

Phenotypic diversity in *Stevia rebaudiana* Bertoni in Doon valley ecosystem

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Abstract

Genetic variation in sixteen morphometric traits associated with thirty six accessions of *Stevia rebaudiana* was examined for selection of higher herb yielding accessions. ANOVA exhibited highly significant ($p < 0.01$) differences between thirteen traits. Correlation analysis resulted highest significant positive interaction between fresh branch weight-fresh stem weight (0.955**) and highest significant negative interaction between fresh stem weight- leaf/stem (dry weight wise) ratio (-0.484**). The most economic character fresh herb weight/plant exhibited high significant correlation between number of branches (0.818**). The path coefficient under study revealed that the highest direct contribution to total fresh herb weight/plant was made by number of branches (0.756). Indirect contribution of spread of plant via number of branches (0.595) for fresh herb weight/plant was large. Therefore, the traits like number of branches, fresh stem weight, dry weight of branch and dry herb weight/plant may be used as a better selection criterion for improvement of fresh herb weight/plant.

Keywords: ANOVA, correlation, genetic variation, path analysis, *Stevia rebaudiana*.

Introduction

Stevia rebaudiana Bertoni ($2n=22$), a promising medicinal and aromatic perennial crop from Paraguay (Lewis, 1992) [5] belonging to Asteraceae became increasingly popular within industrial and scientific vistas in the last 30 years, representing a suitable nutritional alternative to sucrose and artificial sweeteners. Stevia (Figure 1) has food and pharmaceutical applications; however, the main agronomic and industrial interest lies in the accumulation of steviol glycosides in leaves which are 300 times sweeter to sucrose (Bridel & Lavieille, 1931) [2]. Two steviol glycosides are mainly found in leaves – stevioside and rebaudioside A (Ceunen & Geuns, 2013) [3]. The leaves (Figure 2) therefore are the most valuable part of the crop (Angelini *et al.*, 2018) [1]. Bioactive compounds like phenolics, flavonoids, vitamins, polyphenols, and carotenoids are also present which are useful for production of nutraceuticals and functional foods.



Fig 1: *Stevia rebaudiana* in vegetative growth phase.

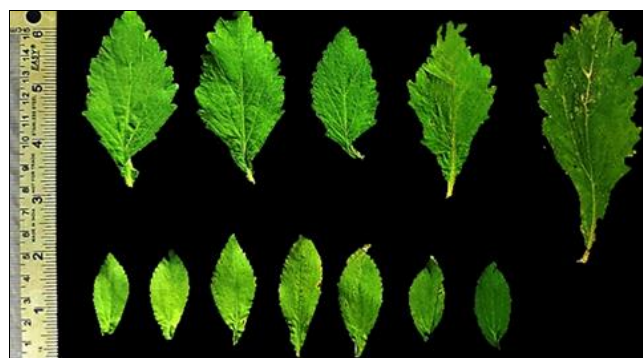


Fig 2: Variation in leaves of *Stevia rebaudiana*.

Diabetes in India has risen from 7.1% in 2009 to 8.9% in 2019. India currently ranks second in the world after China in the global diabetes epidemic registering 77 million people suffering with this disease. Among these, 12.1 million belong to senior citizen group (aged >60 years), which is assumed to elevate to 27.5 million by 2045 (Pradeepa & Mohan, 2021) [6]. In such condition, it is quite evident that intake of sugar will definitely not be in the diet plan for patients who are diabetic. However, due to age old habit and practice it will also be almost impossible to cut off sugar intake from the diet, so, artificial sweeteners are suggested and prescribed which in the long run may lead to side-effects. Here, the herbal alternative as Stevia can play a vital role in preserving the sweetness in diet plan as well as consumption of it will have minimum or no such side-effects in diabetic patients.

The increasing number of scientific publications regarding Stevia related to plant sciences, agronomy and food science technology since 2010 exhibit its importance towards alternative medicine. There is an urgent need to elaborate the studies in phenotypic diversity coupled with genetics for the genetic improvement of Stevia. The present study evaluated the morphometric performances of thirty-six accessions of Stevia collected from Doon valley, propagated in open field conditions under the climatic conditions of

Dehradun, Uttarakhand, INDIA (Figure1). A total of sixteen traits (Table 1) were used to describe this plant. The findings depicted high variability among the accessions in terms of morphometric traits that are a prerequisite for any crop improvement (varietal development) program.

