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Research Article
Evaluation of Seed Germinability and Seedlings Performance in *Amaranthus* Accessions Collected in the Eastern Cape Province, South Africa

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Abstract
Background and Objective: *Amaranthus* is among the most popular consumed indigenous leafy vegetables in the Eastern Cape Province of South Africa that can be domesticated. It is propagated from seed annually; however, lack of quality seed is one factor that prohibits its domestication. This study aimed to investigate seed viability and seedling vigour of *Amaranthus* accessions and their influence on early seedling establishment. **Materials and Methods:** Laboratory and greenhouse experiments were conducted to determine seed viability and seedling vigour of seeds from four *Amaranthus* accessions. In laboratory experiment, 25 seeds of each accession were used to determine germination while in the greenhouse experiment, emergence percentage and seedling vigour were established. Both experiments were arranged in a Complete Randomized Design (CRD) and replicated four times. Data collected were subjected to Analysis of Variance (ANOVA) using Genstat® 14th edition. **Results:** In summer and autumn, the highest final germination percentage was observed from *Amaranthus retroflexus* 2 (85%) while *Amaranthus hypochondriacus* produced the highest seedling vigour (2.00) in the laboratory experiment. A high positive correlation between seed weight and germination was observed in summer (R^2 = 0.91) and autumn (R^2 = 0.92). In the greenhouse experiment, the emergence percentage (93%) and seedling vigour (1.24) were significantly higher in *A. hypochondriacus* in summer. A negative correlation in summer (R^2 = 0.58) and autumn (R^2 = 0.62) was observed between seed weight and seedling vigour. **Conclusion:** *The A. retroflexus* 2 and *A. hypochondriacus* are recommended owing to their significant response in germination, emergence and seedling vigour.

Key words: *Amaranthus* accessions, climate change, indigenous leafy vegetables, germination percentage, seed quality

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Amaranthus L. is a member of the Amaranthaceae plant family comprising between 70-75 species[1] of which more than 50 are consumed as leafy vegetables worldwide while others are considered weeds[2]. According to Omondi *et al.*[3] *Amaranthus* species, A. *dubious* L., *A. hybridus* and *A. tricolor* L. are consumed as leafy vegetables, while *A. caudatus* L., *A. cruentus* L. and *A. hypochondriacus* L, are regarded as grain amaranth[4]. Other species such as *A. retroflexus* L. (redroot pigweed), *A. albus* L. (tumbleweed), *A. palmeri* S. Wats. (Palmer amaranth) and *A. spinosus* L. (Spiny amaranth) are regarded as weeds[5].

In South Africa, different species of *Amaranthus* are consumed as leafy vegetables across the country, including the Eastern Cape Province, except in the arid South-Western areas[6]. Even though *Amaranthus* is among the popular indigenous leafy vegetables in the Eastern Cape, it is rarely cultivated but gathered from the wild hence there is a need for its domestication[7]. However, lack of high-quality seed is one of the main constraints that may prohibit its full domestication as it is propagated from seeds annually[8].

Seeds are such important inputs in crop production and their quality determines crop productivity as well as the ultimate yield[9]. Planting good quality seeds is an initial step towards optimizing crop production. Seeds are regarded as the reproductive components of plants, hence seeds must be able to germinate, emerge and establish healthy seedlings to develop into productive plants[10]. Seed germination is considered the first physiological stage in the life cycle of a plant[11]. Seeds that can germinate are considered viable and the term viability is defined as the ability of the embryo to germinate when exposed to favourable germination conditions[12]. The concept of good quality seeds is reflected by the rate and uniformity of seed germination, emergence, growth and vigorous plants resulting in good plant stand and final yield[13,14]. Good quality seed is based on various attributes such as seed viability, genetic purity, physical purity, seed health, seed vigour and moisture content[15].

Seed vigour plays a vital role in germination and early seedling establishment. Improved seed germination and seedling vigour are crucial concerning the success of stand establishment of crops. Seedling vigour is characterized by the rapid growth of seedlings during cotyledon and early true leaf stages. According to Tyagi *et al.*[16] seedling vigour is vital in improving stand establishment in several crops. Seedling vigour is an intricate issue and is governed by various physiological parameters such as an important aspect of seed vigour. Also, seed weight is among the most important principles in seed quality determination. It confers an advantage to developing seedlings by increasing germination rate, seedling survival and enhancing seedling and plant growth[17]. Therefore, it is widely accepted that higher seed weight positively correlates with better early seedling performance[18]. Little attention has been paid to the germinability and seedling vigour of *Amaranthus* seeds in the Eastern Cape even though the crop has a high potential to be developed and cultivated as a vegetable. This could be achieved through the collection and conservation of seeds from the wild or cultivated fields for future usage. This study therefore aimed at investigating the seed viability and seedling vigour of *Amaranthus* accessions and their influence on the early seedling establishment.

