NIST libraries and comparison with literature data, ***nd* :** not detected.

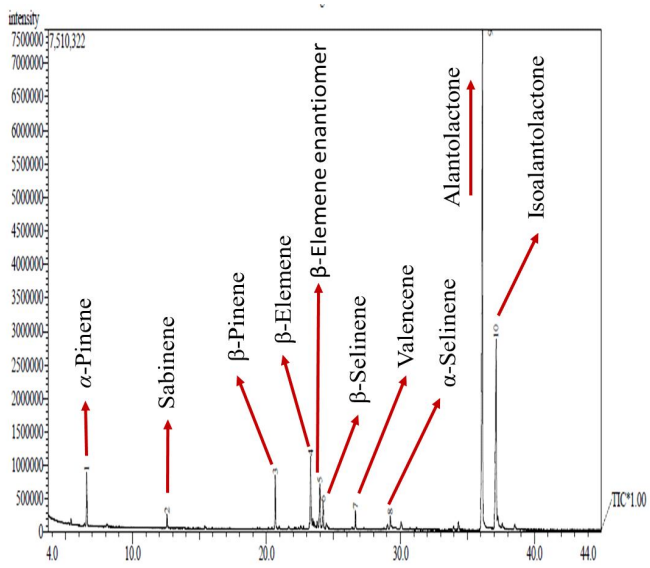

Fig. 5 : Representative chromatograph of essential oil compounds present in *Inula racemosa*.

stability analysis in plant breeding (Eberhart and Russell, 1966). The stable genotype has a regression coefficient close to unity and a small deviation from the mean indicates consistent performance across environments. The identification of superior and stable genotypes of *Inula racemosa* have utilization in terms of commercial cultivation under Western Himalayas. The other approach is GGE (Genotype + Genotype × Environment) biplot analysis, which is a graphical depiction for visualizing genotype performance across different environments. GGE biplot utilizes principle component analysis (PCA) to create a visual representation of genotype and environment interaction. The biplot graphically represents the genotypic main effect and Genotype × Environment interaction effects, making it easier to identify superior genotypes (Yan *et al*., 2000).

In this study, both these methods have been used to differentiate stable genotypes and favorable environments for higher root biomass and essential oil content in *Inula racemosa*. The partitioning of the total variance was done through Analysis of variance (ANOVA) based on Eberhart and Russell's model. ANOVA is used to partition the total sum of squares into components such as genotype (G), environment (E) and genotype by environment (G×E) interaction (Table 5). The results suggest that genotype and environment linear components are significant for all the traits studied. This finding allows further analysis of stability parameters for economically important traits in *Inula racemosa* (*i.e.*, root biomass and essential oil content).

Eberhart and Russel model

Stability variables such as regression coefficient (b_i), deviation from regression coefficient (S^2d_i), and stability response (R) for higher root biomass and essential oil content were calculated to estimate the stability response of the genotypes (Table 6). The regression co-efficient for root biomass ranges from 0.41 to 1.40. Out of ten genotypes, the two genotypes, CSIR-IHBT-IR-01 and CSIR-IHBT-IR-09 were recorded with non-significant to unity regression coefficient with minimum deviation from the regression. This indicates that these genotypes have stable response under varied environments of Western Himalaya.

Three genotypes namely, CSIR-IHBT-IR-05, CSIR-IHBT-IR-06 and CSIR-IHBT-IR-08 have regression coefficient less than unity and minimum deviation from regression coefficient indicating that these genotypes are suitable for unfavorable environments. Five genotypes, CSIR-IHBT-IR-02, CSIR-IHBT-IR-03, CSIR-IHBT-IR-04, CSIR-IHBT-IR-07 and CSIR-IHBT-IR-10 have the regression coefficient greater than unity. These genotypes are suitable for favourable conditions only. Overall, the genotype CSIR-IHBT-IR-09 shows the highest mean performance and stable response over the diversified climates of Western Himalaya. This genotype can be recommended for varietal development programs of *Inula racemosa*.

