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Evaluating the relationship between stem and leaf biomass as well as stem length and leaf surface area of amaranth cultivars in improving food plant

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ABSTRACT

Amaranth is now cultivated almost all over the World for multiple purposes, such as a vegetable, seed food, feed for animals, medicine, and for industry uses. They are highly nutritious, with vitamins and minerals. Leaf area and leaf dry biomass are key parameters linked to plant growth and production. The relationship between leaf biomass and leaf surface area, as well as stem biomass and stem length, is important to our understanding of plant scaling relationships because of their relationship to plant survival. This paper aimed to evaluate the relationship between and within the leaf and stem parameters of nine different amaranth cultivars. The fresh leaves and stems were weighed in their fresh masses; the surface area for leaves was calculated by drawing a full leaf in square quadrats and then counting the square quadrats occupied by a leaf and the stem length was measured using a ruler. Our results indicated that there was a clear relationship between leaf fresh mass and leaf dry mass but a negative correlation between stem fresh mass and stem dry mass due to different internal contents such as water and substances. A low significance was obtained between leaf biomass and leaf surface area, stem biomass and stem length, and leaf biomass and stems biomass as well as leaf surface area and stem length. Our results revealed that this variability of values causes the disproportional ratio of fresh mass to dry mass due to the differentiation quantity of water and solutes within the leaves and stems.

Contribution/Originality: Since amaranth is an underutilized food, many scholars have explored its leaf and grain nutrition. Thus, almost all consumers used leaves and grains for food, while stems remained without critical importance. In this line, we focused on the stem biomass to project the utilization of stem as feed.

1. INTRODUCTION

Both the surface area and dry biomass of leaves could be satisfactorily estimated from simple dimension measurements (Niamjit, 2014). Through photosynthesis, the plant leaf plays a crucial role in transferring solar energy to biological energy (Huang et al., 2019). Leaf weight and area measures of leaf size were demonstrated to follow a power-law relationship (Milla & Reich, 2007; Niklas et al., 2007). However, foliar water content (which is equal to the difference between leaf fresh weight and dry weight) plays an important role in photosynthesis (Niklas et al., 2007).

In other words, for different individual leaves, the ratios of leaf dry weight to leaf fresh weight are different. Nevertheless, numerous researchers have employed the measurement of leaf dry weight as a surrogate for leaf biomass in their investigations pertaining to the scaling relationship between leaf biomass and area. The proportion of leaf mass and leaf area could directly influence the amount of light-capturing surface area to investigate dry biomass (Milla & Reich, 2007). Differently from the effect of leaf shape on the computing relationships of leaf mass and area, this gap shows that leaf shape is an important factor influencing the fitness of plants (Lin et al., 2020).

In plants to be grown, the stems provide mechanical support and a hydraulic pathway (Poorter et al., 2012). In competitive situations, the stem mass fraction increases to a smaller extent than the specific stem length (Poorter & Sack, 2012). The plant has to balance the allocation to leaves, stems, and roots in a way that matches the physiological activities and functions performed by these organs (Poorter et al., 2012).

Amaranthus is one of the most planted leafy vegetables in Africa, and it is also cultivated and consumed globally (Kumar & Arya, 2018). Amaranthus is a multi-purpose crop, thus consumable in both its grain (cereal) and vegetable form (leaves) (Sarker & Oba, 2019). It is also known for its medicinal, industrial benefits, and economic purposes (Caselato-Sousa & Amaya-Farfan, 2012; Magdalena et al., 2019). It is a good source of protein, vitamins, and minerals in its leaves and stems (Sarker & Oba, 2019).

The amaranth species has not only C4 photosynthesis but also high water use efficiency, long taproots, and an indeterminate flowering habit to provide tolerance to adverse environmental conditions (Magdalena et al., 2019).

Due to their potential economic value, amaranth cultivation has been subjected to great interest among stakeholders and farmers. Unfortunately, these pseudo-cereal species with significant food potential remain underutilized due to a lack of coherent strategies for their evaluation and development (Aderibigbe et al., 2022).

