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MULTIVARIATE ANALYSIS TO ACCESS VARIATION AMONG MORPHOLOGICAL TRAITS IN *SETARIA VIRIDIS*

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Abstract *Setaria viridis (L) Beauv, or green foxtail, is local to Eurasia and is the putative precursor of foxtail millet. Because of the beneficial hereditary qualities of S. viridis, as a species of C4 plant, it serves as a model. This study delves into the morphological characteristics and reproductive strategies of Setaria viridis, a notable grass species. A prescribed study was conducted to evaluate the morphological characteristics of* Setaria viridis *across three distinct environmental places. Findings demonstrated a significant and positive correlation among various traits, including height, dry weight of plants, fresh weight of plants, leaf area of plants, leaf length of plants, leaf width of plants, and root length of plants. Particularly noteworthy was the influential role of leaf width in determining plant height. Location one was identified as exceptionally conducive to the robust growth and development of Setaria viridis. In light of potential yield losses in crop plants, this study emphasizes the need for timely control or removal of Setaria viridis and underscores the importance of effective population management*.

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Keywords: *Setaria viridis; leaf width; environmental places; hereditary; foxtail millet*

Introduction

Setaria viridis (L) Beauv is a grass species from the Poaceae family that goes by many names, including green foxtail, green bristlegrass, and wild foxtail millet *(*Gabriel et al., 2002*)*. *Setaria viridis* is a yearly diploid species thought to be native to Eurasia and the presumed ancestor of foxtail millet. *S. viridis* has attracted the attention of plant researchers due to its intrinsic C4 qualities *(*Langdale, 2011*)*. *S. viridis* is an ideal genetic model because of its small size (10-15 cm), short life cycle (6-9 weeks depending on photoperiod conditions), and prolific seed production (13,000 seeds per plant). Plants are self-compatible and can be transformed via *Agrobacterium*-mediated methods (Li et al., 2010). The family Setaria includes approximately 125 species, with *S. viridis* considered the ancestral heap of this type (Darmency and Dekker, 2010). Through the allozyme analysis, it was found that the foxtail millet (*S. italica*) was the restrained form of *S. viridis*. At present, a few millet types are part of the significant Poaceae family evolution process that has taken place over time. Pearl millet, foxtail millet, proso millet, and finger millet are some of the primary or main-grown millets (Radivojac et al., 2013). Thus, the green foxtail millet is a troublemaker because it is an annual weed and it propagates by seeds that are known to be long-lived. It was only revealed recently too that *S .viridis* has greatly advanced in its attempt to develop resistance to herbicide use. This increases the chance of discovering the genetic basis of the progression of herbicide resistance by one more perspective.

S. viridis has recently been employed for investigations of a variety of aspects of plant biology, such as flowering time, shoot development, and the evolution of photosynthesis (Brezzi et al., 1985; Li and Brutnell, 2011). The leaf blades of *Setaria viridis* contain tillers, and on average, their length can reach up to 40 cm and the width can be up to 2.5 cm; the leaf surface is glabrous. The inflorescence is a densely aggregated, compact cylindrical spike, up to 20 cm long, erect, or slightly contracted at the apex. This caryopsis is less than 5mm long with spikelets measuring 1.8-2.2 mm long. All of which are accompanied by as many as three stiff bristles (Reed, 1970). Glumes are the outermost bracts of the spikelets proving protection. Inside the glumes, specialized bracts called lemma paleas encase the florets. Each spikelet contains one or more florets, serving as the reproductive flowers. The florets of *Setaria viridis* are hermaphroditic, with both male and female reproductive parts. Spikelets are arranged along the stem in a bottlebrush pattern. Successful pollination leads to the development of small seeds within the spikelets (Zhao et al., 2014).