Materials and Methods

1. Plant material and field experiment

Thirty six accessions of *Stevia rebaudiana* used in this study were collected from the Doon valley of Uttarakhand and were planted in the experimental blocks 50cm×50cm p-p and r-r distance at Centre for Aromatic Plants (CAP), Selaqui Industrial Area, Uttarakhand – 248011, INDIA. Normal agricultural practices were performed all-round the year and the data for morphometric observations were recorded just before flowering.

The experimental area is located at N30.21.812' latitude and E77.51' longitude having an altitude 487m at the foot of the Himalayas. During summers, the temperature ranges between 36°C and 16.7°C and in winters, the temperature lies between 23.4°C and 5.2°C with an average rainfall of 2073.3mm annually. Maximum rainfall occurs between

June and September; however, in December and January the region also receives winter rainfall.

2. Statistical analysis

The mean data for sixteen traits of thirty-six treatments were offered to statistical analysis using statistical software ver. 0.3 based on (Singh & Choudhary, 1979; Panse and Sukhatme, 1967; Dewey & Lu, 1959) ^[7, 13, 4] for ANOVA, correlation and path analysis.

Results and discussion.

Variation among the mean data of thirty-six diverse genetic stocks were highly significant ($p < 0.01$) for the thirteen traits examined (Table 1 and 2). The meticulous study for analysis of variance, revealed highly significant differences among the genotypes for plant height, number of branches, spread of plant, number of leaves/branch, fresh branch weight, fresh leaves weight/branch, fresh stem weight, dry weight of stem, dry weight of branch, number of nodes/branch, length of internode/branch, dry herb weight/plant, fresh herb weight/plant indicating existence of considerable genetic variability among the genotypes (Tables 3).

Table 1: Selection of traits and description.

Sl. No.	Traits	Description	Code
1.	Plant height(cm)	The length of the plant from soil surface to the tip of the shoot.	CP1
2.	Number of branches/plant	Total number of branches in the plant.	CP2
3.	Spread of plant(cm ²)	Maximum herb spread of the plant.	CP3
4.	Number of leaves/branch	Count of the individual leaves present in a branch.	CP4
5.	Fresh branch weight(g)	Weight of the branch containing leaves and stem in undried condition.	CP5
6.	Fresh leaves weight/branch(g)	Weight of the undried leaves without the stem present in a branch.	CP6
7.	Fresh stem weight(g)	Weight of the undried stem without leaves present in a branch.	CP7
8.	Leaf/stem (fresh weight wise) ratio	Ratio of the leaf weight and stem weight in undried condition.	CP8
9.	Dry weight of leaves(g)	Weight of the leaves without the stem present in a branch in dried condition.	CP9
10.	Dry weight of stem(g)	Weight of the stem without the leaves present in a branch in dried condition.	CP10
11.	Dry weight of branch(g)	Weight of the branch containing leaves and stem in dried condition.	CP11
12.	Leaf/stem (dry weight wise) ratio	Ratio of the leaf weight and stem weight in dried condition.	CP12
13.	Number of nodes/branch	Count of the nodes present in a branch.	CP13
14.	Length of internode/branch(cm)	Length of the two adjoining nodes present in the same branch.	CP14
15.	Dry herb weight/plant(g)	Weight of the green part of the plant in dried condition.	CP15
16.	Fresh herb weight/plant(g)	Weight of the green part of the plant in undried condition.	CP16

