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Feasibility of Cultivating Artemisia afra Jacq. ex Willd in Côte d'Ivoire (Daloa) and Evaluation of Its Genetic Diversity on the Basis of Phenotypic Variations

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Abstract
In the southern African regions, *Artemisia afra* Jacq. ex Willd is one of the most popular and commonly used herbal medicines. In recent years, *A. afra* has received much attention from the scientific community and its use is being investigated in the modern diseases like diabetes, cardiovascular diseases, cancer, and respiratory diseases. This growth in popularity could pose a threat to the species due to intensive harvesting. Indeed, overexploitation is a growing problem for many medicinal species in Africa. To sustain the production and availability of *A. afra*, cultivation seems to be a good strategy and an alternative to collecting in the wild. Unlike *A. annua* L. (source of artemisinin), little information is available on the cultivation of *A. Afra* in West African countries. In this study, feasibility of cultivating *A. Afra* in Côte d'Ivoire was evaluated and the extent of its genetic diversity was assessed based on morphological variations. *A. annua* L. was used as control. The result showed for *A. afra*, 30 and 28.02% nursery and field mortality respectively, and 27.77% and 0% for *A. annua*. *A. annua* showed faster growth and development kinetics during the first 90 days after field transplantation. *A. annua* was relatively earlier (83 days to flowering on average) than *A. afra* (207.20 days to flowering on average). Contrary to *A. annua*, *A. afra* was sterile and did not give viable seeds, which poses a major problem of acclimatization in the environmental conditions of Côte d'Ivoire. Assessment of morphological traits revealed significant variations within and between species. Multivariate analysis showed important intra and interspecific genetic diversity. The plants of *A. afra* and *A. annua* were grouped separately and six major clusters were found: two clusters in *A. annua* (cluster I and II) and four clusters in *A. afra* (cluster III, IV, V and VI). These results show that further studies need to be considered to make cultivation of *A. afra* possible in Côte d'Ivoire with superior and genetically stable genotypes.
Keywords: *Artemisia afra*, *Artemisia annua*, genetic diversity, cultivation
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1. Introduction

The genus *Artemisia* belongs to the family *Compositae* (*Asteracea*) which is one of the most important family of plants in the world (more than 23000 species from about 1300 genera) (Bremer 1994). This genus consists of about 500 species, occurring throughout the world. Some very important drug leads have been discovered from this genus, notably artemisinin, the well known anti-malarial drug isolated in the past 36 years from the Chinese herb *Artemisia annua* L. (sweet wormwood) (Liu *et al.* 2009). More recently, in the past decade, new medicinal benefits were reported for another species of *Artemisia*, viz. *A. afra* Jacq. ex Willd with potential benefits to human health. *A. afra*, commonly known as the African wormwood, is an herb widely distributed in the southern parts of Africa (especially South Africa), where it is one of the important and most widely used herbs in the traditional medicine (Patil *et al.* 2012). It is used for a variety of ailments like fever, gastro intestinal disorders, respiratory tract related problems, skin afflictions, gynecological problems, malaria (Koehorst *et al.* 2010, Sunmonu & Afolayan 2012, Kena & Lepheana 2016). Different parts of this plant (roots, stems and leaves) are used in many different ways and taken as enemas, poultices, infusions, body washes, lotions, smoked, snuffed or drunk as a tea (Koehorst *et al.* 2010).

In recent years, *A. afra* has received much attention of the scientific community and pharmaceutical industries and several studies have been carried out so as to verify or support the traditional use of this herb. Thus, many chemical compounds (α–thujone, ß-thujone, artemisyl acetate, artemisia ketone etc.) and several activities (anti-bacterial, anti-tuberculotic, anti-spasmolytic, anti-histaminic, anti-oxidant, narcotic, analgesic, anti-malarial etc.) were reported (Koehorst *et al.* 2010, Patil *et al.* 2012, Kena & Lepheana 2016). Moreover, the use of this plant is also being investigated in the modern diseases like diabetes, cardiovascular diseases, cancer, respiratory diseases etc.; and even utility patents have been given in United States and in Europe for the use of *A. afra* in diabetes, cancer, diabetes and cardiovascular disease (Patil *et al.* 2012).

A. afra could be a promising source for the development of novel strategies to cure fatal maladies and there

is international interest in its chemical properties. However, this dramatic growth in popularity of this herb, the reliance and extensive demands of pharmaceutical industries, and other anthropogenic activities can pose a threat to the species. Indeed, when the effectiveness of a medicinal plant is more widely acknowledged and accepted, over harvesting can result. Overexploitation is a growing problem for many medicinal species in Africa. Hence, serious measures should be taken to sustain the production and availability of *A. afra*, and cultivation is a good strategy and an alternative to collection in the wild. It can be a solution to not only meet increased demand for medicinal plants, but also a tool for biodiversity conservation and poverty alleviation.