The paradoxical question is why, despite its high nutritional value, drought resistance characteristics, and high productivity, amaranth is poorly cultivated and its production is low in Rwanda. Moreover, the prevalence of concealed undernutrition has been associated with the issue of inadequate nutrition, which is a concern about the realm of food security. Infants, young children, and young women of the age-bearing group in middle- and low-income countries are at risk of devastating effects that impair vision, intellect, and retarded development, as well as inflicting morbidity that limits the livelihood of persons, especially smallholder farmers in rural areas (Sarker & Oba, 2019). The study has explored that amaranths have a great contribution to food and nutrition security and consequential benefits that are aimed at improving well-being and livelihood, but the crop is being cultivated by a few farmers mostly for subsistence use and not for commercial purposes. Thus, morphological traits influence plant growth, reproduction, tolerance, and plant distribution in the ecosystem. The goal of this research is to explore the relationship between leaf mass and surface area and stem mass and stem length to improve food plants.

2. MATERIALS AND METHODS

2.1. Leaf & Stem Biomass, Leaf Surface Area, and Stem Length Determination

The seeds were obtained in the Botanical Garden of Ural Federal University; the amaranth leaves and stems used were grown in indoor controlled pots in the biological laboratory of the Institute of Natural Sciences and Mathematics, Ural Federal University.

Then, two months after transplantation, five leaves and five stems for each cultivar of about nine Amaranth cultivars Table 1 were collected. Leaves and stems were selected based on their health (uniform color for each cultivar) and size (bigger and larger).

Table 1. Different nine Amaranth cultivars were assessed during the study.

No	Species	Cultivar	Origin	Registration number
1	*Amarantus caudatus L.*	cv. Edulis	Germany	49406-16
		f. Yellow brown	Germany	45378-16
		R-124	Austria	28893-95-05-16
2	*Amarantus cruentus L*	cv. Hopi RED DYe	France	29844-97-04
		cv. Nodoja	Romania	44628-09-10-16
		cv. Pygmy & TORCh	Romania	49471-16
3	*Amarantus hybridus L.*	cv. Oeschberg	Germany	41398-03-08-12-16
4	*Amarantus hypochondriacus L.*	Unknown	Poland	49785-18
		cv. Black leaved	Germany	47668-16

The fresh leaves and stems were weighed to record the fresh masses. The leaf surface area and stem length were measured by pressing the leaf on a quadrat square, then counting the quadrats and using a ruler, respectively. Then the leaves and stems were dried in an oven at UN 75 at 80°C for two hours and four hours, respectively. Lastly, the dry masses for both were weighed.

2.2. Data Analysis

The mean values and standard errors were used to analyze the data. The regression statistics and analysis of variance (ANOVA) within Microsoft Excel 2010 were used to provide mathematical analyses.

3. RESULTS

We found that there existed a significant relationship between leaf dry mass and fresh mass for all cultivars investigated (Figure 1). In general, the ratios of leaf fresh mass and leaf dry mass were found to be unequal, suggesting the presence of substantial allometric correlations between these two variables across the majority of the examined cultivars. In other words, for most cultivars, leaf fresh mass is not proportional to leaf dry mass, and the estimates of the ratios between these parameters are as follows: 10.67:1; 11.24:1; 10.37:1; 13.06:1; 10.27:1; 10.12:1; 9.78:1; 9.19:1, and 9.65:1 for A.ca R-124, A.ca Ed, A.ca Yb, A. cru HRD, A. cru N, A. cru PT, A. hyb O, A. hypo P, and A. hypo Bl (Table 2 and Figure 2). Correlations between the leaf's parameters were all significant: LFM was strongly and weakly correlated to LDM and LSA ($P = 0.00002$, $R = 0.98$ & $R^2 = 0.97$ and $P = 0.006$, $R = 0.82$ & $R^2 = 0.68$, respectively), and LDM was also weakly correlated to LSA ($P = 0.007$, $R = 0.82$ & $R^2 = 0.67$) (Figure 1).

Table 2. The mean values of leaves and stems parameters and their standard errors (± SE), all masses in milligrams, surface areas in square centimeters, and lengths in centimeters.

