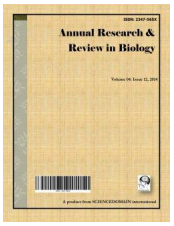

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Impact of Altitude on Morphological Traits-based Phenotypic Variability in *Bidens pilosa* L. from Three Natural Regions of Burundi

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Authors' contributions

This work was carried out in collaboration between all authors. Author FN designed the study, performed the statistical analysis, wrote the protocol and first draft of the manuscript. Authors LM and EM managed the analyses of the study. Author FN managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To examine the possible influence of varying altitudes on the morphological traits-based phenotypic variability in *Bidens pilosa* L.
Study Design: Randomly selected plants from each natural region.
Place and Duration of Study: Three natural regions of Burundi, i.e. IMBO (842 m), KIRIMIRO (1645 m), and MUGAMBA (2075 m), between November 2015 and May 2016.
Methodology: We randomly selected 36 plants (12 plants from each of the three sites of seed sowing and plant development) for which we measured plant life cycle stages such as the period of germination, the period of leaf formation, flowering period, fruit formation period and complete maturity period. Plant height, internode length, leaf length, leaf width, inflorescence length, achene length, inflorescence number, node number, achene number per inflorescence and achene number per plant, after plant complete maturity were also recorded. Obtained data were statistically analyzed.

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Results: *Bidens pilosa* from IMBO (842 m) showed the lowest periods of germination, leaf formation, flowering, fruit formation and complete maturity, while longest periods were observed in MUGAMBA (2075 m) region. In addition, one-way analysis of variance showed that phenotypic variation in *Bidens pilosa* was highly significant within and across populations (P = 0.000) for the studied quantitative morphological traits except the achene number/inflorescence (P = .887). Some traits increased with increase in altitude while others decreased with increase in altitude. Moreover, internode length, leaf length, inflorescence number and achene number per plant significantly differentiated the three populations from the three regions (P < 0.01). Cluster analysis revealed also that IMBO (842 m) population was different from KIRIMIRO (1645 m) and MUGAMBA (2075 m) which are tending to be closer.
Conclusion: Altitudes significantly influenced phenotypic variability of *Bidens pilosa*. However, further studies on a wide range of morphological traits and altitudes are needed as well as biochemical and molecular analyses.

Keywords: Altitude; Bidens pilosa L.; morphological traits; natural region; phenotypic variability.

1. INTRODUCTION

Bidens pilosa is a worldwide distributed weed [1] and multi-purpose medicinal plant [2] involved in various health aspects including anti-diabetic action [3], protection from oxidative damage [4], adipogenesis inhibition [5], and antibacterial activity [6]. It can also be used in phytoremediation process due to its cadmium-hyperaccumulating activity [7,8]. In some localities of Burundi, it is also used in domestic animal feeding.

Belonging to the Asteraceae family, it is an erect herb with a minimum 60 cm and maximum 150 cm height [2]. In Burundi, *B. pilosa* whose local name is ICANDA is distributed throughout the 11 natural regions, thus in different altitudes and climatic conditions.

It has been documented that altitudes have an impact on plant morphological characteristics [9,10,11,12,13]. Impact of altitude on morphological features in various medicinal plant species including *Ferula jaeschkeana* Vatke [14], *Origanum vulgare* L. [15], *Hypericum perforatum* L. [16], *Pluchea indica*, *Ageratum conyzoides* and *Elephantopus scaber* [17], and *Aconitum heterophyllum* Wall. [18] has been evaluated.

In addition, morphological traits have been widely used in the assessment of variability within different plant species including rice [19], *Carthamus tinctorius* L. [20], *Iris* [21], tomato [22], wheat [23], *Psidium guajava* L. [24], *Capsicum sp*. [25], *Solanum macrocarpon* L. [26], *Arachis hypogaea* L. [27] and common bean [28].

Although the altitude influence on morphological variability has been documented in many plant species, no work on *Bidens pilosa* in this aspect has yet been conducted, particularly in Burundi. The present work was aimed at the assessment of the possible influence of different altitudes on the morphological traits-based phenotypic variability in *Bidens pilosa*.

