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Full Length Research Paper

Morphogenesis and plant regeneration from *Anthurium andreanum* cv Calypso leaf explant

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Anthuriums are among the most attractive ornamental plants; however, the commercial production of these plants is limited by the slow propagation methods presently in use. This situation can be resolved with the application of *in vitro* culture techniques which allow massive plant propagation through morphogenic processes. Plant growth regulators (PGR) and the composition of the basal media comprising the culture medium are among the factors influencing the induction of morphogenesis. Optical and electron microscopy analysis suggested that the morphogenic routes induced were organogenesis and somatic embryogenesis. This report presents three protocols of morphogenesis, two for adventitious shoot organogenesis and one via somatic embryogenesis. The treatments which induced adventitious shoot organogenesis were Murashige and Skoog medium at half ionic strength supplemented with 0.2 µM of thidiazuron (TDZ) and 2.2 µM of 6-benzylaminopurine (BAP); 0.2 µM of TDZ and 6.6 µM of zeatin (ZEA); the treatment which induced somatic embryogenesis was 0.4 µM of TDZ and 0.5 µM of 2, 4-dichlorophenoxyacetic acid (2, 4-D).

Key words: *Anthuriums andreanum*, morphogenesis, thidiazuron, 2, 4-dichlorophenoxyacetic acid (2, 4-D), organogenesis, somatic embryogenesis, electron microscopy.

INTRODUCTION

Anthurium andreanum is a member of the Araceae family and the species is native to the tropics of Central and South America (Gantait and Mandal, 2010). The high demand of *A. andreanum* for its commercialization as cut flowers, potted plants and garden plants, requires highly efficient propagation methods. *In vitro* culture is an attractive alternative for the multiplication of cultivars with high commercial values, allowing the production of high quality planting material in large quantities (Desai et al., 2015). The micro-propagation of ornamental plants is performed mainly through tissue culture in commercial laboratories all over the world, producing approximately 200,000 *in vitro* plantlets a week (Bhowmik and Matsuiz, 2001). *In vitro* culture permits the establishment of morphogenesis in plants. Morphogenesis refers to the development of organs (shoots, roots or flowers) giving rise to the form and general structure of the plant (Ramage and Williams, 2002). The development of efficient regeneration protocols through morphogenic processes is also important for the genetic transformation

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and development of transgenic plants with new features, such as new shapes of leaves and flowers. The aim of this work was to develop morphogenic processes for the rapid and massive propagation of *A. andreanum* cv. Calypso. Morphological and histological evaluations of cultures were performed during the induction and the development using optical microscopy and scanning electron microscopy.

MATERIALS AND METHODS

Explant source

Leaves were obtained from six-month-old plants of *A. andreanum* var. Calypso for the induction of morphogenesis. Anthurium plants were sprayed with a fungicidal and bactericidal solution over a period of 15 days prior to cutting the leaves. The selected explants were young leaves which were cut after the light green color had disappeared and the leaves presented a light brown color; the leaf was cut leaving a petiole of 5 to 8 cm. The freshly cut leaves were submerged in a fungicidal and bactericidal solution for 2 h, after which they were washed under running tap water and Extran ®. In a laminar flow station, the leaves were sprayed with a solution of ethanol at 96% (v/v) and rinsed once with distilled water; they were then soaked in a solution of NaOCl at 1% followed by two rinses with sterile distilled water. Once disinfected, the leaves were cut transversely to 0.5 cm^2.

Preparation of the medium and culture conditions

All the treatments were supplemented with sucrose at 3%. The pH of the culture medium was adjusted to 5.8 using potassium hydroxide (1 N) or hydrochloric acid (1 N), before the addition of gel rite. The culture medium was subjected to autoclaving at a pressure of 1.05 kg cm^2 at 121°C for 20 min.

For the induction of morphogenesis, the cultures were maintained at 25 ± 2°C, in darkness. For regeneration, the cultures were allowed to grow in the culture room at 25 ± 2°C under a 16/8-h (light/dark) photoperiod; the light was administered with white LED tube lamps with an irradiation of 60 µmol $m^{-2}s^{-1}$.

Experimental design and statistical analyses

The morphogenic treatments were designed using a factorial design with two factors. The first factor was the ionic strength of the basal culture medium: Murashige and Skoog medium (MS) at 2.2 gL^{-1} (1/2 MS) and 4.4 gL^{-1} (MS); the second factor was the combination of plant growth regulators (PGR). With respect to the PGR, thidiazuron (TDZ) at three concentrations was used, combining it with three auxins: naphthalene acetic acid (NAA), indoleacetic acid (IAA) and 2, 4-D; TDZ at three concentrations combined with three cytokinins: 6-benzylaminopurine (BAP), zeatin (ZEA), and Kinetin (KIN). The design gave a total of 60 treatments (Table 1). The concentrations evaluated varied, taking into consideration the range of action of each PGR. The controls were evaluated using MS and ½ MS without PGR. The results were analyzed with the program of statistical graphs XVI and the significance level was determined as P = 0.05. The average values of the treatments were compared with the Tukey HSD intervals.

Histological analysis

Samples of the foliar explants obtained from the treatments for morphogenesis induction were collected every three days and the sampling was conducted over a period of 60 days. For the stage of tissue fixation, the samples were placed in glass vials and submerged in formaldehyde/acetic acid/alcohol at 70% (v/v) under vacuum for 24 h, according to the protocol of Johansen (1940). Subsequently, a gradual dehydration of the samples was carried out in different concentrations of ethanol (30 to 100%), followed by paraffin inclusion, after which dewaxing was performed with xylene. The sample embedded in paraffin was serially sectioned, performing cuts of more than 5 µm using a KEEDE rotary microtome. The cut sections were placed on a microscope slide and were acidified with periodic acid × 20 min; they were then washed, dried and stained with the Schiff reactive × 15 min, after which naphthol blue was applied for 8 min; finally, the sections were washed and dried, and a solution of Permount TM mounting medium was applied. Observations and photographic records were registered with a Nikon microscope equipped with a camera and infinite analysis software 5.0.3.

Electronic microscopy

Samples of calluses from the cultures in the morphogenesis induction medium were collected. The samples were sectioned in 1 mm square-shaped pieces which were then fixed in glutaraldehyde at 3% and maintained at 4°C for one night. Subsequently, the samples were washed in potassium phosphate buffer 0.05 M (pH 7.0), dehydrated in a graduated series of ethanol: 40, 50, 70, 80, 95 and 100%; dried to a critical point with liquid carbon dioxide, fixed on aluminium plates and covered with gold/palladium. The samples were then placed in a metal support, using an adhesive, and were metalized with a thin film of gold. The callus was examined by scanning electron microscopy JSM6369LV. The results were observed in high resolution and the images were captured digitally.

RESULTS AND DISCUSSION

Morphogenesis and plant regeneration

The induction of organogenesis from adventitious shoots in leaf explant of *A. andreanum* cv. Calypso was induced in two treatments: (1) 1/2 MS supplemented with 0.2 µ M TDZ and 2.2 µ M BAP; (2) 1/2 MS supplemented with 0.2 Mm of TDZ and 6.6 Mm of ZEA. In *A. andreanum* cv., the induction and development of the adventitious shoots and rooting ocurred in the same treatment. Similarly, Ramage and Williams (2002) reported that it is possible to use only one formulation for all the stages of morphogenesis; however, the formulation must be established depending on the species and variety.

In *A. andreanum* cv. Calypso, after 45 days of culture in darkness, it was possible to observe the formation of yellow callus on the edge of the leaf explant in treatment with 1/2 MS supplemented with 0.2 µM of TDZ, 2.2 µM of BAP (Figure 1A to C). For the development and regeneration of the callus, the explants were cultured in photoperiod and after 60 days the formation of leaves and roots was observed (Figure 1D). Organogenesis was also obtained in the treatment with 1/2 MS supplemented with 0.2 µM of TDZ and 6.6 µM of ZEA. The treatment that allowed the greatest number of adventitious shoots per explant was 1/2 MS supplemented with 0.2 µM of

Table 1. Average number of shoots or somatic embryos formed from leaf explants of *A. andreanum* cv Calypso as a result of different PGR and ionic strength of the MS basal medium.

