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Seed germination and morphological characterization of traditional leafy vegetables

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Traditional leafy vegetables provide affordable sources of micronutrients to many people in Zimbabwe. They are produced by smallholder farmers with limited knowledge on the agronomic value of high-quality seed and the seed is rarely tested to determine its quality. This study was therefore conducted to evaluate the germination of seeds of three traditional leafy vegetables sourced from five different farmers and the morphological characteristics of the seedlings. The seeds were collected in the growing season of 2015 to 2016, stored at room temperature and tested for germination in the laboratory at regular monthly intervals during storage. Field germination tests were conducted only once. The seeds tested were classified as normal seedlings, abnormal seedlings, fresh non-germinated seeds or dead seeds. Morphological analysis was conducted at seedling stage, including seven qualitative characters and one quantitative character. Mean separation based on the least significance difference at 5% indicated that percentage germination, purity and 1000-seeds weight for all three species significantly differed among sources. The results showed that *Cleome gynandra* had lowest germination percentages, *Amaranthus hybridus* was intermediate and *Bidens pilosa* had highest values throughout the tests. *C. gynandra* seed exhibited dormancy, showing high levels of fresh non-germinated seed percentage after each test, which is potentially disastrous for the farmers. The combination of pre-chilling, light, potassium nitrate and alternating temperatures as a way of improving germination for *C. gynandra* needs further validation.

Key words: Seed quality, purity, germination, morphological characteristics of seedlings

INTRODUCTION

Traditional leafy vegetables (TLVs) of Africa refers to plant species which originate on the continent and have a long history of cultivation, domestication and use in African conditions (Ambrose-Oji, 2009). Only recently, TLVs' cultivation, domestication and use are tremendous across Sub-Saharan Africa (Oniang'o et al., 2006), partly

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because of their wide use offering important socio-economic benefits. TLVs' consumption and marketing across Africa have increased steadily with the growing demand from consumers as well (Ojiewo et al., 2013). For example, in Zimbabwe, more than 75% of the population eat them when available (DFID project 2309, 2003); most commonly consumed are: spider flower (*Cleome gynandra*), pumpkin leaves (*Cucurbita* spp.), pigweed (*Amaranthus* spp.), cowpea leaves (*Vigna unguiculata*), black jack (*Bidens pilosa*), okra (*Abelmoschus esculentus*) and jute leaves (*Corchorus olitorius*).

The production of TLVs is challenged by many factors including unavailability of quality seeds (Abukutsa-Onyango, 2007), low germination rates of seed (Cernansky, 2015), the problems of purity (Karanja et al., 2012), limited knowledge on the agronomic value of high-quality seed among smallholder farmers (Biemond et al., 2012) and poor seed production skills by smallholder farmers as they rely on traditional seed production technologies to produce their seed (Schippers, 2000). Notably, over three quarters of the farmers that venture into TLVs production use saved seed for planting their crops in Zimbabwe (Schippers, 2000) and its quality is rarely known, risk the poor germination and poor crop stand causing low production and productivity, which directly affects their yields (Karanja et al., 2012) and quality (Ayana et al., 2014) of their produce; therefore reducing their profits. Farmers see very low profits as they fail to fetch premium prices on both vegetables and seed markets (Cromwell et al., 1992), because their seeds have not gone through any certification process and the resulting produce may be of poor quality. The seed markets remain localised, because the seed lack quality declaration documentation such as seed testing certificates, which are required for regional and international seed trade (Cromwell et al., 1992), thus denying the smallholder farmers a variety. When planting the seeds, the farmers are at times forced to use large quantities of seed to compensate for the poor germination rates and this becomes less economically viable. Furthermore, at harvesting, because the variety admixtures are emanating from the use of less pure seed, the farmers tend to selectively harvest, which is cumbersome and time consuming. All these challenges have constrained the ability of farmers to meet the demand and to deliver quality produce to consumers (Afari-Sefa et al., 2012).

This can be addressed by regular checks on the quality of the seed, getting the required quality declaration documentation before the seeds are distributed or sold. Such evaluations are done in the laboratory under optimal conditions. In some cases, skilled farmers do regular checks on quality of their seeds before planting. The commonly used method by these farmers is testing germination by putting just a few seeds in the soil. This justifies the adoption of the study on farmers saved seed which aimed at (1) evaluating the germination of traditional leafy vegetable seeds and (2) determining the morphological characters of seedlings from five sources.

The research question is:

Do percentage germination, qualitative and quantitative morphological characteristics vary by seed source?

Therefore the hypotheses tested include:

i) Percentage germination varies among seed sources.
ii) Qualitative and quantitative morphological characteristics vary by seed sources.

METHODOLOGY

The study uses three traditional leafy vegetable species, namely *C. gynandra*, *B. pilosa* and *A. hybridus* as examples. *C. gynandra* and *A. hybridus* are C_4 plants (Imbamba et al., 1977; Schippers, 2000), while *B, pilosa* is a C_3 plant.

Origin of the seed sources

Seeds of three species were sourced from four farmers and a research organization. These seed sources were chosen using a snow ball sampling technique, with the traditional leafy vegetable markets as entry point. Only farmers who had seed stocks from the previous season showing that they had been producing TLV seed were chosen. The research organization was included for comparison purposes since most of the seed is produced by smallholder farmers with limited knowledge, as alluded earlier.

During the seed development time, factors that might influence seed quality including weather variation were also recorded weekly (Annex 1 contains for full results). About 200 g of seed per species was sourced from each farmer to be used for germination tests and morphological characterisation. The seed collection constituted of 15 samples (samples of three species per seed source). The seed was stored at room temperature and seed germination in the laboratory was analysed at regular time intervals during storage. The germination tests were conducted from August to November 2016 and in February 2017. The laboratory tests were conducted at the Seed Services in the Department of Research and Specialist Services under the Ministry of Agriculture, Mechanisation and Irrigation Development. Field germination tests were conducted in December 2016 only once.

The seed was sourced from three different agro ecological regions of Zimbabwe which entailed: Mashonaland Central, Mashonaland West and Mashonaland East. The five seed sources entailed: Chinhoyi (Farmer 1), Murehwa – two farmers (Farmers 2 and 3), Shamva (Farmer 4) and research organization (Farmer 5). Geographical coordinates of the seed origin sites are as shown in **Error! Reference source not found.**.

