Biological Forum – An International Journal 16(5): 64-69(2024)

ISSN No. (Print): 0975-1130
ISSN No. (Online): 2249-3239

Stability Analysis for Biomass and Essential Oil Content of Rose Scented Geranium Mutant Lines under Western Himalayan Condition

Romika Thakur[1], Ajay Kumar[1,2], Rahul Dev Gautam[1,2], Ramesh Chauhan[1,2], Ashok Kumar[1], Satbeer Singh[1] and Sanatsujat Singh[1,2]*

[1]*Agrotechnology Division, Council of Scientific and Industrial Research Institute of Himalayan Bioresource Technology, Palampur (Himachal Pradesh), India.*
[2]*Academy of Scientific and Innovative Research, Ghaziabad (Uttar Pradesh), India.*

(Corresponding author: Sanatsujat Singh)*
(Received: 02 March 2024; Revised: 24 March 2024; Accepted: 15 April 2024; Published: 15 May 2024)
(Published by Research Trend)

ABSTRACT: Rose scented geranium (*Pelargonium graveolens*) is one of the most important aromatic plants. Essential oil is rich in citronellol, geraniol and linalool and extensively used in perfumery and cosmetic industries. Eight mutant lines along with check variety Bourbon were tested among four different environments. Two factor analysis of variation revealed considerable significant variation due to genotype and G × E interaction for almost all the traits. The CSIR-IHBT-PG-05 has highest biomass (1195.08 g/plant) and essential oil content (0.20%). Eberhart and Russell model of stability analysis was performed to predict the significant influence of different environments on mutant lines which further revealed that line CSIR-IHBT-PG-05 has stable performance among the four environments studied. The present investigation provides a strong basis for identifying stable genotype and to carry out the process of selection and cultivar development in rose scented geranium.

Keywords: Biomass, Essential oil, Genotypes, Scented rose, Stability.

INTRODUCTION

Rose scented geranium (*Pelargonium graveolens*) is one of the most important aromatic plants that have originated from South Africa (Harish *et al*., 2018). The genera Pelargonium has 700 spp. and out of those rose scented geranium is commercially the most important (Shawl *et al*., 2006).The main constituents of its essential oils are geraniol and citronellol (Kaul and Rao 1999). In India, the two most important cultivars of geranium are Algerian and Bourbon (Ram *et al*., 2003). It is cultivated in different parts of India to obtain higher quality essential oil. Most of essential oil demands are fulfilled by countries like China, Arabian countries and Europe (Anonymous, 1996). Small quantity of biomass is produced from different states of India and rest of demand is fulfilled by import from foreign countries. It has antioxidant, antibacterial, insecticidal and antifungal properties (Farukh *et al*., 2014). It is also used to prevent blood clotting, dermis disorders, injury contamination, ulcers and diarrhoea (Harish *et al*., 2014). It is largely used in the fragrance, pharmaceutical and cosmetic manufacturer (Narayana *et al*., 1986; Rao, 2002). India has a great potential to increase its cultivated area due to the high price and demand for oil (Rao, 2015). In India, the biomass yield fluctuates between 300 and 500 quintals of biomass per hectare, and essential oil yield between 30 and 50kg of oil per hectare (Singh *et al*., 2000). The quantity of biomass and quality of essential oil is of greater importance in geranium. Therefore, there is need of successful breeding program for the development and improvement of these two important phenotypic traits *i.e.,* biomass and essential oil. The difference in essential oil constituents in different environment regions of India were studied by Bhaskaruni *et al*. (1990) and they observed that the biomass (essential oil) obtained from medium to the higher altitude climate site was richer in menthone, nerol citronellol, and geraniol, while the essential oil produced from plants that are cultivated at lower environment were richer in linalool and other compounds. It is vegetative propagated crop. Vegetative propagated crops contribute to greater variability on account of recessive mutations under different environment conditions (Shawl *et al*., 2006). Hamouda *et al*. (2009) observed that plants obtained from multi environment differed in essential oil constituents. Higher biomass yield has direct positive correlation with essential oil(Jain *et al*., 2001). Western Himalayan region mostly remains unexplored for its cultivation. Therefore, the present study was undertaken to study genotype × environment interactions and stability of superior mutant lines to access the performance of lines among multi-environments of Western Himalayas. The primary goal of this research work is to select the most promising stable genotype of geranium for biomass and essential oil yield suitable for cultivation in variable environments. Multiple environment evaluation approach was used to study the performance of geranium lines in terms of yield and quality. In this study, we present the multi-environment performance of genotypes for two years with respect to the biomass and essential oil in Western Himalayan region.