2. MATERIALS AND METHODS

2.1 Study Sites

Burundi has 11 eco-climatic regions commonly named natural regions, i.e. IMBO, MUMIRWA, MUGAMBA, BUTUTSI, BURAGANE, KIRIMIRO, BUYENZI, BUYOGOMA, MOSO, BWERU and BUGESERA. Mature achenes from *Bidens pilosa* L. were sampled from three of the 11 regions, i.e., IMBO, KIRIMIRO and MUGAMBA selected for the present work (Fig. 1) and whose coordinates are displayed in Table 1.

2.2 Culture Method and Morphological Characterization

Several achenes for each studied site were sown (December 07, 2015 in IMBO, December 22, 2015 in MUGAMBA, and November 10, 2015 in KIRIMIRO) in a well prepared land containing natural organic fertilizer. After germination, elimination of other small plants was often conducted for a better growth and development of *B. pilosa* plants, i.e. three times in IMBO, four times in KIRIMIRO and five times in MUGAMBA. From germination stage to complete maturity of 12 randomly selected plants for each site, measurements of various morphological traits were carried out according to Table 2.

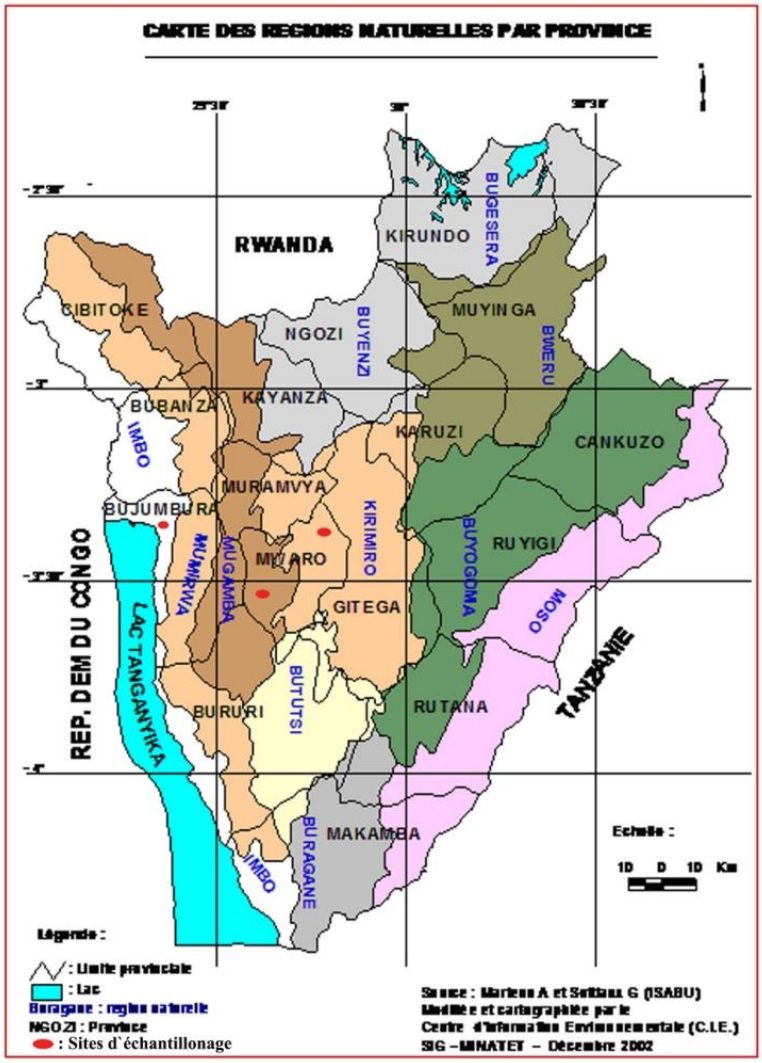

Fig. 1. Collection sites (in red color)

Table 1. Geographical coordinates of selected sites for the present work

Coordinates	Populations		
	IMBO	**KIRIMIRO**	**MUGAMBA**
Altitude (m)	842	1645	2075
Latitude	$03^0$20.522'N	$03^0$24.889'N	$03^0$34.665'N
Longitude	029^o23.247'W	029^o50.757'W	$029^0$41.492'W

2.3 Data Analysis

The data were expressed as mean for each trait. Mean ± standard deviation and difference between groups considered significant at $p < 0.05$ and analyzed by one-way analysis of variance (ANOVA); mean comparisons between groups were considered significant at $p \leq 0.05$ and analyzed by student *t*-test for independent samples. These calculations and hierarchical classification were made using SPSS 22.0 version for Windows. Hierarchical classification of the three populations using UPGMA method (Unweighted Pair Group Method using Arithmetic averages) from MSVP version 3.22.

