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Enhancing growth and leaf yield in *Gynandropsis gynandra* (L.) Briq. (Cleomaceae) using agronomic practices to accelerate crop domestication

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ABSTRACT

Gynandropsis gynandra (Spider plant) is an African leafy vegetable with several nutritional benefits, considered as a weed or cultivated crop. The species is of interest for local communities though knowledge on agronomic practices need to be improved. This study assessed the effects of two seedling ages at transplanting (two weeks and three weeks after sowing), three planting densities (444,444; 250,000 and 166,666 plants ha^{-1}), three second harvest timings (one week, two weeks and three weeks after the first harvest) and three cutting heights ($\leq$10 cm; between 10 and 15 cm and $\geq$15 cm) on growth and yield in *Gynandropsis gynandra*. The results revealed that two weeks and three weeks old seedlings could be used for the species cultivation. Seedling age, planting density and consecutive cutting time had significant effects on growth and biomass yield. Increasing planting density decreased plant growth but increased edible biomass yield. Planting density of 444,444 plants ha^{-1} gave the highest biomass yield (29 t ha^{-1}). Cutting height greater than 15 cm favored a better regrowth and higher biomass yield. Harvesting plants two weeks after the first harvest gave more biomass yield but yield decreased from the first harvest to the second one. These results offer new insights into horticultural practices and the expanding of spider plant cultivation in urban and periurban areas.

1. Introduction

Traditional leafy vegetables including *Gynandropsis gynandra* (Spider plant) contribute better to the balance of micronutrients in the diets of local populations (Schönfeldt and Pretorius, 2011; Smith and Eyzaguirre, 2007; van Jaarsveld et al., 2014). They reduce more efficiently hidden hunger and malnutrition than major crops like cereals, pulses, tubers (Yang and Keding, 2009). They are also adapted to local agro-ecological conditions and constitute a prime source of income mainly for rural populations. However, most of those African traditional leafy vegetables have been neglected and underutilized since they have been viewed as the poor man's food (Padulosi et al., 2013). This perception has been quickly changing and there is an increasing interest in African leafy vegetables. Consequently, in *Gynandropsis gynandra* for instance, there are growing research efforts to document agronomy (Gonye et al., 2017; Onyango et al., 2016) and achieve breeding (Omondi et al., 2017a; Wu et al., 2017).

Gynandropsis gynandra thrives in Africa and Asia where it grows abundantly during the rainy season. The species is found near human settlements or roadsides in wild populations but also cultivated in home gardens or in urban and periurban agriculture (Achigan-Dako et al., 2010; Chweya and Mnzava, 1997; Kiebre et al., 2015; Mnzava and Chigumira, 2004; Weinberger and Pichop, 2009). In Eastern and Southern Africa, the species is cultivated and sold in urban markets and supermarkets (Schippers, 2004) but also grows as a weed. In Zimbabwe, *Gynandropsis gynandra* is the most edible weed used by the local communities (Maroyi, 2013). In Eastern Cape Province, South Africa, the species is still regarded as wild (Sowunmi, 2015), while in the rural areas of Limpopo in South Africa, *G. gynandra* is collected from wild but also cultivated and sold (Faber et al., 2010). In West Africa, the species is spontaneous and widely collected but also cultivated by local communities in home gardens (Achigan-Dako et al., 2010; Kiebre et al., 2015).

The species is reported to contain high values of minerals including potassium, calcium, magnesium, phosphorus, iron, manganese and zinc (Koua et al., 2015; Omondi et al., 2017b; van Jaarsveld et al., 2014). *Gynandropsis gynandra* is also a rich source of vitamin C, vitamin A, vitamin B1, vitamin B2, vitamin B9, β-Carotene and proteins (Neugart et al., 2017; Schönfeldt and Pretorius, 2011; van Jaarsveld et al., 2014). Increased consumption of the species will be beneficial for human health.

Up-scaling the production of *Gynandropsis gynandra* requires the

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development of adequate agronomic practices for yield improvement (Onyango et al., 2013). Agronomic practices include planting density, fertilization, pest management, sowing or transplanting date, stage of transplanting (Momoh and Zhou, 2001), irrigation and harvest techniques that affect crop yield. For instance, transplanting time and planting density were raised as important factors affecting onion productivity (Caruso et al., 2014) and cabbage yield under plastic mulch (Paranhos et al., 2016). Investigations on agronomic practices in *G. gynandra* mostly focused on the effect of fertilizer and deflowering in growth, leaf yield and nutrient content (Garjila et al., 2017; Gonye et al., 2017; Masinde and Agong, 2011; Mauyo et al., 2008; Mavengahama, 2013; Mutua et al., 2015; Seeiso and Materechera, 2012; Wangolo et al., 2015). Application of high dose of nitrogen fertilizer (300 kg ha^{-1} of NPK, 300 kg ha^{-1} of Lime ammonium nitrate) combined with daily deflowering increased leaf yield (Mavengahama, 2013; Mutua et al., 2015). Those doses may not be standard while optimal nitrogen dose depend on soils conditions and seasons (Masinde and Agong, 2011). However, other agronomic practices such as planting density, harvesting frequency and techniques, cutting height, irrigation, planting date are less investigated.

Diverse plant spacing has been recommended for the species and include 30–50 cm between rows and 15–20 cm within rows (Oluoch et al., 2009). In South Africa, it was recommended to use 30 cm as inter-row spacing and 10–15 cm between plants (Department of Agriculture Forestry and Fisheries, 2014). Moreover, people from Adja communities in Southern Benin broadcasted seeds on the plots showing no specific planting density (Matro, 2015). In 2004, AVRDC recommended 20 cm × 20 cm. Wangolo et al. (2015) included planting density (30 cm × 20 cm, 30 cm × 15 cm, 20 cm × 15 cm) as factor in their study but did not show the effect of this factor. This situation may be explained by the sowing method currently used which is direct sowing. Direct sowing is made by spreading the seeds in rows and thinning is done three weeks later to have planting spacing. With this, it is clear that there is a need to assess the effect of planting density in order to recommend a strong one.