Cultivar	LFM ± SE	LDM ± SE	LSA ± SE	SFM ± SE	SDM ± SE	SL± SE
A.ca R-124	392.8 ± 1.4	36.8 ± 2.5	74.7 ± 5.9	2301.0 ± 10.7	135.8 ± 1.3	25.8 ± 0.6
A.ca Ed	377.8 ± 2.7	33.6 ± 3.5	86.7 ± 3.9	3547.8 ± 20.0	384.0 ± 9.0	32.6 ± 0.4
A.ca Yb	394.2 ± 1.2	38.0 ± 3.9	96.0 ± 3.5	2336.6 ± 6.1	147.0 ± 5.6	23.1 ± 0.4
A.cru HRD	173.7 ± 4.0	13.3 ± 4.5	60.0 ± 8.8	543.5 ± 2.5	25.0 ± 2.0	14.0 ± 2.0
A.cru N	394.2 ± 1.0	38.4 ± 2.4	104.3 ± 4.3	2762.4 ± 17.3	819.2 ± 6.4	34.2 ± 2.0
A.cru PT	295.4 ± 2.6	29.2 ± 4.0	83.5 ± 7.8	1861.6 ± 25.0	256.6 ± 12.3	25.4 ± 1.3
A.hyb O	539.6 ± 6.7	55.2 ± 4.0	108.5 ± 7.3	2545.2 ± 30.6	141.6 ± 3.0	30.1 ± 1.9
A.hypo P	404.2 ± 2.7	44.0 ± 2.0	87.6 ± 4.4	2206.2 ± 8.7	182.4 ± 2.4	31.0 ± 1.1
A.hypo Bl	457.6 ± 3.1	47.4 ± 3.3	91.7 ± 5.4	2786.8 ± 24.1	168.0 ± 3.0	23.6 ± 0.5

Note: LFM stands for leaf fresh mass, LDM: Leaf dry mass, LSA: Leaf surface area, SFM: Stem fresh mass, SDM: Stem dry mass, SL: Stem length, and SE: Standard error.

The LFM, LDM, and LSA ranged from 173.7 ± 4.0, 13.3 ± 4.5, and 60.0 ± 8.8 for A. cru HRD to 539.6 ± 6.7, 55.2 ± 4.0, and 108.5 ± 7.3 for A. hyb O, respectively. It is clear that a cultivar with lower or higher leaf fresh and dry mass also has a smaller leaf surface area Table 2, but this observation presents some exceptions (as the correlation between parameters is not strong at all).

Between the cultivars of the same species, the study showed that those of *Amaranthus hypochondriacus* had close quantities of LFM, LDM, and LSA and occupied the second rank after A. hyb O, a cultivar of *Amaranthus hybridus*. On the third rank, we have the cultivars of *Amaranthus caudatus* with close quantities of LFM, LDM, and LSA. The last ones are the cultivars of *Amaranthus cruentus* with non-proportional quantities of LFM, LDM, and LSA.