Treatment	MS g/L	TDZ (µM)	2,4-D (µM)	AIA (µM)	ANA (µM)	BAP (µM)	ZEA (µM)	KIN (µM)	Somatic embryos or adventitious shoots
1	4.4	0.20	0.10	-	-	-	-	-	0.00±0.000[a]
2	4.4	2.20	1.00	-	-	-	-	-	0.00±0.000[a]
3	4.4	0.20	1.00	-	-	-	-	-	0.00±0.000[a]
4	4.4	2.20	0.10	-	-	-	-	-	0.00±0.000[a]
5	4.4	0.40	0.50	-	-	-	-	-	0.00±0.000[a]
6	4.4	0.20	-	0.10	-	-	-	-	0.00±0.000[a]
7	4.4	2.20	-	1.00	-	-	-	-	0.00±0.000[a]
8	4.4	0.20	-	1.00	-	-	-	-	0.00±0.000[a]
9	4.4	2.20	-	0.10	-	-	-	-	0.00±0.000[a]
10	4.4	0.40	-	0.50	-	-	-	-	0.00±0.000[a]
11	4.4	0.20	-	-	0.10	-	-	-	0.00±0.000[a]
12	4.4	2.20	-	-	1.00	-	-	-	0.00±0.000[a]
13	4.4	0.20	-	-	1.00	-	-	-	0.00±0.000[a]
14	4.4	2.20	-	-	0.10	-	-	-	0.00±0.000[a]
15	4.4	0.40	-	-	0.50	-	-	-	0.00±0.000[a]
16	4.4	0.20	-	-	-	2.20	-	-	0.00±0.000[a]
17	4.4	2.20	-	-	-	6.60	-	-	0.00±0.000[a]
18	4.4	0.20	-	-	-	6.60	-	-	0.00±0.000[a]
19	4.4	2.20	-	-	-	2.20	-	-	0.00±0.000[a]
20	4.4	0.40	-	-	-	4.40	-	-	0.00±0.000[a]
21	4.4	0.20	-	-	-	-	2.20	-	0.00±0.000[a]
22	4.4	2.20	-	-	-	-	6.60	-	0.00±0.000[a]
23	4.4	0.20	-	-	-	-	6.60	-	0.00±0.000[a]
24	4.4	2.20	-	-	-	-	2.20	-	0.00±0.000[a]
25	4.4	0.40	-	-	-	-	4.40	-	0.00±0.000[a]
26	4.4	0.20	-	-	-	-	-	2.20	0.00±0.000[a]
27	4.4	2.20	-	-	-	-	-	6.60	0.00±0.000[a]
28	4.4	0.20	-	-	-	-	-	6.60	0.00±0.000[a]
29	4.4	2.20	-	-	-	-	-	2.20	0.00±0.000[a]
30	4.4	0.40	-	-	-	-	-	4.40	0.00±0.000[a]
31	2.2	0.20	0.10	-	-	-	-	-	0.00±0.000[a]
32	2.2	2.20	1.00	-	-	-	-	-	0.00±0.000[a]
33	2.2	0.20	1.00	-	-	-	-	-	0.00±0.000[a]
34	2.2	2.20	0.10	-	-	-	-	-	0.00±0.000[a]; 0.00[a]
35	2.2	0.40	0.50	-	-	-	-	-	19.00±0.520 [c]
36	2.2	0.20	-	0.10	-	-	-	-	0.00±0.000[a]
37	2.2	2.20	-	1.00	-	-	-	-	0.00±0.000[a]
38	2.2	0.20	-	1.00	-	-	-	-	0.00±0.000[a]
39	2.2	2.20	-	0.10	-	-	-	-	0.00±0.000[a]
40	2.2	0.40	-	0.50	-	-	-	-	0.00±0.000[a]
41	2.2	0.20	-	-	0.10	-	-	-	0.00±0.000[a]
42	2.2	2.20	-	-	1.00	-	-	-	0.00±0.000[a]
43	2.2	0.20	-	-	1.00	-	-	-	0.00±0.000[a]
44	2.2	2.20	-	-	0.10	-	-	-	0.00±0.000[a]
45	2.2	0.40	-	-	0.50	-	-	-	0.00±0.000[a]
46	2.2	0.20	-	-	-	2.20	-	-	24.33±0.436a
47	2.2	2.20	-	-	-	6.60	-	-	0.00±0.000[a]
48	2.2	0.20	-	-	-	6.60	-	-	0.00±0.000[a]
49	2.2	2.20	-	-	-	2.20	-	-	0.00±0.000[a]

Table 1. Contd.

50	2.2	0.40	-	-	-	4.40	-	-	0.00±0.000[a]
51	2.2	0.20	-	-	-	-	2.20	-	0.00±0.000[a]
52	2.2	2.20	-	-	-	-	6.60	-	0.00±0.000[a]
53	2.2	0.20	-	-	-	-	6.60	-	10.66±0.3.32[b]
54	2.2	2.20	-	-	-	-	2.20	-	0.00±0.000[a]
55	2.2	0.40	-	-	-	-	4.40	-	0.00±0.000[a]
56	2.2	0.20	-	-	-	-	-	2.20	0.00±0.000[a]
57	2.2	2.20	-	-	-	-	-	6.60	0.00±0.000[a]
58	2.2	0.20	-	-	-	-	-	6.60	10.00±0.225[a]
59	2.2	2.20	-	-	-	-	-	2.20	0.00±0.000[a]
60	2.2	0.40	-	-	-	-	-	4.40	0.00±0.000[a]

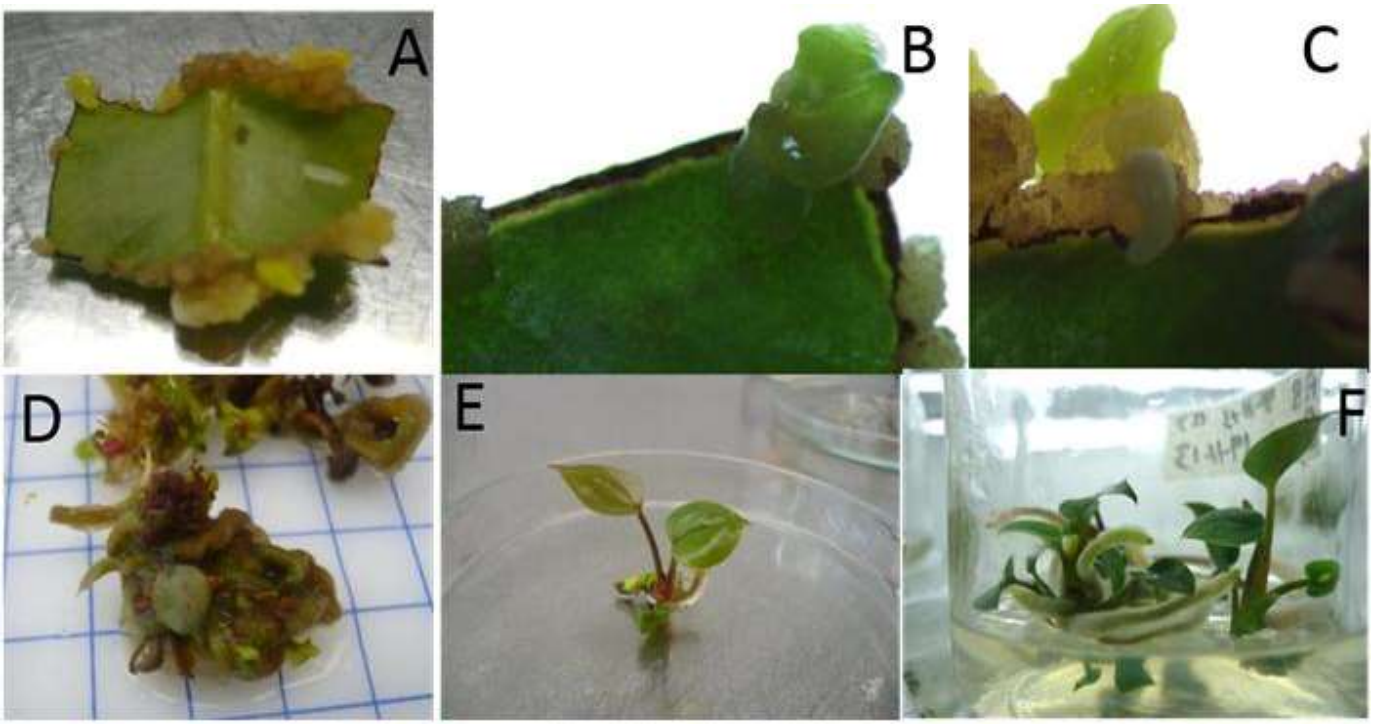

Figure 1. Morphogenesis of *A. andreanum* cv Calypso from leaf explant cultured in ½ MS media with 0.2 µM TDZ and 2.2 µM of BAP. (A-C) The formation of yellow calluses and shoots can be observed on the edges; (D) First leaves and roots; (E) Seven-month-old *in vitro* plantlet; (F) Proliferation of plantlets.

TDZ and 2.2 µM of BAP (Table 1). In both treatments, the time required from the morphogenic induction to the procurement of completely developed plantlets was seven months.