Transplanting allows selection of vigorous seedlings in horticultural crops and may not be suitable for all species. For most leafy vegetables, it is recommended to transplant seedlings at three weeks after sowing in nursery. In *Gynandropsis gynandra*, transplanted plants increased leaf yield than direct seeding (Orchard and Ngwerume, 2009). However, transplanting is not common in *Gynandropsis gynandra* (Mnzava and Chigumira, 2004) and was attributed to the root systems. The root systems consists of long taproot with few secondary roots and slow production of new roots (Chweya and Mnzava, 1997; Mnzava and Chigumira, 2004). In spite of that the species could into some extent well respond to transplanting while roots established by direct seeding plants differ from that developed by transplanted seedlings (Leskovar and Stoffella, 1995). For instance, in pepper (*Capsicum annuum* L.) taprooted species, strong tap root observed in direct seeding plants was modified into lateral and basal roots in transplanted plants (Leskovar et al., 1990; Leskovar and Stoffella, 1995) which allow seedlings to easily grow. The ability of seedlings to overcome shock during transplanting depends on the amount of roots retained during the transplanting, water absorption capacity of the retained roots and the rate of new root formation as well as soil moisture (Leskovar and Stoffella, 1995; Sadhu, 1989).

In leafy vegetable production, harvesting practices including cutting, defoliation (picking individual leaves or leafy branches at frequent intervals), uprooting whole plant, topping (pinching/deflowering) also affect crop yield (Chweya and Mnzava, 1997; Grubben, 1975; Nono-Womdim et al., 2012; Ojo, 2001). In *Gynandropsis gynandra*, leaves are harvested by cutting every week (Seeiso and Materechera, 2012) or two weeks (Machakaire et al., 1998). There is no comparison of leaf yield for consecutive cutting though the effectiveness of cutting depends on its frequency and height, which can affect the regrowth and hence the yield of the species. To accelerate the domestication of *Gynandropsis gynandra*, it is crucial to improve our knowledge of the effects of planting density, transplanting time and cutting modes on the yield.

This study aims at improving the production of *Gynandropsis gynandra* by developing the most suitable agronomic practices. Specifically, the study assessed: (i) the effects of seedlings age and planting density on the growth and biomass yield in *Gynandropsis gynandra*, and (ii) the effects of cutting height and second harvest timing on regrowth and biomass yield in *Gynandropsis gynandra*. To achieve those specific objectives, we hypothesized that: (i) growth and biomass yield in *Gynandropsis gynandra* decrease with increasing seedling age and planting density; and (ii) cutting height and second harvest time affect regrowth and biomass yield in *Gynandropsis gynandra*.

2. Materials and methods

2.1. Experimental site and plant material

The study was conducted at the experimental site (6°25′00.8″N, 2°20′24.5″E) of the Laboratory of Genetics, Horticulture and Seed Science (GBioS) of the Faculty of Agronomic Sciences (FSA), University of Abomey-Calavi (UAC) during the short rainy season from September 2015 to December 2015. Two independent experiments were implemented, the first experiment from September 2015 to November 2015 and the second one from October 2015 to December 2015. The soil type was ferralitic (Azontondé, 1991; Willaime and Volkoff, 1967). Prior to the experiments, soil samples from 0 to 20 cm depth were analyzed at the Laboratory of Soil Science at the Faculty of Agronomic Sciences, University of Abomey-Calavi for its physico-chemical properties. The soil consisted of 61.98% sand, 25.75% silt and 12.27% clay with pH (KCl) of 5.48 and pH (H_2O) of 5.88. Soil texture was sandy-loam and the percentages of organic carbon and nitrogen (N) were 1.03% and 0.06%, respectively. The soil contained 23.06 mg kg^{-1} of available phosphorus, 811.2 mg kg^{-1} of available potassium, 287.95 mg kg^{-1} of magnesium and 126 mg kg^{-1} of calcium. Soil drainage and permeability were good. The experimental site belongs to the Guinean area with a bimodal rainfall. The annual rainfall was 1,000 mm in 48 days with a mean temperature of 27 °C and a relative humidity up to 80%. Data on climate conditions during the experiments (Table 1) were obtained from the meteorological station of the International Institute of Tropical Agriculture (IITA), Abomey-Calavi, Benin, located at 1 km to the experimental site. During the experiments, the daily average temperature, relative humidity, and solar radiation were 26.91 °C, 82.89% and 15.97 MJ m^{-2} respectively. The total rainfall was 220.6 mm during the experimental period with higher precipitation in October. The water evaporation was low in October and increased over the time with a total of 481.97 mm from September to December 2015.

Table 1
Total rainfall and evaporation, daily average temperature, solar radiation, and relative humidity in Abomey-Calavi from September to December 2015.

Month (in 2015)	Total rainfall (mm)	Total evaporation (mm)	Average solar radiation (MJ m^{-2} day^{-1})	Average temperature (°C)	Average relative humidity (%)
September	87.80	105.01	13.13	26.31	89.38
October	110.50	102.44	16.24	27.04	88.23
November	22.30	117.33	17.86	27.74	86.65
December	0	156.89	16.66	26.56	67.33

The accession ODS-14-002 collected in a home garden in Dogbo municipality (06°45′55.3″N, 001°40′22.3″E) was used in this study. The accession is part of a germplasm collection of *Gynandropsis gynandra* available at the Laboratory of Genetics, Horticulture and Seed Science of the University of Abomey-Calavi in Benin. This accession is characterised by erect branching habit with 5–8 branches. Stem is pigmented and plant can reach 50 cm three weeks after transplanting. Leaves are dark green with five leaflets. The central leaflet has an average 62 mm length and 29 mm width. The petiole is green tinged purple and 98 mm length in average. 50% of plants flower 6 weeks after sowing when transplanted three weeks after sowing. Pods are smooth and yellow at maturity.