Table 2. Quantitative morphological traits recorded for *Bidens pilosa* L.

Trait of interest	Scale	Trait description
Period of germination (PG)	Days	Number of days from seed sowing to primary leaves
Period of leaf formation (PLF)	Days	Number of days between primary leaves and second ones
Flowering period (FP)	Weeks	Number of weeks between seed sowing and appearance of first flowers
Fruit formation period (FFP)	Weeks	Number of weeks from seed sowing to fruit (achene) formation
Complete maturity period (CMP)	Months	Number of months from seed sowing to achene`s maturity
Plant height (PH)	mm	Height of each of 12 random plants from ground level to the apex of the main stem
Internode length (IL)	mm	The distance between two consecutive nods for each plant at fruit formation
Leaf length (LL)	mm	The distance between two ends of the leaf in the length sens
Leaf width (LW)	mm	The distance between two ends of the leaf in the width sens
Inflorescence length (INF.L)	mm	Capitulum length from the stem insertion site to the end of the fruit
Achene length (AL)	mm	Length of each achene on the capitulum
Node number (NN)	-	Total number of a single plant
Inflorescence number (INF.N)	-	Number of capitula per plant
Achene number (AN)	-	Number of achenes per capitulum or per plant

3. RESULTS AND DISCUSSION

3.1 Differences in Vegetative Phases

Vegetative phases comprise of periods of germination and leaf formation, flowering, fruit formation and complete maturity periods. The related results are presented in Table 3.

Germination period in IMBO population was short (4 days) while the long one was observed in MUGAMBA population (9 days), KIRIMIRO population being between the two (7 days). There was also a great difference in the period of leaf formation. After germination, leaves appeared respectively in the ranges of 3-5, 7-10 and 9-14 days in IMBO, KIRIMIRO and MUGAMBA populations. Flowering period varied also among populations. Ranges of 4-6, 8-10 and 12-14 weeks after seed sowing were respectively observed in IMBO, KIRIMIRO and MUGAMBA populations. Fruit formation periods showed also variations among populations. Ranges of 7-8, 13-16, and 18-20 weeks after seed sowing were recorded respectively in IMBO, KIRIMIRO and MUGAMBA populations. At this stage, differences in plant height and other morphological traits such as node number are remarkable through the Fig. 2.

Last, period of complete maturity was of 2, 4, and 5 months respectively in IMBO, KIRIMIRO and MUGAMBA populations. These results imply that *Bidens pilosa* prefers higher altitudes for its growth and development.

3.2 Phenotypic Variability

Phenotypic variation in *Bidens pilosa* manifest a highly significant variability within and across populations (P = 0.000) for the studied quantitative morphological traits except for the achene number/inflorescence (Table 4).

From this table, it is evident that plant height, internode length, leaf length, leaf width, inflorescence number and achene number/plant increased with increase in altitude.

The height for *Bidens pilosa* ranges from 458.33±144.338 mm to 800.83±56.159 mm in all the investigated populations and very highly significantly (P = 0.000). From this finding, it is clear that individuals from KIRIMIRO (1645 m) and MUGAMBA (2075 m) populations displayed longer plant height while the shorter plant height is observed in IMBO (842 m) region.

The present work shows that the average internode length ranges from 45.83±14.314 mm

Fig. 2. Selected samples of *Bidens pilosa* L. from (A): IMBO (842 m); (B): KIRIMIRO (1645 m); (C): MUGAMBA (2075 m) showing plant habit

Table 3. Diversity in vegetative phases

Populations	Vegetative phases				
	PG (days)	PLF (days)	FP (weeks)	FFP (weeks)	CMP (Months)
IMBO (842 m)	4	3-5	4-6	7-8	2
KIRIMIRO (1645 m)	7	7-10	8-10	13-16	4
MUGAMBA (2075 m)	9	9-14	12-14	18-20	5

Tableau 4. Phenotypic variability in morphological quantitative traits of *Bidens pilosa* populations from three natural regions of Burundi