For essential oil content, the regression coefficient ranges from 0.31-4.18. Out of ten genotypes only one genotype *i.e.*, CSIR-IHBT-IR-09 have non-significant unity regression coefficient with least deviation from the regression. Thus, this genotype has shown a stable response over the different test environments. Eight genotypes namely, CSIR-IHBT-IR-01, CSIR-IHBT-IR-02, CSIR-IHBT-IR-03, CSIR-IHBT-IR-04, CSIR-IHBT-IR-05, CSIR-IHBT-IR-06. CSIR-IHBT-IR-07 and CSIR-IHBT-IR-10 were recorded with regression coefficient less than unity and minimum deviation from the regression. These genotypes are suitable for unfavorable environments. Whereas, one genotype CSIR-IHBT-IR-08 was recorded with regression coefficient greater than unity with minimum deviation from regression. This genotype is suitable for favourable environments only. Overall, the genotype CSIR-IHBT-IR-09 have shown the maximum mean performance and stable response for root biomass and essential oil content compared with all genotypes. This genotype has the potential to be promoted for commercial cultivation in suitable locations of the Western Himalayas. Our results are in line with previous reports, where large G×E interaction has shown differences in genotypic performance at different environmental locations and differences in genotype performance over the mega-environment. Similar type of observations based on Eberhart and Russell joint regression model has earlier been performed to identify stable and high-yielding maize hybrids (Alwala *et al*., 2010), rice genotypes (Das *et al*., 2010) and german chamomile genotypes (Shakya *et al*., 2023).

GGE Biplot

The genotype and genotype × environment interactions are the principal source of variation while assessing the genotypes under multi-locations (Khan *et al*., 2021). GGE biplot typically uses principle component analysis (PCA) to reduce the dimensionality of overall data into two components. The plot consists of two axes, firstly the data was cantered and then sectionalizing the singular value (SV) into GE scores for individual principal components. This is accompanied by plotting PC1 scores opposite to PC2 scores. A higher value of PC1 indicates higher yield ability while a lesser PC2 value indicates more stability.

In the present investigation, two major patterns of GGE biplot have been depicted using biplot analysis (Fig. 6). The first one is the "which-won-where' view that depicts the G × E interaction based on the correlation between genotypes and environment (Fig. 6A). The other pattern discussed is "descriminitiveness vs. representativeness" patterns of the GGE biplot that provides information for the selection of genotypes stable over the environments (Fig. 6B).

"Which-won-where view" of GGE biplot for fresh root biomass plotted with two main principle components PC1 and PC2 contributing 94.54% of the total variation (individual contribution 82.18% and 12.36%, respectively). The genotype × environment interaction under mega-environment is depicted using a polygon view of the GGE biplot. Four genotypes, CSIR-IHBT-IR-05, CSIR-IHBT-IR-08, CSIR-IHBT-IR-09 and CSIR-IHBT-IR-10 were at the vertices of the asymmetrical polygon. The perpendicular stripes/ lines running vertically from the origin of the biplot to each side of the polygon separate the biplot into six sectors. The genotypes at the corner of the polygon are the best performers in different environments. Out of six sectors, the two sectors have all the tested environments. Env-1, Env-3 and Env-4 were grouped into one sector while Env-2 forms a separate sector. All the studied environments have cumulatively made a mega-environment for root biomass. The genotype IR-9 (winning genotype) have shown a stable response across all the tested environments.

For essential oil content the first two principle components of biplot contribute 99.97% of total variation (98.84% by PC1 and 1.13% by PC2, individually) (Fig. 6B). Four genotypes namely, CSIR-IHBT-IR- 04, CSIR-IHBT-IR- 06, CSIR-IHBT-IR- 08 and CSIR-IHBT-IR-09 were found to be at the vertexes of the polygon. For essential oil content, only one sector has all the test environments where genotype, CSIR-IHBT-IR-09 was best performer. All the test environments in the present investigation cumulatively made a mega-environment for essential oil content and the genotype CSIR-IHB-SS-09 (winning genotype) has shown a stable response. Overall the results of "which-won-where" view for root biomass and essential oil content are in consistence with the findings of Eberhart and Russell's regression model where the genotype, CSIR-IHBT-IR-09 performed better in mega-environment including all the test environments. Thus, this genotype has the potential to be promoted for commercial cultivation in suitable locations (environments) of the Western Himalayas. Identification of stable genotypes over the locations have earlier been successfully done utilizing which "won-where pattern of GGE biplot in lavender and german chamomile for commercial cultivation in the western Himalayan regions (Gupta *et al*., 2023; Shakya *et al*., 2023). In one more study, Khan *et al*. (2021) identified three winning genotypes based on the "which-won-where pattern" of the GGE biplot during the multi-environment trial evaluation of *Vigna subterranea* L. Verdc. (bamabara groundnut) genotypes.