2.2. Experimental design

Seeds were sown in trays containing sterilized sand for seedlings production. Treatments consisted in factorial combination of (i) two seedling ages (A1: two weeks old from sowing and A2: three weeks old from sowing), (ii) three planting densities (D1: 166,666 (30 cm × 20 cm); D2: 250,000 (20 cm × 20 cm) and D3: 444,444 (15 cm × 15 cm) plants ha^{-1}) and (iii) three cutting times (C1: one week; C2: two weeks and C3: three weeks after the first cutting) making a total of 18 treatments. They were assessed in a randomized complete block with three replicates. Each plot was 2 m x 1 m (2 m^2) in size and separated by 0.5 m within block while blocks were 1 m apart. Three weeks after transplanting, the first harvest was done following three cutting heights (i.e. $H1 \leq 10$ cm; $10\ cm < H2 \leq 15$ cm; and $H3 > 15$ cm) on each plot. The cutting heights of $H1 \leq 10$ cm, $10\ cm < H2 \leq 15$ cm and $H3 > 15$ cm mean that plant stand was cut just after the second, third and fourth nodes above ground level respectively.

Poultry manure was applied at a dose of 30 t ha^{-1} with 20 t ha^{-1} applied one week before transplanting and 10 t ha^{-1} two weeks after transplanting. Poultry manure contained 1.3% of nitrogen (N), 0.1% of phosphorus (P) and 0.3% of potassium (K). The applied dose of 30 t ha^{-1} was equivalent to 390 kg ha^{-1} of nitrogen (N), 30 kg ha^{-1} of phosphorus (P) and 90 kg ha^{-1} of potassium (K). Pesticides such as Alpha-cyhalothrin and Acetamiprid were used to control caterpillars while Mancozeb and Metalaxyl were used against fungi responsible of damping off, soil-borne diseases, downy mildews such as *Pythium* spp, *Sclerotium* spp and *Phytophtora* spp and *Fusarium* spp. Each plot was watered with 11 L of water twice a day, making a total of 22 L per day.

2.3. Data collection

Seven days after transplanting, the number of seedlings on each plot was recorded in order to determine seedlings survival rate. Daily observations were done to record the time to 50% flowering in each plot. Three plants were selected on inner rows in each plot for growth data collection. Growth parameters including stem diameter, plant height, number of leaves, leaf width, leaf length and leaf area were collected at the first harvest (twenty-one days after transplanting) and at the subsequent harvest. Three fully developed leaves were randomly selected per plant for leaf length and leaf width measurements. They were measured using ruler and subsequently, mean for each trait was calculated. For leaf area measurement, three other fully developed leaves were randomly selected and their areas were determined using the equation, leaf area (cm^2) = x/y, where is x the weight (g) of the area covered by the leaf outline on a millimeter graph paper, and y is the weight of one cm^2 of the same graph paper (Pandey and Singh, 2011).

At each harvest, total fresh biomass and edible fresh biomass were weighed for the three selected plants for individual plant biomass determination whereas total fresh biomass and edible fresh biomass were weighed for the sample size of 0.5 m × 0.5 m (0.25 m^2) for yield estimation. A sample of 100 g of edible biomass was oven dried on sterilizer at 65 °C for 72 h for dry matter content (DMC) determination, which was calculated as: DMC = *[(Dry weight)/(Fresh weight)] × 100*. Harvest Index (HI) was also computed as follows: HI = (*Edible fresh biomass weight*)/(*Total fresh biomass weight*). All weight measurements were done using a digital balance "Pioneer Balance MODEL (Item) PA512" which maximum capacity is 510 g and readability is 0.01 g manufactured by OHAUS CORP.

2.4. Data analysis

A proportion test was used to assess the effect of seedling age on seedlings survival rate. Analysis of variance and Kruskall Wallis test were performed to evaluate effects of seedling age, planting density, cutting time, and cutting height on yield, stem diameter, plant height, leaf width, leaf length, and leaf area. For count data such as number of leaves and time to 50% flowering, we performed generalized linear models (GLM) with poisson or quasi-poisson error distribution. We used generalized linear models (GLM) with binomial or quasibinomial error to analyze the effects of the tested factors on the harvest index. A Tukey's honestly significant difference test (Tukey's HSD) at $P \leq 0.05$ was applied to separate treatment means. All statistical analysis were performed with R.3.3.2 software (R Core Team, 2016).

3. Results

Analysis of growth and yield data showed that there was no significant difference between the two experiments ($p > 0.05$) for all measured variables. This could result from the fact that both experiments were carried out in the same environment and approximatively at the same period. Therefore, data reported are means of both experiments. In addition, no significant interaction was observed among seedling age, planting density and cutting time ($p > 0.05$).

3.1. Effects of seedling age and planting density on plant growth and flowering time

The mean seedlings survival rate was of 60% and did not significanlty change with the seedling age ($p = 0.20$). The flowering time ranged from 16 to 21 days, and did not significantly differ among seedling ages ($p = 0.58$) and planting densities ($p = 0.95$).

The effect of seedling age varied according to the harvest rank. At 21 days after transplanting seedling age significantly affected the stem diameter (Table 2) and the number of leaves (Fig. 1A). The stem diameter and the number of leaves were higher in seedlings transplanted at two weeks old than those transplanted at three weeks old. After the first harvest, the plant height, the leaf length, and the number of leaves significantly increased with seedling age. Seedlings of three weeks were taller (62.96 ± 1.23 cm) with a higher number of leaves (197 ± 8) than those transplanted at two weeks old (54.64 ± 1.44 cm and 150 ± 7 respectively for plant height and number of leaves). When both harvests were cumulated, three weeks old seedlings yielded higher number of leaves than two weeks old seedlings (Fig. 1A).