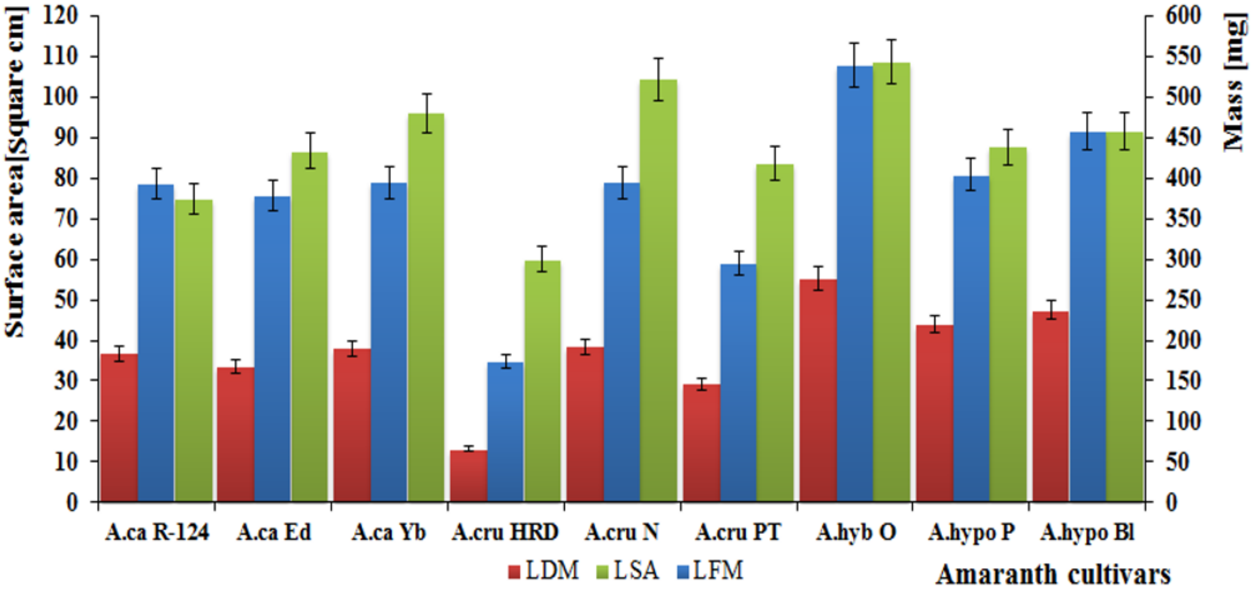

Figure 1. Amaranth leaves parameters such as leaf surface area on the left Y axis (Yellow-green graphs), leaf fresh mass, and leaf dry mass on the right Y axis (Blue and red graphs respectively).

The study found a weak link between the amaranth leaf surface area and amaranth stem length ($P = 0.034$, $R = 0.70$ & $R^2 = 0.50$). There was a moderate link and a moderate between amaranth leaf fresh mass and amaranth stem mass ($P = 0.029$, $R = 0.72$ & $R^2 = 0.52$). A.cru HRD showed the smaller traits of leaves as described above, and this cultivar showed the smaller traits of stems, but differently for A.hyb O with good traits of leaves, A. ca Ed with good stem fresh mass, and A. cru N with good stem dry mass and stem length.

The relationship between the stem fresh mass and stem length was weak with $P = 0.0094$, $R = 0.80$, and $R^2 = 0.64$ as well as very weak between stem dry mass and stem length ($P = 0.04$, $R = 0.69$, and $R^2 = 0.47$) because the ability to give the dry mass from the fresh mass was not proportional equally to the same (Figure 2). As Freschet, Kichenin, and Wardle (2015) stated, coordination among traits is often expressed by positive and negative correlations, representing trade-offs and allometries based on biomechanical and physiological requirements in response to environmental conditions. So with a negative correlation between stem fresh mass and stem dry mass ($P = 0.18$, $R = 0.50$, and $R^2 = 0.25$) (Figure 2), the ratios of stem fresh mass and stem dry mass are 16.94:1; 9.24:1; 15.90:1; 21.74:1; 3.37:1; 7.25:1; 17.97:1; 12.10:1 and 16.59:1 for A.ca R-124, A.ca Ed, A.ca Yb, A. cru HRD, A. cru N, A. cru PT, A. hyb O, A. hypo P, and A. hypo Bl (Table 2 & Figure 3).

In terms of stem traits, between the cultivars of the same species, the results showed that A. ca Ed had a high level of SFM, the second level of SDM, and the second level of SL, while the others of the same species, *Amaranthus caudatus*, had proportional quantities at all but low if compared to this. The close quantities of SFM, SDM, and SL were recorded in cultivars of *Amaranthus hypochondriacus* and *Amaranthus hybridus*. The cultivars of *Amaranthus cruentus* had lower quantities in general, but A. cru N presented an exception with the third level of SFM, the first level of SDM, and the first level of SL, as well as A. cru PT with low SFM but a high level of SDM (third level with 256.6 ± 122.3).