Morphological traits	Populations (means ± standard deviations)			F	*P*
	IMBO (842 m)	KIRIMIRO (1645 m)	MUGAMBA (2075 m)		
PH	458.33±144.338	800.83±56.159	800.83±32.039	34.422	0.000
IL	45.83±14.314	77.00±10.401	93.250±12.8863	43.626	0.000
LL	65.50±1.883	75.83±9.731	87.50±7.538	28.127	0.000
LW	48.25±6.047	75.83±6.686	75.83±6.686	72.483	0.000
INF.L	15.08±1.505	12.25±0.965	11.67±1.155	26.543	0.000
AL	14.08±0.900	8.58±0.634	8.575±0.3019	278.955	0.000
ANI	38.00±5.752	38.83±4.239	38.83±4.239	0.121	.887
NN	10.67±1.875	10.50±1.446	8.58±1.311	6.586	0.000
INF.N	20.33±5.774	40.92±2.999	33.92±7.585	39.485	0.000
ANP	770.58±277.652	1588.67±209.634	1302.00±243.248	34.422	0.000

PH: plant height; IL: Internode length; LL: leaf length; LW: leaf width; INF.L: inflorescence length; AL: achene length; ANI: achene number per inflorescence; NN: node number; INF.N: inflorescence number; ANP: achene number per plant

to 93.250±12.8863 mm in the studied populations, individuals from MUGAMBA (2075 m) showing the highest internode length while those from IMBO (842 m) region depict the lowest internode length. As for the plant height, the internode length increases with the increase in altitude and varies highly significantly (*P* = 0.000).

The average for leaf length ranges from 65.50±1.883 mm in IMBO (842 m) to 75.83±9.731 mm in KIRIMIRO (1645 m) and 87.50±7.538 mm in MUGAMBA population. The result depicted here shows that leaf length was short in IMBO (842 m) individuals while those from MUGAMBA population show a high leaf length. It is then clear that the leaf length

increases with an increase in altitude and varies highly significantly (*P* = 0.000).

The leaf width varies from 48.25±6.047 mm in IMBO (842 m) to 75.83±6.686 mm in KIRIMIRO (1645 m) and MUGAMBA (2075 m). This indicates that leaf width increases with altitude and varies highly significantly (*P* = 0.000) but does not vary between KIRIMIRO and MUGAMBA populations. The leaf width also increases with the increase in altitude.

Inflorescence number varies from 20.33±5,774 in IMBO (842 m) to 40.92±2.999 in KIRIMIRO (1645 m) and 33.92±7.585 in MUGAMBA (2075 m) population. Although the highest number is observed in KIRIMIRO population, this morphological trait increases with altitude and varies highly significantly across populations (*P* = 0.000).

Achene number per plant varies from 770.58±277.652 in IMBO (842 m) population to 1588.67±209.634 and 1302.00±243.248 respectively in KIRIMIRO (1645 m) and MUGAMBA (2075 m) populations. Despite the highest number is observed in KIRIMIRO population there is an increasing number with altitude a highly significant variation across populations (*P* = 0.000).

Our findings are different from those in other plant species such as *Euphorbia macrostegia* [29], *Inula racemosa*) [30], *Achillea aucheri* [31] and *Skimmia anquetilia* [9] where plant height, leaf dimensions and numbers of flowers were found to decrease with an increasing altitude.

This indicates that *Bidens pilosa* finds optimum conditions for its development at higher altitudes for the considered morphological traits. [32] found that plants from higher altitude present higher nitrogen content in leaves. Nitrogen significantly stimulates plant morphological traits such as plant height or the number of leaves [33]. This speculation is supported by the fact that in this species, it was found that its two varieties, i.e., *minor* and *radiata*, had significantly higher chlorophyll and nitrogen contents in a mid-altitude compared to low-altitude [34].

In contrary, inflorescence length, achene length and node number decreased with increase in altitude, while achene number/inflorescence was static across altitudes.

Inflorescence length varied highly significantly (*P* = 0.000) and decreases with the increase in altitude. Indeed, IMBO (842 m) population showed 15.08±1.505 mm, while KIRIMIRO (1645 m) and MUGAMBA (2075 m) respectively show averages of 12.25±0.965 mm and 11.67±1.155 mm.

Achene length varies from 14.08±0.900 mm in IMBO (842 m) population to 8.58±0.634 mm and 8.575±0.3019 mm in KIRIMIRO (1645 m) and MUGAMBA (2075 m) populations. Again, this morphological trait decreases with altitude and varied highly across populations (*P* = 0.000).

The node number varies from 10.67±1.875 in IMBO (842 m) to 10.50±1.446 in KIRIMIRO (1645 m) and 8.58±1.311 in MUGAMBA (2075 m) population. It varies highly significantly across populations (*P* = 0.000) and decreases with the altitude.