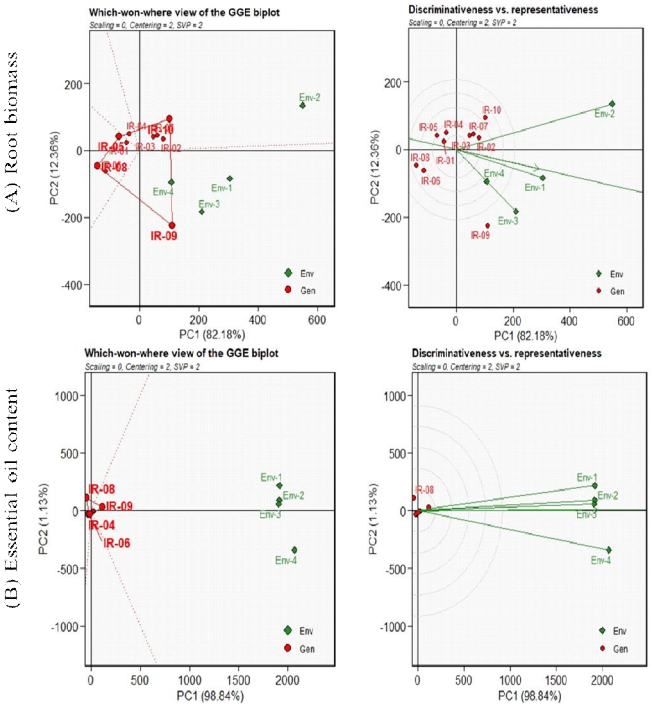

Fig. 6 : "Which-Won-Where' and "Descriminitiveness vs, representativeness" pattern of GGE biplot's for root biomass (A) and essential oil content (B) representing the genotypes and environment interaction.

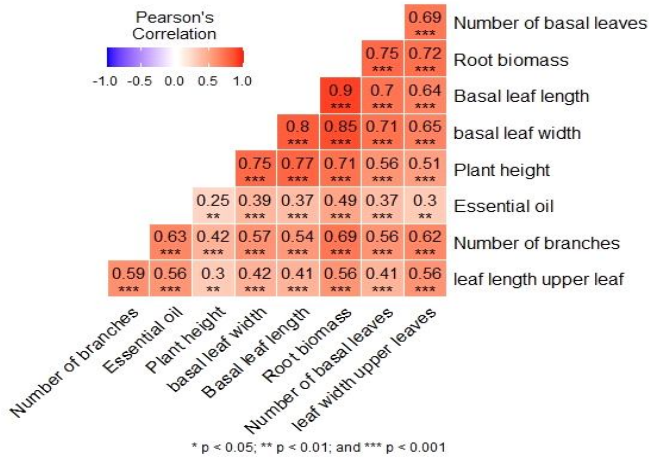

Fig. 7 : Pearson's correlation coefficients among all the traits.

Descriminitiveness vs. Representativeness view of GGE biplot provides information for the selection of genotypes stable over the environments. Descriminitiveness is the ability of biplot to distinguish between genotypes or environments, while representativeness refers to how well the biplot represents the relationship between genotypes and environments. The "descriminitiveness vs. representativeness" view of GGE biplot for root biomass (Fig. 6A) revealed that the Environment with long vector (*i.e.*, Env-2) was most prominent in differentiating the genotypes. Moreover, the environment consisting of a long vector that forms a small angle with the average environment coordinate (AEC) vertical line is considered ideal for the selection of superior genotypes. In the present study, the Env-1 forms the smallest angle with the average environment coordinate (AEC) abscissa line and thus can be considered the most promising location for higher root biomass. The results showed that the genotypes under study identified the most appropriate environment depending on the representativeness and discriminative ability of the test environments. Recently, Hashim *et al*. (2021) reported one environment ideal out of four tested environments, for the selection of superior genotypesbased on yield per hectare using "descriminitiveness vs. representativeness" view of GGE Biplot.

For essential oil content, the Env-4 with long vector was most differentiating (Fig. 6B). However, the Env-1, with long vector that forms smallest angle with AEC abscissa line can be considered more suitable location for essential oil content. The presence of an arrow on the AEC abscissa line depicts its direction, while a small concentric circle indicates the mean value of the environments. Whereas, the length of the test environment vector elucidates the differentiation ability. Overall, the Env-1, with long vector and forming smallest angle with AEC abscissa line is considered as most prominent location for higher root biomass and essential oil content. The results showed that the genotypes under study identified the most appropriate environment depending on the representativeness and discriminative ability. Based on the length of environment vectors for their discrimination ability, genotypes of mutant rice (Oladosu *et al*., 2017) and proso millet (Zhang *et al*., 2016) have been successfully differentiated in the tested environments.

Selection strategy

The genetic improvement for a target trait requires a direct or indirect selection of the contributing traits simultaneously in a single breeding program. The identification of component traits and their genetic parameters can be utilized to develop a selection strategy for the higher root biomass and essential oil content in *Inula racemosa*.