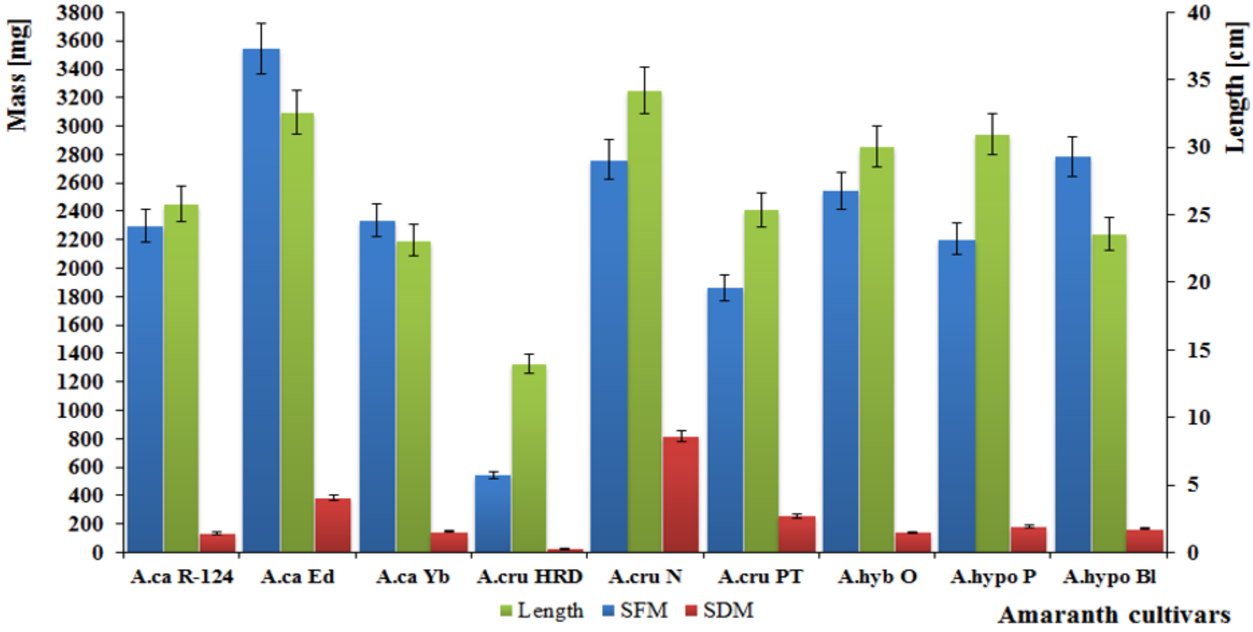

Figure 2. Amaranth stem parameters such as stem fresh mass and stem dry mass on the left Y axis (blue and red graphs respectively) and stem length on the right Y axis (Yellow-green graphs).