MATERIALS AND METHODS

Study site: Laboratory and greenhouse experiments were conducted from December, 2018 to January, 2019 (summer season) and April, 2019 (autumn season) at Döhne Agricultural Development Institute (DADI) which is geographically located at -32.52833, 27.46165. The mean minimum and maximum weekly temperatures during the experimental period within the greenhouse were presented in Table 1.

Plant material: Seeds of 4 *Amaranthus* accessions were collected as follows: *A. hypochondriacus*, *A. retroflexus* 1, from Xhorhana Village in Mqanduli and City of Mthatha (O.R. Tambo District), respectively. While, *A. retroflexus* 2 and *A. hybridus* were obtained from Döhne Agricultural Development Institute (DADI) experimental farm, Stutterheim, (Amathole District).

Experimental procedure: All collected seeds were sundried, cleaned and kept at room temperature until the period of use. An experiment that aimed to determine seed viability was established in the Agronomy laboratory while the seedling emergence experiment was conducted in the greenhouse. The experiments were conducted in the summer and autumn seasons of 2018 and 2019, respectively. In the laboratory, before the experiment, Petri dishes (9×1.5 cm) and laboratory workbench were disinfected using 70% ethanol and allowed to dry before use. The 100 seeds of each of the four *Amaranthus* accessions were weighed using a weighing balance (Adam ACBplus-600H Max 600 g d = 0.01 g) and their weights were recorded. Twenty-five seeds of each *Amaranthus* accession were placed in a 9 cm Petri-dish lined with a double layer of Whatman No. 1 filter paper and moistened with distilled water. After planting, the Petri dishes were placed on a laboratory workbench at ambient temperature (24 ± 2°C) until the experiment was

Table 1: Mean minimum and maximum weekly temperatures recorded within the greenhouse in summer (December 2018-January 2019) and autumn (April 2019)

	Summer		Autumn	
Weeks	Mean min. (T°C)	Mean max. (T°C)	Mean min. (T°C)	Mean max. (T°C)
1	12.6	29.7	6.5	23.2
2	13.4	28.2	11.3	30.2
3	13.7	28.7	10.2	25.2
4	13.2	31.4	10.3	27.1
Mean	13.2	29.5	9.6	26.4

Table 2: Final germination percentage, seedling vigour, radicle length and coefficient of velocity of germination of the four *Amaranthus* accessions

Accessions	FGP (%)	SV (Score 1-5)	RL (mm)	CVG
Summer				
Amaranthus hybridus	42.00±2.58[c]	2.97±0.12[a]	17.10±0.50[a]	13.50±0.00[a]
Amaranthus retroflexus 2	85.00±1.00[a]	2.16±0.09[b]	19.12±0.97[a]	13.50±0.00[a]
Amaranthus retroflexus 1	34.00±3.46[c]	3.24±0.05[a]	19.33±0.79[a]	13.70±0.20[a]
Amaranthus hypochondriacus	75.00±3.42[b]	2.00±0.13[b]	19.15±0.99[a]	13.53±0.02[a]
LSD	9.54	0.39	2.62	0.33
CV (%)	10.10	9.4	8.8	1.5
Autumn				
Amaranthus hybridus	42.25±3.57[b]	2.98±0.05[a]	17.92±0.19[a]	13.52±0.05[a]
Amaranthus retroflexus 2	87.75±1.03[a]	2.18±0.13[b]	20.12±1.02[a]	13.50±0.00[a]
Amaranthus retroflexus 1	36.00±4.45[b]	3.24±0.04[a]	19.90±0.75[a]	13.57±0.02[a]
Amaranthus hypochondriacus	77.50±4.29[a]	2.01±0.15[b]	19.32±1.02[a]	13.52±0.02[a]
LSD	11.85	0.35	2.82	0.10
CV (%)	12.2	8.5	9.1	0.5

Means in each column with the same letters is not significantly different (p<0.05), FGP: Final germination percentage, CVG: Coefficient of velocity of germination, RL: Radicle length, SV: Seedling vigour, Scores 1-Very high vigour, Scores 2: High vigour, Scores 3: Moderate vigour, Scores 4: Poor vigour, Scores 5: Very poor vigour and SE: Mean±Standard Error

terminated on day 21. In greenhouse experiment, the seeds of each accession were sown in a 200-celled polystyrene cavity seedling tray using a hygromix commercial growing medium. Both experiments were arranged in a Complete Randomized Design (CRD) and replicated four times.