Increasing planting density in *Gynandropsis gynandra* significantly decreased plant growth (Table 2, Fig. 1B). The effect of planting density was more noticeable on growth traits after the first harvest. It significantly affected stem diameter and leaf width only at the first harvest (21 days after transplanting), whereas at the second harvest (after one week, two weeks and three weeks) it significantly affected stem diameter, leaf width, leaf length and leaf area. At the first harvest, planting density of 166,666 plants ha^{-1} performed better than planting densities of 250,000 and 444,444 plants ha^{-1} for growth traits. However, apart from the stem diameter, all other growth parameters including plant height, leaf width, leaf length and leaf area decreased from the first harvest to the second harvest (Table 2). At the second harvest, the planting density of 166,666 plants ha^{-1} exhibited the highest number of leaves (369 ± 11) which represented respectively 18% and 62% more leaves than planting densities of 250,000 and 444,444 plants

Table 2
Growth data (mean ± standard error) of *Gynandropsis gynandra* plants according to seedling age and planting density for two consecutive harvests. Values within a column followed by no letters or the same letter are not significantly different according to Tukey's HSD at P ≤ 0.05.

	Stem diameter (mm)		Plant height (cm)		Leaf width (cm)		Leaf length (cm)		Leaf area (cm^2)	
	Harvest 1	Harvest 2	Harvest 1	Harvest 2	Harvest 1	Harvest 2	Harvest 1	Harvest 2	Harvest 1	Harvest 2
Seedling age										
two weeks old	11.97 ± 0.22 a	13.55 ± 0.31	74.95 ± 0.90	54.64 ± 1.44 a	9.42 ± 0.13	5.55 ± 0.09	6.41 ± 0.08	3.79 ± 0.06 b	50.90 ± 1.53	17.11 ± 0.62
three weeks old	11.14 ± 0.21 b	13.39 ± 0.29	74.38 ± 0.92	62.96 ± 1.23 b	9.34 ± 0.13	5.74 ± 0.08	6.43 ± 0.08	4.00 ± 0.05 a	48.98 ± 1.39	17.74 ± 0.55
Planting density										
166,666 plants ha^{-1}	12.41 ± 0.26 a	14.93 ± 0.39 a	74.42 ± 1.12	59.70 ± 1.66	9.63 ± 0.17 a	5.82 ± 0.10 a	6.57 ± 0.10	4.05 ± 0.06 a	53.39 ± 1.90	18.70 ± 0.65 a
250,000 plants ha^{-1}	11.55 ± 0.25 b	13.60 ± 0.29 b	73.74 ± 1.09	58.81 ± 1.60	9.51 ± 0.14 ab	5.72 ± 0.10 ab	6.45 ± 0.09	3.90 ± 0.07 ab	48.69 ± 1.54	17.32 ± 0.78 ab
444,444 plants ha^{-1}	10.70 ± 0.27 b	11.88 ± 0.32 c	74.33 ± 1.13	57.90 ± 1.81	8.99 ± 0.16 b	5.40 ± 0.12 b	6.23 ± 0.09	3.74 ± 0.08 b	47.57 ± 1.85	16.11 ± 0.69 b

ha^{-1} (Fig. 1B). Regardless of the planting density, we also observed a 12% increase in the number of leaves from the first harvest to the second harvest (Fig. 1B).

3.2. Effects of cutting time and cutting height on plant regrowth

The time of second harvest affected regrowth traits in *G. gynandra* (Table 3). The time of second harvest significantly affected plant height, leaf width, leaf length (Table 3) and the number of leaves (Fig. 2A). The number of leaves increased with the time of second harvest (Fig. 2A). Plants cut at three weeks after the first harvest exhibited higher number of leaves (201 ± 10.69; $p < 0.001$).

Regrowth in *G. gynandra* after the first harvest significantly depended on cutting height (Table 3). Cutting height above 15 cm allowed a better regrowth in *G. gynandra*. Plants cut above 15 cm height had larger leaves than plants cut below 15 cm height. Cutting height had also a significant effect on the number of new leaves ($p < 0.001$) (Fig. 2B). Leaf production increased with higher cutting height. Plants cut above 15 cm height produced on average 217 leaves, representing 77% and 24% more leaves than those cut at height below 10 cm and between 10 and 15 cm respectively.

3.3. Effect of seedling age and planting density on edible biomass yield and attributes

Edible biomass yield and plant harvest index were significantly affected by seedling age (Tables 4 and 5). At the first harvest, two weeks old seedlings produced on average 47.92 ± 1.84 g biomass per plant, which was 21% higher than the biomass from three weeks old seedlings (Table 4). This difference was reflected in the edible biomass yield with two weeks old seedlings showing higher yield than that of three weeks old seedlings (Table 5). At the second harvest, seedlings of three weeks old gave 15% more biomass than seedlings transplanted at two weeks old (Table 4). However, this gain in edible plant biomass at the second harvest was not enough to inverse the trend observed at the first harvest when both harvests were cumulated. Two weeks old seedlings had 13% higher biomass for cumulated harvest than three weeks old seedlings. However, cumulated edible biomass yield was not significantly different between both seedling ages (Table 5). Edible biomass decreased from the first harvest to the second one. At the first harvest, the plant harvest index did not significantly change between both seedling ages. However, at the second harvest, two weeks old plants showed a slightly higher harvest index (0.41) than three weeks old one (0.35) (Table 4). No changes were observed between both seedling ages when harvests were cumulated.

Dry matter content in *G. gynandra* leaves ranged from 18% to 24% and depended on the harvest rank. Dry matter content showed 24% increase from the first harvest to the second one. Three weeks old seedlings transplanted contained 17% more dry matter than two weeks old seedlings at the second harvest.