Laboratory and field experiments

The seed was sub-sampled to working samples using the hand-halving method as described by the International Seed Testing Association (ISTA, 2016). Thereafter, the purity analysis was conducted on each working sample. The findings were classified as pure seed, inert matter (comprising broken seed, sand, chaff,

stalks) and other seeds. All the purity analyses were done in accordance with the *pure seed definition* provided for each crop species (ISTA, 2016) and procedures applicable to *Tagetes tenuifolia* were used for *B. pilosa*. Other seeds were identified with the aid of reference samples (seed of other crop species collected and filed according to ISTA requirements at Seed Services). Thereafter, each component was weighed using the same balance as on determination of working sample and the percentage weights were calculated using the formula presented below. Also, for morphological quantitative analysis, 1000 seeds were randomly counted from each sample and weighed. The weights ranged from 0.24-0.38 g, 1.03-1.18 g and 1.33-1.74 g, for *A. hybridus, C. gynandra* and *B. pilosa*, respectively, depending on source.

$$Percentage\ pure\ seed = \frac{Weight\ of\ pure\ seed\ after\ purity\ analysis}{Weight\ of\ working\ sample} x\ 100$$

The germination tests were carried out on a working sample of 200 seeds randomly picked from a working sample (ISTA, 2016) and only intact seeds (that is, showing no signs of damage or fungal attack, not shrivelled) were used. Hundred seeds were evenly spread on moistened filter papers- 2 replicates, laid out in a Randomised Complete Block Design on the Jacobsen tank. Plastic funnel caps were placed on each filter paper, to maintain the humidity at high levels around each seed. For *A. hybridus* and *C. gynandra* seeds, filter papers were moistened using potassium nitrate (KNO_3); 100 seeds were evenly spread, then placed in petri dishes and pre-chilled at 5-10°C for seven days (ISTA, 2016). For *B. pilosa* seeds, filter papers were moistened using water and placed directly on the Jacobsen tank without pre-chilling. Germination tests were done according to ISTA standards (temperature requirements, light intensity, alternating temperatures, pre-chilling). The experiments were conducted under light (two fluorescence lamps, 40W) at 8 h light and 16 h darkness, alternating temperatures of 20<=>30°C, using the same Jacobsen tank throughout. Temperatures for the Jacobsen tank were monitored with readings taken at 8 am, 12 pm and 4 pm daily except for weekends and public holidays. Thereafter, seedling evaluation was done on different days depending on the prescribed requirements of ISTA and ISTA (2009) Seedling Evaluation Handbook. Germinated seeds – seedlings were counted by destructive method, with all the seedlings and diseased seeds removed on each day of counting. This included assessing each individual germinated seedling, recording on the card and classifying all normal seedlings, abnormal seedlings, fresh non-germinated, and dead seeds. Annex 3 shows the seedling evaluation intervals. Further, to substantiate whether the combination of pre-chilling and KNO_3 damaged the seed, laboratory tests were conducted for *A. hybridus* and *C. gynandra* seeds in February 2017, which included testing the *A. hybridus* seed without pre-chilling, along with the use of KNO_3 and pre-soaking of the *C. gynandra* seed for 24 and 48 h to mimic the farmer's environment and dried at ambient temperature; then even spread of the seed on filter papers before placing them on Jacobsen tank for germination. Thereafter, similar seedling evaluation procedure described earlier was followed.

Field germination tests were conducted in Seke rural (18° 1' 4" S; 31° 31' 29" E and 1790 m above sea level) in December, 2016. The experiment was laid out in a randomised complete block design. The field was prepared and each treatment was clearly labelled. 100 seeds replicated twice were sown in a bed of 1 m × 0.50 m length, thereafter guard rows were made. The seeds were placed in rows and then thinly covered with soil. The experiment was conducted under rain-fed condition with no application of additional water through irrigation. Seedling evaluation for *B. pilosa* was done on day 7 and 14; *A. hybridus* and *C. gynandra* were evaluated on days 7, 14 and 21; and weeds were removed by hand.

Germination results were calculated using a formula derived from ISTA (2016) with modification and the ratio is then multiplied by 100:

$$Percentage\ normal\ seedling = \frac{Normal\ seeds\ germinated}{Total\ number\ of\ seeds\ per\ replicate} x\ 100$$

Morphological characters evaluation of three species

The experiment was conducted in the greenhouse at University of Zimbabwe from 17 February to 7 March, 2017. A randomized complete block design with three blocks was used. The pots were labelled, randomly arranged and filled to approximately 6 cm under the rim. The soil was sterilised at 70°C for 24 h and allowed to cool. A mixture of soil and pine bark at ratio 1:3 was made to reduce soil compaction and placed in pots. Proper levelling of the growing media was done before approximately 1000 seeds were evenly distributed per pot. The seeds were then covered with 1.5 cm thick layer of the growing media. Water as needed was added to the pots. By 20 February, seeds from most pots with *A. hybridus* and *B. pilosa* had emerged, but not those with *C. gynandra* seeds. On 7 March, seedling emergence was high for all pots expect for *C. gynandra* pots with seed from Farmers 2 and 3 which showed very low germination. The presence of diseases on seedlings was also recorded in the greenhouse (e.g. damping off). The seedlings were thinned randomly from the pots which had high emergence. Later, observations and recordings were conducted on the plants without uprooting them.

Morphological evaluation was done on 7 March at seedling development stage. UPOV- International Union for the Protection of New Varieties of Plants (2002) standard guides was used for categorising the essential plant parts that can be used for morphological evaluation. The essential plant parts subjected to evaluation differed at different plant developmental stages. For instance at seedling stage, hypocotyl, colour, and pubescence can be used for morphological evaluation; when plants are full grown, the following characteristics can be used: growth habit, crown shape, root characteristics (shape, surface, flesh colour, system, leaf characteristics (e.g. blade, petiole, stipule), inflorescence, flower (e.g. calyx, sepal, corolla, petal, stamen, pistil), fruit and seed or grain. The chronological order (order of development) included seedling stage, vegetative phase, reproductive phase, pre-harvest and postharvest. UPOV (2002) guideline shows that morphological qualitative and quantitative characters of certain crop can be measured at seedling developmental stage.