Table 2: Specific qualitative description of *Stevia* accessions

Accessions	Description
SC1	Leaves –light green colour, medium-large size, medium fringed margins.
SC2	Leaves – small size, medium fringed margins; Plant – short height; Stem hairy.
SC3	Leaves – bright green colour, less fringed margins, small size; Plant – short height; Stem very less hairy.
SC4	Leaves – light green colour, more fringed margins, medium-large size; Stem hairy. Opposite (cyclic) arrangement of leaves.
SC5	Plant –tall; Leaves – light green colour, broader lamina; Stem more hairy.
SC6	Leaves – light green colour, more fringed margins, medium-large size; bushy appearance.
SC7	Plant –tall, spreading, bushy, more branches; Leaves – light green colour, more fringed margins, medium-large size.
SC8	Plant –tall, spreading, bushy, more branches; Leaves – light green colour, less fringed margins, small-medium-large size.
SC9	Plant –tall; Leaves – longer than wide, light green colour; Stem hairy.
SC10	Plant –tall, bushy, spreading, more branches; Leaves – medium-large size, light green colour; Stem hairy.
SC11	Leaves – small, light green colour, more fringed margins; Stem less hairy.
SC12	Leaf – extra broad, more fringed margins, light green colour; Stem hairy.
SC13	Leaf – extra broad and long, light green colour, more fringed margins; Stem very less hairy; Shoot apex turned yellow.
SC14	Plant –tall, more branches; Leaves – dark green colour, small size; Stem hairy; Opposite (cyclic) arrangement of leaves.
SC15	Plant –apical part turned yellow; Leaves –medium-large size; Stem hairy.
SC16	Plant –tall; Leaves – medium fringed, dark green colour; Stem hairy.
SC17	Plant –tall; Leaves – small size, rosette arrangement.
SC18	Leaves- extra long, extra broad,opposite (cyclic) arrangement, dark green colour, less fringed margins; Stem hairy.
SC19	Plant –tall, bushy; Leaves- extra long, dark green colour, less fringed margins; Stem hairy.
SC20	Plant –tall, bushy; Leaves- extra long, dark green colour, less fringed margins; Stem hairy.

SC21	Plant – bushy, spreading, more branches; Leaves- medium –large size, light green colour, medium fringed margins; Stem hairy.
SC22	Plant – bushy, more branches; Leaves- very dark green coloured, opposite (cyclic) arrangement, more fringed margins, Stem-hairy.
SC23	Leaves- extra broad, extra long, rosette appearance from top view, Stem –hairy.
SC24	Plant – spreading; Leaves – light green colour, broad, medium fringed leaves; Stem – hairy.
SC25	Plant – tall, spreading; Leaves –dark green colour, less fringed margins; Stem – less hairy.
SC26	Leaves –dark green colour, medium fringed margins, small-medium size.
SC27	Plant – bushy; Leaves –dark green colour, less fringed margins, small-medium size; Stem – less hairy.
SC28	Plant – bushy; Leaves –some dark green colour other light green, less fringed margins, small-medium size; Stem – less hairy.
SC29	Leaves- extra long leaves, dark green coloured, opposite (cyclic) arrangement.
SC30	Plant – Tall, bushy, spreading, more branches; Leaves- medium –large size, light green colour, medium fringed margins; Stem hairy.
SC31	Leaves- extra long leaves, light green coloured, less fringed margins; Stem –hairy.
SC32	Leaves- extra long leaves, less fringed margins; Stem –very hairy.
SC33	Leaves- extra long leaves, less fringed margins; Stem –very hairy.
SC34	Leaves- dark green colour, opposite (cyclic) arrangement, medium-large size, medium fringed leaf margins; Stem – less hairy.
SC35	Leaves – light green colour, opposite (cyclic) arrangement, medium fringed margins; Stem – less hairy.
SC36	Plant – tall; Leaves- medium size, opposite (cyclic) arrangement, less fringed margins, light green colour;

Table 3: Analysis of variance for various morphological traits of *Stevia rebaudiana* for sixteen traits in thirty six accessions.