Planting density had a significant effect on edible biomass per plant (Table 4) and overall edible biomass yield (Table 5). Edible biomass per plant significantly decreased as planting density increased (Table 4) while edible biomass yield significantly increased with planting density (Table 5). That trend remained unchanged at the first and second harvests, and for cumulated harvest. For instance, planting at 444,444 plants ha^{-1} resulted in low biomass per individual plant (59.45 ± 2.91 g) while planting density of 166,666 plants ha^{-1} showed higher edible plant biomass (97.51 ± 4.65 g) for cumulated harvest (Table 4). However, the edible biomass yield of higher planting density (444,444 plants ha^{-1}) averaged 29.26 t ha^{-1} for cumulated harvest, which represented 28% and 40% more yield than lower planting densities of 250,000 and 166,666 plants ha^{-1} respectively. In spite of the fact that the proportion of edible plant part increased from the first harvest (33%) to the second harvest (38%), it was statistically similar across planting densities at the first and second harvest (Tables

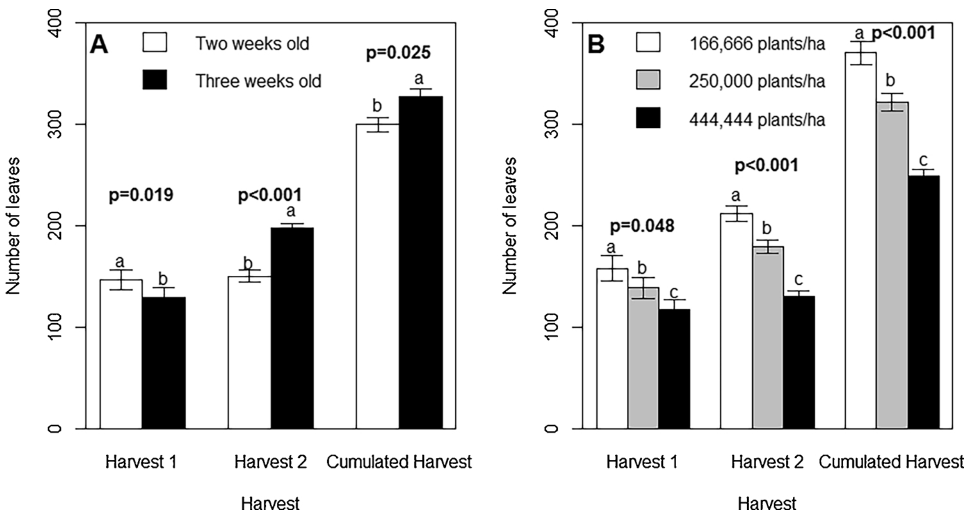

Fig. 1. Number of leaves per plant at the two first consecutive harvests and for cumulated harvest by (A) seedling age and (B) planting density.

Table 3
Regrowth traits (mean ± standard error) in *Gynandropsis gynandra* at different cutting time and by cutting height applied 21 days after transplanting. Values within a column followed by no letters or the same letter are not significantly different according to Turkey's HSD at P ≤ 0.05.

	Diameter (cm)	Plant height (cm)	Leaf width (cm)	Leaf length (cm)	Leaf area (cm²)
Cutting time					
one week	12.83 ± 0.37	41.71 ± 1.10 c	5.43 ± 0.11 b	3.65 ± 0.07 b	16.68 ± 0.73
two weeks	13.98 ± 0.38	62.64 ± 1.25 b	5.85 ± 0.12 a	4.01 ± 0.08 a	18.42 ± 0.81
three weeks	13.61 ± 0.32	72.05 ± 1.14 a	5.66 ± 0.09 ab	4.03 ± 0.05 a	17.17 ± 0.59
Cutting height					
≤10 cm	12.21 ± 0.46 c	53.81 ± 1.17 b	5.55 ± 0.14 b	3.80 ± 0.10 b	18.34 ± 0.98 a
]10;15] cm	13.41 ± 0.29 b	57.19 ± 1.48 b	5.50 ± 0.08 b	3.78 ± 0.05 b	15.69 ± 0.50 b
> 15 cm	14.59 ± 0.35 a	65.51 ± 1.55 a	5.93 ± 0.12 a	4.16 ± 0.07 a	19.46 ± 0.80 a

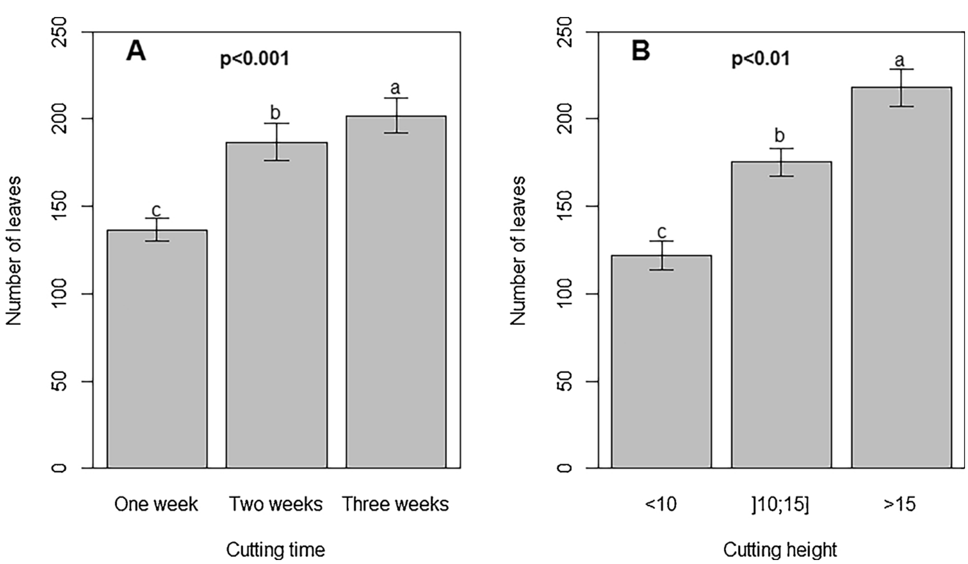

Fig. 2. Number of leaves per plant at the second harvest according to (A) cutting time and (B) cutting height.