The morphological characters for each species were validated using herbarium specimens in the Zimbabwe National Herbarium and Botanic Garden Institute, descriptors of PROTA (Grubben and Denton, 2004) and the International Plant Genetic Resources Institute and descriptors listed by Wasonga et al. (2015). Only data for the seedling stage was assessed using three replicates of each species. Since the plant species were not commercial varieties like the improved maize varieties, these plant species tended to be heterogeneous (e.g. germination and population characteristics tend to vary); hence the most frequently occurring characteristics observed in the population were recorded. For example, in the presence of anthocyanin colouration on leaf base dominated in the population, evaluation that species was done on that basis. Similar criteria are used by ISTA when a client submitted a mixed seed sample without declaring the name. The observations on the most frequently occurring characteristics covered seven qualitative

characters - including the leaf shape, leaf size and leaf colour, stem colour and near ground stem colour, the presence of anthocyanin colouration on leaf base, presence of diseases, and one quantitative character: 1000-seed weight measured before the seed was planted. Some of these qualitative morphological characters have also been used by Blas et al. (2008) and listed by Wasonga et al. (2015) in morphological characterisation of wild *Arracacia* species and *C. gynandra*, respectively.

Data analysis

The analysis of variance (ANOVA) in GenStat 18th edition was performed on percentage germination, abnormal seedling, fresh non-germinated seed, dead seed and 1000-seed weights and also to test the significance of the differences among the seed sources per species. All the results were transformed using LOG10 plus 1, except for 1000-seed weights and purity of the seed. The transformation was done because some of the data sets had zero values. All the statistical analyses for germination results were carried out on the transformed data. Means of the treatments within each species, among seed sources by species were separated by Duncan's multiple range least significance difference at 5% ($LSD_{0.05}$). The independent variables included: age, source, versus percentage germination, abnormal seedling, fresh non-germinated and dead, as dependent variables. For purity analysis, results were visually compared among the seed sources per species. For morphological analysis, only the quantitative morphological character (1000-seed weight) data was subjected to ANOVA and means separation.

RESULTS

The Seeds Rules and Regulations (1971) of Zimbabwe do not have guidelines for seed certification for these plant species; they are only regarded as weeds (Seeds Act 1971). Hence, there were no prescribed standards for these plant species to compare with. Normal standards are presented on Seeds [certification scheme] Notice (2000), e.g. maize percentage germination and purity is 90 and 99%, respectively. With the case of TLV seeds not covered, but from the farmer's perspective, seed with high germination is preferable. Seed samples sourced from farmers and research organizations were tested for germination in both laboratory and field. The percentage germination and purity were compared among the seed sources and the tests and results are presented in figure and table form. Generally, *C. gynandra* germination was poor; the species had difficulties in germination and was characterised by high occurrence of fresh non-germinated seed at the end of each germination test.

Table 1 shows the percentage pure seed for traditional leafy vegetable species by farmer. Highest percentage pure seed was observed on seed sourced from Farmer 4 for *A. hybridus*. The lowest percentage pure seed for *C. gynandra* was observed for Farmer 1, followed by Farmer 4 and highest for Farmer 3. For *B. pilosa,* the highest percentage pure seed was detected for Farmer 3 and least for Farmer 1. However, the differences among farmers are very small for all three species.

Table 2 depicts the percentage mean dead seed for traditional leafy vegetable species by farmer. There were no significant differences on dead seed percentage across all the seed sources by species.

Table 3 shows the percentage mean fresh non-germinated seed for traditional leafy vegetable species by farmer. The percentage fresh non-germinated seed differed among the farmers but not for *C. gynandra*, which did not show any significant differences across all the seed sources. For *A. hybridus*, Farmers 2, 3, 4 and 5 showed highest percentage mean fresh non-germinated seed and did not significantly differ among themselves. The lowest percentage fresh non-germinated seed was observed for Farmer 1, but did not significantly differ from Farmer 5. The results for the percentage fresh non-germinated seeds for *B. pilosa* also showed that Farmers 2, 3, 4, and 5 were not significantly different and least. However, Farmers 1 and 2 did not differ significantly and depicted highest percentage fresh non-germinated seed.

Percentage germination for the traditional leafy vegetable species

The germination for the traditional leafy vegetable species by farmers was evaluated and results are presented in Figure 1 to 3. For all species, the percentage germination refers to the count of normal seedlings classified according to ISTA (2016) Seedling Evaluation Handbook. Seedlings are regarded as normal if they are defects free, and have the ability to grow into a healthy plant under conducive environments. The percentage germination for all the traditional leafy vegetable species increased at each time interval as storage duration increased from August to November. In the case of each species, the percentage germination varied among farmers. The details for each species are presented later.

For *A. hybridus*, seed sourced from Farmer 3 consistently showed the lowest germination percentage across all time intervals, except for February. In comparison among farmers, Farmers 2, 3, 4 and 5, had considerably very low percentage germination during the first four months of storage. The highest percentage germination was observed for Farmer 1. Interestingly, for Farmer 1, the percentage germination drastically went down, whereas for Farmers 3, 4 and 5, a sharp increase was realised in the seventh month during storage. The standard deviation for each farmer is as follows: Farmer 1 is 9, Farmer 2 is 12, Farmer 3 is 35, Farmer 4 is 16 and Farmer 5 is 18.

Figure 2 depicts very low but variable percentages of germination for *C. gynandra*. The highest percentage germination was consistently observed for Farmer 5, intermediate for Farmer 4 and least for Farmers 2 and 3.

Table 1. The percentage pure seed for traditional leafy vegetables from five farmers (in parentheses the transformed values).

Source	*Amaranthus hybridus*	*Cleome gynandra*	*Bidens pilosa*
Farmer 1	95.3 (1.98)	94.2 (1.97)	97.7 (1.99)
Farmer 2	89.3 (1.95)	99.8 (2.00)	99.6 (2.00)
Farmer 3	85.7 (1.93)	99.9 (2.00)	99.9 (2.00)
Farmer 4	99.6 (2.00)	96.4 (1.98)	99.6 (2.00)
Farmer 5	90.6 (1.96)	99.8 (2.00)	98.0 (1.99)

Table 2. The mean percentage dead seeds for traditional leafy vegetable from five farmers (in parentheses the transformed values and the mean separation).

Source	*Amaranthus hybridus*	*Cleome gynandra*	*Bidens pilosa*
Farmer 1	20 (1.06)	17 (1.03)	13 (1.08)
Farmer 2	22 (1.14)	19 (1.04)	13 (1.08)
Farmer 3	15 (0.96)	20 (1.04)	10 (0.94)
Farmer 4	14 (0.97)	16 (0.97)	9 (0.94)
Farmer 5	13 (0.89)	12 (0.86)	8 (0.92)
$LSD_{5\%}$	0.35 (NS)	0.30 (NS)	0.18 (NS)

There was a sharp increase in percentage germination for Farmers 4 and 5 between November and February. The percentage germination for Farmer 1 showed consistent trends, maintaining a slight increase by month, throughout the tests. The standard deviations are presented by farmer with the following values; Farmer 1 is 1, Farmers 2 and 3, is 0, Farmer 4 is 7 and Farmer 5 is 13.