Unlike *A. annua* which is reported to be cultivated in many West African countries, very little information is available on the cultivation of *A. Afra* in West Africa (especially in Côte d'Ivoire). Hence, the objective of this study is to assess the feasibility of cultivating *A. Afra* in Côte d'Ivoire and to evaluate the extent of its genetic diversity based on phenotypic characters.

2. Materials and methods

2.1 Experimental Site

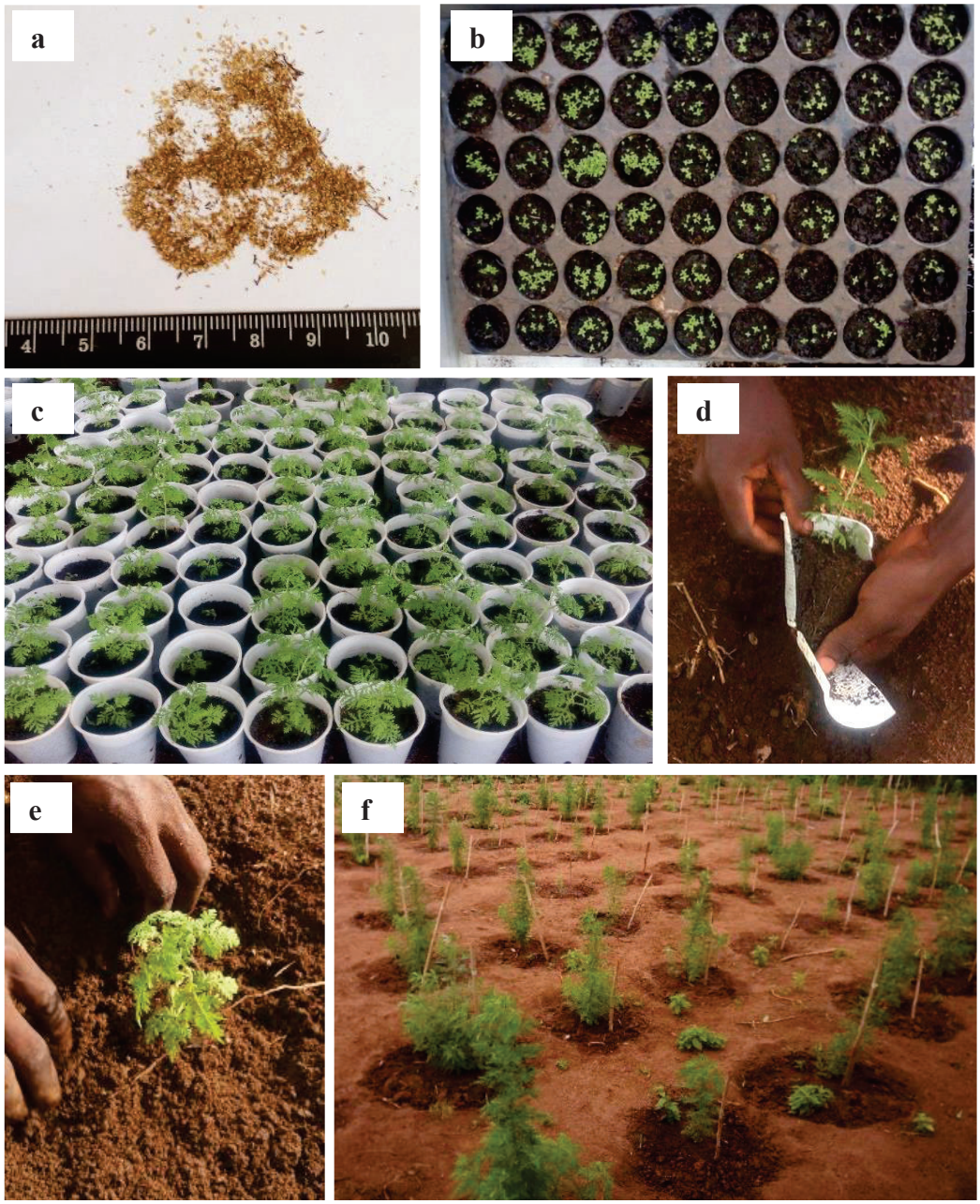
The work was carried out at the experimental farm of the Crop Improvement Laboratory of Jean Lorougnon Guédé University, located in the town of Daloa in the center-west of Côte d'Ivoire. Daloa is one of the most agricultural regions of Côte d'Ivoire. The area was under humid tropical conditions with 1317 mm of rainfall per year and relative dense forest vegetation. The soil of the plot was sandy loam texture with good fertility, properly leveled and well drained. The mean temperature and relative humidity during the experiment period ranged from 24°C to 29 °C and 70 to 87%, respectively.

2.2 Plant Materials

The plant material used in this study consisted of 280 plants of *A. Afra* obtained from seeds originating in Malawi and supplied by the Laboratory of Tropical Agroecology of Gembloux Agro-Bio Tech (University of Liège, Belgium). Eighteen plants of *A. annua* were used as controls and were obtained from seeds provided by the same Laboratory of Liège University.