In this report, it was possible to observe that the leaf explant of *A. andreanum* cv Calypso has a high totipotent capacity given that it also acquired embryogenic competency. When the leaf explant was cultured in the treatment with 1/2 MS supplemented with TDZ 0.4 µM and 0.5 µM 2, 4-D in the dark, the formation of white embryogenic callus with a friable consistency was observed. Somatic embryogenesis has been described from nodal segments of *A. andreanum* cv. Eidibel through culture in Pierik medium supplemented with 10 µM α-naphthalene (ANA) (Pinheiro, 2014). In *A. andreanum* Calypso, the 2, 4-D induced somatic embryogenesis in combination with TDZ. It is known that 2, 4-D is a powerful PGR which has been reported in different species for the induction of somatic embryogenesis (Dhillon and Gosal, 2012; Pinheiro, 2013; Asthana et al.,

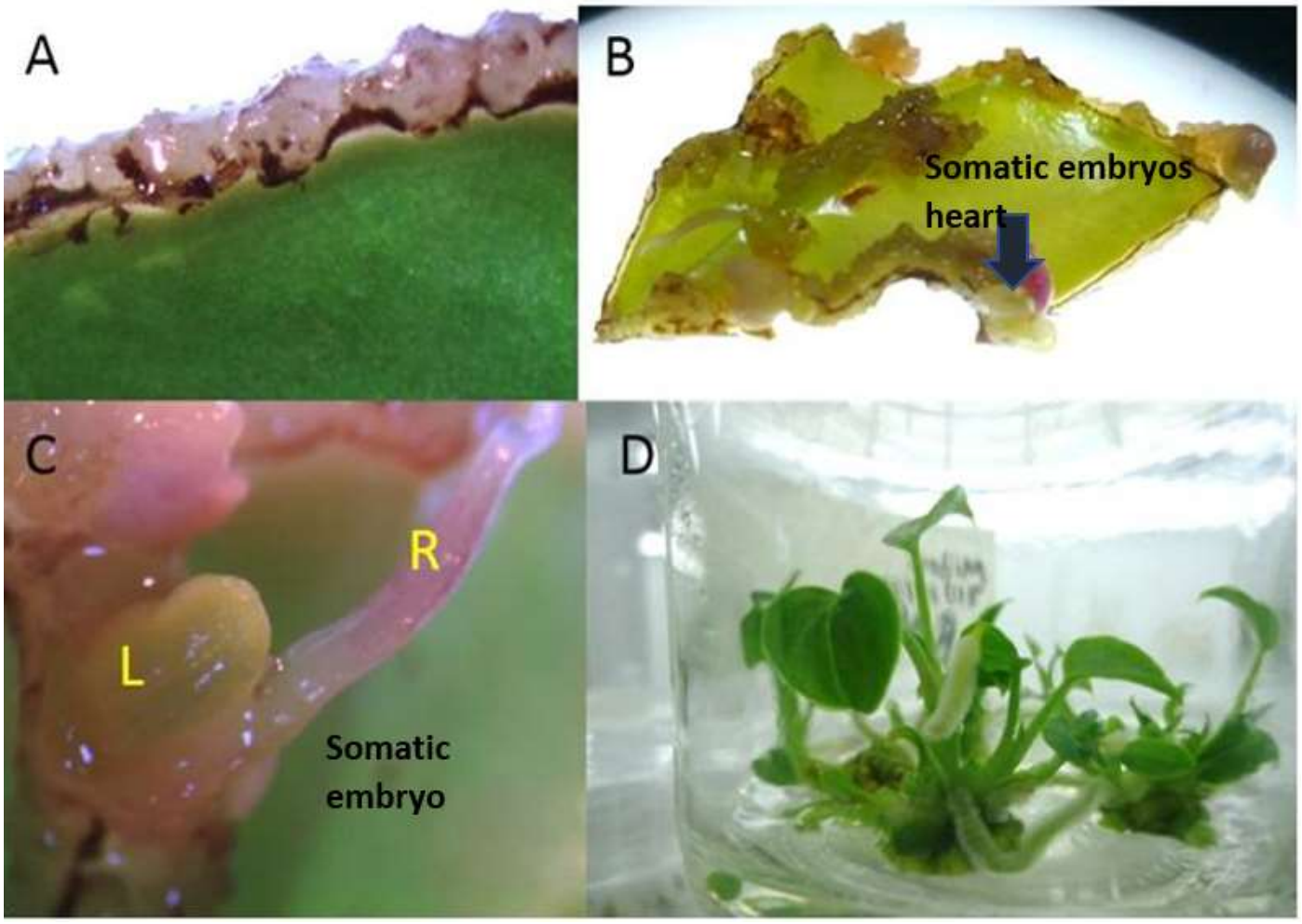

Figure 2. Morphogenesis in *A. andreanum* cv Calypso from leaf explant cultured in ½ MS supplemented with 0.4 µ M TDZ and 0.5 µM 2, 4-D. (A) Formation of white friable callus on the edges of the explant; (B) Somatic embryos on the edges of the explant; (C) Germinated somatic embryo with leaves (L) and roots (R) at 2 months of culture; (D) Four month old regenerated plantlets.

2017). There are reports which indicate that 2,4-D alone or combined induces morphogenetic response, but it has an inhibitory effect on elongation or rooting (Nissen and Minocha, 1993; López-Puc et al., 2006); therefore, 2,4-D must be eliminated (Raghavan, 2004; Fehér, 2015). In this report, 2,4-D did not interfere in the development of morphogenesis, given that the same treatment that induced callus formation, also allowed the complete regeneration, forming somatic embryos which continued to develop until plantlets were obtained (Figure 2A to D).

The ionic strength of the basal medium MS used in this study had a significant influence on the morphogenesis and the regeneration response; this is due to the fact that the minerals are the main components of the culture media. A number of researchers have examined the process involved in the administration of minerals and the results suggest a complex network of interactions between the explant and the culture medium (Ramage and Williams, 2002). In *A. andreanum,* adventitious shoots of Calypso were obtained in 1/2 MS, a result which is similar to those obtained in *Anthurium antioquiense* (Murillo-Gómez et al., 2014), *A. andreanum* cv Tinora Red and Senator (Martin et al., 2003), *A. andreanum* cv flamingo (Viégas et al., 2007) and *A. andreanum* (Jahan et al., 2009). There are a number of reports in which the induction of shoots in *A. andreanum* has been possible using MS at 100% of its ionic strength (Sedaghati et al., 2012); this is possible due to the fact that the morphogenic response varies according to the genotype, which would suggest the need to develop protocols for each variety (Gantait and Mandal, 2010). It is well known that the success of morphogenesis is due, in part, to the correct selection of the type and concentration of the PGR. Teixeira et al. (2015) reported that the adequate concentration of PGRs or their combinations differed for different varieties, explants or culture stages, including, most importantly, the differentiation and proliferation of shoots for callus

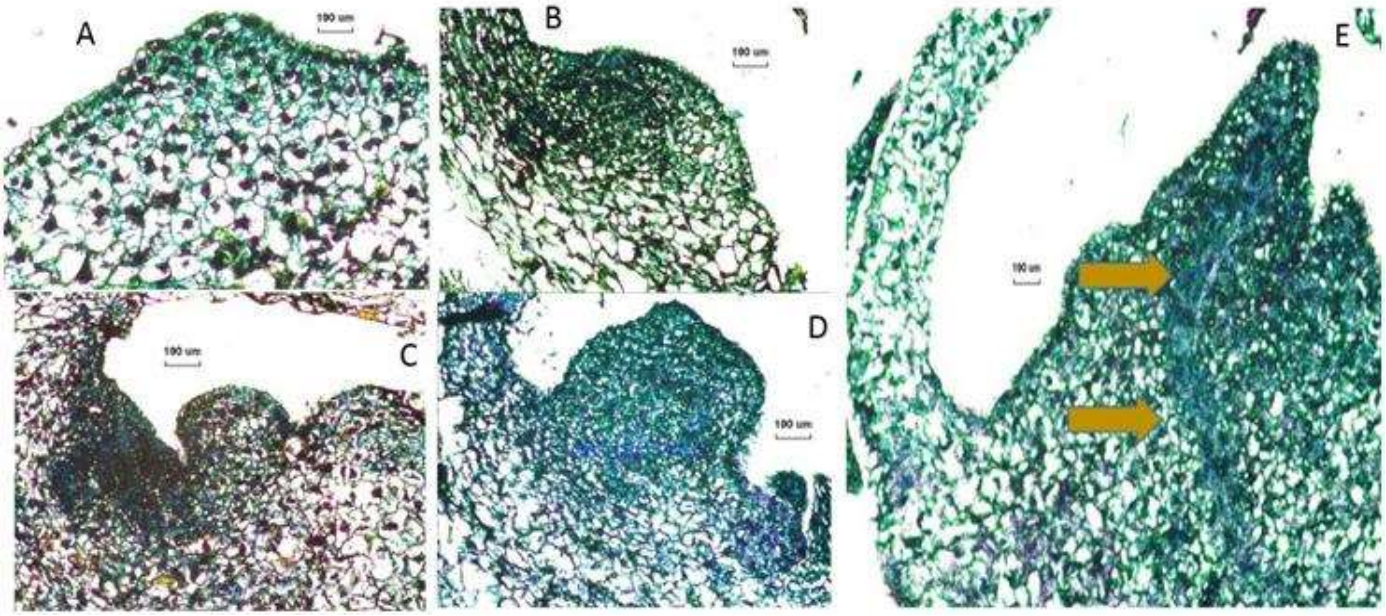

Figure 3. Histological analyses of the leaf explant from *A. andreanum* cv. Calypso. (A) The cells with dense nuclei showing meristemic activity in the sub-epidermal areas; (B-D) Development of shoots at 12, 15 and 30 days, respectively; (E) Caulinar apex of a shoot in advanced phase of development which shows the procambium (arrows).

induction, formation of roots and other organogenic processes. In this report, TDZ was evaluated given that it has demonstrated its effectiveness in the induction of morphogenesis (Gopale et al., 2013). TDZ presents activity similar to that of a cytokinin and it has also been suggested that it may be a modulator of endogenous auxin levels. There is experimental evidence that TDZ stimulates the synthesis of *de novo* auxins, increasing the levels of IAA and its precursor, tryptophan (Murthy et al., 1995). In some cases, it is necessary to transfer the shoots to a treatment with a lower concentration of TDZ so as not to affect rooting (Cari and Preece, 1993). In this study, the presence of TDZ had no effect on the development for the formation of plantlets; this may be due to the fact that a low concentration was used. Gu et al. (2012) obtained 24 adventitious shoots in leaf explant of *A. andreanum* in the cultivars Alabama and Sierra when they were cultured in ½ MS supplemented with 1.82 µM TDZ and for the rooting, it was necessary to apply 0.98 µM of indole-3-butyric acid (IBA). In *A. andreanum* cv Calypso, the use of TDZ as the only growth regulator was unable to produce morphogenic response. However, the combined use of TDZ with another PGR allowed the formation of callus, regeneration of shoots and the formation of complete plantlets; although, it was necessary to combine TDZ with another regulator in *A. andreanum* Calypso, it is important to note the advantage in the fact that it the whole morphogenic process was carried out in the same treatment and there was no need to develop other formulations, as is usually the case in most of the reports on anthurium.