Genetic variability in population is important for adaptation to different environments. The parameters like the genotypic coefficient of variance (GCV), phenotypic coefficient of variance (PCV), heritability and genetic advance for all the traits along with correlation analysis were executed to plan a selection strategy for genetic improvement of higher root biomass and essential oil content through component traits in *Inula racemosa* (Table 7). The GCV was recorded as lower compared

Table 5 : Analysis of variance for the stability of all the traits based on Eberhart and Russel model.

Source of variation	Degree of freedom	Mean sum of squares							
		Plant height	Number of branches	Basal leaf length	Basal leaf width	Number of basal leaves	Leaf length upper leaf	Leaf width upper leaf	Root biomass	Essential oil
Genotype (G)	9	216.41*	2.21*	202.88*	6.01*	3.73*	23.59*	1.91*	39,121.49*	16,90,367.01*
Environment (E)	3	2,267.35*	2.91*	3,355.84*	69.34*	49.71*	26.87*	19.86*	2,90,515.46*	49,018.38*
G × E	27	238.26*	0.32*	28.26*	2.91*	2.31*	4.46*	0.80*	7,072.20*	7,296.55*
E+ G × E	30	441.17	0.57	361.01	9.55	7.05	6.70	2.71	35,416.52	11,468.73
E (Linear)	1	6,802.05*	8.73*	10,067.53*	208.04*	149.13	80.63*	59.59*	8,71,546.38*	1,47,055.14*
G × E (Linear)	9	103.17*	0.36*	28.78*	1.46*	2.69*	2.07*	1.34*	9,096.88*	19,100.09*
Pooled deviation	20	275.23	0.26	25.19	3.27	1.90	5.09	0.487	5,453.87	1,255.30
Pooled error	72	18.42	0.26	2.206	1.26	0.37	1.12	0.507	2.892	3.892

* significant at $p \leq 0.05$

with PCV for all the traits, indicating a significant effect of locations. The significant effect of environment on traits indicates that the selection must be done over multi-location trials to improve the traits associated with overall yield (Biswas *et al*., 2023). High GCV (>40%), PCV (> 60%), heritability (>50%) and genetic advance as percentage of mean (> 60%) were found for number of branches, root biomass and essential oil content. Additionally, a medium GCV (>20%), PCV (>55%), heritability (<30%) and genetic advance as percentage of mean (< 25%) was recorded for number of basal leaves. A low GCV (<20%), PCV (<30%), heritability (<30%) and genetic advance as percentage of mean (<20%) were recorded for basal leaf length, basal leaf width and leaf width of upper leaf. Additionally, minimum GCV (< 10%), PCV (<15%), heritability (<20%) and genetic advance as percent of mean (<10%) was recorded for Plant height.

Number of branches (74.88%), leaf length of upper leaf (77.12%), root biomass (50.02%) and essential oil content in particular have very high heritability (>100%) that suggest a lesser influence of environments and thus can give maximum gain under direct selection. High heritability of such quantitative traits might be due to the different components of variation including mixed effects of genotypes and environments from multi-location investigations (Shakya *et al*., 2023). The selection of independent traits such as number of branches, leaf length upper leaf and essential oil content can be utilized with dependent traits (*i.e.*, higher root biomass). Earlier reports on heritability and genetic parameters in other crops revealed that high heritability is used for the selection and improvement of traits (Kangai Munene *et al*., 2018; Schmidt *et al*., 2019; Shakya *et al*., 2023).

The association of morphological traits and essential oil content was analysed using Pearson's correlation coefficient. All the traits have shown a positive correlation with the essential oil content. However, the essential oil content has positive and significant correlation with root biomass (Fig. 7). The finding suggests that the direct selection for root biomass will improve the essential oil yield. Overall the two economically important traits of *Inula racemosa* (*i.e.*, Root biomass and essential oil content) can be improved simultaneously in a single breeding program due to their moderate positive correlation (r = 0.49).

The basal leaf length, basal leaf width, plant height, number of branches and leaf length of upper leaf and essential oil content are the contributing traits for higher root biomass in *Inula racemosa*. Direct selection for essential oil content and indirect selection through component traits such as plant height, number of branches, basal leaf length and width would be a better selection strategy for improving root biomass yield of *Inula racemosa*.

The breeding behavior of the plant is cross-pollinated and the base population of the present study has a heterogeneous genetic make-up. The Half-sib selection approach is being used to enhance the frequency of desirable alleles in the progeny lines which may further be improved through the selection of progeny lines with high general combining ability. The high activity of insects during flowering may degrade the genetic purity of stable genotypes through cross-pollination events. Thus, the stable genotype needs to be evaluated after regular intervals under multilocation trials

Table 6 : Stability parameters for root biomass and essential oil content.