2.3 Nursery and transplantation in field

The seeds of *A. afra* and *A. annua* are very small (less than a millimeter) (Figure 1a). For this reason, they were sown by sprinkling them on the surface of growing trays filled with moist soil. These trays were placed in tanks filled with water to ensure the continual humidification of the soil (Figure 1b). Seedlings at the 2- to 4-leaf stage were transplanted into 200 ml pots and placed under shade (Figure 1c). Then, seedlings in pot of 6- to 10-leaf stage were transplanted in rows on the experimental plot, with a row-to-row spacing of 100 cm and plant-to-plant spacing of 80 cm (Figure 1de). Seeding depth was 50 mm below the soil. Transplanting was done in the afternoon, when the weather was cool, in order to limit heat stress to seedlings and promote their good and fast recovery.



Figue 1. Activities for setting up the nursery and transplanting *A. Afra* in the field: a) Seeds of *A. Afra*; b) Growing trays, used for sowing, showing young emerged seedlings of *A. afra*; c) Seedlings of 2- to 4-leaf stage transplanted into 200 ml pots; d, e) Transplanting a seedling into the field; f) Experimental field showing plants of *A. afra* transplanted in the experimental field.

2.4 Field Managements

Standard agronomic and management practices were adopted, but no fertilizers and insecticides were applied. Daily watering using a watering can was applied uniformly to all plots when necessary. Manual weeding by hoeing and handpicking were carried out to avoid any competition between the crop and the weeds.

2.5 Data Collection

Data collected concerned the mortality rate, the plant fertility, the survival rate after cuttings, and variation in morphological traits:

- *Evaluation of mortality rate*

The mortality rate was determined by the percentage of dead plants in the nursery and in the field relative to the total number of plants transplanted in pot and in field, respectively.

- *Evaluation of plant fertility*

To have an indication of pollen quality, about 50 pollen grains per plant were analyzed. Flowers were collected in the morning of the day of anthesis (Figure 2a). Pollen grains were dipped in a drop of 1.5% acetic-carmine solution on a slide for 30 minutes (Figure 2b) and were analyzed under a stereomicroscope (Figure 2c). Only fully stained and large pollen grains were scored as viable and non-aborted (Konan *et al.* 2020). The quantity of viable pollen was estimated as the percentage of stained pollen.

In addition, the fertility of the plants was evaluated by checking the ability of the seeds, harvested from the studied plants, to germinate after sowing in culture trays.

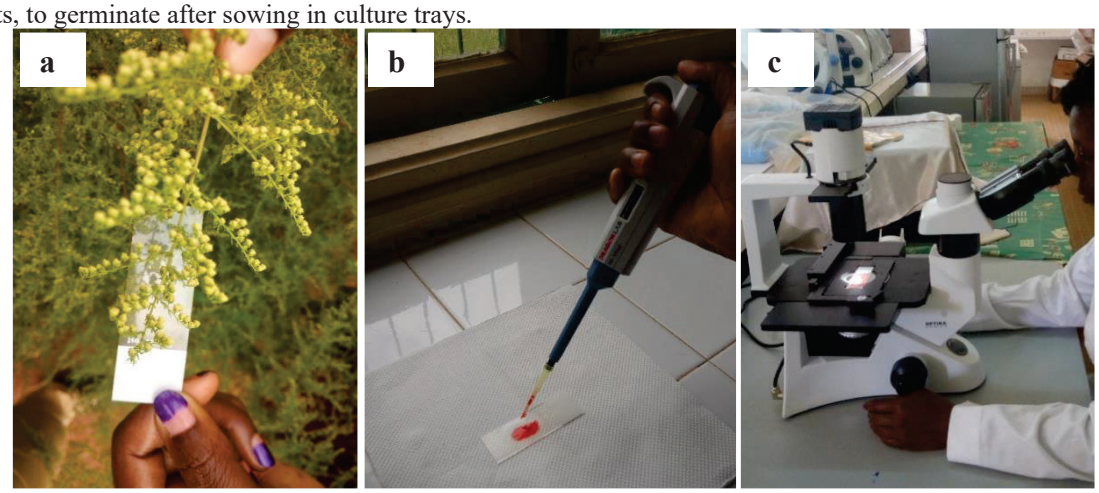


Figure 2. Pollen viability assessment activities: a) Flower and pollen grain collection; b) Addition of an acetic-carmine solution on pollen grains collected on a slide; c) Examination of the slide under a microscope.

- *Survival rate after cuttings*

A cuttings test was carried out to assess the possibility of vegetative propagation of the studied Artemisia species. This was carried out on 10 plants of each species. Branches were cut then refreshed at the base leaving 4 leaves at the upper end (Figure 3abc). Each branch was planted in a pot filled with soil (Figure 3d). The number of successful cuttings was then determined and the success rate calculated.