From the histological analyses, morphological changes during the morphogenesis were observed in the leaf explant of *A. andreanum* Calypso when it was cultured in the induction treatment with ½ MS with 0.2 µM of TDZ and 2.2 µM of BAP. The formation of small protrusions with dense nuclei was observed at 12, 15 and 30 days (Figure 3A to C). Emergent organogenic structures were observed at 30 days (Figure 3D) and apical formation of the meristems in the shoot was obtained at 60 days of induction (Figure 3E). Vargas et al. (2007) reported anatomic studies which showed green callus with organogenic potential and their results coincide with our findings; organogenesis was confirmed by the histological evaluations during the induction and the development. For genetic transformation studies, it is important to identify the areas where cellular divisions are produced, giving rise to morphogenesis.

With the use of scanning electron microscopy (SEM), structures regenerated from the leaf explant were visualized (Figure 4A to B). For the treatment in 1/2 MS supplemented with 0.2 µM TDZ and 2.2 µM BAP adventitious shoots of high resolution were observed. The samples of leaf explant cultured in ½ MS supplemented with 0.4 µM TDZ and 0.5 µM 2, 4-D presented somatic embryo formation (Figure 4C). Observations in the SEM revealed that the morphogenic calluses formed structures with organogenic and embryogenic potential.

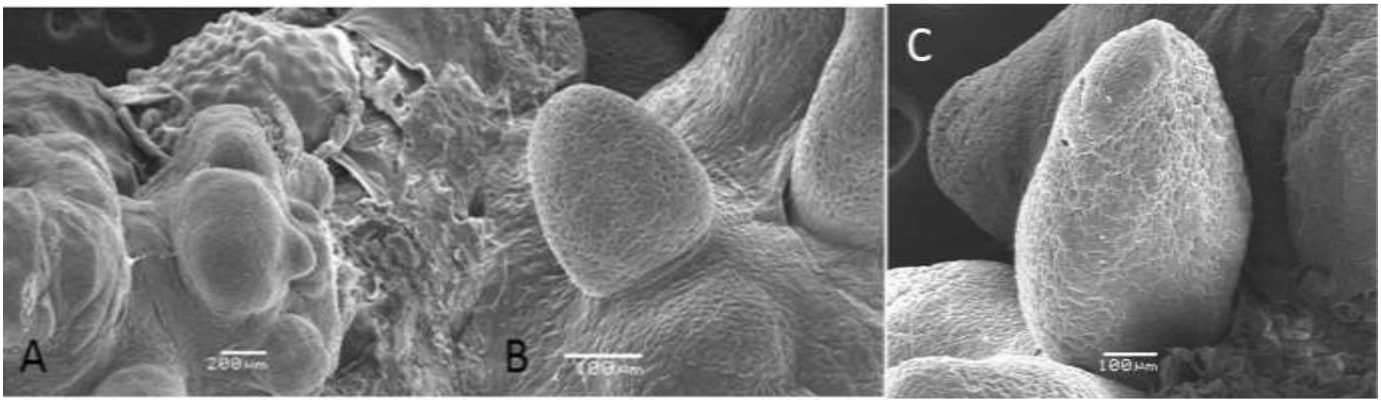

Figure 4. Scanning electron micrographs of organogenic and embryogenic structures formed in leaf explant of *A. andreanum* cv Calypso. (A) Group of structures on the surface of the callus which represent the early stage of organogenesis; (B) Formation of adventitious shoots in leaf explant after 2 months of induction; (C) Somatic embryo in torpedo stage.

Conclusion

Three successful protocols were established for the culture of *A. andreanum* cv Calypso tissue, two for shoot organogenesis and one through somatic embryogenesis, which would allow the clonal propagation of this plant for the floriculture market. Moreover, these protocols represent efficient methods for the regeneration of genetically transformed plants.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Genetic variability and character association of some local wheat varieties (*Triticum* species) using agromorphological traits grown in South Gondar zone, Ethiopia

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Wheat is the most important cereal crop in Ethiopia ranking third in terms of area after teff and maize and second in terms of production after maize. Six local wheat varieties cultivated in South Gondar, one of the 11 zones found in Amhara region, were collected from the local farmers to study their variability, heritability and trait associations using 12 agro-morphological traits. The studied genotypes were grown in main rain season of 2014/2015 at Farta district in a complete randomized block design with three replications. Analysis of variance (ANOVA) revealed the presence of highly significant difference that revealed the presence of high genetic variability of wheat in the study area. The highest yield was recorded from the local variety Ferno with 1957 kg/ha followed by Chekole (1588.33 kg) and Canada Sendie (1580.7 kg). Higher value of genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV) were recorded in most of the studied traits indicating the selection may be effective from these traits and phenotypic expression would be good indication of the genotypic potential. Broad sense heritability estimates were very high for most traits signifying the possibility of success in the selection. Correlation study revealed that the number of tillers per plant, number of seeds per plant and harvest index had positive and highly significant correlation with grain yield. The present investigation confirms the presence of high genetic variability in the study area which deserves conservation and formulation of breeding strategy for improving the productivity of wheat in the country.

Key words: Local wheat varieties, agro-morphological traits, genetic variability, correlation.

INTRODUCTION

Wheat is one of the most important cereals world-wide and it is grown in many areas (Briggle and Curtis, 1987). It has been described as the 'King of Cereals' because of the acreage it occupies, high productivity and the prominent position it holds in the international food grain trade (Food and Agriculture Organization [FAO], 2004).

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During the past four decades, wheat has made a significant contribution to the increase in global food production. This is due to the use of higher-yielding, water and fertilizer responsive, and disease resistant cultivars, combined with strengthened input delivery systems, tailored management practices and improved marketing (Ortiz et al., 2008; Dixon et al., 2006).

Wheat is the most important small cereal crops in Ethiopia (Central Statistical Agency [CSA], 2007). Both durum (*Triticum turgidum L.* var *durum)* and bread wheat (*Triticum aestivum* L.) species are widely cultivated in the country, although other species are cultivated to a lesser extent (Amsal, 2001). Wheat is a temperate crop, even currently, it is also becoming the most important cereals grown on a large scale in the tropical and subtropical regions of the world (Onwueme and Sinha, 1999). This makes it the most suitable and commonly cultivated crop in South Gondar highland areas. According to South Gondar Agriculture and Rural Development Office, wheat has been the leading cultivated crop in the zone for longer years.

Variability is the occurrence of differences among individuals due to differences in their genetic composition and/or the environment in which they are raised (Allard, 1960; Falconer and Mackay, 1996). If the character expression of two individuals could be measured in an environment identical for both, differences in expression would result from genetic control and hence such variation is called genetic variation (Falconer and Mackay, 1996). Information on the nature and magnitude of genetic variability present in a crop species is important for developing effective crop improvement program. Heritability in broad sense refers to the portion of phenotypically expressed variation, within a given environment and it measures the degree to which a trait can be modified by selection (Christiansen and Lewis, 1982).

Grain yield is the most complex trait, because it is influenced by all factors (known and unknown) that determine productivity (Araus et al., 2001). Consequently, the inheritance and interrelationships of grain yield and of characters influencing grain yield are highly important. It is, therefore, imperative to estimate the magnitudes of correlations between grain yield and its components.

Despite the fact that extensive wheat research practices have been conducted in different parts of the country, further cooperative investigations from different institutions is still needed to exploit the existing genetic resource which enables the nation to meet the goal of self sufficiency. Therefore, this research work was aimed at collecting the existing local wheat varieties from local farmers in South Gondar zone and investigating their extent of variability, heritability and correlations using some agro-morphological traits.

MATERIALS AND METHODS

Description of the study area

All the studied genotypes were collected from local farmers living in different districts of South Gondar zone. The experiment was conducted at Farta district which is found in South Gondar zone, Amhara Regional State, Ethiopia. The study area is located at 660 km Northwest of Addis Ababa, capital city of Ethiopia and lies between the coordinates of 11°32' to 12°03' N latitude and 37°31' to 38°43' E longitude with an estimated area of 1118 km^2. The entire area of the study district has a topography characterized by extremely high relief in the upper watershed of Blue Nile River system. The altitude of the study area varies between 1920 and 4235 m above mean sea level with topography of gentle to undulating. The average annual minimum, maximum and mean temperatures are 9.7, 22, and 15.5°C, respectively. The rainfall pattern is uni-modal, stretching from May to September. The annual rainfall ranges between 1097 and 1954 mm with a long term average of 1448 mm (Debretabor University, 2013 Survey Result).