MATERIAL AND METHODS

Eight superior mutant lines developed through chemical mutagenesis and maintained at CSIR-IHBT, Palampur (Table 1) were investigated along with check variety Bourbon in the open field conditions at four different locations (Table 2). The experimental sites come under the sub-tropical low hills zone to temperate and dry temperate regions of Western Himalayas of Himachal Pradesh. The soil is silt clay loam, acidic in nature (pH ranged from 5.00 to 6.00) and enriched with available nitrogen phosphorus and potassium. The climate is generally sub humid to temperate, characterized by cold winters. The phenotypic data was observed on plant height (cm), plant spread, number of leaves, number of branches, biomass (g/plant) and essential oil (%). A total of 300 gram of biomass sample was used for essential oil extraction. Essential oil was obtained by using hydro distillation Clevenger apparatus operated for a period of 3 hours. The essential oil obtained in vials were dried by chemical sodium sulphate and stored at 4°C in a refrigerator until further analysis. The data were analyzed by two factor ANOVA and stability (Eberhart and Russell method) analysis. Two factors ANOVA was performed by exploiting dual components *i.e.,* environments and genotypes and the stability analysis by making use of Eberhart and Russell model (Eberhart and Russell 1966). All the analysis was performed by using software OPSTAT (Sheoran *et al.*, 1998).

Table 1: Details of genotypes used in the study.

Sr. No.	Genotype (Mutant lines)
1.	CSIR-IHBT-PG-01
2.	CSIR-IHBT-PG-02
3.	CSIR-IHBT-PG-03
4.	CSIR-IHBT-PG-04
5.	CSIR-IHBT-PG-05
6.	CSIR-IHBT-PG-06
7.	CSIR-IHBT-PG-07
8.	CSIR-IHBT-PG-08
9.	Bourbon

Table 2: Details of different locations, its average annual weather parameters and soil type.

Sr. No.	Parameters	Sihunta (Chamba)	Bajaura (Kullu)	Palampur (Kangra)	Bilaspur (Ghumarwin)
1.	Location code	Env-1	Env-2	Env-3	Env-4
2.	Agro-climatic Zone	Mid-hill sub humid	High-hill Temperate wet	Mid-hill sub-humid	Low hill sub tropical
3.	Altitude (m)	1410	1679	1219	597
4.	Average minimum temperature (°C)	6.67	8.9	11.83	19.7
5.	Average maximum temperature (°C)	26.85	21.5	21.70	34.9
6.	Total rainfall (mm)	1421	1306	1578	1112
7.	Relative humidity (%)	41.5	36.8	62.58	48.05
8.	Soil texture	Clay Loam	Silt loam	Silty clay loam	Alluvial
9.	Soil pH	6.12-6.78	6.42-6.96	5.23 -6.10	6.38-6.61
10.	Soil type	Slightly acidic	Acidic to Neutral	Acidic	Slightly acidic