Seed dormancy, which prevents seedling germination, can be caused by a variety of physiological and developmental mechanisms (Bewley, 1997; Finch-Savage and Leubner-Metzger, 2006). In nature, several factors can frequently work together to prevent germination until a favorable condition is met. When *S. viridis* seeds are freshly collected and planted in soil or tissue culture, they frequently show close to 0% germination rates. The average germination rate gradually increases from 0 to 42% 55 days after seed harvest and can reach 56% after 115 days. *Setaria viridis* thrives in well-drained loamy soils with a pH of 6.0 to 7.5. It thrives in soils with moderate to high levels of nitrogen, phosphorus, and potassium. Adequate levels of micronutrients, such as iron and zinc, are essential. Organic matter-rich soils with good drainage support healthy growth. Regular soil testing helps determine and maintain the necessary nutrient levels for successful cultivation. *Setaria viridis*, commonly known as green foxtail, displays remarkable adaptability to diverse environmental and soil conditions. Herbicides such as glyphosate and atrazine commonly used to control *Setaria viridis (green foxtail)* in agricultural settings and lawns. They work by inhibiting the growth of the weed, allowing for better management of desirable plants. It can help control the growth of *Setaria viridis*, an invasive weed, which can benefit native plant species and crops *(*Zhong et al., 2022*)*. *S. viridis*, a tufted annual grass, can be easily suppressed normally by conventional tillage operations. Where these do not suffice and other non-chemical methods are required early planting is one of the most recommended strategies for controlling S. viridis in cereal crops in North America since wheat can germinate at relatively low temperatures to that of the weed (Khan et al., 1996). Again, high planting density and higher nutrient levels are beneficial as in the case of Wheat and Barley crops. Higher density is also planned for herbicide-free maize production in Ukraine (Bobro and Bachassi, 1994). Crop rotation is suggested for the management of the S. viridis seed bank in the United States *(*Jordan et al., 2003*)*.

Materials and Method

Collection of plant material

The specimens of *Setaria Viridis* were assembled for this study from three distinct locations at the backfield of the faculty of agricultural sciences, University of the Punjab, Lahore. Each location had three replications of the specimens, for a total of nine replications. We dug up the soil next to the weed plant to collect a representative sample. After that, the entire plant material was dried in the shade.

Leaf Length (cm)

To measure the leaf length, three leaves were haphazardly chosen from each plant, and their length was recorded utilizing a cm scale. The average value of the three measurements was calculated.

Leaf Width (cm)

Leaf width was estimated at three distinct focuses on each leaf: the base, focus, and tip. There were three leaves randomly chosen from each plant, and the width at each point was recorded. The average value of the three measurements was then calculated.

Leaf Area (cm^2)

The leaf area was calculated by multiplying the length of the leaf, by the leaf width and a correction factor of 0.74 cm. The formula for calculating the leaf area was as follows:

Leaf area = leaf width × leaf length × 0.74

Plant Height (cm)

To quantify the plant level, the estimation interaction began from the place of connection of the stem to the root (stem base), and the longest shoot on each plant was estimated.

Fresh weight (g)

The fresh weight of the plant was estimated immediately after the weed sample was eliminated from the field to keep the sample from drying out. The electronic weight balance was utilized to measure the weight of the sample, both with and without the inflorescence.

Dry Weight (g)

To get the dry weight, the sample was dried on a stove. Each sample was pressed independently in envelopes and passed on them to dry for 8-13 hours. After the samples were taken from the stove, they were weighed on a balance, both with and without inflorescence.

Moisture Percentage (%)

The total plant moisture percentage both with and without the inflorescence was recorded using the following formula:

Moisture percentage = (fresh weight of plant – dry weight of plant)/fresh weight of plant×100

Results and Discussion

Findings (from Table 1) indicate that there were notable variations observed among all the places and studied traits of *Setaria viridis*. Specifically, the average plant height was measured to be approximately (82.9 ± 8.011 cm), leave width (0.8767± 0.0549 cm), leave length (33.626±0.4961cm), leave area (16.277 ± 2.340cm2), and root length (6.7444±0.7324 cm). Fresh weight (21.513±0.5795g), dry weight (7.0411±0.4552g), and moisture (67.378 ± 1.3603%) for *Setaria viridis* plants collected from three different places. *Setaria viridis* exhibits a better limit than persevere through cruel climatic circumstances, as proven by its higher plant weight and dampness rate. In any case, it is critical to eliminate these plants from crop fields because of their cutthroat nature. They drink fundamental assets like food, minerals, water, and space, prompting diminished crop yield by outcompeting the developed plants and making them dry out. We still need to get rid of these herbs close to vegetation because they are very competitive and absorb many nutrients from nearby plants, which lowers crop yield.