Source of Variation	d.o.f	CP1	CP2	CP3	CP4	CP5	CP6	CP7	CP8	CP9	CP10	CP11	CP12	CP13	CP14	CP15	CP16
Replications (MSS)	2	13.12	16.26	82.53	63.82	1.24	2.29	0.38	0.44	0.16	0.01	0.63	0.40	10.86	0.09	487.63	30728.46
Treatments (MSS)	35	236.72**	6217.51**	4084.53**	1463.55**	93.03**	26.00**	17.56**	0.28	1.42	4.16**	10.02**	0.50	20.69**	5.20**	13339.73**	268967.48**
Error (MSS)	70	30.28	9.18	24.79	10.33	0.81	0.51	0.23	0.05	0.05	0.04	0.11	0.02	0.57	0.14	495.23	20478.02

** = $p < 0.01$, respectively.

Where, CP1-Plant height (cm), CP2-Number of branches, CP3- Spread of plant(cm^2), CP4- Number of leaves/branch, CP5- Fresh branch weight(g), CP6- Fresh leaves weight/branch (g), CP7- Fresh stem weight(g), CP8- Leaf/stem (fresh weight wise) ratio, CP9- Dry weight of leaves(g), CP10- Dry weight of stem(g), CP11- Dry weight of branch(g), CP12- Leaf/stem (dry weight wise) ratio, CP13- Number of nodes/branch, CP14- Length of internode/branch(cm), CP15- Dry herb weight/plant(g), CP16- Fresh herb weight/plant(g).

The correlation study (Table 4) provided relevant information about the nature and magnitude of association of different component characters. The highest significant positive interaction was represented between fresh branch weight-fresh stem weight(0.955**), followed by higher significant positive interactions between fresh branch weight-fresh leaves weight/branch (0.948**), dry weight of stem-dry weight of branch(0.942**), dry weight of leaves-dry weight of branch(0.913**), followed by high significant positive interactions between fresh leaves weight/branch-fresh stem weight(0.895**), fresh stem weight-dry weight of branch (0.886**), fresh branch weight/dry weight of branch (0.889**), fresh leaves weight/branch-dry weight of

branch (0.856**), followed by significant positive interactions between number of branches-spread of plant (0.788**), number of branches-dry herb weight/plant (0.760**), spread of plant-dry herb weight/plant (0.692**), spread of plant-fresh herb weight/plant (0.692**). The most economic character fresh herb weight/plant exhibited high significant correlation between number of branches (0.818**), dry herb weight/plant (0.747**), spread of plant (0.692**), length of internode/branch (0.430**) and plant height (0.392**) whereas negative significant correlation between leaf/stem (dry weight wise) ratio (-0.273**) and leaf/stem (fresh weight wise) ratio (-0.249**). The correlation study also represented highest significant negative interaction between fresh stem weight- leaf/stem (dry weight wise) ratio (-0.484**), followed by fresh branch weight- leaf/stem (dry weight wise) ratio (-0.460**) and dry weight of stem- leaf/stem (dry weight wise) ratio (-0.459**). Similar observations were also recorded in *Plantago ovata* by Sarkar and Lal, 2015 [8]; in *Lavandula angustifolia* by Sarkar, 2020 [9]; in *Cymbopogon* sp by Sarkar et al, 2021 [11]; in *Cymbopogon jwarancusa* by Sarkar, 2022 [10]; in *Lavandula angustifolia* by Sarkar and Sharma, 2022 [12].

Table 4: Phenotypic correlation coefficients among fresh herb weight per plant and attributing morphological traits in *Stevia rebaudiana*.

	CP1	CP2	CP3	CP4	CP5	CP6	CP7	CP8	CP9	CP10	CP11	CP12	CP13	CP14	CP15	CP16
CP1	1															
CP2	0.268**	1														
CP3	0.513**	0.788**	1													
CP4	0.084	-0.326**	-0.129	1												
CP5	0.209*	-0.311**	-0.081	0.718**	1											
CP6	0.204*	-0.319**	-0.119	0.685**	0.948**	1										
CP7	0.280**	-0.315**	-0.054	0.676**	0.955**	0.895**	1									
CP8	-0.365**	-0.231*	-0.420**	-0.079	-0.198*	-0.096	-0.277**	1								
CP9	0.183	-0.288**	-0.072	0.609**	0.842**	0.818**	0.829**	-0.131	1							
CP10	0.099	-0.310**	-0.142	0.538**	0.844**	0.784**	0.867**	-0.200*	0.813**	1						
CP11	0.108	-0.340**	-0.139	0.623**	0.889**	0.856**	0.886**	-0.166	0.913**	0.942**	1					