For *B. pilosa* (Figure 3), percentages germination were higher across all the seed sources compared to other species; and the age effect did not show very consistent and distinctive trends, especially not for Farmers 3, 4 and 5. However, distinctive trends were observed for Farmers 1 and 2. The standard deviation is presented in the figure by farmer and included the following values: Farmer 1 is 13, Farmer 2 is 6, Farmer 3 has 4, Farmers 4 and 5 is 1.

The two-way interactions between time and method on percentage germination is shown in Annexes 6 to 8. The results of a two-way ANOVA for *A. hybridus* (Annex 6) did not show a clear trend on the percentage germination. However, pre-chilling + KNO_3 and water in February gave the highest percentage germination; these values were, however, not statistically significant from those of October and November. The results obtained from the field were significantly lower than the values from laboratory tests, but were not statistically significantly different from the results obtained in August, September, October and November where pre-chilling and KNO_3 were used. For *C. gynandra* (Annex 7), pre-soaking the seed for 24 and 48 h before planting led to highest percentage germination in February. The ranking of percentage germination using pre-chilling and KNO_3 was consistent among the planting times, but February gave highest percentage germination, and least in August. The field results for *C. gynandra* gave statistically significantly higher percentages germination over pre-chilling and KNO_3. The difference between methods and time on percentage germination for *B. pilosa* (Annex 8) was statistically significant. The field tests gave significantly lower results comparable to the results obtained in laboratory tests for August and September.

Qualitative and quantitative morphological characters evaluation of three species of traditional leafy vegetables

The qualitative morphological characters of each traditional leafy vegetable species were examined among the farmers presented (Annex 5). We observed high morphological character variations in *A. hybridus* seed. Only *A. hybridus* seed for Farmer 2 had narrow, lanceolate leaves while the remaining four seed sources had broad, ovate leaves. Farmer 3 had a majority of seedlings with red purple stem. The majority of farmers had seedlings without anthocyanin colouration on leaf base except for Farmer 4 who had seed producing plants that had anthocyanin colouration on the leaf base. Damping off was observed on *A. hybridus* and *C. gynandra* on seed from Farmer 4 only. No morphological

Table 3. The mean percentage fresh non-germinated seeds for traditional leafy vegetables from five farmers (in parentheses the transformed values and the mean separation).

Source	*Amaranthus hybridus*	*Cleome gynandra*	*Bidens pilosa*
Farmer 1	4 (0.63^{a})	74 (1.68)	11 (0.84^{b})
Farmer 2	26 (1.16^{b})	77 (1.71)	6 (0.60^{ab})
Farmer 3	50 (1.31^{b})	77 (1.70)	3 (0.43^{a})
Farmer 4	24 (1.12^{b})	70 (1.66)	2 (0.44^{a})
Farmer 5	23 (1.09^{ab})	68 (1.65)	2 (0.36^{a})
$LSD_{5\%}$	0.46	0.47 (NS)	0.34

The mean separation indicates per species the differences among the five farmers. N.S is not significant.

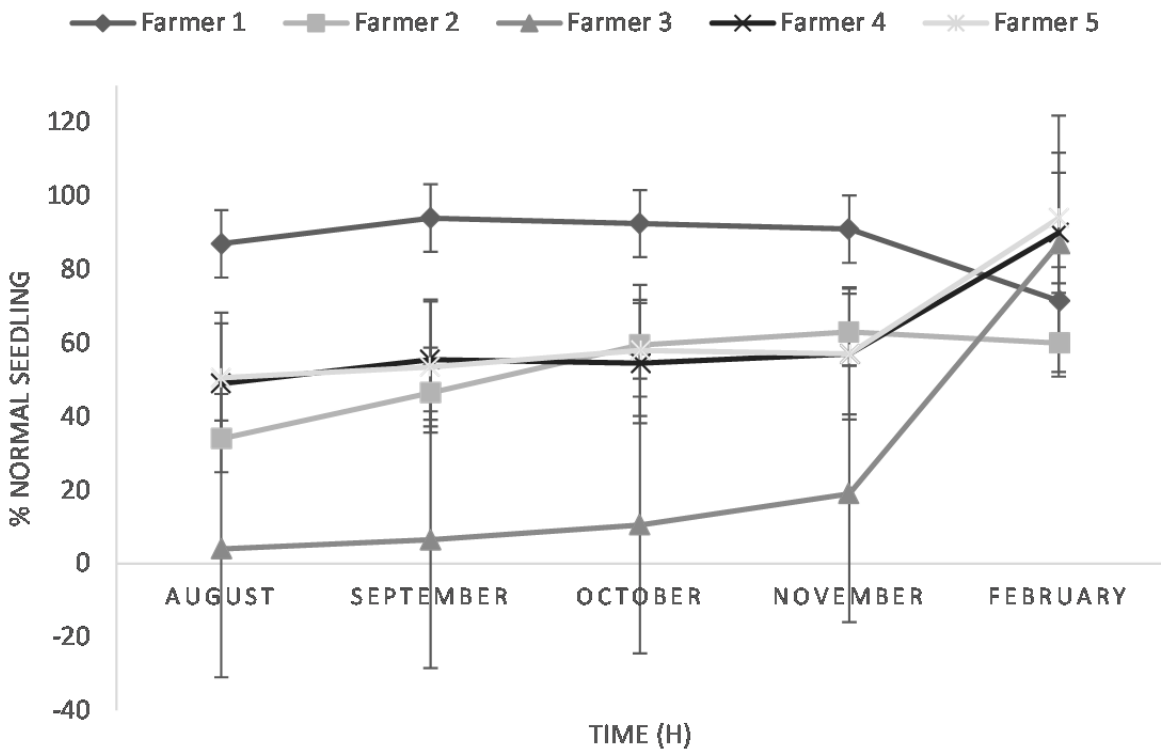

Figure 1. The percentage germination for *Amaranthus hybridus* from five farmers.

character differences were observed for seed from the five farmers on *C. gynandra* and *B. pilosa*.