Table 1. Analysis of variance for the morphological traits of *Setaria viridis*

SOV	Height	Leave width	Leave Length	Leave Area	Root Length	Fresh Weight	Dry Wight	Moisture
Locations	200.963*	0.0327*	0.46753*	16.8929*	1.87444*	5.69943*	3.90654*	29.9464*
Error	192.533	0.00905	0.73827	16.4331	1.60944	1.00763	0.62151	5.5509
Grand Mean	82.9	0.8767	33.626	16.277	6.7444	21.513	7.0411	67.378
CV	16.74	10.85	2.56	24.91	18.81	4.67	11.2	3.5
Standard Error	8.0111	0.0549	0.4961	2.3404	0.7324	0.5795	0.4552	1.3603

*=Significant at the 5% probability level, CV=Coefficient of variance

The purpose of the correlation analysis was to examine the relationship between the various morphological traits of *Seteria viridis* (Table 2; Figure 1). The outcomes uncovered that all concentrated-on attributes displayed huge and positive relationships with one another, showing the plants momentous ability to endure cruel and hot ecological circumstances. This flexibility is reasonably credited to the plant's higher photosynthetic rate and gathering of natural builds in its body, which advance vigorous development and improvement. However, it is important to note that there were exceptions in the correlation between fresh weight, dry weight, overall weight, and moisture. These species characteristics didn't show a critical connection, recommending a more many-sided connection between them. To forestall potential yield misfortune in crop plants, it is basic to control the development of *Setaria viridis*. Manual evacuation and prudent utilization of synthetics can be viable strategies in dealing with these obtrusive plants. On the other hand, utilizing transgenic crop plants with glyphosate herbicides can be applied using sprayers also be considered as a strategy to control the growth of weeds. By executing these actions, ranchers can shield their harvest and upgrade generally speaking rural efficiency.

Table 2. Correlation among morphological traits of Setaria viridis

Traits	Dry Weight	Fresh Weight	Height	Leave Area	Leave Length	Leave Width	Root Length
Fresh Weight	0.4572*						
Height	-0.3834	-0.0468					
Leave Area	0.8414*	-0.0575	-0.5221				
Leave Length	0.346	0.9697*	-0.1343	-0.1163			
Leave Width	0.7679*	0.2371	0.2121	0.6690*	0.1051		
Root Length	0.2621	0.9556*	0.1863	-0.2928	0.9006*	0.1401	
Moisture	0.9330*	0.1788*	0.5051*	0.9710*	0.1174	0.7334*	-0.0649

*=Significant at 5% probability level

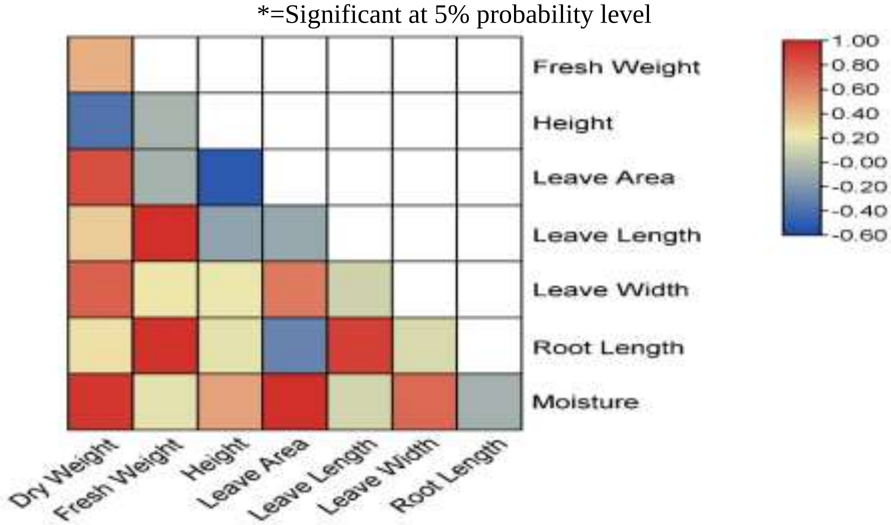

Figure 1: Correlation between different parameters of *Setaria viridis*

Regression analysis showed that a larger height contribution was noted for dry weight (3.056), root length (0.021) and moisture (0.666) (Table 3). The regress equation was recorded as; Y = 12.273 + 3.056(dry weight) - 0.136(leaf width) + 0.666(moisture) - 0.011(leaf length) + 0.021(root length) - 0.004(leaf area) - 0.001(height) - 44.383(fresh weight)

Regression analysis plays a crucial role in plant trait research, particularly when studying key traits in weeds like *Setaria viridis*. Researchers employ regression to understand the relationships between various environmental factors, like as soil composition, temperature, and water content, and the growth and behavior of *Setaria viridis*. By collecting data on these traits and conducting regression analyses, researchers can identify significant predictors and quantify the impact of each factor on weed growth and development. Also, the investigation demonstrated that area 1 is especially good for the development and advancement of *Setaria viridis* plants, as it showed the most elevated efficiency. It has been found that the location 1 was more suitable for plant growth. Thus, it is feasible to moderate potential yield misfortunes in crop plants and guarantee ideal development conditions for *Setaria viridis*. This approach would prompt more effective farming practices and further develop crop efficiency (Khalid and Amjad, 2018; Sami et al., 2023).