Average of achene number per inflorescence does not significantly vary across populations (*P* =.887). It is 38.00±5.752 in IMBO (842 m), 38.83±4.239 in KIRIMIRO (1645 m) and 38.83±4.239 in MUGAMBA (2075 m) population.

This is similar to the finding in *Euphorbia macrostegia* [31], *Inula racemosa*) [32], *Achillea aucheri* [33] and *Skimmia anquetilia* [9] where morphological characters decreased with increasing altitude but different from the result in *Popuplus cathayan* [35] for inflorescence length in which it increases with increase in altitude. Considered together, the results show a possible phenotypic plasticity in response to altitudinal variation in *B. pilosa*.

Phenotypic variability was also depicted by mean comparisons of the three natural regions based on quantitative morphological traits by means of student *t*-test for independent samples (Table 5).

This analysis showed that internode length, leaf length, inflorescence number and achene number per plant significantly differentiated IMBO (842 m), KIRIMIRO (1645 m) and MUGAMBA (2075 m) populations, while plant height, leaf width, inflorescence length, and achene length significantly differentiated IMBO and KIRIMIRO, and IMBO and MUGAMBA populations but not KIRIMIRO and MUGAMBA populations. Node number significantly differentiated IMBO and MUGAMBA, and KIRIMIRO and MUGAMBA populations but not IMBO and MUGAMBA populations. In contrary, achene number per inflorescence is a morphological trait which was not able to differentiate the three populations.

Table 5. **Mean comparisons of the three natural regions based on quantitative morphological traits by means of student *t*-test for independent samples**

Morphological traits	Comparisons		
	IMBO-KIRIMIRO	IMBO-MUGAMBA	KIRIMIRO-MUGAMBA
PH	t = 7.661 ; *P* = 0.000	t = 8.025 ; *P* = 0.000	t = 0.000 ; *P* = 1.000
IL	t = 6.102 ; *P* = 0.000	t = 8.528 ; *P* = 0.000	t = 3.399 ; *P* = 0.003
LL	t = 3.611 ; *P* = 0.002	t = 9.809 ; *P* = 0.000	t = 3.283 ; *P* = 0.003
LW	t = 10.599 ; *P* = 0.000	t = 10.599 ; *P* = 0.000	t = 0.000 ; *P* = 1.000
INF.L	t = 5.889 ; *P* = 0.000	t = 6.239 ; *P* = 0.000	t = 1.343 ; *P* = .193
AL	t = 17.305 ; *P* = 0.000	t = 20.094 ; *P* = 0.000	t = 0.041 ; *P* = .968
ANI	t = 0.404 ; *P* = .690	t = 0.404 ; *P* = .690	t = 0.000 ; *P* = 1.000
NN	t = 0.244 ; *P* = .810	t = 3.154 ; *P* = 0.005	t = 3.401 ; *P* = 0.003
INF.N	t = 10.960 ; *P* = 0.000	t = 4.936 ; *P* = 0.000	t = 2.973 ; *P* = 0.007
ANP	t = 8.146 ; *P* = 0.000	t = 4.987 ; *P* = 0.000	t = 3.092 ; *P* = 0.005

PH: plant height; IL: Internode length; LL: leaf length; LW: leaf width; INF.L: inflorescence length; AL: achene length; ANI: achene number per inflorescence; NN: node number; INF.N: inflorescence number; ANP: achene number per plant

Using Euclidian distances based on internode length, leaf length, inflorescence number and achene number per plant, a dendrogram was generated showing that IMBO (842 m) population is tending to be different from KIRIMIRO (1645 m) and MUGAMBA (2075 m) populations which are closer (Fig. 3).

Moreover, a highly significant positive correlation was observed between plant height and internode length, leaf length, leaf width, inflorescence number and achene number per inflorescence. The same positive correlation was depicted between internode length and leaf length, leaf width, inflorescence number and achene number per plant; leaf length and leaf width and achene number per plant; leaf width and inflorescence number, and achene number per plant; inflorescence length and achene length; and inflorescence and achene number per plant. The positive significant correlation was also observed between leaf length and inflorescence number, and achene number per inflorescence and achene number per plant (Table 6).