RESULT AND DISCUSSION

A. Combined analysis of variance (ANOVA)

Combined analysis of variance for all traits is presented in Table 3. Environment, genotype and G×E interaction were found significant for number of branches, number of leaves, biomass yield and essential oil. Plant spread was found highly significant for genotype main effect and G × E interaction. Plant height has showed high significance for genotype main effect. The results showed that these traits have wider variation and indicating the greater effect of environment on all the quantitative traits. The significant genotype and environment interaction (G × E) indicated that genotypes behave distinctly in diverse environments. Therefore, genotypes should be examined in multi-environments to facilitate the selection of stable genotype and release of new varieties commercially. However, combined analysis of variance was found highly significant for economic traits such as biomass and essential oil which further allow stability analysis of these economic traits. The results of the combined analysis were found to be in line with (Abd El-Salam *et al.*, 2010). They observed a significant mean square for number of branches, and biomass in geranium due to the environment, which directly indicates that the environment has a great influence on the yield of all traits. The influence of environment, genotype, and (G × E) were observed for traits such as biomass and essential oil of geranium by El-tahawey and associates (2020) and they reported that variations were present in all traits. These results indicated that genotypes behave distinctively in multiple environments and stability analysis should be performed to identify the most stable genotypes from all environments. All the above mentioned previous reports match our current research study.

Table 3: Combined analysis of variance (2020-2021) for the yield and related traits.

Source of Variation	Mean Sum of squares						
	Degree of freedom	Plant height (cm)	No. of leaves	No. of branches	Plant spread	Biomass yield (g/plant)	Essential oil (%)
Replication	2						
Environment(E)	3	11.88	136.95*	130.43*	14.92	106764.23*	0.13
Genotypes (G)	8	47.97*	168.94*	158.26*	34.13*	432283.46*	0.02*
G × E	24	22.09	34.76*	16.13*	20.22*	47522.33*	$2.0e^{4}$*
Error	70	16.71	17.31	4.69	7.86	115.09	$8.5e^{5}$

*significant at $p \leq 0.05$

B. Mean performance of genotypes and environments

Mean comparison of genotypes and environments was performed and presented in Table 4 and Fig. 1. The environment Env-1 and Env-4 have showed maximum mean performance. Env-1 has showed maximum significant mean performance for the traits biomass. The results of mean performance among environments revealed that Env-1 is most suitable site for geranium cultivation followed by other environments. The variation in mean performance of traits among environments might be due to different climatic conditions and soil factors (Salamon, 2017). On the basis of overall mean performance of genotype, the genotype CSIR-IHBT-PG-05 has showed maximum mean value for traits like number of leaves, number of branches, plant spread, biomass and essential oil content. Therefore, genotype CSIR-IHBT-PG-05 could be further used in breeding improvement programs of geranium. The variation in traits like biomass and essential oil content in geranium genotypes was mainly due to different climatic conditions of locations (El-tahawey *et al*., 2020; Naragund and Divakar 1983). Earlier the same model of mean comparison of genotypes and environments for different traits was followed in geranium by Harish and co-workers (2018) and they revealed environment influence on genotypes. All the above-mentioned earlier reports confirm our present research findings. A similar strategy of mean comparison between genotypes and environments for traits like crucial oil and biomass was followed in palmarosa (Pareek and Maheshwari 1990), and vetiver (Patra *et al*., 2000).

Table 4: Mean comparison of genotypes and environments.

Genotype/ Environment	Plant height (cm)	Number of leaves	Number of branches	Plant spread	Biomass yield (g/plant)	Essential oil content (%)
CSIR-IHBT-PG-01	79.583	79.38	53.58	80.88	915.75	0.16
CSIR -IHBT-PG-02	79.292	80.58	55.58	81.17	846.79	0.14
CSIR-IHBT-PG-03	78.542	75.54	56.25	80.75	535.00	0.08
CSIR-IHBT-PG-04	78.958	79.08	56.13	78.71	1028.92	0.12
CSIR-IHBT-PG-05	81.375	88.13	65.88	84.21	1195.08	0.20
CSIR-IHBT-PG-06	79.292	80.63	55.46	79.83	712.54	0.06
CSIR-IHBT-PG-07	74.708	81.83	54.46	79.17	921.50	0.11
CSIR-IHBT-PG-08	80.083	75.83	56.08	79.83	856.63	0.16
Bourbon	81.583	77.96	54.88	82.25	746.13	0.10
Env-1	78.31	76.78	53.82	80.09	955.48	0.13
Env-2	79.35	79.82	59.02	80.13	821.80	0.12
Env-3	79.59	80.96	57.22	81.41	842.37	0.13
Env-4	79.81	81.98	55.85	81.39	828.50	0.12
Overall Mean	79.27	79.88	56.48	80.75	862.04	0.13
CD (environments)	NA	2.26	1.18	0.00	5.84	NA
CD (genotypes)	3.33	3.40	1.77	2.29	8.75	0.01