CP12	-0.300**	-0.108	-0.271**	-0.295**	-0.460**	-0.380**	-0.484**	0.622**	-0.210*	-0.459**	-0.392**	1				
CP13	-0.022	-0.336**	-0.031	0.441**	0.454**	0.381**	0.454**	-0.194*	0.499**	0.474**	0.509**	-0.329**	1			
CP14	0.441**	0.502**	0.515**	-0.303**	-0.323**	-0.350**	-0.242*	-0.239*	-0.397**	-0.308**	-0.373**	-0.118	-0.402**	1		
CP15	0.341**	0.760**	0.692**	-0.048	0.028	-0.01	0.043	-0.348**	0.148	0.051	0.07	-0.252**	0.034	0.297**	1	
CP16	0.392**	0.818**	0.692**	-0.065	-0.004	-0.013	0.021	-0.249**	-0.074	-0.075	-0.08	-0.273**	-0.203*	0.430**	0.747**	1

* = $p < 0.05$; ** = $p < 0.01$, respectively.

Where: CP1-Plant height (cm), CP2-Number of branches, CP3- Spread of plant(cm^2), CP4- Number of leaves/branch, CP5- Fresh branch weight(g), CP6- Fresh leaves weight/branch (g), CP7- Fresh stem weight(g), CP8- Leaf/stem (fresh weight wise) ratio, CP9- Dry weight of leaves(g), CP10- Dry weight of stem(g), CP11- Dry weight of branch(g), CP12- Leaf/stem (dry weight wise) ratio, CP13- Number of nodes/branch, CP14- Length of internode/branch(cm), CP15- Dry herb weight/plant(g), CP16- Fresh herb weight/plant(g).

The path coefficient (Figure 3) under study (Table 5) revealed that the highest direct contribution to total oil yield was made by number of branches (0.756) followed by fresh stem weight (0.679), dry weight of branch (0.321) and dry herb weight/plant (0.28). Direct contribution of dry weight

of leaves and dry weight of stem to fresh herb weight/plant was negative but the indirect contribution was large via number of branches as well as through length of internode/branch, although residual effect was 0.18251. Indirect contribution of spread of plant via number of branches (0.595) for fresh herb weight/plant was large. Indirect contribution of dry weight of leaves (0.563), dry weight of stem(0.589) and dry weight of branch(0.602) via fresh stem weight for fresh herb weight/plant was also large. Therefore, the choice of most economic traits, number of branches, fresh stem weight, dry weight of branch and dry herb weight/plant may be used as a better selection criterion for improvement of fresh herb weight/plant. Similar observations were also recorded in *Plantago ovata* by Sarkar and Lal, 2015 [8]; in *Cymbopogon* sp by Sarkar *et al*, 2021 [11].