Experimental and design

The trial was established on May 20, 2014 at Farta district on farmer's field. Six local wheat varieties which were commonly cultivated in different districts of South Gondar zone were collected from the field and used for this study (Table 1). These local genotypes were certified and released varieties from research centers, but cultivated for more than 10 years in the study area. The experiment was conducted in randomized complete block design with three replications. The total plot size was 2 × 3 m consisting of 10 rows per plot and net plot size of 2 × 2.8 m with six harvestable rows. Distance of 20 and 10 cm were used for between rows and plants, respectively. The seed rate was 150 kg/ha and sowed by hand drilling at 20 cm row spacing and fertilizer rates (diammonium phosphate [DAP] and urea) were applied as recommended. All other pre and post-stand establishment management practices were done by following the recommended wheat husbandry practices.

Data collection

Data was collected on phenological and yield components based on 12 agro-morphological characters, namely, days from plant emergence to heading, days from sowing to physiological maturity, grain filling period, plant height (cm), number of fertile tillers per plant, number of spikes per m^2, spike length (cm), number of grains per spike, number of spikelets per spike, biomass yield per plot (g), grain yield per plot (g) and harvest index per plot (%).

Plant height, number of fertile tillers, spikelets per spike, number of grains per spike and spike length were determined on the basis of 10 randomly chosen plants per plot (Geleta and Grausgruber, 2013). Days to heading was counted from the date of sowing till 75% of the heads emerged while days to maturity was recorded from the date of sowing till 75% of the plant will get matured. A plant is physiologically matured when 75% of the glumes of the primary spike turned yellow. Biomass and grain yield was recorded on a per plot basis, and harvest index was determined from the ratio of grain yield to biomass. Biomass and grain yield per plot recorded was converted into kg/ha.

Statistical analysis

Mean data collected from the field was subjected to analysis of variance using appropriate procedures of the statistical analysis system (SAS) software version 9.2 (SAS Institute Inc., 2008). The treatment effects were compared using least significant difference (LSD) test at 5 and 1% probability level. Descriptive statistics was used to observe the existing variability among the studied genotypes for each agro-morphological trait. Pearson's correlation

Table 1. List of the six local wheat varieties used for this study.

Given code	Local name	Meaning of the local name
LC1	Canada Sendie	Indicating the name of the country where the variety is brought from
LC2	Chekole	Fast matured variety/needs short period of time for cultivation
LC3	Key Sendie	Signifying color of this variety is red
LC4	Wond alfa	Indicating the variety needs intensive work to cultivate
LC5	Gomadie	indicates lack of awn on the seed
LC6	Ferno	Notifying the seed color is white

Table 2. Analysis of variance for the agro-morphological trait.

Trait	Mean squares		
	Genotypes	Error	P value
DTH	412.63 **	9.11	<0.0001
DTM	165.29**	16.78	<0.0006
DGFP	407.70**	8.89	<0.0001
PHT	962.20**	47.26	<0.0001
TPP	2.36*	0.50	0.0127
SPM	8379.43*	2488.28	0.0394
SL	11.90**	0.32	<0.0001
SkPS	21.95**	2.78	0.0017
NGPS	367.52**	15.76	<0.0001
BMY	3038131.52**	164040.61	<0.0001
GY	1086806.27**	14621.056	<0.0001
HI	169.83**	1.58	<0.0001

*Significant at 0.05 level of significance, **Significant at 0.01 level of significance. DTH: Days from sowing to heading; DTM: days from sowing to physiological maturity; DGFP: days of grain filling period; PHT: plant height (cm), TPP: number of fertile tillers/plant; SPM: number of spikes/m^2; SL: spike length (cm); SkPS: spikelets per spike; NGPS: number of grains/spike; BMY: biomass/plot (kg/ha); GY: grain yield (kg/ha); HI: harvest index.

coefficient was used to see the association between traits.

The variability present in the genotypes was estimated by phenotypic and genotypic variances and coefficient of variations using the procedure suggested by Burton and De Vane (1953).

$\delta^2 g = \frac{MSg - MSe}{r}$ and $\delta^2 p = \delta^2 g + \delta^2 e$

Where, $\delta^2 g$ = genotypic variance, $\delta^2 p$ = phenotypic variance, $\delta^2 e$ = environmental (error) variance or error mean square, MSg = mean square due genotype, MSe = mean square of error (environmental variance) and r = number of replication.

Phenotypic coefficient of variance (PCV), genotypic coefficient of variance (GCV) and broad sense heritability (h^2) was calculated as follow:

$PCV = \frac{\sqrt{\delta 2\, p}}{X} \times 100$,

$GCV = \frac{\sqrt{\delta 2\, g}}{X} \times 100$

$h^2 = \frac{\delta 2\, g}{\delta 2\, p} \times 100$

Where, x = population mean of the character.

RESULTS

Analysis of variance

Mean square of genotypes for all the studied characters, except number of fertile tillers per plant, have highly significant ($P < 0.0001$) differences among the studied local wheat varieties (Table 2) indicating the existence of sufficient genetic variability within different genotypes to be exploited in the breeding programs that was also reflected in the broad ranges observed for each traits.

Mean performance of genotypes

The shortest time for heading was recorded for Canada Sendie variety (65.67 days) and got matured earlier than other studied varieties (122.67 days), while Ferno took the longest time for heading (94 days) and matured after 144 days (Table 4). The highest number of fertile tillers per plant was recorded from Chekole variety (5.70), while

Table 3. Agro-morphological variations recorded in the studied local wheat varieties.

Trait	Range (min.-max.)	Mean ± SE	SD	CV (%)	Median	GCV (%)	PCV (%)	H^2 (%)
DTH	64 - 97	82.17 ± 2.66	11.3	13.76	86.5	14.11	14.42	95.68
DTM	121 - 152	135.89 ± 1.83	7.78	5.72	137.5	5.15	5.73	81.56
DGFP	84 - 117	102.22 ± 2.64	11.23	10.99	106.5	11.28	11.53	95.73
PHT	80 - 142.2	102.53 ± 4.19	17.79	17.35	98.2	17.03	17.89	90.63
TPP	2.8 - 6.2	4.68 ± 0.24	1.02	21.86	4.9	16.82	20.82	65.26
SPM	224 - 440	328.5 ± 15.31	64.97	19.78	317.5	13.49	18.32	54.20
SL	5.3 - 11.8	9.57 ± 0.45	1.93	20.17	10.4	20.53	21.08	94.84
SkPS	14 - 24	18.78 ± 0.68	2.9	15.45	18	13.46	15.29	77.54
NGPS	36.2 - 68.2	52.55 ± 2.57	10.91	20.78	55.10	20.61	21.51	91.77
BMY	2100 - 5300	4135 ± 236.8	1004.67	24.29	4495	23.67	24.98	89.75
GY	467 - 2001	1205.7 ± 135.39	574.43	47.64	1236.5	49.58	50.25	97.34
HI	10.3 - 43.5	29.11 ± 2.68	11.36	39.02	30.13	25.72	25.97	98.14

GCV: Genotypic coefficient of variance; PCV: phenotypic coefficient of variance; H2: broad sense heritability; DTH: days from sowing to heading; DTM: days from sowing to physiological maturity; DGFP: days of grain filling period; PHT: plant height (cm); TPP: number of fertile tillers/plant; SPM: number of spikes/m2; SL: spike length (cm), SkPS: spikelets per spike; NGPS: number of grains/spike; BMY: biomass/plot (kg/ha), GY: grain yield (kg/ha), HI: harvest index; SD: standard variation; SE: standard error; coefficient of variation..

Key Sendie had the least number (3.13). The highest yield was recorded by Ferno variety (1957kg/ha) followed by Chekole (1588.33 kg) and Canada Sendie (1580.7 kg) while the lowest yield was found in Gomadie variety (495 kg).

Phenotypic and genotypic variations

The amount of genotypic and phenotypic variability that exists in a species is of utmost importance in breeding better varieties and in initiating a breeding program. Genotypic and phenotypic coefficients of variation are used to measure the variability that exists in a given population (Burton and Devane, 1988). In general, PCV values were greater than GCV values, although the differences were small. This indicated that the environmental effect was small for the expression of most characters. The GCV ranged from 5.15 for days taken for maturity to 49.58 for grain yield, while PCV ranged from 5.73 for days taken for maturity to 50.25 for grain yield (Table 3). Deshmukh et al. (1986) classified PCV and GCV values as high (>20%), medium (10 to 20%) and low (<10%). Accordingly, high PCV and GCV were observed in traits spike length, numbers of grains per spike, grain yield and harvest index, while others scored medium PCV and GCV except days taken for maturity which scored low. The high PCV and GCV indicated that selection may be effective based on these traits and their phenotypic expression would be good indication of the genotypic potential (Singh et al., 1994), while low PCV and GCV implies less scope of selection as they are under the influence of environment.

Heritability

Generally, heritability indicates the effectiveness with which selection of genotypes can be based on phenotypic performance. In this study, the heritability estimate ranged from 54.20% for number of spikes/m^2 to 89.08% for harvest index (Table 3). The heritability was larger for most of the traits due to smaller phenotypic variances. According to Pramoda and Gangaprasad (2007), heritability estimates can be low (<40%), medium (40 to 59%), moderately high (60 to 79%), and very high (>79%). Accordingly, heritability estimates were very high for nine traits from the total of 12 studied traits (Table 3) indicating the possibility of success in selection, because there would be a close correspondence between genotype and phenotype due to a relatively smaller contribution of environment to phenotype.