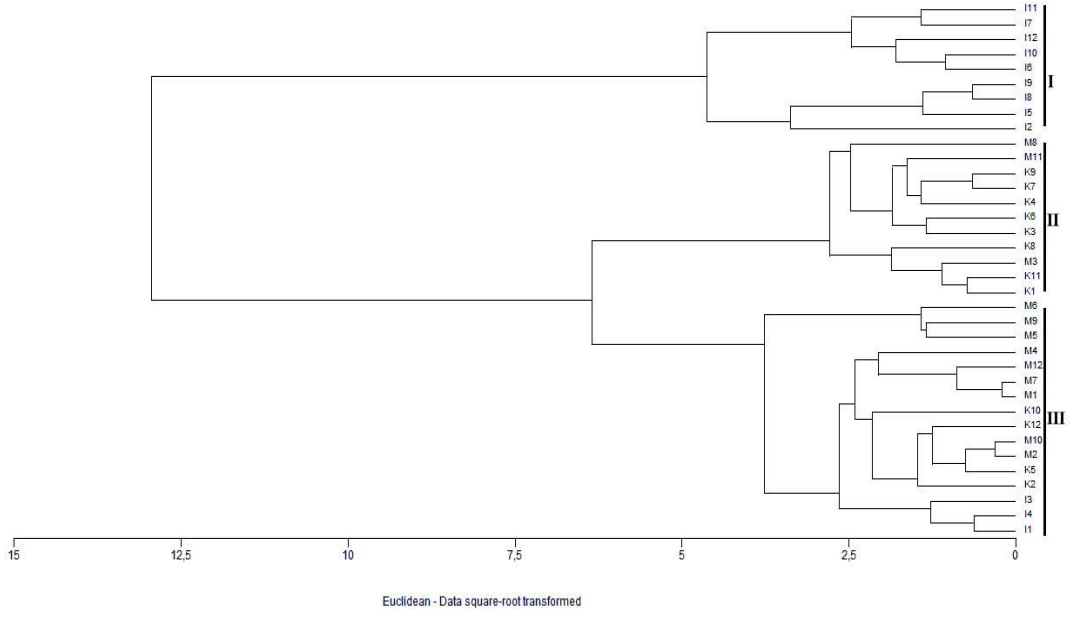

Fig. 3. Clustering of 36 plants of *Bidens pilosa* from IMBO (I1-I12), KIRIMIRO (K1-K12) and MUGAMBA (M1-M12); I: initial for IMBO; K: initial for KIRIMIRO; M: initial for MUGAMBA

Table 6. Pearson correlation coefficients for the ten morphological traits

	PH	IL	LL	LW	INF.L	AL	ANI	NN	INF.N	ANP
PH	1									
IL	0.816**	1								
LL	0.621**	0.635**	1							
LW	0.839**	0.755**	0.692**	1						
INF.L	-0.653**	-0.599**	-0.586**	-0.752**	1					
AL	-0.835**	-0.729**	-0.669**	-0.892**	0.821**	1				
ANI	0.096	0.085	0.195	0.214	-0.152	-0.185	1			
NN	-0.096	-0.597**	-0.271	-0.191	0.231	0.234	0.030	1		
INF.N	0.828**	0.605**	0.402*	0.790**	-0.599**	-0.750**	-0.017	0.010	1	
ANP	0.797**	0.595**	0.445**	0.824**	-0.593**	-0.762**	0.334*	0.007	0.930**	1

*PH: plant height; IL: Internode length; LL: leaf length; LW: leaf width; INF.L: inflorescence length; AL: achene length; ANI: achene number per inflorescence; NN: node number; INF.N: inflorescence number; ANP: achene number per plant; * significant at 0.05 level; ** significant at 0.01 level.*

This indicates that plant height has an impact on other morphological traits as recently found by [14]. It implies also that some of the morphological traits can be taken into account in the breeding program for this important plant species, such as plant height, internode length, leaf length, leaf width, inflorescence number and achene number per inflorescence.