CD-critical difference at $p \leq 0.05$

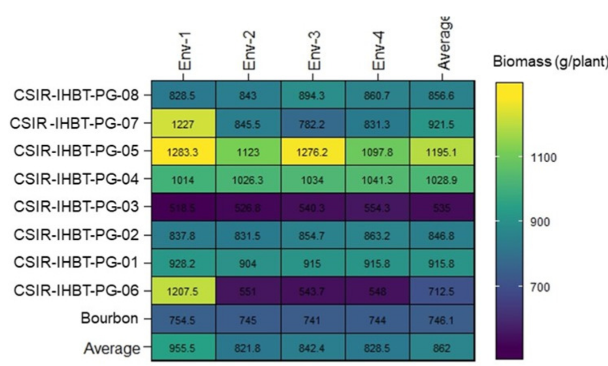

Fig. 1. Biplots of genotype over environment performances for biomass yield.

C. Stability analysis Eberhart and Russell model

The biomass and essential oil are economically most important traits in rose scented geranium. The response of variable environment for genotypes can be explored by using stability parameters (Lal *et al*., 2021). The different stability parameters of Eberhart and Russell model related to biomass and essential oil are computed and presented in Table 5. The two important stability parameters like regression coefficient and deviation from regression coefficient were used to identify adaptable genotypes among multiple environments. The bi value of distinct genotypes for biomass ranged from -0.15 to 5.18. Among all the nine accessions, the genotype CSIR-IHBT-PG-05 had the highest mean performance, zero divergence and unity regression coefficient. Therefore, this genotype has revealed stable response in multiple environments. The mean performance of CSIR-IHBT-PG-06 and CSIR-IHBT-PG-07 genotypes is above average, the regression coefficient is greater than unity (bi>1) and the mean deviation was zero (sd=0), which showed that these genotypes are considered suitable only for favourable environments. Six genotype CSIR-IHBT-PG-01, CSIR-IHBT-PG-02 CSIR-IHBT-PG-03 CSIR-IHBT-PG-04, CSIR-IHBT-PG-08 and Bourbon have showed above average mean performance, regression coefficient less than one (bi<1) and mean deviation equal to zero (sd=0), which revealed that these genotypes were considered best for adverse and stressful environment. Overall, genotype CSIR-IHBT-PG-05 has revealed superior and stable performance in different environment and it could be used as cultivars for commercial cultivation in Western Himalayan conditions.

Table 5: Stability parameters of biomass and essential oil by using Eberhart and Russell model.

Genotype	Biomass (g/plant)				Essential oil (%)			
	Mean	bi	S^2di	SR	Mean	bi	S^2di	SR
CSIR-IHBT-PG-01	915.75	0.14	0.01	U	0.16	1.80	2.51	F
CSIR-IHBT-PG-02	846.79	-0.07	0.05	U	0.14	2.56	1.83	F
CSIR-IHBT-PG-03	535.00	-0.15	0.04	U	0.08	-1.51	3.99	U
CSIR-IHBT-PG-04	1028.92	-0.14	0.01	U	0.12	-2.38	0.08	U
CSIR-IHBT-PG-05	1195.08	1.05	0.44	S	0.20	1.05	5.18	S
CSIR-IHBT-PG-06	712.54	5.18	0.64	F	0.06	7.43	116.17	F
CSIR-IHBT-PG-07	921.50	3.20	0.39	F	0.11	3.31	53.32	F
CSIR-IHBT-PG-08	856.63	-0.29	0.12	U	0.16	-0.70	7.88	U
Bourbon	746.13	0.09	0.00	U	0.10	-2.56	22.74	U

bi, regression coefficient; S^2di deviation from regression; S*R*, stability responses (*F*: favourable environment; *U*: unsuitable for favourable environment; suitable for stressful environment and *S*, stable in multi environments)