4 and 5).

3.4. Effect of cutting time and cutting height on edible biomass yield and attributes

The edible plant biomass was lower when the second harvest occurred just one week after the first harvest compared with harvests two and three weeks after the first one (Fig. 3A). Plants harvested two weeks after the first harvest produced 36.56 ± 2.58 g, representing 73% and 16% more biomass than when the second harvest occurred one and three weeks later respectively. This trend applied for overall edible biomass yield (Fig. 4A). When the second harvest occurred in the second week after the first harvest; it yielded on average 13.03 t ha^{-1} edible biomass, representing 0.94 t ha^{-1} to 4.58 t ha^{-1} gain over plants harvests in the first week and third week respectively. The plant harvest index significantly decreased with cutting time (Fig. 4B). The proportion of edible plant part was 56% when the second harvest occurred one week after the first one.

Average edible biomass from plant cut above 15 cm was 36.89 g, representing 54% and 30% gain over plant cut below 10 cm and between 10 and 15 cm respectively. Edible biomass produced of individual plant significantly increased with increasing cutting height

Table 4
Edible biomass per individual plant and harvested index in *Gynandropsis gynandra* by seedling age and planting density at the first, second and cumulated harvests. Values (mean ± standard error) within a column followed by no letters or the same letter are not significantly different according to Turkey's HSD at P ≤ 0.05.

	First harvest		Second harvest		Cumulated harvest	
	Edible biomass per plant (g)	Harvest index per plant	Edible biomass per plant (g)	Harvest index per plant	Edible biomass per plant (g)	Harvest index per plant
Seedling age						
two weeks old	47.92 ± 1.84 a	0,33 ± 0.00	25.39 ± 1.31 b	0.41 ± 0.01 a	83.73 ± 3.47 a	0,34 ± 0.00
three weeks old	39.42 ± 1.54 b	0.32 ± 0.00	29.30 ± 1.48 a	0.35 ± 0.01 b	73.64 ± 3.01 b	0.33 ± 0.00
Planting density						
444,444 plants ha^{-1}	37.41 ± 2.17 b	0.33 ± 0.00	20.48 ± 1.28 b	0.37 ± 0.01	59.45 ± 2.91 c	0.33 ± 0.00
250,000 plants ha^{-1}	43.41 ± 2.00 ab	0.33 ± 0.00	28.78 ± 1.64 a	0.39 ± 0.01	78.42 ± 2.34 b	0.34 ± 0.00
166,666 plants ha^{-1}	49.92 ± 2.01 a	0.33 ± 0.00	32.85 ± 2.00 a	0.38 ± 0.01	97.51 ± 4.65 a	0.33 ± 0.00

(Fig. 3B). Cutting above 15 cm at the first harvest gave at the second harvest a significantly higher edible biomass than cutting below 15 cm. Leaf dry matter content increased with cutting time at the second harvest. Higher dry matter content was observed at the second harvest when cutting occurred at three weeks (27.35 ± 0.99%).

4. Discussion

4.1. Seedling survival, growth and regrowth in Gynandropsis gynandra

In the present study, the survival rate of two and three weeks old seedlings was not significantly different. This result suggests that *Gynandropsis gynandra* can be sown in nursery and transplanted from two to three weeks' time. Two weeks old seedlings of *G. gynandra* had at least four true leaves. Such a number of leaves was reported to be sufficient enough to favor regrowth, for instance in *Amaranthus cruentus*, another C_4 plant (Lal and Edwards, 1996; Tazoe et al., 2006) like *Gynandropsis gynandra*. Seeiso and Materechera (2012) used transplanting in their study but did not mention the age of the seedlings. They only mentioned that transplanted seedlings had five or six leaves while four weeks old seedlings were reported to have at least five leaves (Sowunmi, 2015). The success of transplanting of two weeks and three weeks old seedling could explained by the fact that up to three weeks seedlings have not old for transplanting. Those seedlings do not have yet suberized and cutinized roots and are able to initiated lateral and/or basal roots development while old seedlings have vigorous roots which are less able to initiate new root development (Forbes and Watson, 1992). The water supplied ensure soil moisture which are crucial to maintain root-soil contact and allow absorption for nutrients uptake (Forbes and Watson, 1992; Schrader, 2000). Survival of seedlings may also due to the seedling production system which is cell/plug trays and has been reported to allow better seedlings establishment in tomato (Javanmardi and Moradiani, 2017).

Growth in *Gynandropsis gynandra* significantly decreased with increasing seedling age and planting density. The growth of three weeks old seedlings was lower during the first three weeks after transplanting. However, they responded well to cutting and grew faster after cutting. This may be explained by the fact that average net assimilation rate increased in *Gynandropsis gynandra* with plant age (Rajendrudu and Das, 1982). Seedlings transplanted at three weeks could be the most appropriated stage while seedlings was at the best physiological stage such as water use efficiency like reported in *Solanum macrocarpum* L. for growth performance (Gaveh et al., 2011). Moreover, *Gynandropsis gynandra* grew better when planted at low density as reported for *Amaranthus cruentus*, *Corchorus olitorius*, and *Celosia argentea* (Grubben, 1975; Maseko et al., 2015). At low density, the area available for each plant is high and the competition for space, light and nutrients is limited.

Regrowth in the species depends on cutting height as reported in other leafy vegetable species such as *Celosia argentea*, *Amaranthus cruentus* (Grubben, 1975) and *Moringa oleifera* L. (Zheng et al., 2016). We observed that regeneration ability increased with cutting height. Cutting above 15 cm height favored more new branches, higher number of leaves and biomass per plant in *Gynandropsis gynandra*. The same cutting height was also reported by Ojo (2001) for *Celosia argentea* at 40,000 plants ha^{-1}. For *Amaranthus cruentus*, cutting at 25 cm height favored better regeneration than at 15 cm (Grubben, 1975). The rapid regrowth observed with high cutting height may probably be due to the high residual leaf area. Simon et al. (2004) reported that residual leaf area after cutting played an important role in *Medicago sativa* L. regrowth.