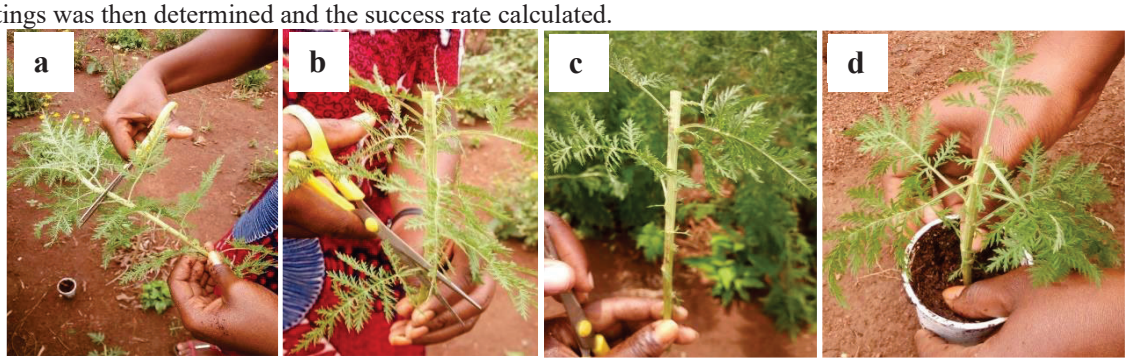


Figure 3. Cuttings of *A. Afra*: a, b) Preparation of a branch for cuttings; d) A branch ready for cuttings; d) Planting of the prepared branch in a pot filled with soil.

- *Variation in morphological traits*

Thirty *A. afra* plants and 10 *A. annua* plants were selected at random and used for the assessment of variation in morphological characters. This evaluation concerned 12 phenotypic descriptors which are: Days to emergence (DE), Days to flowering (DF), Number of leaves on the main stem (NL), Number of leaflets (Nl), Number of branches connected to the main stem (NB), Plant height at flowering (PH), Stem diameter at base at flowering (SD), Leaf width (LW), Leaf length (LL), Fresh leaf weight (WfL), Dry leaf weight (WdL).

2.6 Statistical Analysis of Data

Microsoft Excel Software (2007 edition) was used to compile the data and the software Statistica 7.1 was used for statistical analyses. The means, the standard errors, and variation coefficients were calculated and the data were subjected to Analysis of Variance (ANOVA) to determine the presence of statistically significant differences among the means. A p-value of 0.05 or less was considered statistically significant. The least significant difference (LSD) test was used to separate significantly different means. The morphological variables were also subjected to two methods of multivariate analyses: Principal Component Analysis (PCA) and Cluster Analysis (CA). PCA was done to transform the original variables into a limited number of uncorrelated new variables and to allow the visualization of differences among genotypes, and the identification of groups. The Eigen Values and Eigen

Vectors were computed, which represent the variance and the loadings of the corresponding principal components (PCs). A biplot analysis was carried out based on the two most important PCs to visualize the pattern of total diversity within the germplasm studied. CA was used to group the genotypes into various clusters according to genetic distance. The clustering was performed using the Euclidian genetic distances. The distance matrix was used to construct a dendrogram based on the Unweighted Pair-group Method with Arithmetic Means (UPGMA). The grouping of the genotypes into clusters based on their genetic relationships was determined and analyzed.

3. Results

3.1 Mortality rate in the nursery and in the field

Table 1 presents the results of mortality rates observed in the nursery and in the field. In the nursery, a total of 84 plants out of 280 (30%) and 5 plants out of 18 (27.77%) died respectively in *A. afra* and *A. annua* respectively. In the field, 44 out of 157 plants (28.02) died *in A. afra*, while 0 out of 13 plants (0%) died in *A. annua*.

Table 1. Results of mortality rates observed in the nursery and in the field for *A. afra* and *A. annua*.

	Species	Nb of plants transplanted	Nb of dead plants	Mortality rate (%)
Nursery	*A. annua*	18	5	27.77
	A. afra	280	84	30
Field	*A. annua*	13	0	0
	A. afra	157	44	28.02

3.2 Plant fertility and survival rate after cuttings

The results of pollen fertility test showed good viable pollen for all the plants of *A. annua* tested. The harvested seeds from the studied plants of this species germinated easily. And even, under the studied plants of *A. annua*, many seedlings were found growing from fallen seeds. On the other hand, all *A. afra* plants were sterile; and for the germination test of the harvested seeds, no seeds germinated. Under the plants of *A. Afra*, no seedlings growing from fallen seeds were observed.

For the cuttings trial, 90% survival rate was obtained for *A. afra*, while for *A. annua* 50% survival rate was found.

3.3 Growth and development kinetics

Figure 4 shows the average kinetics of growth, branching and leaf production during 90 days after transplanting the *A. afra* and *A. annua* plants into the field.