The weights of 1000 randomly selected seeds per plant species by farmer are presented in Table 4 and were used to determine the quantitative morphological characters by performing analysis of variance and separation of means using Duncan's multiple range method in GenStat ($LSD_{5\%}$).

There were significant differences among the mean weights of 1000 seeds of *A. hybridus*, *C. gynandra* and *B. pilosa* for farmers (Table 4). For both *A. hybridus* and *C. gynandra* species, seeds from Farmer 1 had the lightest ones (0.24 g and 1.03 g, respectively). For *B. pilosa,* Farmer 3 had the lightest weight of 1.33 g.

DISCUSSION

Seed is the initial link in the food production chain (Shiva, 2016); and good seed perpetuates to more yield and yields better-quality products (Louwaars and Boef, 2012). Seed also symbolizes food security attainment (Shiva, 2016). Good seed comprises many different attributes that add to the performance of the seed ranging from

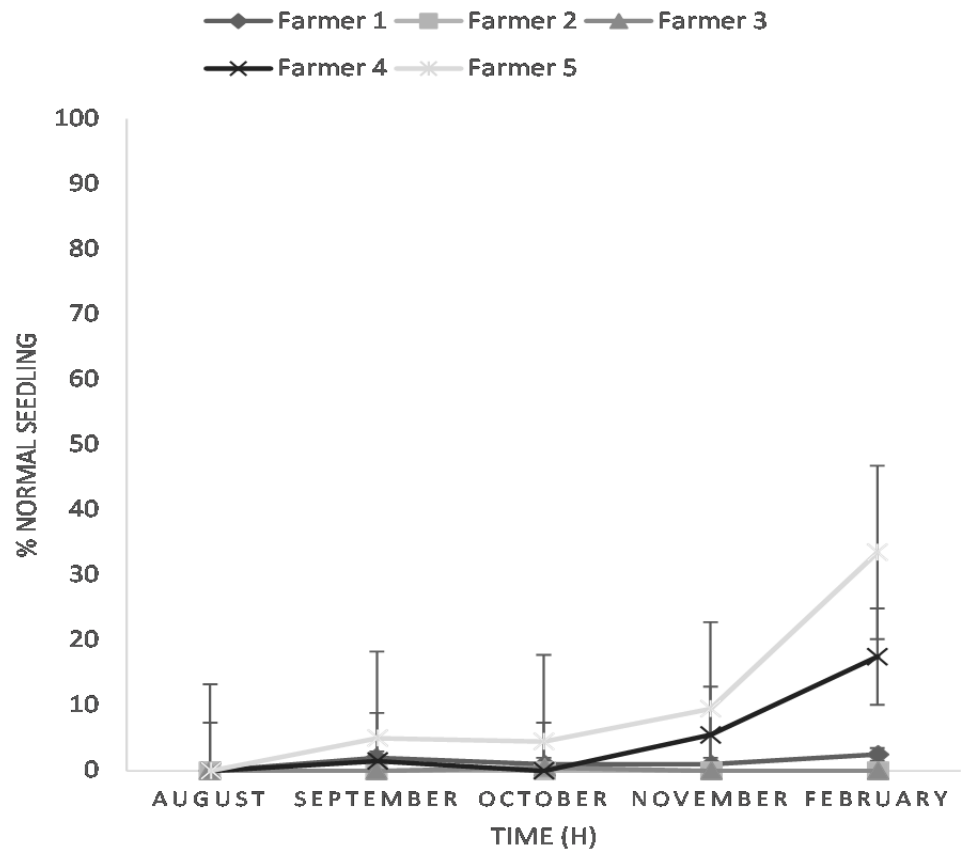

Figure 2. The percentage germination for *Cleome gynandra* from five farmers.

being of proper age, being able to give a vigorous and uniform crop stand and producing vigorous plants that give an optimal yield, being genetically pure to avoid an uneven crop stand and difficulties with harvest if crop plants mature unevenly (Madamba, 2002; Dube and Mujaju, 2013), enabling farmers to meet the requirements of the market (Karanja et al., 2012), e.g. regarding taste, market preference and other quality aspects. In this study, the term seed quality refers to the physiological characteristics, purity and the uniformity of the plants.

The least percentage germination was observed for *C. gynandra* throughout the tests; intermediate values were observed for *A. hybridus* and highest values for *B. pilosa* seed. Throughout the tests, *C. gynandra* seed showed high levels of dormancy, a feature which is potentially disastrous to farmers. All results showed that the germination percentage increased with age and varied with the test method used when no dormancy was present.

On average, using KNO_3 solution to moisten filter papers and pre-chilling *C. gynandra* seed for seven days at 5 to 10°C gave lower results than the field test, pre-soaking seed for 24 and 48 h. According to Kim et al. (2008), light induces the reduction of abscisic acid (ABA) which in turn promotes germination in many plant species. For *C. gynandra* seed, it was observed that such effect did not promote germination, rather induced dormancy. Similar results were reported by Ochuodho and Modi (2007), who observed that the germination of *C. gynandra* seed tested at 20°C was inhibited by light, and gave 0% germination. According to Copeland and McDonald (1995), seeds that are sensitive to light are also sensitive to KNO_3. The seed from all farmers showed very low significant percentage germination. Taken together, our results show that *C. gynandra* seed presents both KNO_3 and light sensitivity throughout the tests. Furthermore, for *C. gynandra*, it was observed that pre-chilling seed for seven days at 5-10°C would not improve percentage germination. According to ISTA (2016), pre-chilling is a way of breaking the seed dormancy, thereby improving percentage germination in many crop species including *C. gynandra*. There are reports of pre-chilling sensitivity in seed of wild plant species, such as *Withania somnifera* (Kambizi et al., 2006) and texasweed seed (Koger et al., 2004). Koger et al. (2004) found that pre-chilling the texasweed seed for 1 day resulted in a decrease in germination to 17% compared to higher percentage germination obtained