Delayed second harvest increased plant regrowth characteristics. While the number of leaves increased with the second harvest timing there was no significant difference between harvest at two and at three weeks after the first harvest. This is in contrast with the trend observed in *Amaranthus hybridus* by Materechera and Medupe (2006) who

Table 5
Edible biomass yield per ha and harvest index of *Gynandropsis gynandra* plants by seedling age and planting density at the first, second and cumulated harvests. Values (mean ± standard error) within a column followed by no letters or the same letter are not significantly different according to Turkey's HSD at P ≤ 0.05.

	First harvest		Second harvest		Cumulated harvest	
	Edible biomass yield ($t\,ha^{-1}$)	Harvest Index	Edible biomass yield ($t\,ha^{-1}$)	Harvest Index	Edible biomass yield ($t\,ha^{-1}$)	Harvest Index
Seedling age						
two weeks old	14.96 ± 0.67 a	0.34 ± 0.01	10.02 ± 0.59	0.41 ± 0.02	24.97 ± 0.92	0.33 ± 0.00
three weeks old	11.57 ± 0.64 b	0.33 ± 0.00	10.35 ± 0.77	0.35 ± 0.01	23.59 ± 1.15	0.33 ± 0.00
Planting density						
166,666 plants ha^{-1}	11.20 ± 0.74 b	0.32 ± 0.00	9.72 ± 0.87 b	0.38 ± 0.02	20.92 ± 1.20 b	0.33 ± 0.00
250,000 plants ha^{-1}	12.58 ± 0.88 b	0.34 ± 0.01	10.59 ± 0.80 ab	0.38 ± 0.03	22.71 ± 1.16 b	0.33 ± 0.01
444,444 plants ha^{-1}	16.07 ± 0.73 a	0.33 ± 0.00	13.33 ± 0.79 a	0.38 ± 0.02	29.26 ± 1.05 a	0.33 ± 0.01

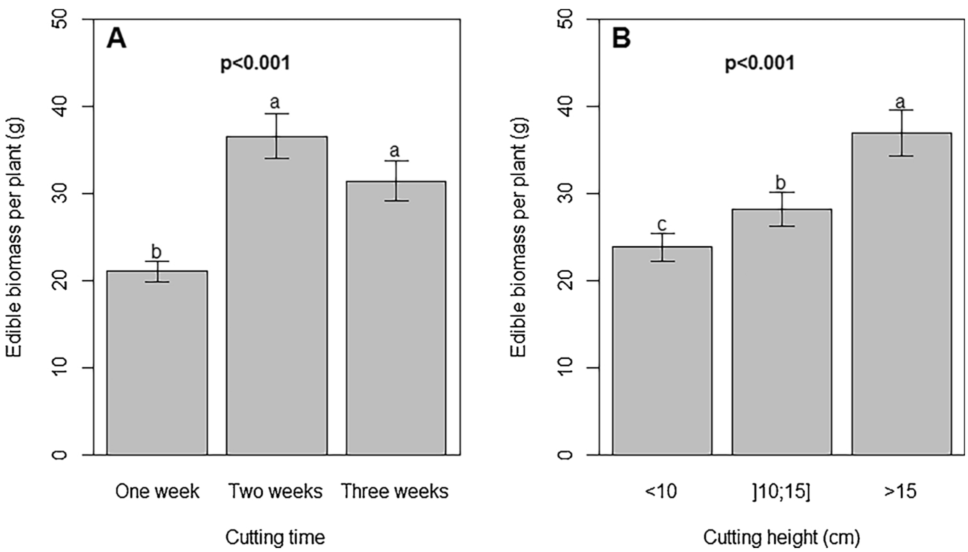

Fig. 3. Edible biomass per plant at the second harvest according to (A) cutting time and (B) stem cutting height.

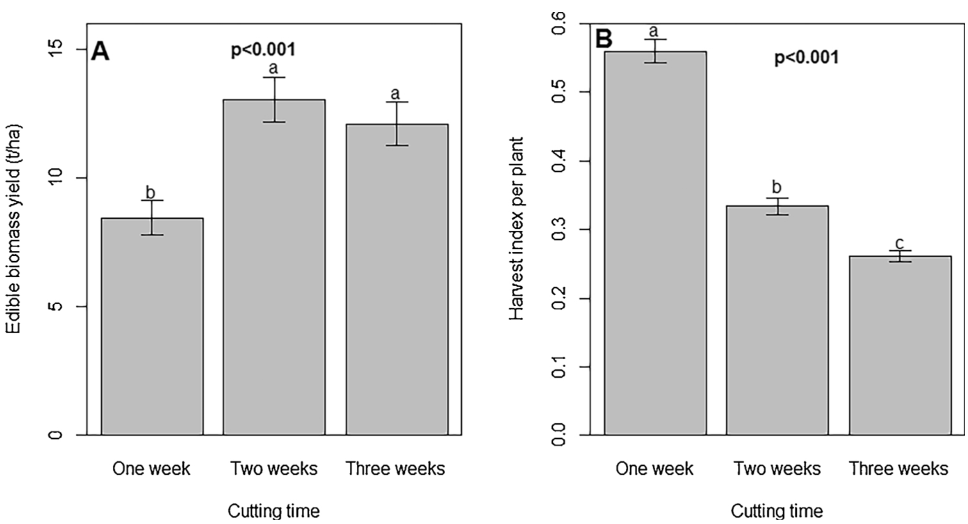

Fig. 4. Edible biomass yield (A) and harvest index (B) of the second harvest according to cutting time interval.

reported that the number of leaves decreased when increasing cutting interval. In *Amaranthus hybridus*, the number of leaves cut one week after the first cutting was higher than those cut two and three weeks; however, leaves cut every week were not marketable. In *Gynandropsis gynandra*, plants reached optimum leaf size around two weeks old. After two weeks, flowering became active as plants allocated more assimilates to the terminal apex which bore the inflorescence for fruit development as reported by Oluoch et al. (2009).