4. CONCLUSION

The present work reveals that *Bidens pilosa* manifests significant phenotypic variability across the three populations from the three natural regions with varying altitudes, indicating an impact of altitude on phenotypic variability based on morphological traits considered in this species. Although it is not yet investigated, differences in temperatures, rainfall and soil structure across the three populations are supposed to underlay the exhibited phenotypic variability. Nonetheless, some environmental parameters such as water availability in soil, pH and nitrogen content of the soil shall be monitored for each eco-climatic region as well as additional morphological traits such root length and biomass to emphasize the present findings.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Potawale SE, Shinde VM, Harle UN, Borade SB, Anandi L, Dhalawat HJ, and Deshmukh RS. *Bidens pilosa* L.: A comprehensive review. Pharmacology Online. 2008;2:185-196.
2. Bartolome AP, Villaseñor IM, Yang WC. *Bidens pilosa* L. (Asteraceae): Botanical properties, traditional, uses, phytochemistry, and pharmacology. Evidence-Based Compl. Alternat. Med. 2013;1-51.
3. Yang WC. Botanical, pharmacological, phytochemical and toxicological aspects of the antidiabetic plant *Bidens pilosa* L.", evidence-based compl. Alternat. Med. 2014;1-14.
4. Yang HL, Chen SC, Chang NW, Chang JM, Lee ML, Tsai PC, Fu HH, Kao WW, Chiang HCh, Wang HH, and Hseu YCh. Protection from oxidative damage using *Bidens pilosa* extracts in normal human erythrocytes. Food and Chem. Toxicol, 2006;44:1513-1521.
5. Liang YC, Yang MT, Lin CJ, Chang CLT, Yang WC. *Bidens pilosa* and its active compound inhibit adipogenesis and lipid accumulation via down-modulation of the C/EBP and PPARγ pathways. Scientific Reports. 2016;6:1-10.
6. Lawal OA, Amisu KO, Akinyemi SK., Sanni AA, Simelane MBC, Mosa RA, Opoku AR. *In vitro* antibacterial activity of aqueous extracts of *Bidens pilosa* L. (Asteraceae) from Nigeria. British Microbiol. Res. J. 2015;8(4):525-531.
7. Sun Y, Zhou Q, Wang L, Liu W. Cadmium tolerance and accumulation characteristics of *Bidens pilosa* L. as a potential Cd-hyperaccumulator. J. Haz. Mat. 2008;161: 808-814.
8. Dai H, Wei S, Twardowska I, Han R, Xu L. Hyper accumulating potential of *Bidens pilosa* L. for Cd and elucidation of its translocation behavior based on cell membrane permeability. Environ. Sci. Pollut. Res. Internat. 2017;24(29):23161-23167.
9. Nissar S, Nawchoo IA, Mohi-Ud-Din GG, Majid N. Impact of altitude and habitat variability on morphological attributes and resource allocation patterns in *Skimmia anquetilia* in Kashmir Himalaya. Imper. J. Interdisci. Res. 2016;2(12):1443-1448.
10. Demissie A, Bjornstad A. Phenotypic diversity of Ethiopian barleys in relation to geographical regions, altitudinal range, and agro-ecological zones: As an aid to germplasm collection and conservation strategy. Hereditas. 1996;124:17-29.
11. Jacquemyn H, Micheneau C, Roberts DL, Pailler T. Elevational gradients of species diversity, breeding system and floral traits of orchid species on Réunion Island. J. Biogeogr. 2005;32:1751-1761.
12. Maliníková E, Kukla J, Kuklová M, Balážová M. Altitudinal variation of plant traits: morphological characteristics in *Fragaria vesca* L. (Rosaceae). Ann. Forest Res, 2013;56 (1):79-89.
13. Zhong M, Wang J, Liu K, Wu R, Liu Y, Wei X, Pan D, Shao X. Leaf morphology shift of three dominant species along altitudinal gradient in an alpine meadow of the Qinghai-Tibetan plateau, Pol. J. Ecol. 2014;62:639-648.
14. Yaqoob U, Nawchoo IA. Impact of habitat variability and altitude on growth dynamics

and reproductive allocation in *Ferula jaeschkeana* Vatke. J. King Saud Univ-Sci. 2017;29:19-27.