Data collection: The data collected in the laboratory study included final germination percentage, radicle length, coefficient of the velocity of germination, seedling vigour (measured visually using the scale of 1-5 on day 14) and the effect of seed weight on germination and seedling vigour was also investigated. While in the greenhouse study, data was collected on the emergence percentage and seedling vigour.

The Germination percentage was determined by counting the number of seeds that have germinated starting from the 5th day after sowing until day 14. Percent germination was calculated for each replicate using the following formula described by Mangena and Mokwala[19]:

$$\text{Germination (\%)} = \frac{\text{Number of seeds germinated}}{\text{Total number of seeds}} \times 100$$

The coefficient of velocity of germination in each treatment was calculated as per equation[20]:

$$\text{CVG} = \frac{\sum \text{Ni}}{\sum \text{NiTi}} \times 100$$

Where:

Ni = Number of germinated seeds per day

Ti = Number of days from the start of the experiment

Data analysis: All collected data were subjected to statistical analysis using Genstat® 14th edition. Analysis of Variance (ANOVA) followed by least significant difference (LSD) was used to test the differences among treatments. In cases where treatment means were found to be significant, the Duncan Multiple Range Test (DMRT) was used to separate them at $p \leq 0.05$.

RESULTS

Determination of seed germination and seedling vigour of four local *Amaranthus* accessions (*A. hypochondriacus*, *A. hybridus*, *A. retroflexus* 1 and *A. retroflexus* 2) in summer and autumn

Final germination percentage (FGP): The highest germination percentage in summer was attained from *A. retroflexus* 2 (85%) followed by *A. hypochondriacus* (75%)

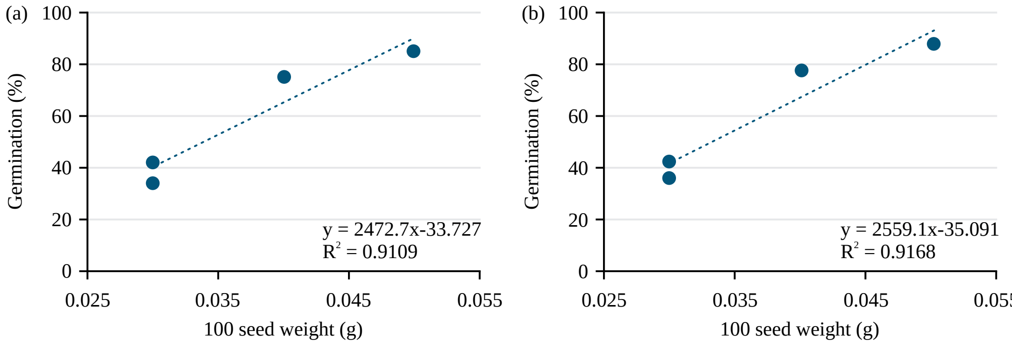

Fig. 1(a-b): Relationship between seed weight and final germination percentage in (a) Summer and (b) Autumn

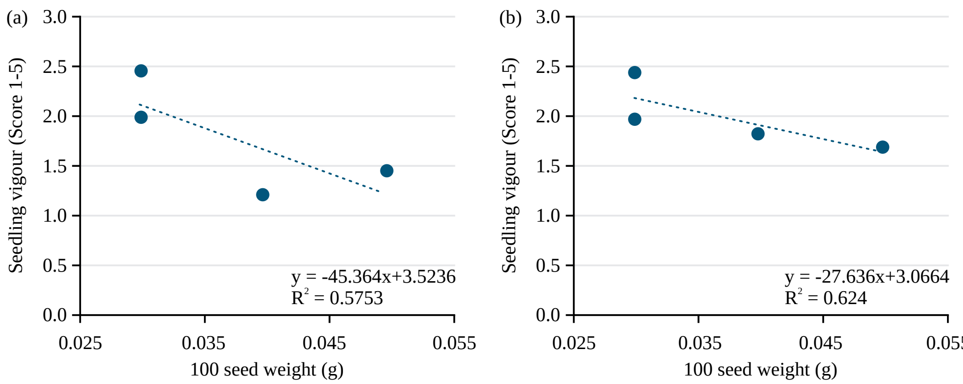

Fig. 2(a-b): Relationship between seed weight and seedling vigour in (a) Summer and (b) Autumn

and *A. hybridus* at 42% while the lowest was observed from *A. retroflexus* 1 (34%). In autumn, *A. retroflexus* 2 outperformed all the other accessions with a final germination percentage of 87.75% followed by *A. hypochondriacus* (77.50%) and *A. hybridus* (42.25%) whilst *A. retroflexus* 1 (36%) yielded the least germination percentage (Table 2).