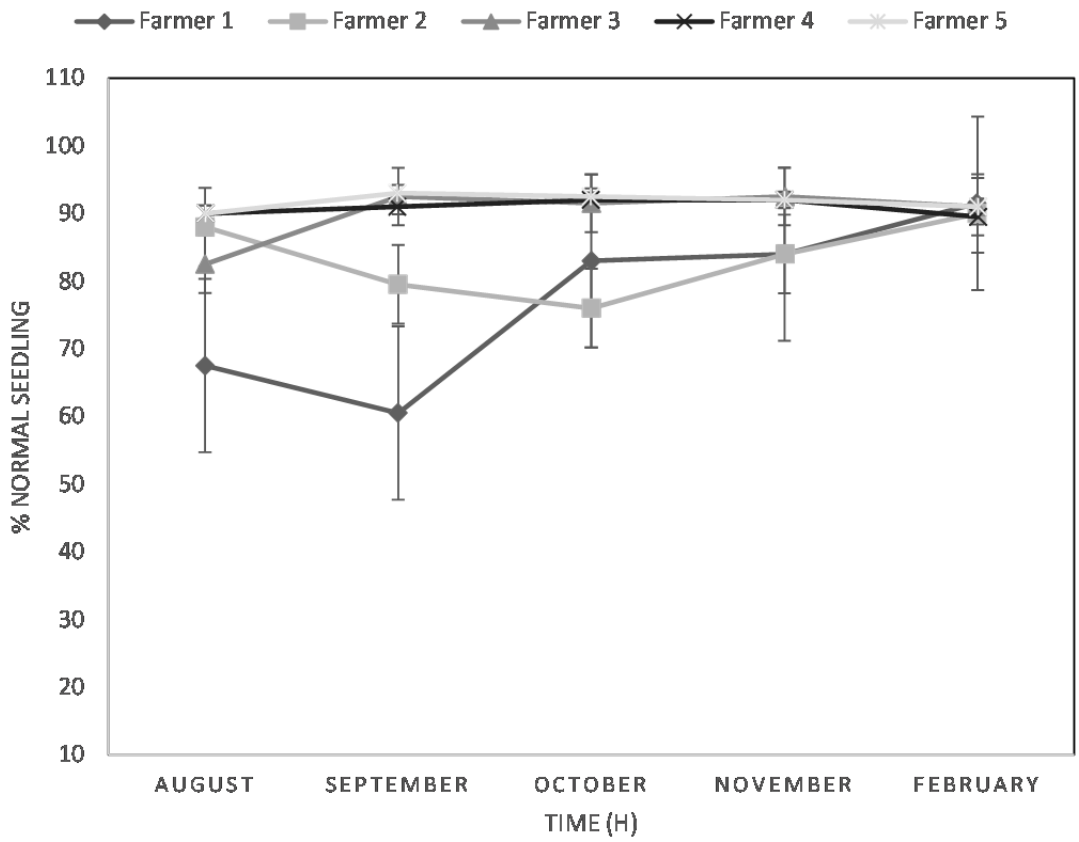

Figure 3. The percentage germination for *Bidens pilosa* from five farmers.

without any pre-chilling. Pre-chilling *C. gynandra* seed for seven days at 5-10°C and hydration for four hours significantly reduced the germination (Ochuodho and Modi, 2005). In comparison between the tests, seed pre-soaked for 24 or 48 h in water, though relatively low, had significantly higher percentage germination than observed in the KNO_3 treatment. Although our findings for the field tests were very low compared to the results reported by Abukutsa-Onyango (2007), they were significantly higher than in the laboratory tests where a combination of KNO_3, pre-chilling, light and alternating temperatures of 20<=>30°C were used. The field results also significantly differed among the seed sources. Abukutsa-Onyango (2007) reported 90 and 30% germination of *C. gynandra* seed tested in the field and laboratory, respectively. *C. gynandra* seeds from Farmers 5, 4, and 1, in that order, for instance showed significantly higher percentages of germination for all tests, along with no significant variation on percentage fresh non-germinated seed, implying that this phenomenon cannot be merely attributed to species but is also influenced by environmental effects. Our results can be partially explained in findings by Ochuodho and Modi (2005) and de Barros et al. (2017) who confirmed that seed dormancy relates to different habitats from where the seeds were collected. Also, there was germination variation among seeds sown in the greenhouse for morphological analysis (results not shown), confirming our results from the laboratory. Seed sourced from Farmers 2 and 3 had very low germination; and hardly 20 plants per pot among all three replicates, although approximately 1000 seeds were used per each pot.

For *A. hybridus* seed, moistening filter papers with water gave comparable results with pre-chilling for seven days at 5-10°C with filter papers moistened by a solution of KNO_3. Germination percentages were significantly higher than in the field test. The average percentage germination varied among farmers and test method used. The higher variations in *A. hybridus* seed can be the result of environmental effects and genetic variation as already shown for the morphological evaluations conducted. The results for the morphological evaluations showed a high diversity among the *A. hybridus* species. The magnitude of morphological variations among the seed sources suggests that the population must be heterogeneous, since we recorded the most frequently occurring characteristics from the observation. This finding can partially explain the observed variation in

Table 4. Quantitative morphological characters of three species of traditional leafy vegetables.

Source	Mean weight for 1000 seeds
Amaranthus hybridus	
Farmer 1	0.24[a]
Farmer 2	0.38[c]
Farmer 3	0.35[b]
Farmer 4	0.38[c]
Farmer 5	0.37[c]
$LSD_{5\%}$	**0.01**
Cleome gynandra	
Farmer 1	1.03[a]
Farmer 2	1.18[b]
Farmer 3	1.16[b]
Farmer 4	1.17[b]
Farmer 5	1.17[b]
$LSD_{5\%}$	**0.02**
Bidens pilosa	
Farmer 1	1.74[d]
Farmer 2	1.45[b]
Farmer 3	1.33[a]
Farmer 4	1.68[c]
Farmer 5	1.68[c]
$LSD_{5\%}$	**0.04**

For each factor, different letters indicate significant differences according to Duncan's multiple range least significant difference test ($LSD_{5\%}$) for each plant species.

percentage germination as well.

For *B. pilosa*, laboratory tests gave significantly higher results than the field test. Generally, *B. pilosa* seed germination is not problematic. The low percentage germination observed in the field test could have been caused by excessive rains received just after planting (over 100 mm down pour on that day). The average percentages germination was statistically significant among farmers. *B. pilosa* seed germination depends on seed origin (de Barros et al., 2017). The average percentage germination and purity of the seed across all seed sources were higher than earlier reported by Dube and Mujaju (2013) for *B. pilosa*. These authors reported the minimum germination and purity standards to be 88 and 99% respectively.