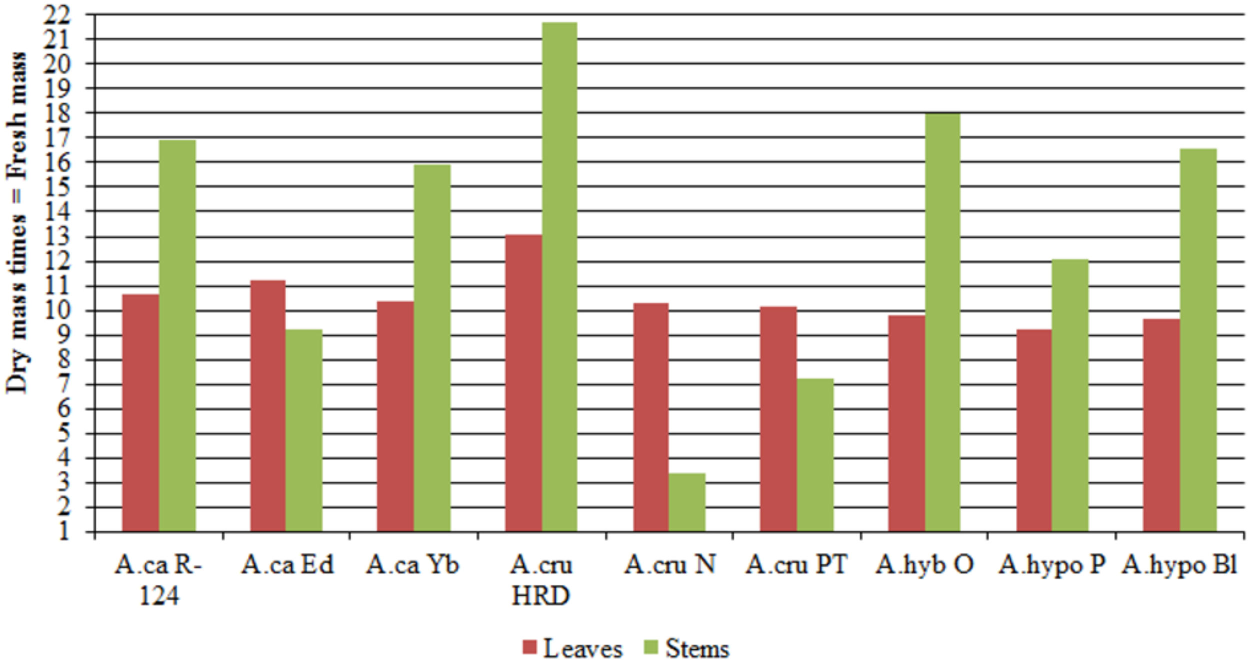

Figure 3. Representative ratios at which the fresh masses were dried to provide the dry masses.

4. DISCUSSION

The results obtained in this study suggest that there was large variability in the values of leaf functional traits and stem functional traits among the nine amaranth cultivars. For instance, according to Hamidzadeh et al. (2021), morphological and biochemical traits of *Amaranthus retroflexus* L differed significantly within species.

In our results, LFM, LDM, and LSA values varied between 173.7 ± 4.0 mg to 539.6 ± 6.7 mg, 13.3 ± 4.5 mg to 55.2 ± 4.0 mg, and 60.0 ± 8.8 cm^2 to 108.5 ± 7.3 cm^2, respectively, for one side. On the other side, SFM, SDM, and

SL values varied between 543.57 ± 2.5 mg to 3547.8 ± 20.0 mg, 25.0 ± 2.0 mg to 819.2 ± 6.4 mg, and 14.0 ± 2.0 cm to 34.2 ± 2.0 cm, respectively.

Our results clearly showed that this functional variation in the leaf has provided a strong correlation between LFM and LDM but a weak correlation between LFM and LSA as well as LDM and LSA. Similarly, Abe, Jansen van Rensburg, and Adebola (2015) reported that in *Amaranthus* species, leaf area showed a moderate correlation with fresh biomass and dry biomass. According to Niamjit (2014), correction factors showed a rather narrow variation for leaf area and leaf biomass in his study.

Huang et al. (2019), in their study of broad-leaved plants, concluded that leaf fresh mass might be more valuable to reflect the physiological functions of leaves associated with photosynthesis and respiration than leaf dry mass. Leaf dry mass and leaf surface area are important leaf traits of plants to describe 'power law (Niklas et al., 2007).

Pan et al. (2013), demonstrated the relationship between leaf size and leaf surface area for the understanding of how leaves maintain a positive carbon balance and influence whole-plant fitness. Several studies have shown diminishing returns (not always leaves increase in mass at the same time in surface area) (Niklas et al., 2007). Within the leaves, there are at least two components: productive tissues (lamina) and support tissues. The growth rate of plants exhibits a positive correlation with the mass fraction found in the leaf lamina while displaying a negative relationship with the fraction of support tissues. Some studies indicate that leaf size modifies the distribution of leaf biomass between productive and support tissues, which further leads to the underlying allometric proportion relationships between leaf dry mass and leaf surface area (Huang et al., 2019). According to Niklas et al. (2007), in particular, large leaves tend to have a larger fractional biomass investment in support structure relative to small ones.