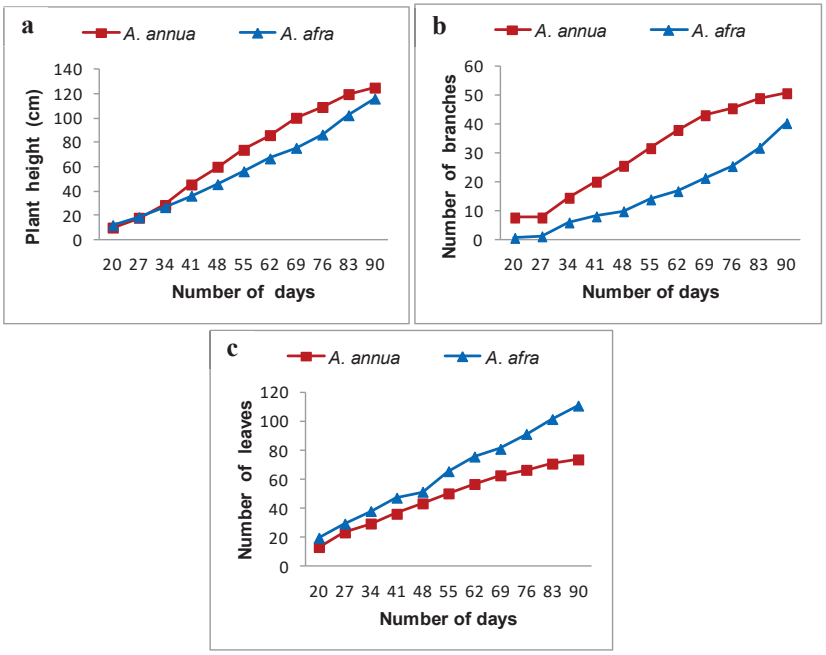


Figure 4. Average kinetics of growth and development during 90 days after transplanting into the field of *A. afra* and *A. annua* plants: a) Growth kinetics; b) Branching kinetics; c) Kinetics of leaf production.

The analysis of these kinetics showed differences between the two species. In terms of growth and branching, *A. annua* grew faster than *A. afra* during the first 90 days after transplanting. In contrast, in terms of leaf production, *A. afra* produced more leaves than *A. annua* during this period. It should be noted that after 90 days *A. afra* exceeded *A. annua* for these three characters.

3.4 Variation in morphological traits

The results of the variation in morphological traits are presented in Table 2. In terms of plant phenology, the days before seedling emergence were on average 4 and 6 for *A. afra* and *A. annua* respectively. The number of days to flowering ranged from 179 to 273 days with an average of 207.20 for *A. Afra*, while it varied from 51 to 111 with an average of 83 for *A. annua*. For growth trait, plant height values showed significant variations. Plant height at flowering ranged from 180 to 337 cm and from 66 to 189 cm for *A. afra* and *A. annua* respectively. The values of the plant development parameters also showed variations. Thus, the stem diameter for *A. afra* ranged from 1.9 to 4.9 cm, while for *A. annua* it ranged from 1.5 to 3 cm. The size of the leaves of *A. Afra* ranged from 8.3 cm to 14 cm long with an average of 11.06 cm and 4.5 to 10 cm wide with an average of 6.66 cm. For *A. annua*, these values ranged from 2 to 9 cm long with a mean of 5.96 and 1.5 to 9 cm wide with a mean of 5.27. *A. Afra* had a number of leaflets varying from 11 to 23 with a mean of 17.07; while this parameter varied in *A. annua* from 5 to 17 with an average of 12.80. The weight of 20 fresh leaves varied in *A. afra* from 3.08 to 18.28 g with an average of 8.60 while in *A. annua* it varied from 0.79 to 4.01 g with an average of 2.65. For the weight of 20 dry leaves, it varied for *A. afra* from 0.49 to 5.95 with a mean of 2.49 and for *A. annua* from 0.19 to 1.55 with a mean of 0.75.

Table 2. Variation in morphological traits studied for *A. afra* and *A. annua* plants.

Species		PH (cm)	SD (cm)	DF (day)	LL (cm)	LW (cm)	Nl	WfL (g)	WdL (g)
	Mean	235.23[b]	3.11[b]	207.20[b]	11.06[b]	6.66[b]	17.07[b]	8.60[b]	2.49[b]
	Min	180	1.9	179	8.3	4.5	11	3.08	0.49
A. afra	Max	337	4.9	273	14	10	23	18.28	5.95
	sd	34.26	0.55	24.13	1.46	1.45	2.80	3.37	1.17
	vc (%)	14.56	17.51	11.64	13.24	21.79	16.42	39.21	46.94
	Mean	124.85[a]	2.38[a]	83.00[a]	5.96[a]	5.27[a]	12.80[a]	2.65[a]	0.75[a]
	Min	66	1.5	51	2	1.5	5	0.76	0.19
A. annua	Max	189	3	111	9	9	17	4.01	1.55
	sd	39.36	0.62	17.67	2.20	2.26	3.94	1.01	0.42
	vc (%)	31.53	25.89	21.29	36.91	42.83	30.77	38.02	56.48

PH: Plant height at flowering; SD: Stem diameter at flowering; DF: Days to flowering; LL: Leaf length; LW: Leaf width; Nl: Number of leaflets; WfL: 20-Fresh leaf weight; WdL: 20-Dry leaf weight. sd: standard error; vc: variation coefficient.