There were significant differences in the weight of 1000 seeds between farmers and between species. For *C. gynandra,* the weight for 1000 seeds ranged from 1.03 g (Farmer 1) to 1.18 g (Farmer 2) and weights statistically differed by farmer (*p=0.05%*). Seed weight for 1000 seeds of *A. hybridus* was statistically significant (*p=0.05%*) among all farmers; lowest weight observed was 0.24 g (Farmer 1) and heaviest was 0.38 g (Farmer 2). Also, 1000-seed weight of *B. pilosa* was statistically significantly different (*p=0.05%*) among all farmers, ranging from 1.33 g (Farmer 3) to 1.74 g (Farmer 1). The observed 1000-seed weights for *C. gynandra* are within the range to those obtained from seed sourced in communities in western Kenya (Abukutsa-Onyango, 2007). *B. pilosa* seed produced under optimum conditions had heavier weights (de Barros et al., 2017). These 1000-seed weight differences can be attributed to the different agro ecological conditions and other environmental conditions prevailing during seed formation, but could also be due to different harvest dates, to genetic variability between the populations as already confirmed by the qualitative morphological analysis and also varying seed moisture content. In our study, we did not test for moisture content, though. For instance, for Farmer 5, *A. hybridus* and *B. pilosa* were planted early December, and the flowering stage could have coincided with low rainfall experienced beginning from January onwards.

The classification of species according to stem colour

has been reported to be very useful to the farmers and consumers. The purple pigment on the stem is very useful and has been reported to play a role in deterring pests (Schippers, 2000). The *C. gynandra* with purple stem is less attractive to pests, drought tolerant and more medicinal than the green stemmed varieties. The author further reported that the purple pigments provide quite a range of health benefits to people, including much needed antioxidants. Other people preferred a green stem because of its tenderness and less bitter taste compared to the purple stem which is bitterer and takes more time to cook. Another important attribute concerning the green stemmed type is that it regenerates better than the purple type, with the latter having a problem of drying up or decaying when the major shoots have been harvested only once or twice (Schippers, 2000).

Conclusions

C. gynandra seeds germinated better after pre-soaking for 24 and 48 h and in field conditions. Hence, pre-soaking *C. gynandra* seeds can be important to kick start the germination process and is one of the good standard procedures to test the quality of this species. The method is relatively inexpensive and even used by resource-poor smallholder farmers. For *B. pilosa* seeds, applying more water than required whether in the field or in the germination trays in the laboratory poses risks of poor percentage germination. However, water can be used to moisten the filter papers instead of KNO_3 treatment. *A. hybridus* seeds have comparably moderate percentage germination with large variations among the seed sources, but higher percentage germination is obtained after longer storage periods. Likewise, water can be used to treat the filter papers instead of KNO_3 and pre-chilling is required to improve germination. This study hypothesizes that percentage germination varies among seed sources. For *C. gynandra* and *B. pilosa* seeds, seeds sourced from Farmer 5 gave highest percentage germination, showing that the seeds were of good quality and the superiority of a research organization. *A. hybridus* seeds from Farmer 1 had highest percentage germination. The heterogeneous population observed for *A. hybridus* seed indicates the rich genetic pool that can be used for breeding purposes and improvement of the species through selection at farmers' level.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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ANNEX

The climatic conditions during crop growth and geographical coordinates of the seed origin sites are presented in **Error! Reference source not found.**. Same seed sources used for the paper http://academicjournals.org/journal/AJAR/article-abstract/308A49E56621 are also used in this paper. There are only a few farmers who produce TLV seeds.

Annex 1. Geographical coordinates and climatic data during crop growth of the origin sites of the seed.

Farmer	Origin	Agro ecological region[a]	Mean annual temperature	Temperature range during crop growth	Geographical coordinates	Altitude (m)	Annual rainfall (mm)	Amount of rainfall during crop growth (mm)
1	Mashonaland West	2B	22-29°C	31-22°C	17°22′00″ S; 30°11′59″ E	1153	700-800	73-0.31
2	Mashonaland East	3	18-29°C	31-21°C	17° 38' 35.59" S; 31° 47' 2.40" E	1400	800-850	100-0.8
3	Mashonaland East	3	18-29°C	31-21°C	17° 38' 35.59" S; 31° 47' 2.40" E	1400	800-850	100-0.8
4	Mashonaland Central	3	21-31°C	31-21°C	17° 18' 56" S; 31° 34' 14" E	953	800-850	110-1.5
5	Mashonaland East	2B	18-29°C	29-27°C	18° 11' 6.97" S; 31° 33' 6.95" E)	1688	850-900	105.7-24.8

Agro ecological classification also known as natural region (NR) classification of Zimbabwe, divided the country into five regions based on mean annual rainfall and was done in the 1960s (Vincent and Thomas, 1960).
Source: Authors (https://www.worldweatheronline.com Historical average weather 20/04/2017).

Annex 2. Average monthly weather data (temperature, rainfall, cloud cover, humidity and bees activity.

Weather	Dec	Jan	Feb	March	April	May	June
				Farmer 1			
Temp (°C)	31	29	30	28	26	23	22
Rain (mm)	73	56	49	98	68.5	11.2	0.31
% Cloud cover	24	36	27	33	25	17	5
% Humidity	57	69	72	81	68	61	56
Bees activity	-	-	Mod	Mod	High	Low	-
Planting				Sole crop			
				Farmer 2			
Temp (°C)	31	29	30	28	26	22	21
Rain (mm)	100	49	46	85	29.5	17	0.8
% Cloud cover	-	39	33	38	22	20	13
% Humidity	-	67	76	79	69	67	63
Bees activity	-	-	-	Mod	High	Low	-

Annex 2. Cont'd

Planting	Intercropped with maize						
				Farmer 3			
Temp (°C)	31	29	30	28	26	22	21
Rain (mm)	100	49	46	85	29.5	17	0.8
% Cloud cover	-	39	33	38	22	20	13
% Humidity	-	67	76	79	69	67	63
Bees activity	-	-	Mod	Mod	High	Low	-
Planting	Intercropped with maize						
				Farmer 4			
Temp (°C)	31	29	30	28	26	22	21
Rain (mm)	110	57	63	29	15	10	1.5
% Cloud cover	-	39	33	38	22	20	13
% Humidity	-	67	76	79	69	67	63
Bees activity	-	-	Mod	Mod	High	-	-
Planting	Sole crop						
				Farmer 5			
Temp (°C)	28.9	27.2	28.4	26.7	-	-	-
Rain (mm)	105.7	14.5	16.7	24.8	-	-	-
% Cloud cover	24	33	30	36	29	21	17
% Humidity	57	68	67	77	70	69	64
Bees activity	-	-	-	-	-	-	-
Planting	Sole crop						