4.2. Flowering time in Gynandropsis gynandra

Early flowering in *Gynandropsis gynandra* represents an important constraint for growers (Onyango et al., 2013). Flowering can be managed by using appropriate agronomic practices or selection. The agronomic practices investigated in the present study showed that transplanting two and three weeks old seedlings at 15 cm × 15 cm, 20 cm × 20 cm and 30 cm × 20 cm spacing did not affect flowering time in the species. A study on four selected spider plant (*Gynandropsis gynandra*) morphotypes from Zimbabwe and Kenya showed that time to 50% flowering varied from one accession to another (Masuka et al., 2012). Authors reported that morphotypes with purple stem type flowered later than the green stem ones. The same findings were reported for some Kenyan and South African accessions (Wasonga, 2014). The absence of significant difference between seedling ages at transplanting may certainly be due to a stress caused by transplanting that induced flowering. Transplanting certainly accelerated or delayed flowering irrespective to seedling age and planting density. Including multiple accessions with contrasting flowering times in the study would have been useful to confirm such a hypothesis.

4.3. Yield, yield components, and dry matter accumulation in Gynandropsis gynandra

Increasing planting density increased yield in *Gynandropsis gynandra*. A similar trend between planting density and yield was also reported in *Amaranthus cruentus* (Grubben, 1975; Maseko et al., 2015), *Corchorus olitorius* (AVRDC, 2004; Maseko et al., 2015), *Celosia argentea* (Grubben, 1975), *Crotalaria* spp (AVRDC, 2004) and *Moringa oleifera* (Zheng et al., 2016). This is due to the high number of plant per area unit rather than the plant biomass that decreased with increasing planting density. Out of the three tested densities, higher edible biomass yield (29.26 ± 1.05 t ha^{-1}) was obtained with 444,444 plants ha^{-1} (15 × 15 cm). This expands knowledge on the effect on planting density on *G. gynandra* yield. According to AVRDC (2004) leaf yield of *G. gynandra* in 20 × 20 cm spacing was 166.7 t ha^{-1}. Yield found by AVRDC (2004) was five time higher than our own (29.26 ± 1.05 t ha^{-1}), which rather complied with other reports indicating 30 t ha^{-1} for cumulative leaf yield (Chweya and Mnzava, 1997; Ochuodho et al., 2012; Oluoch et al., 2009). The discrepancy between our finding and that of AVRDC might reflect differences in genotypes used in the studies and the application of fertilizer (Seeiso and Materechera, 2012).

The harvest index decreased with the second harvest timing in contrast to the dry matter content, which increased with the second harvest timing. This could be explained by the fact that lignification is associated with dry matter accumulation causing decrease in the proportion of tender plant parts. The rapid dry matter accumulation in *Gynandropsis gynandra* may be partly associated to diaheliotropic leaf movements (Kolberg, 2001). In addition, the leaf area decreased over harvests. This trend has been observed by Rajendrudu and Das (1982) who reported that at sixth week after sowing, the mean leaf area per plant in the species decreased significantly due to its C_4 photosynthetic activity and allocation of assimilates. Moreover, in our study, the trend observed is mainly due to the effects of frequent defoliation on roots assimilation. In *Moringa oleifera*, for instance, frequent defoliation inhibited the possibility of photosynthesis and reduced nutrient assimilation and consequently the carbohydrate reserve, which in turn influenced the leaf area development and affected the growth rate of the plant (Sánchez et al., 2006).

Harvesting tender stems, leaves and flowers was reported to give the highest economic leaf yield per plant in *Gynandropsis gynandra* (Oluoch et al., 2009). Cutting stem up to the second node from the ground level is better than harvesting tips (Orchard and Ngwerume, 2009). However, in Benin farmers usually harvest leafy vegetables through frequent cuttings. The number of harvest can be higher when done weekly. In our case, the maximum number of harvests was three. This was lower than that reported by some authors (Mnzava and Chigumira, 2004; Seeiso and Materechera, 2012). Seeiso and Materechera (2012) found that when cutting all leaves or only edible tender leaves, one could harvest up to five times before yield declines. Irrespective of this fact, a second harvest at the second week after the first harvest gave higher biomass yield. This corroborated findings of Chweya and Mnzava (1997) who reported that biweekly harvest enabled ease regeneration in the species.

5. Conclusion

Agronomic practices used, especially planting density, seedlings age at transplanting, cutting height and second harvest timing, affected growth and yield in *Gynandropsis gynandra*. Leaf yield in the species increased with planting density. Density of 444,444 plants ha^{-1} (spacing of 15 cm × 15 cm) appeared as the most promising for leaf yield. Seedlings transplanted at two weeks and three weeks old after sowing did not affect flowering and reacted differently to cutting. Three weeks old seedlings regrew better after cutting but their leaf yield was similar to the two weeks ones. Moreover, regrowth ability in the species depends on the cutting height and second harvest timings and affects the species leaf yield. Harvest at height greater than 15 cm (beyond the fourth node above the ground level) in combination to second harvest at two weeks after the first harvest allowed a better regrowth as well as a higher leaf yield. The average higher yield was 29.26 t ha^{-1} in 8–9 weeks which was comparable to the reported yield (30 t ha^{-1}). Those identified agronomic practices improved the species cultivation especially its introduction into urban and periurban market garden systems and therefore accelerated its domestication. The yield potential and regeneration ability of more genotypes under the present agronomic practices should be assessed in order to select the best ones.

Conflict of interest

The authors declared no conflict of interest.

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