The regression coefficient of essential oil was ranged from -0.70 to 7.43. Out of all genotypes, only one genotype CSIR-IHBT-PG-05 had high mean performance, unity regression coefficient and zero divergence. Therefore, the results of stability parameters for genotype CSIR-IHBT-PG-05 have revealed that this genotype is stable for essential oil in multi environments. Four genotypes CSIR-IHBT-PG-01, CSIR-IHBT-PG-02, CSIR-IHBT-PG-06 and CSIR-IHBT-PG-07 have above average mean performance, regression coefficient is greater than unity (bi>1) along with zero mean deviation (sd = 0), which showed that these genotypes are considered suitable only for favourable environment. The genotypes CSIR-IHBT-PG-03, CSIR-IHBT-PG-04, CSIR-IHBT-PG-06 and CSIR-IHBT-PG-07 have shown best performance for unfavourable environments as these genotypes have above average mean performance, regression coefficient less than one (bi<1) and mean deviation equal to zero (sd=0). Therefore, the results of stability parameters for essential oil revealed that genotype CSIR-IHBT-PG-05 has showed stable performance among multi environments and could be used for commercial cultivation in western Himalayas. The results of stability analysis were found in accordance with (El-tahawey *et al*., 2020) where the stability parameters for economic traits like fresh inflorescence and essential oil have been reported based on Eberhart and Russell model. A stability analysis of biomass and essential oil was performed by Harish *et al*. (2018) and they revealed the most stable genotype from multiple environments in geranium. A multi-location investigation was carried out by Bhaskar and co-workers (1998) and they found that larger differences exist in biomass yield and essential oil content of different genotypes of geranium and indicated that genotypes behave differently in various environments but some genotypes were able to perform better and stable in all environments due to their genetic makeup. Mutant lines of rose scented geranium were evaluated in northern plains and showed that the mutant lines resulted in higher biomass yield and essential oil as compared to parental lines (Saxena and Rahman 2008). Thus, it is clear from this study that the genetic makeup of the genotype is responsible for higher biomass yield and essential oil. A multilocation breeding approach was adopted for the selection of mutant lines superior to the parental lines in biomass and essential oil traits. The superior mutant lines showed better adaptability to different areas of cultivation (Saxena *et al*., 2000; Saxena and Rahman 2008). These above mentioned results are as per our present research work. Similar methodology for stability analysis has earlier been successfully utilized in German chamomile (Shakya *et al*., 2023), lavender (Gupta *et al*., 2023), basil (Panwar *et al*., 2011), and in legume crops (Mondal *et al*., 2011; Salgotra *et al*., 2020).

CONCLUSIONS

The primary goal of this study was to identify stable genotype over multi-environments and for that two factor ANOVA and stability analysis had been performed. The traits with significant environment main effect, genotypic main effect and genotype × environment interaction were further allowed for stability analysis. On the basis of stability parameters, the most stable genotype was selected from multi environments. It was concluded that genotype CSIR-IHBT-PG-05 has superior and stable performance for biomass and essential oil over all environment.

FUTURE SCOPE

It is clear from our study that genotype CSIR-IHBT-PG-05 will improve the biomass yield and essential oil content in geranium over different environments. Higher economic yield and adaptability will directly enhance the commercialization of geranium and will improve the economy of farmers. It will also enhance the livelihood of smallholder farmers which lead to greater benefits for future generations.

Acknowledgement. The authors are grateful to the Director of CSIR-IHBT Palampur India for his unwavering support and providing necessary facilities required for this work. The authors acknowledge the Council of Scientific and Industrial Research (CSIR) Government of India for financial support. This research work has been undertaken under the CSIR network project HCP-0007. The CSIR-IHBT communication number of this paper is 5584.

Conflict of Interest. None.

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How to cite this article: Romika Thakur, Ajay Kumar, Rahul Dev Gautam, Ramesh Chauhan, Ashok Kumar, Satbeer Singh and Sanatsujat Singh (2024). Stability Analysis for Biomass and Essential Oil Content of Rose Scented Geranium Mutant Lines under Western Himalayan Condition. *Biological Forum – An International Journal, 16*(5): 64-69.