15. Kofidis G, Bosabalidis AM, Moustakas M. Contemporary seasonal and altitudinal variations of leaf structure features in Oregano (*Origanum vulgare* L.). Ann. Bot. 2003;92:635-645.
16. Roblek M, Germ M, Sedej TT, Gaberscik A. Morphological and biochemical variations in St. John's wort, *Hypericum perforatum* L., growing over altitudinal and UV-B radiation gradients. Period. Biolog. 2008;110(3):257-262.
17. Yuliani Soemarno, Yanuwiadi B, Leksono AS. The relationship between habitat altitude, enviromental factors and morphological characteristics of *Pluchea Indica*, *Ageratum Conyzoides* and *Elephantopus Scaber*. OnLine J. Biologic. Sci. 2015;15(3):143-151.
18. Jeelani SM, Siddique MAA, Rani S. Variations of morphology, ecology and chromosomes of *Aconitum heterophyllum* Wall., an endangered Alpine medicinal plant in Himalayas. Caryologia: Internat. J. Cytol., Cytosyst. Cytogenet. 2016;68(4): 294-305.
19. Caldo RA, Sebastian LS, Hernandez JE. Morphological-based genetic diversity analysis of ancestral lines of Philippine rice cultivars. Philipp. J. Crop Sci. 1996;21(3): 86-92.
20. Shinwari ZK, Rehman H, Rabbani MA. Morphological traits based genetic diversity in sawfflower (*Carthamus tinctorius* L.). Pak. J. Bot. 2014;46(4):1389-1395.
21. Azimi MH, Tahernezhad Z, Zamani MJ. Genetic variation within iranian iris species using morphological traits. Internat. J. Horticult. Sci. Technol. 2016;3(1):89-98.
22. Yadav A, Tandan N, Singh R. Morphological based genetic variability analysis and clustering of tomato. World J. Pharmaceut. Med. Res. 2017;3(6):255-261.
23. Bellatreche A, Mahmad MY, Kaouadji Z, Gaouar SBS. Agro-morphological diversity of some accessions of bread wheat (*Triticuma aestivum*) in western Algeria. Biodiv. 2017;18(1):409-415.
24. Kidaha LM, Alakonya AE, Nyende AB. Morphological characters of guava landraces in Western and Coastal Kenya. Americ. J. Exp. Agricult. 2015;9(6):1-11.
25. Agyare RY, Akromah R, Abdulai MS. Assessment of genetic diversity in pepper (*Capsicum* sp.) landraces from Ghana using agro-morphological characters. Americ. J. Exp. Agricult. 2016;12(1):1-16.
26. Saka M, Ahoton LE, Ezin V, Quenum F, Ayena M. Agro-morphological assessment of local cultivars of nightshade (*Solanum macrocarpon* L.) in Benin. J. Exp. Agricult. Internat. 2017;15(3):1-14.
27. Neya FB, Koita K, Elise S, Zagre BM, Nana AT, Burow MD, Sankara P. Agro-morphological diversity of six peanut (*Arachis hypogaea* L.) breeding lines from three geographical areas. J. Exp. Agricult. Internat. 2018;19(5):1-12.
28. Boros L, Wawer A, Borucka K. Morphological, phonological and agronomical characterisation of variability among common bean (*Phaseolus vulgaris* L.) local populations from the national center for plant genetic ressources: Polish genebank. J. Horticult. Res. 2014;22(2): 123-130.
29. Keveshk NM. Investigation of effect of altitude in some morphological characteristics and yield in *Euphorbia macrostegia*. Internat. J. Agricult. Crop Sci. 2013;5(16):1779-1783.
30. Shabir PA, Nawchoo IA, Wani AA. Among and within population variation in growth dynamics and floral sex ratios in *Inula racemosa*; A critically endangered medicinal herb of N. W. Himalayas. Internat. J. Biodiv. Conserv. 2013;5(12): 796-802.
31. Sardrodi AF, Kheyri A, Soleymani A, Zibaseresht R. Evaluation of morphological traits and oil contents of *Achillea aucheri* from different altitudes. J. Med. Plants By-products. 2014;2:219-223.
32. Korner C. The nutritional status of plants from high altitudes: A worldwide comparison. Oecologia. 1989;81:379-391.
33. Singh M, Khan MMA, Naeem M. Effect of nitrogen on growth, nutrient assimilation, essential oil content, yield and quality attributes in *Zingiber officinale* Rosc. J. Saudi Society Agricult. Sci. 2014;15:171-178.
34. Huang HL, Huang YL, Wu TC, Kao WY. Phenotypic variation and germination behavior between two altitudinal populations of two varieties of Bidens pilosa in Taiwan. Taiw. 2015;60(4):194-202.

35. Asmare B, Demeke S, Tolemariam T, Tegegne F, Haile A, Wamatu J. Effects of altitude and harvesting dates on morphological characteristics, yield and nutritive value of desho grass (*Pennisetum pedicellatum* Trin.) in Ethiopia. Agri. Nat. Res. 2017;51:148-153.

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