In this present study, in globalization the results showed that between leaf parameters and stem parameters, there was a low significance; thus, we found a moderate correlation between amaranth leaf fresh mass and amaranth stem fresh mass, as well as a weak correlation between amaranth leaf surface area and amaranth stem length. Above, we described the variability of the values in stem functional traits, and by doing so, we found that stem fresh mass had a weak correlation with stem length and a very weak correlation between stem dry mass and stem length. Unfortunately, the stem fresh mass was negatively correlated with the stem dry mass. This antagonist relationship within the amaranth stem was defined by Hamidzadeh et al. (2021) in the study of morphological and biochemical traits of *Amaranthus retroflexus*, where the species differed when they grew in the same conditions.

Contrary to the findings of Thanapornpoonpong, Somsak, Pawelzik, and Vearasilp (2007), who reported a significant and positive correlation between plant height and fresh biomass. Moreover, these authors found positive and significant correlations between plant height and dry biomass in Thailand. Thanapornpoonpong et al. (2007), also reported that there were significant and positive correlations between plant yield and plant height, as well as dry biomass. Abe et al. (2015), also reported that plant height was positively correlated with fresh biomass and dry biomass. Thus, the study was conducted in the Middle Ural of the Russian Federation, Ekaterinburg city, Russia (56°50′ N and 60°36′ E, 255 m above sea level) with a continental climate of temperate latitudes and temperatures between 10°C and 15°C (meteorological summer). It could be the subjected projection of Rwanda's agricultural improvement of food amaranth because Rwanda has a tropical climate characterized by its hilly landscape stretching from east to west with a latitude of 1.9403° S and longitude of 29.8739° E and annual temperatures between 16 - 25°C.

The ratios of leaf dry mass and leaf fresh mass, as well as stem dry mass and stem fresh mass, were disproportional between the cultivars studied in general and between the cultivars of the same species (Figure 3). According to Niklas et al. (2007), such plant-environment interactions can result in adaptive plant strategies reflected in morphology, anatomy, and physiology. However, according to Niklas et al. (2007), it is not the principle that the increase in foliar water content is proportional to the increase in leaf dry weight. They also stated that in the same species, the ratios of leaf dry weight to leaf fresh weight are not constant. A higher ratio was obtained between the stem parameters than the leaf parameters (Figure 3). The observed phenomena may be attributed to the elevated solute concentration and increased water content present in the stem of amaranth plants. The stems play a supportive role in plant aerial parts, and they are the hydraulic pathway of those parts, so they have nutrient stocks and are involved in conducting them to the aerial parts of the plant (Poorter et al., 2012).

5. CONCLUSION

Biomass estimation is a critical characteristic for understanding plant productivity and quality. In this study, we used leaf fresh mass and stem fresh mass to represent leaf and stem biomass, respectively. For the investigated cultivars, the leaf and stem mass were obtained in an undefined manner. However, within a cultivar, there were no proportional measures between leaf and stem parameters, and among all cultivars, A. cru HRD represented the biggest difference between fresh and dry mass and the lowest measures of leaf surface area and stem length. In Rwanda, the amaranth could be cultivated by modernization for feeding animals, and the biomass technique is an easy way to conserve food for them. This pseudo-cereal genus as a supplement food for cereals requires considering it as a non-underutilized crop to make the healthy future crop of the World. Because all investigated species are found in Rwanda, this study is an opportunity that can be exploited to enhance Amaranth adoption levels and farmers' access to information in the production of food Amaranth.

Abbreviations

A.ca R-124:	Amaranthus Caudatus R-124.
A.ca Ed:	Amaranthus Caudatus cv. Edulis.
A.ca Yb:	Amaranthus Caudatus f. Yellow Brown.
A.cru HRD:	Amaranthus Cruentus cv. Hopi Red Dye.
A.cru N:	Amaranthus Cruentus cv.Nodoja.
A.cru PT:	Amaranthus Cruentus cv.Pygmy & Torch.
A.hyb O:	Amaranthus Hybridus cv. Oeschberg.
A.hypo P:	Amaranthus Hypochondriacus.
A.hypo Bl:	Amaranthus Hypochondriacus cv. Black leaved.

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Data Availability Statement: The corresponding author can provide the supporting data of this study upon a reasonable request.
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