3.5 Principal Component Analysis (PCA)

The principal component analysis (PCA) transformed the raw set of data into 8 factors loadings or principal components, with the first principal component (PC1) contributing the most variability (63.27%) and the last principal component (PC8) contributing the lowest variability (0.16%). Eigen values are often used to determine how many Principal Components to retain. Usually Components with Eigen values less than 1 are excluded. The first two PC (PC1, PC2) had Eigen values greater than 1, and showed therefore high significant variability compared to the rest of the PCs which had Eigen values less than 1. These latter PCs had not been considered as they were not significantly influencing the variability among the genotypes. The percentage of variation explained by the first two PCs, their Eigen value and the factor scores for the traits studied are presented in Table 3. These two PCs cumulatively explained a variation of 76.80%, including PC1 (63.27% variation) with a greater weightage on the number of days to flowering, the height of the plants, the number of leaves, the leaf size and the number of leaflets; and PC2 (13.53% variation) with greater weightage on stem diameter and leaf weight.

Table 3. Principal component analysis of morphological traits in *A. afra* and *A. annua* showing Eigen vectors, Eigen values, total and cumulative percentage of variance explained by the first two PC axes

Trait	PC 1	PC 2
LL	**-0.42**	-0.06
LW	**-0.32**	0.30
Nl	**-0.31**	-0.17
WfL	-0.38	**0.43**
WdL	-0.36	**0.46**
DF	**-0.37**	-0.31
PH	**-0.39**	-0.21
SD	-0.25	**-0.59**
Eigen value	5.06	1.08
Total variance (%)	63.27	13.53
Cumulative variance (%)	63.27	76.80

PH: Plant height at flowering; SD: Stem diameter at flowering; DF: Days to flowering; LL: Leaf length; LW: Leaf width; Nl: Number of leaflets; WfL: 20-Fresh leaf weight; WdL: 20-Dry leaf weight.

Biplot analysis was carried out based on the first two PCs. Traits and genotypes were shown on the biplots

(Figure 5 and Figure 6, respectively) to clearly visualize their associations and differences. The scatter plot of the genotypes (Figure 6) showed the pattern of diversity of the species studied. PCA showed a separation between the plants of *A. afra* and those of *A. annua*.

3.6 Cluster Analysis

A dendrogram (Figure 7) was constructed using the Euclidian distance and based on the Unweighted Pair-group Method with Arithmetic means (UPGMA). This dendrogram showed the relationships between the plants studied and grouped them into 6 major clusters at 58% level of similarity. ClusterI included 3 plants of *A. annua*, ClusterII 7 plants of *A. annua*, ClusterIII one plant of *A. afra*, ClusterIV 4 plants of *A. afra*, ClusterV 15 plants of *A. afra* and ClusterVI 10 plants of *A. afra.*

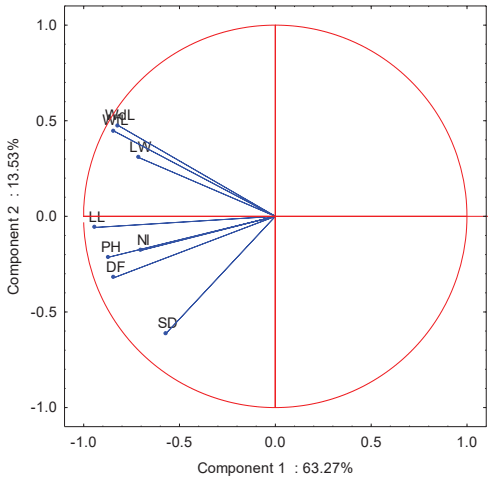


Figure 5. Plot of components weight of the morphological traits studied in *A. afra* and *A. annua*.

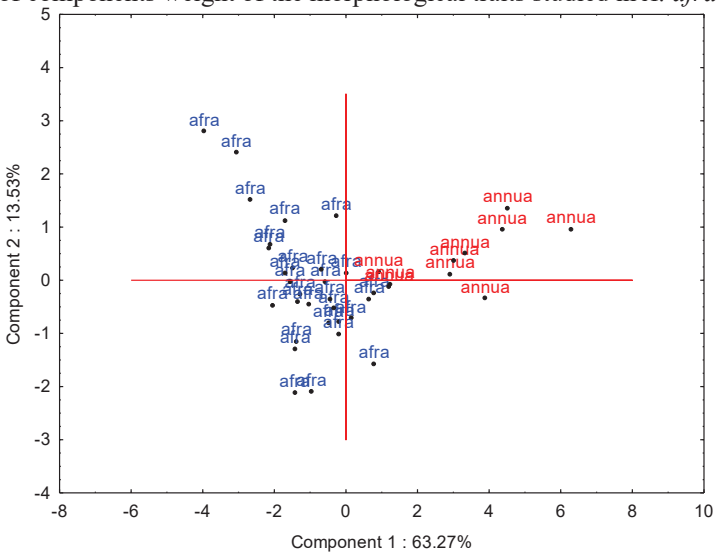


Figure 6. Scatter plot of the first and second principal component analysis for the plant of *A. afra* and *A. annua*.