Source: Authors and world weather online.com.
(authors, https://www.worldweatheronline.com Historical average weather 20/04/2017)

The research organization is a government institute located in Mashonaland East province and is under agro ecological region 2B (18° 11' 6.97" S: 31° 33' 6.95" E) at an elevation of 1688 m above sea level. The mean annual rainfall for the area ranges from 850-900 mm. The mean annual temperature ranges from 18-29°C. During seed crop growth temperatures and rainfall ranged (December 2015- March 2016) 28.9-26.7°C and 105.7-24.8 mm, respectively. Shamva is in Mashonaland Central province and located under agro ecological region 3 (17° 18' 56" S: 31° 34' 14" E) at an elevation of 953 m above sea level, receiving the annual rainfall of the range 800-850 mm. The mean temperature for Shamva ranges between 21-31°C. During seed crop growth temperatures and rainfall ranged (December 2015 - June 2016) 31-21°C and 110-1.5 mm, respectively (Annex 1). Murehwa is located in Mashonaland East province under agro ecological region 3 (17° 38' 35.59" S: 31° 47' 2.40" E) at an elevation of 1400 m above sea level. The mean annual rainfall ranges from 800-850 mm. The mean annual temperature ranges from 18-29°C. During seed crop growth, temperatures and rainfall were: (December 2015 - June 2016) 31-21°C and 100 - 0.8 mm, respectively (Annex 2). Chinhoyi is located in Mashonaland West province under agro ecological region 2B (17°22′00″ S: 30°11′59″ E) at an elevation of 1153 m above sea level. The mean annual rainfall and temperature ranges from 700-800 mm and 22-29°C, respectively. During seed crop growth, temperatures and rainfall ranged (December 2015- June 2016) from 31 - 22°C and 73 - 0.31 mm, respectively (Annex 2), (Authors, https://www.worldweatheronline.com Historical average weather 20/04/2017).

Annex 3. Overview of TLV seed samples harvested and collected from farmers.

Farmer/ Organization	Research organization (Farmer 5)	Farmer 4	Farmer 3	Farmer 2	Farmer 1
February, 2016	*Cleome gynandra*	-	-	-	-
March, 2016	-	-	-	-	*Cleome gynandra*
May, 2016	-	-	*Amaranthus hybridus*	-	*Amaranthus hybridus*
May, 2016	-	-	*Bidens pilosa*	-	*Bidens pilosa*
May, 2016	-	-	*Cleome gynandra*	-	-
June, 2016	*Bidens pilosa*	*Bidens pilosa*	-	*Bidens pilosa*	-
June, 2016	*Amaranthus hybridus*	*Amaranthus hybridus*	-	*Amaranthus hybridus*	-
June, 2016	-	*Cleome gynandra*	-	*Cleome gynandra*	-

No harvests were carried out.

Annex 4. Seedling evaluation intervals (ISTA, 2016).

Seedling evaluation days after planting	TLV species		
	Bidens pilosa	***Amaranthus hybridus***	***Cleome gynandra***
Three	+	-	-
Seven	+	+	+
Fourteen (final count)	+	-	-
Fourteen	-	+	+
Twenty one	-	+	+
Twenty eight (final)	-	+	+

+ Seedling evaluation day, - No seedling evaluation done.
Source: Annex Table 4, is (ISTA (2009)

Annex 5. Qualitative morphological characters of three species of traditional leafy vegetables.

Seed source	Colour of stem	Colour of leaf	Anthocyanin colouration on leaf base	Leaf size	Shape of leaf	Colour of near ground stem	Damping off
			Amaranthus hybridus				
Farmer 1	Green and purple	Light green	Absent	Broad	Ovate	Purple	None
Farmer 2	Light green	Light green	Absent	Narrow	Lanceolate	Green	None
Farmer 3	Red purple	Green and purple	Absent	Broad	Ovate	Red purple	None
Farmer 4	Green and purple	Light green	Light purple	Broad	Ovate	Purple	Present
Farmer 5	Green and purple	Light green	Absent	Broad	Ovate	Purple	None
			Cleome gynandra				
Farmer 1	Light green	Light	Absent	Broad	Ovate	Light green	None
Farmer 2	Light green	Light	Absent	Broad	Ovate	Light green	None
Farmer 3	Light green	Light	Absent	Broad	Ovate	Light green	None
Farmer 4	Light green	Light	Absent	Broad	Ovate	Light green	Present
Farmer 5	Light green	Light	Absent	Broad	Ovate	Light green	None
			Bidens pilosa				
Farmer 1	Purple and green	Green	Absent	Intermediate	Oblanceolate	Red purple	None
Farmer 2	Purple and green	Green	Absent	Intermediate	Oblanceolate	Red purple	None
Farmer 3	Purple and green	Green	Absent	Intermediate	Oblanceolate	Red purple	None
Farmer 4	Purple and green	Green	Absent	Intermediate	Oblanceolate	Red purple	None
Farmer 5	Purple and green	Green	Absent	Intermediate	Oblanceolate	Red purple	None

Annex 6. The two-way interactions between media and time percentage germination of *Amaranthus hybridus.*

Media × Time	Mean
Prechill + KNO_3 August	45 (1.52^{a})
Prechill + KNO_3 September	51 (1.60^{a})
Prechill + KNO_3 October	55 (1.67^{ab})
Prechill + KNO_3 November	57 (1.72^{ab})
Prechill + KNO_3 February	81 (1.91^{b})
Field December	36 (1.60^{a})
Water February	80 (1.90^{b})
LSD 5%	0.25

Annex 7. The two-way interactions between media and time percentage germination of *Cleome gynandra.*

Media × Time	Mean
Prechill + KNO_3 August	0 (0.00^{a})
Prechill + KNO_3 October	1 (0.24^{ab})
Prechill + KNO_3 September	2 (0.31^{abc})
Prechill + KNO_3 November	3 (0.41^{bc})
Prechill + KNO_3 February	11(0.66^{cd})
Field December	17 (0.95^{de})
Pre-soak 24 h February	23 (1.33^{ef})
Pre-soak 48 h February	24 (1.36^{f})
LSD 5%	0.38

Annex 8. The two-way interactions between media and time percentage germination of *Bidens pilosa.*

Media × Time	Mean
Field December	76 (1.89[a])
Water September	83 (1.92[ab])
Water August	84 (1.93[ab])
Water October	87 (1.94[b])
Water November	89 (1.95[b])
Water February	91 (1.96[b])
LSD 5%	0.04