Correlation of grain yield and yield related traits

The various characteristics of crop plants are generally interrelated or correlated. Such correlations can either be negative or positive. In plant genetics and breeding studies, correlated characters are of prime importance, because of genetic causes of correlations through pleiotropic action or developmental interactions of genes and changes brought about by a natural or artificial selection (Falconer and Mackay, 1996; Sharma, 1998). Number of grains per spike, number of tillers per plant and harvest index was found to have highly significant positive correlation with grain yield, while it had significant and positive correlation with spikelets per spike and biomass (Table 5).

Table 4. Means of six local wheat varieties for twelve agro-morphological traits.

Genotype	Trait											
	DTH	DTM	PHT	DGFP	TPP	SL	SPM	SkPs	NGPS	BMY	GY	HI
LC1	65.67^{c}	122.67^{c}	82.80^{d}	85.67^{d}	5.00b^{a}	8.33^{c}	381.0^{a}	17.33^{cd}	53.07^{b}	4003.3^{b}	1580.67^{b}	28.19^{a}
LC2	69.67^{c}	137.67^{ba}	110.34^{b}	90.00^{d}	5.70^{a}	9.87^{b}	318.33^{abc}	18.67^{bc}	54.08^{b}	4600.0^{ab}	1588.33^{b}	25.75^{b}
LC3	90.67^{a}	139.67^{ba}	86.67^{cd}	110.67^{ba}	3.13^{c}	6.13^{d}	252.33^{c}	15.67^{d}	40.53^{c}	2166.7^{c}	545.67^{d}	20.09^{c}
LC4	88.67^{a}	133.67^{b}	100.8^{cb}	108.67^{bca}	4.70^{ba}	11.23^{a}	342.67^{ba}	22.67^{a}	62.73^{a}	4885.3^{a}	1067.33^{c}	18.01^{c}
LC5	84.33^{b}	137.00^{ba}	134.00^{a}	104.33^{c}	4.30^{bc}	11.00^{a}	387.33^{a}	17.00^{cd}	39.00^{c}	4554.7^{ab}	495.00^{d}	9.77^{d}
LC6	94.00^{a}	144.67^{a}	97.20^{c}	114a	5.27^{ba}	10.83^{ba}	288.33^{bc}	21.33^{ab}	65.90^{a}	4600.3^{ab}	1957.00^{a}	29.85^{a}
LSD	5.37	7.29	12.23	5.31	1.25	1.00	88.74	2.97	7.06	720.53	215.11	2.23
CV	3.67	3.01	6.705	2.92	15.1	5.89	15.18	8.88	7.56	9.79	10.03	-

DTH: days from sowing to heading; DTM: days from sowing to physiological maturity; DGFP: days of grain filling period; PHT: plant height (cm); TPP: number of fertile tillers/plant; SPM: number of spikes/m^{2}; SL: spike length (cm), SkPS: spikelets per spike; NGPS: number of grains/spike; BMY: biomass/plot (kg/ha), GY: grain yield (kg/ha), HI: harvest index.

Table 5. Pearson's correlation coefficient among yield related traits of local wheat varieties traits.

Trait	DTH	DTM	DGFP	PHT	TPP	SPM	SL	SKPS	NGPS	BMY	GY	HI
DTH	1.00	-	-	-	-	-	-	-	-	-	-	-
DTM	0.729***	1.00	-	-	-	-	-	-	-	-	-	-
DGFP	0.999***	0.733***	1.00	-	-	-	-	-	-	-	-	-
PHT	0.005^{ns}	0.167^{ns}	0.008^{ns}	1.00	-	-	-	-	-	-	-	-
TPP	-0.417^{ns}	-0.143^{ns}	0.412^{ns}	0.195^{ns}	1.00	-	-	-	-	-	-	-
SPM	-0.35^{ns}	-0.403^{ns}	0.357^{ns}	0.337^{ns}	0.004^{ns}	1.00	-	-	-	-	-	-
SL	0.161^{ns}	0.197^{ns}	0.162^{ns}	0.564**	0.46	0.319^{ns}	1.00	-	-	-	-	-
SkPS	0.297^{ns}	0.150^{ns}	0.298^{ns}	-0.115^{ns}	0.097^{ns}	0.036^{ns}	0.664**	1.00	-	-	-	-
NGPS	0.122^{ns}	0.099^{ns}	0.125^{ns}	-0.280^{ns}	0.463*	-0.027^{ns}	0.576*	0.781**	1.00		-	-
BMY	-0.121^{ns}	-0.004^{ns}	-0.119^{ns}	0.510*	0.591**	0.556*	0.852***	0.549*	0.583*	1.00	-	-
GY	-0.259^{ns}	-0.004^{ns}	0.257^{ns}	-0.310^{ns}	0.623**	0.024^{ns}	0.257^{ns}	0.457^{ns}	0.792**	0.556*	1.00	-
HI	-0.269^{ns}	-0.056^{ns}	-0.268^{ns}	-0.657**	0.398^{ns}	-0.248^{ns}	-0.175^{ns}	-0.236^{ns}	0.591**	-0.015^{ns}	0.877***	1.00

*Significant at 0.05 level of significance, **Significant at 0.01 level of significance. DTH: days from sowing to heading; DTM: days from sowing to physiological maturity; DGFP: days of grain filling period; PHT: plant height (cm); TPP: number of fertile tillers/plant; SPM: number of spikes/m^{2}; SL: spike length (cm), SkPS: spikelets per spike; NGPS: number of grains/spike; BMY: biomass/plot (kg/ha), GY: grain yield (kg/ha), HI: harvest index.

DISCUSSION

Six locally cultivated wheat varieties were evaluated for variability, heritability and association of characters using 12 phonological and yield related traits. Highly significant variation was recorded in all traits except for tillers per plant and numbers of spikelets/m^{2} that could indicate the presence of high wheat genetic variability in the study area. This finding agreed with Dawit et al. (2012) which indicated the presence of high genetic diversity of durum wheat genotypes in

Ethiopia. Geleta et al. (2013) concluded that the presence of considerable genetic variation for quantitative morphological and quality traits from different wheat accessions was taken from Ethiopia. This investigation showed that PCV was slightly higher than GCV in all the tested traits that indicates the presence of small environmental influence. Awale et al. (2013) explained the presence of slightly higher PCV value than GCV on some bread wheat genotypes grown in Eastern Ethiopia. Asaye et al. (2013) got the same result after being tested on wheat genotypes from East Gojjam zone. Higher broad sense heritability was recorded in most of the tested traits which is because of smaller phenotypic variance and this is in line with the studies of Khan et al. (2010), Salem et al. (2008) and Awale et al. (2013). Grain yield had a positive and highly significant correlation with number of grains per spike, number of tillers per plant and harvest index that indicates these traits must be considered during selection. Nawaz et al. (2013) also got similar correlation result in some wheat varieties from Pakistan. This study shows that better yield was recorded from local variety Ferno followed by Chekole and Canada Sendie which indicates these varieties should be considered for future wheat yield improvement programs.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Salinity increased vitamins concentration in *Amaranthus cruentus* leaves

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Salt stress is one of the major environmental constraints limiting agricultural productivity and influencing the concentration of bioactive compounds of vegetables. In this study, the effect of NaCl salt stress on nutrient contents of leaves in a cultivar of amaranth, an important leafy vegetable cultivated in some tropical regions worldwide, was evaluated. The experiment was carried out in a screen house at Center for Agricultural Research of Agonkanmey, Benin Republic as a randomized complete block design (RCBD) with three replications. Three weeks old plants were subjected in pots containing a mixture of potting soil and sand, to three concentrations (0, 30 and 90 mM) of NaCl by irrigation every two days. Nutrient contents in leaves were determined at maturity, after four weeks of stress, using standards methods. Proteins, total sugars, reducing sugars, lipids, potassium, calcium, vitamins C and B3 contents were not significantly affected by NaCl. Iron content increased significantly only at 30 mM NaCl but vitamins A, B1 and B2 contents increased significantly with increase in NaCl concentration. Thus, salt stress did not reduce nutritional values of this amaranth cultivar but improved its leaves nutritional quality by increasing vitamins A, B1 and B2 content.

Key words: Amaranth, NaCl, proteins, lipids, sugars, mineral, vitamins.

INTRODUCTION

Amaranthus, collectively known as amaranth, is a cosmopolitan genus of annual or short-lived perennial

plants. Some amaranth species are cultivated as leafy vegetables which are essentially required to safeguard health, particularly by precluding human diseases as they are good source of vitamins, mineral nutrients and antioxidants (Prasad et al., 2014). They exhibit a high nutritive value but also a fascinating ability to adapt to diverse harsh environments (Omami et al., 2006). As tropical leafy vegetables, they are acquiring increasing importance as potential subsidiary food crop considering their excellent quality in protein and endogenous micronutrients content (Devadas and Saroja, 1980; Prakash and Zaidi, 2000). Presently, some amaranth species are cultivated in semi-arid regions, where salinity problem is acute (Bhattacharjee, 2008). Vegetable crops are predominantly cultivated in the southern part of Benin, in urban and suburban areas and in the valley of *Ouémé* (Adorgloh-Hessou, 2006). In Benin, amaranth species are mainly cultivated as leaf vegetable in the cultivable lands of the coastal areas where soil and irrigation water's salinity constitute real problems hampering crop production. Salt stress is one of the major environmental constraints limiting agricultural productivity (Boyer, 1982; Wei et al., 2003). As environmental stress, it may have a strong influence on the concentration of bioactive compounds of vegetables (Prasad et al., 2014). However, despite a substantial amount of literature on responses of plants to salinity stress, data on the effect of salt stress on nutrient contents in leaves of amaranth are lacking. Moreover, only little research work has focused on the response of amaranth cultivars produced in Benin to salt stress. Since amaranths are mainly used in Benin as leafy vegetable, it is important to show if NaCl stress induced modification in leaves nutrient contents. The present study aims at evaluating NaCl stress effects on protein, sugar, lipid, mineral and vitamin concentrations of the main amaranth cultivar grown in Benin.