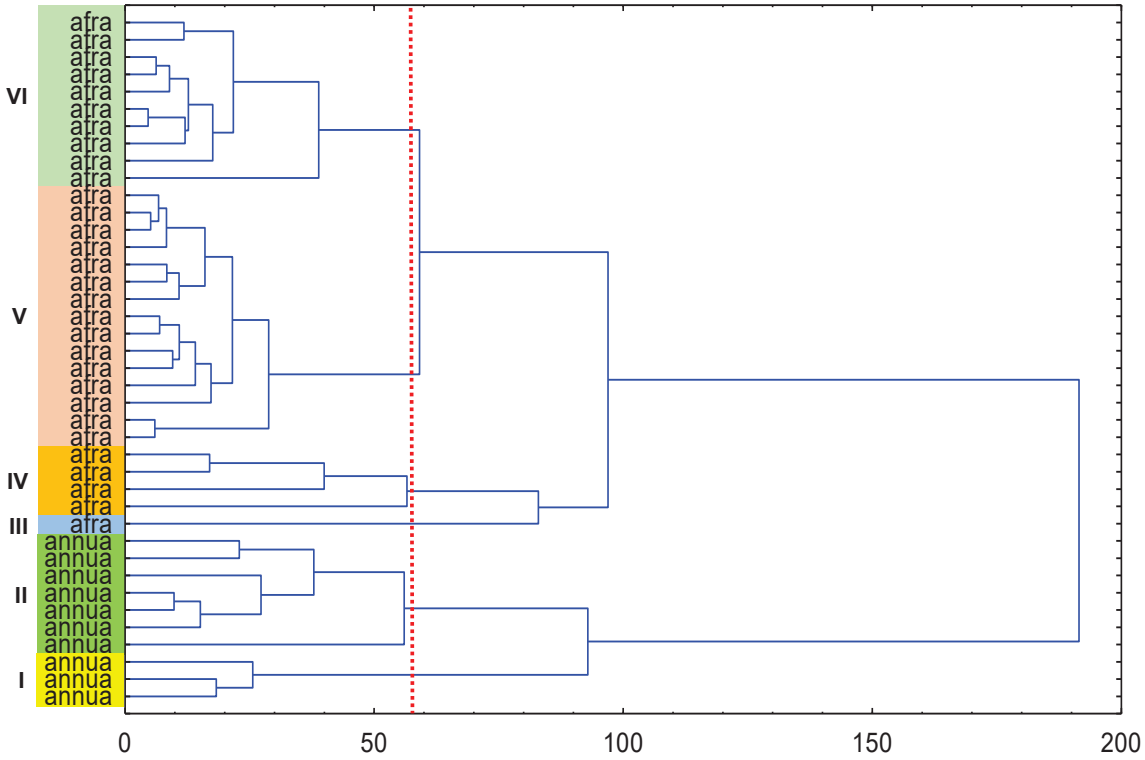


Figure 7. Dendrogram of Euclidian distance illustrating the genetic relationships among the studied plants of *A. afra* and *A. annua* and showing 6 major clusters at 58% level of similarity.

4. Discussion

The natural growth area of *A. afra* is the tropical zone of south-eastern Africa (South Africa, Kenya, Zimbabwe, Malawi, Tanzania, Angola etc.) (Viljoen 2007). The agroecological conditions of this zone are different from those of Côte d'Ivoire (West Africa). Therefore, *A. afra* must acclimatize to these new environmental conditions. The acclimatization of this species in Côte d'Ivoire will result from its ability to continue to ensure its growth, development, flowering and reproduction under the new conditions. The present study was undertaken to evaluate the level of this acclimatization and also to assess the extent of genetic diversity of the population generated from the seeds received. All the results obtained were compared with those of *A. Annua* which was used as control because it grows and reproduces well in West Africa as reported by previous studies (Bambara 2007, Onimus *et al.* 2009, Sounon *et al.* 2009, Mergeai & Sankaré 2016).

The seeds of *A. afra* germinated faster than those of *A. annua* (4 and 6 days on average respectively). In addition, the number of seeds of *A. afra* germinated was significantly higher than that of *A. annua* (more than 280 for *A. afra* against 18 for *A. annua* for the same quantity of seeds sown). This difference in germination time and number of germinated seeds could be explained by a poorer quality of the seeds of *A. annua* received. Indeed, Sounon *et al.* (2009) obtained germination times for *A. annua* of 3 days in Benin with good germination rate close to the result obtained in the present study. This indicates that *A. afra* and *A. annua* can have the same time and rate of germination.

Nursery and field mortality rates were 27.77% and 0% for *A. annua* and 30% and 28.02% for *A. afra*, respectively. The mortality rate is higher in *A. afra* than in *A. annua*. This result suggests that *A. annua* would be more vigorous than *A. afra*. However, the mortality rates of these two species did not exceed 30%, indicating an acceptable level of survival. This result is a good indicator of the possibility of acclimatization of these species (Harris, 1989) in Daloa.