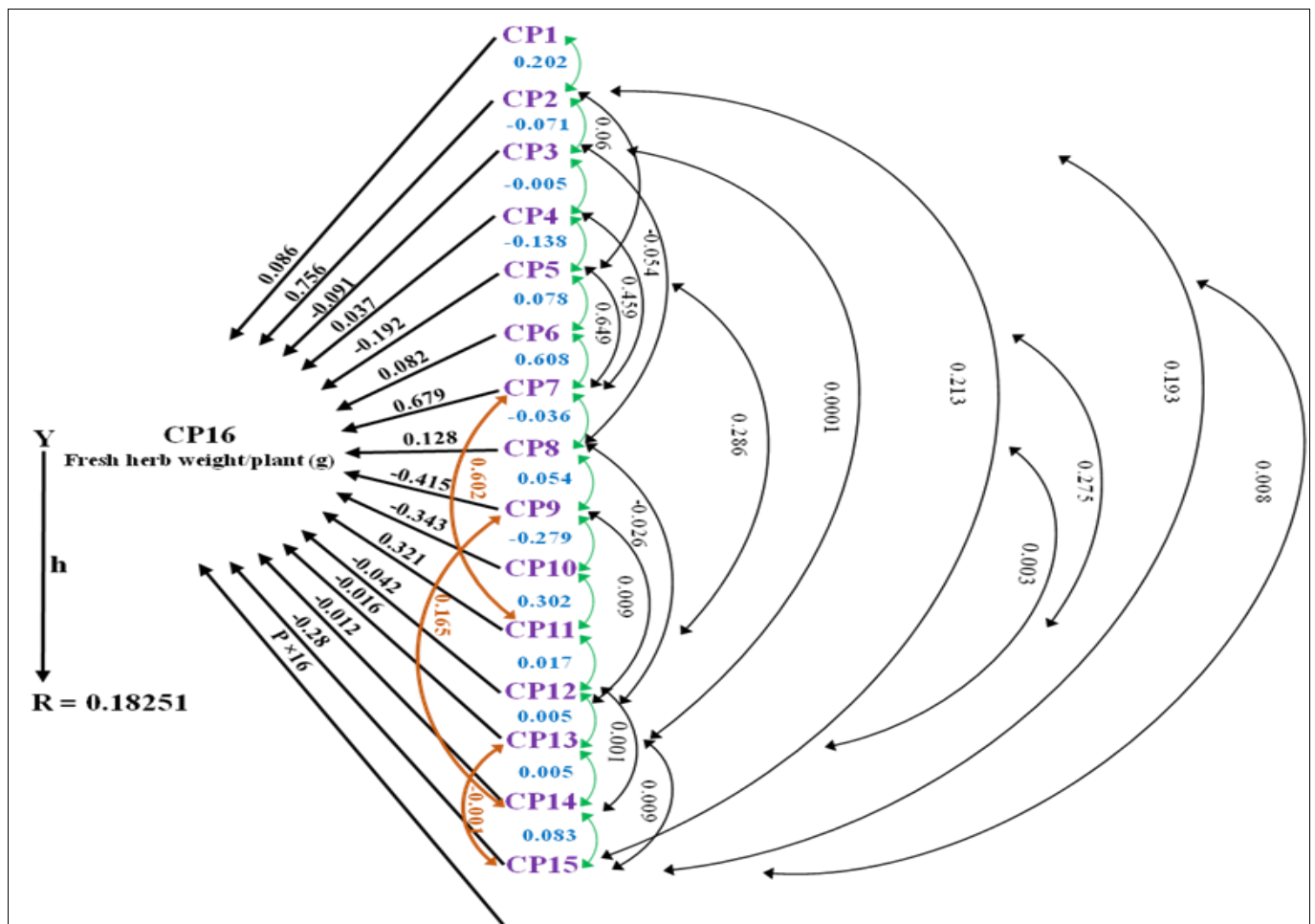


Fig 3: Path diagram in relation to sixteen traits of thirty six accessions of *Stevia rebaudiana*.

Where, CP1-Plant height (cm), CP2-Number of branches, CP3- Spread of plant(cm^2), CP4- Number of leaves/branch, CP5- Fresh branch weight(g), CP6- Fresh leaves weight/branch (g), CP7- Fresh stem weight(g), CP8- Leaf/stem (fresh weight wise) ratio, CP9- Dry weight of

leaves(g), CP10- Dry weight of stem(g), CP11- Dry weight of branch(g), CP12- Leaf/stem (dry weight wise) ratio, CP13- Number of nodes/branch, CP14- Length of internode/branch(cm), CP15- Dry herb weight/plant(g), CP16- Fresh herb weight/plant(g).

Table 5: Phenotypic path matrix reflecting direct (in bold) and indirect effects of morphological traits on fresh herb weight per plant in *Stevia rebaudiana*.