Seedling vigour (SV): In summer and autumn, the highest seedling vigour was attained from *A. hypochondriacus* (2.00 and 2.01, respectively) and compared well with *A. retroflexus* 2 (2.16 and 2.18, respectively). In summer, the lowest seedling vigour was observed from *A. retroflexus* 1 (3.24) and was comparable to *A. hybridus* (2.97). A similar trend was observed in autumn (Table 2).

Radicle length (RL): No significant differences were observed in the radicle length of all four accessions in both seasons (Table 2). However, in summer *A. retroflexus* 1 produced the longest radicle 19.33 mm while in autumn *A. retroflexus* 2 (20.12 mm) was the best performer. The lowest in both seasons was observed from *A. hybridus*.

Coefficient of velocity of germination (CVG): In both seasons, insignificant differences were observed among the four accessions on the coefficient of velocity of germination (Table 2). Nonetheless, *A. retroflexus* 1 was the best performer in both seasons.

Determination of seed emergence and seedling vigour of four local *Amaranthus* accessions (*A. hypochondriacus, A. hybridus, A. retroflexus* 1 and *A. retroflexus* 2)

Emergence percentage (EP): A superior emergence percentage (93%) in summer was attained from *A. hypochondriacus* and was insignificantly different from *A. hybridus* (84.88) and *A. retroflexus* 2. The lowest emergence percentage was observed from *A. retroflexus* 1 (47.50%). In autumn, a remarkable performance was observed from all accessions except for *A. retroflexus* 1 which yielded the lowest emergence percentage (Table 3).

Seedling vigour (SV): A highly significant difference ($p<0.001$) was observed among all the accessions in summer. Very high seedling vigour was attained from

Table 3: Emergence and seedling vigour of the four *Amaranthus* accessions in the greenhouse

	Summer		Autumn	
Accessions	EP (%)	SV (score 1-5)	EP (%)	SV (score 1-5)
Amaranthus hybridus	84.88±2.16[a]	2.04±0.13[b]	83.75±2.17[a]	2.02±0.16[b]
Amaranthus retroflexus 2	88.50±1.60[a]	1.49±0.04[c]	88.38±0.90[a]	1.73±0.04[b]
Amaranthus retroflexus 1	47.50±8.03[b]	2.52±0.12[a]	48.75±8.08[b]	2.50±0.09[a]
Amaranthus hypochondriacus	93.00±2.75[a]	1.24±0.08[c]	83.50±8.32[a]	1.87±0.07[b]
LSD	14.09	0.33	20.08	0.33
CV (%)	11.20	11.3	16.5	10.1

Means in each column with the same letters is not significantly different ($p<0.05$), EP: Emergence percentage, SV: Seedling vigour, Scores 1: Very high vigour, Scores 2: High vigour, Scores 3: Moderate vigour, Scores 4: Poor vigour, Scores 5: Very poor vigour and SE: Mean±Standard Error

Table 4: Average weight of 100 seeds of the four *Amaranthus* accessions used in the experiment

Amaranthus accessions	Seed weight (g/100 seeds)
Amaranthus hybridus	0.03
Amaranthus retroflexus 2	0.05
Amaranthus retroflexus 1	0.03
Amaranthus hypochondriacus	0.04

A. hypochondriacus (1.24) and was comparable to *A. retroflexus* 2 (1.49) while *A. hybridus* and *A. retroflexus* 1 yielded high vigour (2.04 and 2.52, respectively). In autumn, the response of the four accessions was significantly different ($p<0.05$). *The A. retroflexus* 2 (1.73), *A. hypochondriacus* (1.87) and *A. hybridus* (2.02) attained high vigour whilst *A. retroflexus* 1 (2.50) achieved moderate vigour (Table 3).

Relationship between seed weight and final germination percentage as well as seedling vigour: The 100 seed weight of *A. retroflexus* 1, *A. retroflexus* 2, *A. hypochondriacus and A. hybridus* is shown in Table 4. The 100 seed weight of *A. retroflexus* 2 and *A. hypochondriacus* was superior compared to *A. retroflexus* 1 and *A. hybridus.* Accessions that gave the highest seed weight resulted in the highest final germination percentage and showed a positive correlation in summer ($R^2 = 0.9109$) (Fig. 1a) and autumn ($R^2 = 0.9168$) (Fig. 1b). A negative correlation ($R^2 = 0.5753$) in summer (Fig. 2a) and ($R^2 = 0.624$) in autumn (Fig. 2b) was observed between seed weight and seedling vigour, meaning that accessions with bigger seed weight produced more seedling vigour.