The results of the growth kinetics showed a difference between the two species. The growth and branching of *A. annua* plants are faster than those of *A. afra* plants during the first 90 days after transplanting in field. This result implies that *A. afra* is relatively late and this is confirmed by the relative great number of days to flowering observed in this species. However, *A. Afra* quickly produced more leaves than *A. annua*, which is an interesting trait since leaves are the main part of interest in these species.

The good pollen fertility combined with the good viability of seeds harvested from the plants of *A. annua* testifies to the good reproductive capacity of this species under the environmental conditions of Daloa. Such behavior of *A. annua* was reported in other countries of west Africa (Bambara 2007, Onimus *et al.* 2009, Sounon

et al. 2009, Mergeai & Sankaré 2016). For *A. afra*, seeds collected from the plants studied were not viable. This sterility of *A. Afra* in Daloa could probably be due to faulty fertilization. Obviously a problem must make the pollen of *A. afra* non-functional and therefore unfit to fertilize the ovules, hence the sterility observed in the plants of *A. afra*. This result shows a major problem of acclimatization of *A. afra* in the environmental conditions of Daloa. Agroecological conditions in this region did not appear to be favorable for *A. afra* reproduction by seed.

We do not yet know why *A. annua* is fertile and not *A. afra*. But sterility of plants can be associated with disturbances of tapetal development and degeneration (Papini *et al.* 1999, Wang *et al.* 2015). Tapetum is the innermost of the four sporophytic layers of the anther wall that plays an important role in the male fertility of pollen grains. It comes into direct contact with the developing male gametophyte and contains all the nutrients for microspore development and maturation, such as callose, sporopollenin and proteins (Wang et al. 2015). Studies have proven that tapetal tissue has a secretory role, providing nutrients required for microspore and pollen grain development, and defects in tapetal tissue can lead to non-functional pollen (Chiavarin *et al.* 2000, Wang *et al.* 2015). Konan *et al.* (2020) reported that in male sterile plants, pollen grains can be non-functional after their formation because the tapetum in anthers of such plants did not deteriorate and release the food material necessary for the normal development of the pollen grains. There was a possible association of the nonviable character of pollen grains with the nutritive role of tapetum in male sterile plants. Recently, there have been a large number of reports that confirmed this statement (Ma *et al.* 2015, Wang *et al.* 2015, Li *et al.* 2017, Zheng *et al.* 2019). Perhaps the environmental conditions of Daloa favor such a phenomenon in *A. afra*. Pending more in-depth studies to more understand and resolve this problem of sterility, vegetative reproduction by cuttings appears to be a good means of multiplication of *A. afra* in Daloa. Indeed, cutting tests carried out showed recovery rate of 90% for this species. *A. annua* was also quite suitable for cuttings even if the cuttings success rate were lower than those of *A. Afra*.

Evaluation of morphological traits revealed the existence of significant variation in and between species. This result could be explained by the allogamous reproductive system of these species with a great ease of inter-plant crossing (Mergeai & Sankaré 2016).

PCA results showed that 76.80% of the total variability between the plants studied was explained by the first two principal components. This result indicates that a high percentage of the total variance was explained by these two components. PCA showed a distinction between *A. afra* and *A. annua* like the cluster analysis. These results indicate the presence of significant interspecific diversity between these species which implies that *A. afra* and *A. annua* are genetically different with a distinct genetic background. PCA showed that *A. afra* was mainly differentiated from *A. annua* by taller plants, more days to flowering, and larger leaf size with greater number of leaflets; while *A. annua* was characterized by a relative precocity, a small height of plants and small leaves in size. The PCA results also revealed a large dispersion of individuals in each species, suggesting the existence of an important intra-specific diversity. With the cluster analysis (CA), this intra-specific diversity was further clarified thanks to a clear clustering by the dendrogram. Two clusters were found in *A. annua* (cluster I and II) and four clusters in *A. afra* (cluster III, IV, V and VI). This important inter and intra specific diversity revealed by PCA and CA are supported by the large phenotypic variation observed in and between these two species. These results suggest that *A. afra* and *A. annua* are incipiently domesticated species with mostly xenogamic plants, highly heterozygous for most characteristics, which implies a high degree of segregation.

5. Conclusion

At the end of this study, it appears that *A. afra* can grow and flower in Daloa from seeds coming from its area of origin. However, the plants thus obtained present, unlike those of *A. annua*, a major problem of sterility which therefore prevents the reproduction of this species by seed in Daloa. Multi-site tests should be carried out to validate this result and microscopy studies of flower buds, pollen and seeds should be carried out to more understand this problem of sterility.

Moreover, the multivariate analysis revealed the presence of an important intra-specific diversity both in *A. afra* and in *A. annua*. This finding calls for molecular and chemical analysis to assess the extent of this diversity at the molecular and chemical level, which would make it possible to see whether the active principles of interest in these species do not exhibit significant variation in terms of quantity. In this case superior genotype selection should be considered for clonal multiplication by cuttings or other means.

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