Genotypes	Root biomass (g)				Essential oil content (mg/Kg)			
	Mean	b_i	S^2d_i	R	Mean	b_i	S^2d_i	R
CSIR-IHBT-IR-01	338.50	0.96 ns	280.24	S	1566.91	0.56	71.85	U
CSIR-IHBT-IR-02	475.52*	1.23	4,665.81	F	2101.62*	0.89	11.14	U
CSIR-IHBT-IR-03	433.96*	1.22	1,827.66	F	1685.10	0.58	72.97	U
CSIR-IHBT-IR-04	340.17	1.17	561.76	F	1487.17	0.31	78.11	U
CSIR-IHBT-IR-05	307.10	0.74	4,034.77	U	1417.03	0.60	5.69	U
CSIR-IHBT-IR-06	287.10	0.59	12,903.15	U	1343.90	0.43	24.20	U
CSIR-IHBT-IR-07	447.46*	1.26	2,777.48	F	1906.22*	0.88	7.44	U
CSIR-IHBT-IR-08	257.96	0.41	8,525.88	U	991.84	4.18	128.52	F
CSIR-IHBT-IR-09	553.39*	1.03 ns	7,522.55	S	3393.21*	1.04 ns	3.40	S
CSIR-IHBT-IR-10	483.88*	1.40	11,429.77	F	1764.11	0.54	56.61	U

*, higher than the overall mean at $p \leq 0.05$; b_i, regression coefficient; ns, non-significant to 1 (b_i =1); S^2d_i deviation; *R*, stability responses as (*F*, suitable for favourable environment; *U*, suitable for un-favourable environment and *S*, stable).

Table 7 : Genotypic coefficient of variance, phenotypic coefficient of variance, heritability and genetic advance for all the traits pooled over locations and years.

Traits	Genotypic coefficient of variance	Phenotypic coefficient of variance	Heritability (broad sense) (%)	Genetic advance as percent of mean
Plant height (cm)	6.11	14.03	18.99	5.48
Number of branches	52.29	60.43	74.88	93.22
Basal leaf length (cm)	15.27	30.39	25.25	15.80
Basal leaf width (cm)	12.74	25.13	25.71	13.31
Number of basal leaves	25.44	54.85	21.52	24.31
Leaf length upper leaf (cm)	21.98	25.03	77.12	40.00
Leaf width upper leaf (cm)	10.06	18.81	28.63	11.09
Root biomass (g)	43.78	61.90	50.02	63.78
Essential oil (mg/ Kg)	73.56	73.77	99.44	151.11

based on above key traits. This will help in avoiding loss of stability.

Conclusion

The present study was carried out in search of stable and superior genotypes of *Inula racemosa* and to develop a selection strategy based onmulti-environment investigationsin Western Himalaya. The combined ANOVA revealed significant variations in genotypes, environments and their interactions for all the traits. GGE biplots also depicted that all the test environments showed a single mega environment. The stability variables utilizing Eberhart & Russell model and GGE biplot analysis revealed that the genotype CSIR-IHBT-IR-09 was a stable genotype (higher root biomass and essential oil content) across all the environments. In addition, GC-MS studies have revealed that this genotype was unique for the presence of highest alantolactone and isoalantolactone (marker compounds) in the essential oil across all the tested locations. The Env-1 was the best suitable environment for the cultivation of *Inula racemosa*. The clustering differentiates all the genotypes into two groups where the highest performing genotype (CSIR-IHBT-IR-09) was grouped in cluster G-I. The root biomass and essential oil content has high GCV and genetic advance and can be improved simultaneously in a single breeding program. Direct selection for essential oil content and indirect selection through component traits (plant height, number of branches, basal leaf length and width) could be a better strategy for improving root biomass. Conclusively, the present study provides a strong base to identify superior genotypes and selection strategy for future breeding efforts of *Inula racemosa.*

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Author contributions

A.K.: data observations and analysis, manuscript writing. R.D.G.: data recording. R.T.: collection of root sample and essential oil extraction. S.V. and M.K.: GC-MS analysis and calculations. R.C.: implementation of standard agronomic practices in experiments. D.K.: evaluation of marker compounds and manuscript editing. A.K.: trial layout and manuscript editing. Satbeer S.: preparation of graphs and figures, monitoring and manuscript editing. Sanatsujat S.: conceptualization, planning, monitoring of experiment and manuscript editing.

Data availability statement

Data will be made available on request.

Conflict of interest

The authors declare that they have no conflict of interest.

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