MATERIALS AND METHODS

The main *Amaranthus cruentus* cultivar produced in Benin named 'Locale' was used. Seeds were obtained from the Market Gardening Crops Program of the Benin National Institute for Agricultural Research (INRAB).

Experimental conditions

The experiment was carried out in a screen house at Center for Agricultural Research of Agonkanmey (Abomey-Calavi, Benin Republic) from March to May 2016. Plants were cultivated at a temperature of 26/22°C day/night with natural light and a relative humidity of 55%.

Seeds were incubated for germination in tanks filled with potting moistened soil for two weeks. Young seedlings were then transferred to earthen small pots of 5.8 cm diameter and 6 cm height containing a mixture of potting soil and sandy loam soil 50:50 (one plant/pot) and cultivated one week before stress application. Plants of cultivar *Locale* were subjected to salt stress in earthen big pots of 11.3 cm diameter and 14 cm height filled with 3 kg of the same mixture. Treatments consisted of plant irrigation every two days with 100 ml/pot of 0, 30 or 90 mM NaCl solution corresponding respectively to an electric conductivity of 0, 1.91 and 8.39 $dS.m^{-1}$ determined by a conductimeter (VWR; CO310). The experiment was laid out as a randomized complete block design (RCBD) with one factor (NaCl concentrations) and three replications.

Leaf nutrient determination

Nutrient contents were determined at plant maturity after four weeks exposure to stress. Plants were then fifty days old. The mature leaves of these plants were used for estimating proteins, total sugars, reducing sugars, lipids, potassium, calcium, iron, vitamin A, thiamine (vitamin B1), riboflavin (vitamin B2), niacin (vitamin B3) and ascorbic acid (vitamin C) contents. Total and reducing sugars were determined using the method of Dubois et al. (1965) and proteins were assayed with method of Gornall et al. (1949). Total lipids were extracted using Soxhlet modified methods with acetone as solvent (Randall, 1974). Each probe was immersed in the boiling solvent and then rinsed in the cold one. After extraction, the solvent was evaporated and recovered by condensation. The residue of total lipids was determined gravimetrically after drying. For mineral ion determination, leaves were annealed in a muffle furnace at 550°C for 24 h; the ashes thus obtained were dissolved in 5 ml hydrochloric acid 6N which was evaporated on a hot plate at 125°C. The viscous residue obtained is again dissolved and recovered using HNO_3; mineral ions were then determined by atomic absorption spectrophotometer. Vitamin A was determined with the method of Jedlicka and Klimes (2005) and Kini et al. (2008); vitamins B1, B2 and B3 with the method of Benmoussa et al. (2003) and Gregory (1954), and vitamin C with the method of Karboue and Nesrallah (2014).

Statistical analysis

For all parameters, each value was presented in the form of mean ± standard error from three independent samples values per treatment. Analysis of the main effects of stress intensity was based on a one-way analysis of variance (ANOVA). Differences among means were compared through Student, Newman and Keuls (SNK) test. All statistical analyses were performed by SPSS 16.0. (SPSS Inc. Released, 2007).

RESULTS

NaCl effect on sugars, proteins and lipids concentration

NaCl effects on leaf total sugars, reducing sugars, proteins and lipids concentrations are shown in Figure 1. A non-significant increase was observed at 30 mM NaCl

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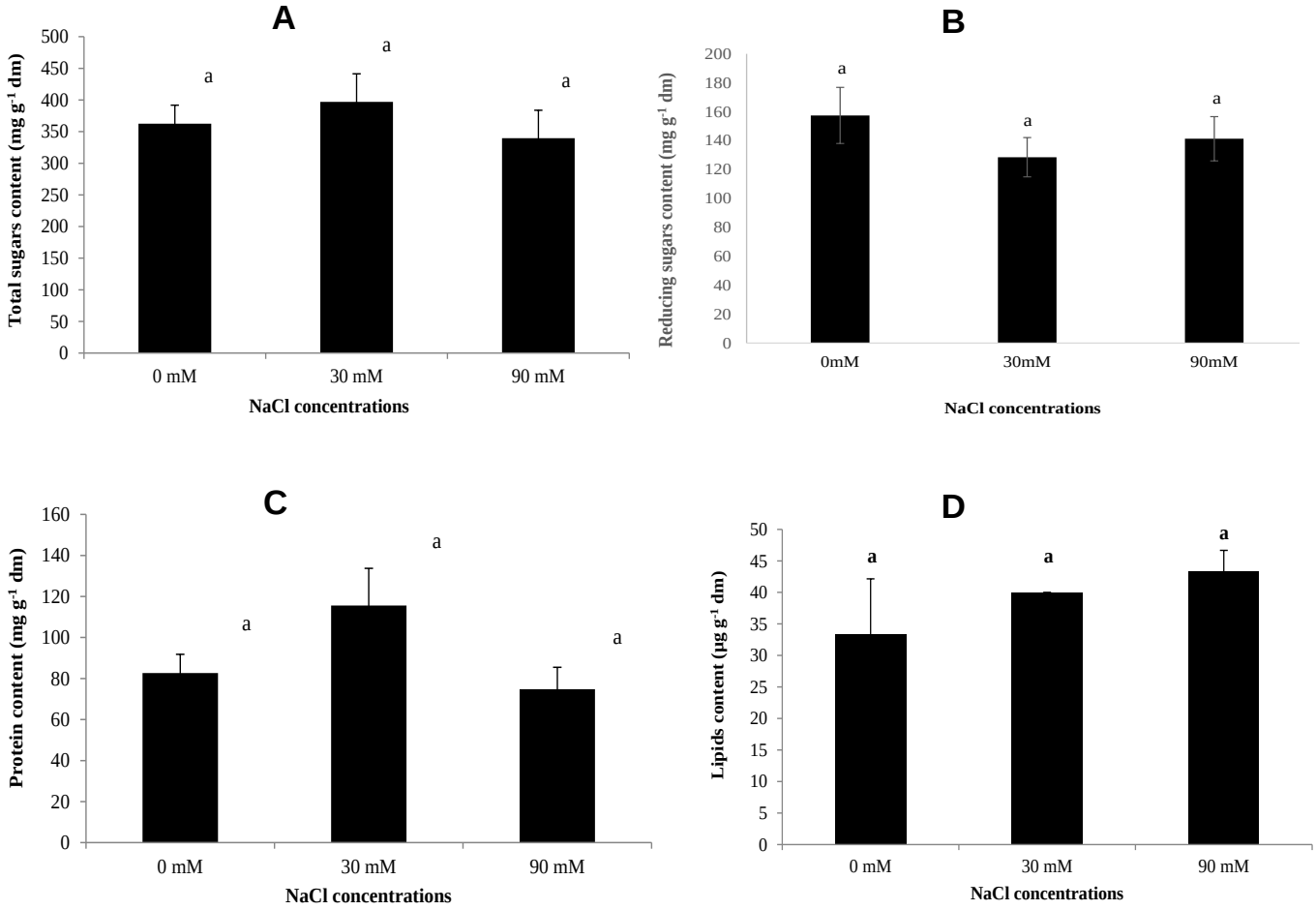

Figure 1. Effect of different NaCl concentrations (0, 30 and 90 mM) on leaf macronutrients contents of *A. cruentus* cv. Locale after four weeks of stress. Values are means ± *SEs*, n = 3. (A) Total sugars, (B) Reducing sugars, (C) Proteins and (D) Lipids. Means with different letters are significantly different (p≤0.05).

Table 1. Effect of different NaCl concentrations (0, 30 and 90 mM) on leaf mineral contents (potassium, calcium and iron: mg g^{-1} dm) of *A. cruentus* cv. 'Locale' after four weeks of stress.

NaCl (mM)	Potassium (K^+)	Calcium (Ca^{2+})	Iron (Fe)
0	39.76±2.72^a	20.26±2.33^a	0.297±0.013^a
30	47.74±3.36^a	21.88±1.92^a	0.358±0.012^b
90	45.61±4.25^a	18.83±2.03^a	0.284±0.004^a

Values are means ± SEs, n = 3. Means with different letters within a column were significantly different (p≤0.05).

for total sugars (Figure 1A) and proteins (Figure 1C) followed by a slight non-significant decrease at 90 mM NaCl. For reducing sugars, a non-significant decrease was observed at 30 and 90 mM NaCl (Figure 1B), whereas for lipids, a non-significant increase was observed at 30 and 90 mM NaCl (Figure 1D). Thus, NaCl effect on leaf total sugars, reducing sugars, proteins and lipids contents remained not significantly affected by the NaCl concentrations used.