DISCUSSION

The substantial response of seeds from *A. retroflexus* 2 and *A. hypochondriacus* to germination suggested that the tested seed was highly viable compared to *A. retroflexus* 1 and *A. hybridus.* It was observed that *A. retroflexus* 2 and *A. hypochondriacus* seed weight was superior to *A. retroflexus* 1 and *A. hybridus* and this could be attributed to the genotypic variations. Similar to Kahu *et al.*[21] who observed variations between *Amaranthus* varieties assessed. A parallel relationship was observed between seed weight and final germination percentage from the four *Amaranthus* accessions assessed, which concurred with Myint *et al.*[22] who reported that seed weight influenced germination on oil palm crosses. The significant difference observed between *A. retroflexus* 1 and *A. retroflexus* 2 could probably be ascribed to the diverse environmental conditions of the maternal plant hence the two species were collected from various agroecological zones. This agreed with Dube and Struik[9] who observed similar results on *A. hybridus* sourced from different farmers from agroecological regions. The better final germination percentage achieved by heavier seeds could result from greater availability of food reserves in heavy seeds[23]. A report by Kimaru *et al.*[15] indicated that seed germination percentage in African Nightshade (*Solanum* spp.) was positively influenced by seed weight. The effect of grain weight on germination and seed vigour of wheat was studied by Shahi *et al.*[23]. Their results contrasted with the observation of the present study. These authors' results showed that the grain weight had no significant effect on germination percentage. The close association observed between seed weight, final germination percentage and seedling vigour affirms the more competitive nature of heavy-weight seeds. Similarly, Thangjam and Sahoo[24] observed a positive relationship between seed weight and seedling dry weight on *Parkia timoriana* (DC.) Merr. Also, Shahi *et al.*[23] confirmed that grain weight significantly affects the seedling vigour. The higher seed weight effect might have accounted for the early comparative growth advantage of the seedlings. Thus, seed weight can be used to predict final germination percentage and seedling vigour.

Concerning the emergence percentage, all accessions tested exhibited substantial response except for *A. retroflexus* 1. An inferior performance of *A. retroflexus* 1 in both seasons may be due to genetic variations. On the other hand, *A. hybridus* performed poorly on final germination percentage in the laboratory, however, its substantial response on emergence in the greenhouse was evident and that could be probably due to the nutritional effect of the growing media used at sowing. A high germination percentage is desirable for the producer and the germination test with a high seedling percentage should indicate the potential germinability which should predict the germination in the field and the final produce.

The overall performance of the four accessions tested ranged on the score of 1-3 which denotes very high to moderate seedling vigour. The substantial performance of *A. hypochondriacus* and *A. retroflexus* 2 to other accessions could be attributable to the seed weight influence. This concurred with Hamza *et al.*[25] who observed the highest average seedling vigour index when Giza-1 with higher seed weight was used. The study revealed that seed weight had a considerable influence on seed germination, emergence and seedling vigour despite the seed source. Also, seed weight had a positive correlation with final germination percentage showing that seed weight is a good predictor for enhanced germination. Therefore, based on the current results, the use of high seed weight of *Amaranthus* is highly recommended for germination purposes to achieve substantial results. Hence, the germination and emergence studies were conducted in the laboratory and greenhouse, respectively, future studies are required that would investigate the germination potential of these tested accessions under field conditions.

CONCLUSION

Amaranthus accessions with the highest seed weight were found to be highly responsive to germination percentage, seedling emergence and seedling vigour compared to those with the lowest seed weight. These four assessed accessions showed enhanced seedling vigour irrespective of the species. However, the response behaviour of *A. retroflexus* 2 and *A. hypochondriacus* in all the measured parameters was prominent, therefore they are highly recommended. The substantial performance of these two accessions would result in an improved seedling establishment which would also increase the yield.

SIGNIFICANCE STATEMENT

This study aims to identify key protocols to be considered when conducting seed selection from local *Amaranthus* accessions for future domestication in the Eastern Cape Province. The study revealed that some *Amaranthus* accessions, even if collected from their natural habitat, have high germinability, emergence percentage and seedling vigour. It was also observed that accessions with bigger seed weights, had better germinability when compared to those with smaller seed weights. Inversely, accessions with bigger weights had poor seedling vigour. It is recommended that bigger seed weights be used for improved germination and better seedling establishment. The future prospective of this study includes evaluating the germination potential and seedling performance of these tested accessions under field conditions.

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