	CP1	CP2	CP3	CP4	CP5	CP6	CP7	CP8	CP9	CP10	CP11	CP12	CP13	CP14	CP15	Correlation with fresh herb weight/ plant(CP16)
CP1	0.086	0.202	-0.046	0.003	-0.04	0.017	0.19	-0.047	-0.076	-0.034	0.035	0.013	0.0001	-0.005	0.095	0.392
CP2	0.023	0.756	-0.071	-0.012	0.06	-0.026	-0.214	-0.03	0.119	0.106	-0.109	0.005	0.005	-0.006	0.213	0.818
CP3	0.044	0.595	-0.091	-0.005	0.016	-0.01	-0.037	-0.054	0.03	0.049	-0.045	0.012	0.0001	-0.006	0.193	0.692
CP4	0.007	-0.247	0.012	0.037	-0.138	0.056	0.459	-0.01	-0.253	-0.185	0.2	0.013	-0.007	0.004	-0.013	-0.065
CP5	0.018	-0.235	0.007	0.027	-0.192	0.078	0.649	-0.025	-0.349	-0.29	0.286	0.02	-0.007	0.004	0.008	-0.004
CP6	0.018	-0.241	0.011	0.025	-0.182	0.082	0.608	-0.012	-0.339	-0.269	0.275	0.016	-0.006	0.004	-0.003	-0.013
CP7	0.024	-0.238	0.005	0.025	-0.184	0.073	0.679	-0.036	-0.344	-0.298	0.284	0.021	-0.007	0.003	0.012	0.021
CP8	-0.031	-0.174	0.038	-0.003	0.038	-0.008	-0.188	0.128	0.054	0.069	-0.053	-0.026	0.003	0.003	-0.097	-0.249
CP9	0.016	-0.217	0.006	0.023	-0.162	0.067	0.563	-0.017	-0.415	-0.279	0.293	0.009	-0.008	0.005	0.041	-0.074
CP10	0.009	-0.234	0.013	0.02	-0.162	0.064	0.589	-0.026	-0.337	-0.343	0.302	0.019	-0.008	0.004	0.014	-0.075
CP11	0.009	-0.257	0.013	0.023	-0.171	0.07	0.602	-0.021	-0.379	-0.323	0.321	0.017	-0.008	0.005	0.02	-0.08
CP12	-0.026	-0.082	0.025	-0.011	0.088	-0.031	-0.329	0.08	0.087	0.157	-0.126	-0.042	0.005	0.001	-0.071	-0.273
CP13	-0.002	-0.254	0.003	0.016	-0.087	0.031	0.309	-0.025	-0.207	-0.163	0.164	0.014	-0.016	0.005	0.009	-0.203
CP14	0.038	0.379	-0.047	-0.011	0.062	-0.029	-0.165	-0.031	0.165	0.106	-0.12	0.005	0.006	-0.012	0.083	0.43
CP15	0.029	0.575	-0.063	-0.002	-0.005	-0.001	0.029	-0.045	-0.061	-0.018	0.022	0.011	-0.001	-0.004	0.28	0.747

Residual effect = 0.18251

Where: CP1-Plant height (cm), CP2-Number of branches, CP3- Spread of plant(cm²), CP4- Number of leaves/branch, CP5- Fresh branch weight(g), CP6- Fresh leaves weight/branch (g), CP7- Fresh stem weight(g), CP8- Leaf/stem (fresh weight wise) ratio, CP9- Dry weight of leaves(g), CP10- Dry weight of stem(g), CP11- Dry weight of branch(g), CP12- Leaf/stem (dry weight wise) ratio, CP13- Number of nodes/branch, CP14- Length of internode/branch(cm), CP15- Dry herb weight/plant(g), CP16- Fresh herb weight/plant(g).

Conclusion

Significant amount of variation existed among the thirty-six diverse genetic stocks for the thirteen traits that were examined in this study. At the phenotypic level, significantly positive association of fresh herb yield/plant with number of branches/plant (0.818) were recorded, however, in negative direction significant correlation was observed with leaf/stem (dry weight wise) ratio (-0.273). Phenotypic path coefficients established that highest positive direct effect on fresh herb weight/plant was by number of branches/plant (0.756) whereas highest negative direct effect was shown by dry weight of leaves (-0.415). The estimates of the residual effect were 0.18251, indicating the presence of some other minor factors that had not been considered in the present study and need to be included in the analysis to bring the desired improvement in fresh herb weight/plant.

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