NaCl effect on mineral concentration

NaCl effect on leaf potassium, calcium and iron contents is shown in Table 1. A non-significant increase was observed at 30 and 90 mM NaCl for potassium, whereas a similar observation was made at 30 mM NaCl followed by a non significant decrease at 90 mM NaCl for calcium. For iron, a significant increase ($p<0.05$) was noted at 30 mM NaCl followed by a non significant decrease at 90

Table 2. Effect of different NaCl concentrations (0, 30 and 90 mM) on leaves vitamins contents (µg g^{-1}fm) of *A. cruentus* cv. 'Locale' after four weeks of stress.

NaCl (mM)	Vitamin A	Vitamin B1	Vitamin B2	Vitamin B3	Vitamin C
0	0.190±0.014^{a}	0.244±0.014^{a}	3.725±0.001^{a}	2.053±0.022^{a}	6.4±0.1^{ab}
30	0.254±0.020^{a}	1.057±0.046^{b}	3.755±0.004^{b}	2.054±0.02^{a}	4.10±1^{a}
90	0.666±0.036^{b}	1.62±0.146^{c}	3.839±0.004^{c}	2.054±0.052^{a}	7.5±0.05^{b}

Means with different letters within a column are significantly different (p≤0.001).

mM NaCl (Table 1). Thus, NaCl effect on leaf micronutrient contents was significant only for iron at 30 mM NaCl.

NaCl effect on vitamins concentrations

NaCl effect on leaf vitamins concentrations is shown in Table 2. A non-significant increase was observed at 30 mM followed by a significant increase ($p<0.001$) at 90 mM NaCl for vitamin A, whereas a non-significant decrease was noted at 30 mM NaCl followed by a non significant increase at 90 mM NaCl for vitamin C in comparison with the control (0 mM NaCl). For vitamins B1 and B2, a significant increase ($p<0.001$) was observed for 30 mM NaCl. For vitamin B3, a slight non-significant increase was observed in the presence of NaCl. Thus, NaCl effect on leaf vitamins contents increase was significant for vitamins A, B1 and B2.

DISCUSSION

Vegetables are known to contain nutritional properties provided by sugars, proteins, lipids, minerals, antioxidants, vitamins, etc. (Prasad et al., 2014). According to these authors, increasing environmental stresses has strong influence on the concentration of bioactive compounds while affecting the valuable constituents of vegetables which are getting deteriorated day by day. The results revealed no modification in total sugars, reducing sugars, proteins and lipids concentrations of leaves. In other vegetables, a positive effect of salt stress on these nutrients was reported. It is the case in watermelon with an increase in fructose, glucose, sucrose and total soluble solids contents in fruits by salt stress (Colla et al., 2006) and in tomato with an increase in sugar and organic acids contents of fruits (Dorais et al., 2001). Minerals play a vital role in plant and animal metabolism (Zargar et al., 2015). Among these minerals, potassium (K), calcium (Ca) and iron (Fe) are three of the main one in leafy vegetables. The results revealed no effect of NaCl on K^+ and Ca^{2+} concentrations in amaranth leaves. Similar results were reported in watermelon fruit for K^+ (Colla et al., 2006) and cucumber fruit for Ca^{2+} (Trajkova et al., 2006). As reported in other plants, salt stress generally resulted in K^+ and/or Ca^{2+} decrease (Stamatakis et al., 2003; Trajkova et al., 2006; Abdelhamid et al., 2013). The results revealed a significant increase of iron content in the presence of 30 mM NaCl followed by a non significant decrease at 90 mM NaCl. In faba bean, Abdelhamid et al. (2013) reported that NaCl decreased shoot iron content. The increase or decrease of potassium and calcium concentration, and the decrease of iron content of amaranth leaves at 90 mM NaCl was not significant, indicating that NaCl salt stress did not reduce mineral concentrations in leaves of amaranth. Studies of salt stress effect on vitamin A content of vegetables are scarce. Carotene concentrations are commonly determined as these vegetable yellow pigments are converted to vitamin A in the human body and are accordingly refer to as provitamin A (Ratnakar and Rai, 2013). Amaranth species are known to be a rich β-carotene source (Gopalan et al., 1971). In the present investigation, vitamin A content in the leaves of *A. cruentus* showed a significant increase with increasing NaCl concentration in the growth medium. In unicellular green alga *Dunaliella,* several authors reported that increasing salt concentration induced increment in β-carotene (Pisal and Lele, 2005; Sarmad et al., 2007; Rad et al., 2011). However, other authors reported a decrease in β-carotene content under salt stress in several species or genera including *Paulownia imperialis* and *Paulownia fortunei* (Ayala-Astorga and Alcaraz-Melendez, 2010), *Chlorella* (Fathi and Asem, 2013) and *Amaranthus polygamous* (Ratnakar and Rai, 2013). B vitamins are the precursors of essential metabolic cofactors but are prone to destruction under stress conditions (Hanson et al., 2016). It is therefore *a priori* reasonable that stressed plants suffer B vitamin deficiencies and certain stress symptoms are metabolic knock-on effects of these deficiencies. Studies on environmental stresses effects on B vitamins in plants are very scarce. In general, the foods that contain carbohydrates as a major source of energy contain a higher level of thiamine (vitamin B1) which is required for carbohydrate metabolism (Gopalan et al., 1971). Thiamine can function to alleviate environmental stresses in plants, as it can directly act as an antioxidant (Tunc-Ozdemir et al., 2009). The results reveal that thiamine content in the leaves of *A. cruentus* increased with salt concentration. Similar trend was

observed in maize under salt stress (Rapala-Kozik et al., 2008) and *Arabidopsis thaliana* seedlings (Tunc-Ozdemir et al., 2009). However, Ratnakar and Rai (2013) observed a decrease of thiamine content in *A. polygamous* leaves under NaCl salinity stress. Riboflavin (vitamin B2) is essential for the metabolism and proper utilization of energy, carbohydrates, proteins and fats (Ratnakar and Rai, 2013). It is also essential for several oxidative processes occurring inside the cell. Green leafy vegetables are a good source of riboflavin (Ratnakar and Rai, 2013). In the present study, a significant increase in riboflavin concentration was recorded in *A. cruentus* leaves under NaCl salt stress. In leaves of *A. polygamous*, Ratnakar and Rai (2013) reported a decrease in riboflavin concentration under NaCl stress. Niacin (nicotinic acid or vitamin B3) is a water-soluble vitamin. Nicotinamide is the derivative of niacin and used by the body to form the coenzymes nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP). Over 400 enzymes require the niacin coenzymes, NAD and NADP, mainly to accept or donate electrons for redox reactions (Penberthy and Kirkland, 2012). NAD functions most often in energy-producing reactions involving degradation (catabolism) of carbohydrates, fats, proteins and alcohol. The niacin coenzyme, NAD, is the substrate (reactant) for at least four classes of enzymes that separate the nicotinamide moiety from NAD and transfer ADP-ribose to acceptors.
The results revealed a non-significant increase in niacin content in *A. cruentus* leaves under NaCl salt stress. Ascorbic acid (vitamin C) is an essential nutrient which occurs widely in crop foods products, especially in fresh fruits and green leafy vegetables (Ratnakar and Rai, 2013). It is a small, water soluble, antioxidant molecule which acts as a primary substrate in the cyclic pathway of enzymatic detoxification of hydrogen peroxide (Beltagi, 2008). Vitamin C also helps in absorption of dietary iron by keeping it in the reduced form (Ratnakar and Rai, 2013). The current results revealed that in the leaves of *A. cruentus* cv. 'Locale', the ascorbic acid content was not significantly affected by NaCl. In other amaranth species (*A. polygamous)*, Ratnakar and Rai (2013) observed a decrease of ascorbic acid content with increase of salt concentration. The same tendency was reported also in wheat (Seth et al., 2007; Mandhania et al., 2010), and in *Linum usitatissimum* plants (Emam and Helal, 2008). However, the increase of ascorbic acid contents under salt stress was reported in tomato fruits (Stamatakis et al., 2003; Kim et al., 2008; Gautier et al., 2010) and in other plants including barley (*Hordeum vulgare*) (Sarwat and El-Sherif, 2007) and *Cicer arietinum* cv. Abrodhi (Mishra et al., 2009). Generally, it is well known that during the onset and development of salinity-stress within a crop plant, major processes such as photosynthesis, protein synthesis, and energy and lipid metabolism are affected, leading to quality and yield losses in most crops (Hasegawa et al., 2000; Hagemann and Erdmann, 1997; Hayashi and Murata, 1998). However, there are several crops with an inherent capacity to withstand salinity-stress, which allows for stable vegetable production and significantly contributes to palatability and food functionality (Sato et al., 2006). Thus, there are several reports on the application of salinity-stress for improvement of the quality of vegetables such as tomato fruits (Auerswald et al., 1999), spinach (Makabe and Tanii, 2008) and strawberry (Keutgen and Pawelzik, 2008). The results of the present study revealed that salt stress improved leaves nutritional quality by mainly increasing some vitamins concentrations.

Conclusion

This study indicated that increasing NaCl concentrations did not reduce total sugars, reducing sugars, proteins, lipids, potassium, calcium, vitamin B3 and vitamin C concentrations in leaves of *A. cruentus,* but rather increased vitamins A, B1 and B2 concentrations.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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