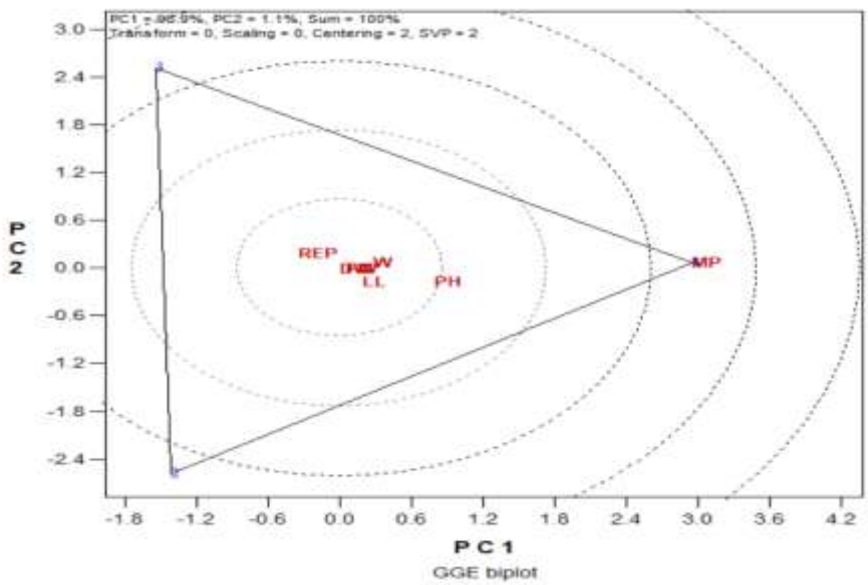

Figure 2: Graphical demonstration of multilinear regression of *S. Viridis*

Table 3. Multiple linear regression stepwise for fresh weight of *Setaria viridis*

Traits	Coefficients	Standard Error	t Stat	P-value	Lower 95%	Upper 95%
Dry Weight	3.056	0.062	49.304	0.013	2.268	3.844
Leaf Width	-0.136	0.101	-1.343	0.407	-1.422	1.150
Moisture	0.666	0.023	28.874	0.022	0.373	0.960
Leaf Length	-0.011	0.037	-0.298	0.815	-0.485	0.463
Root Length	0.021	0.022	0.967	0.511	-0.259	0.302
Leaf Area	-0.004	0.005	-0.846	0.553	-0.069	0.060
Height	-0.001	0.001	-1.058	0.482	-0.017	0.014
Fresh weight	-44.383	1.976	-22.464	0.028	-69.488	-19.279

R^2= 76.23

This GGE biplot visualizes the relationships between different traits (e.g., REP, W, LL, PH, MP) and their contributions to the first two principal components (PC1 and PC2). PC1 explains 98.9% of the total variation, while PC2 accounts for 1.1%, summing to 100% (Afzal et al., 2016; Meeran et al., 2023). The traits are represented by red labels and vectors, where the length of each vector indicates its contribution to the variance. Traits with vectors close to each other (e.g., LL and W) are positively correlated, while those in opposite directions have negative relationships. MP appears to have the strongest influence on PC1 due to its longer vector. The dashed circles provide a visual aid for interpreting the relative magnitude and direction of contributions to the principal components.

Conclusion

Based on the findings, location 1 is favorable for the growth and development of *Setaria viridis*. In light of the review's discoveries, managing the plant population of *Setaria viridis* is suggested to decrease potential yield misfortunes in crop plants.

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Declarations

Author Contribution statement

All authors contributed equally.

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All data generated or analyzed during the study are included in the manuscript.

Ethics approval and consent to participate

Not applicable

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Not applicable

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Conflict of Interest

Regarding conflicts of interest, the authors state that their review was carried out independently without any affiliations or financial ties that could raise concerns about biases.