Ethylene and abscisic acid in the control of development of the rhizome of *Kohleria eriantha* (Benth.) Hanst. (Gesneriaceae)

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Kohleria eriantha has rhizomes which are underground stems with buds enclosed by modified leaves, that store starch. The buds of this rhizome can develop in two morphological patterns: an aerial shoot (similar to the mother plant) or a rhizome, depending on the water level present in the substrate. Development of the shoot was inhibited by low water availability (1 mL) in the substrate. It was verified that ethylene and ABA were involved in controlling the development of the rhizome pattern under low water availability. Treatments with ethrel, PEG and ABA induced shoot development whereas treatments with $AgNO_3$ or AVG inhibited shoot development. Increased ethylene and ABA were observed under conditions that inhibited shoot development. Moreover, it is suggested that ABA may induce the production of ethylene in the sections of the rhizome under low water availability.
Key words: abscisic acid, buds, ethylene, meristem, rhizome.

Etileno e ácido abscisico no controle do desenvolimento do rizoma de *Kohleria eriantha* (Benth.) (Gesneriaceae): *Kohleria eriantha* possui rizomas subterrâneos, caule com gemas envoltas por folhas modificadas que armazenam amido. As gemas desse rizoma podem-se desenvolver em dois padrões morfológicos: o padrão parte aérea (semelhante à planta mãe) ou um rizoma dependendo do nível de água do substrato. O desenvolvimento do padrão parte aérea foi inibido pela baixa disponibilidade de água do substrato (1 mL). Verificou-se que o etileno e o ABA estavam envolvidos com o controle do desenvolvimento do padrão rizoma na ausência ou baixa disponibilidade hídrica. Tratamentos com ethrel, PEG e ABA induziram o desenvolvimento do padrão parte aérea, enquanto os tratamentos com AVG e $AgNO_3$ inibiram o desenvolvimento do padrão parte aérea. Observaram-se aumento de etileno e ABA sob condições que inibiram o desenvolvimento da parte aérea. Sugere-se, também, que ABA pode induzir a produção de etileno em seções do rizoma na ausência ou em baixa disponibilidade hídrica.
Palavras-chave: ácido abscísico, etileno, gemas, meristema, rizoma.

INTRODUCTION

Kohleria eriantha belongs to the family Gesneriaceae and is distributed from southern Mexico down to Peru and Suriname, with its center of diversity in the Colombian highlands. This species produces rhizomes which are underground stems (growing perpendicular to the surface of the ground) that have buds enclosed by modified leaves. These organs store starch (Almeida, 1997; Almeida and Pereira, 2000) and the buds are capable of producing either aerial shoots or additional rhizomes. These organs are known to be an adaptation that aids in survival during the dry season (Anonymous, 1991; Kvist and Skog, 1992).

The development of these buds into rhizomes instead of aerial shoots is a response to low or the lack of water availability (1 mL) (Almeida and Pereira, 2004). Water availability is known to influence ABA responses, and ABA is known to influence ethylene responses (Cracker and Abeles, 1969; Jackson and Osborne, 1975; Goren et al., 1979; Yeh et al., 1995). These observations led to the hypothesis that these growth substances might be involved in the control of development of the rhizome of *K. eriantha* under low water availability.

Low water availability has been shown to be a factor that triggers ethylene production in plants (Ben-Yehoshua and Aloni, 1974; Apelbaum and Yang, 1981; Kapuya and Hall, 1984; Hale and Orcutt, 1987; Hyodo, 1991). 1-aminocyclopropane-1-carboxylic acid (ACC) is the direct precursor of its synthesis (Saftner, 1992; Sarquis et al., 1992; Rothan and Nicolas, 1994). Every plant tissue may produce ethylene (Yu and Yang, 1980; Yang and Hoffman, 1984; Mattoo and White, 1991) but this production may also be indirectly induced by exogenous auxin (Blumenfeld, 1975; Bradford and Yang, 1980; Yoshii and Imaseki, 1981; Yeang and Hillman, 1982; Zimmerman and Wilcoxon, 1935 apud Yang, 1987; Arteca et al., 1988; Smulders et al., 1990; Hoson et al., 1990; Kim et al., 1992).

Endogenous ABA content may also increase in plants exposed to lack of water (Trewavas, 1981; Zhang and Davies, 1987), as verified in *Salix viminalis* (Barros and Neill, 1987), *Craterostigma plantagineum* (Bartels et al., 1990), *Glycine max* (Creelman et al., 1990), *Funaria hygrometrica* (Werner et al., 1991), *Oriza sativa* (Grossmann and Kwiatrowski, 1993a), *Helianthus annuus* (Grossmann et al., 1993b), *Solanum tuberosum* (Leone et al., 1994) and *Fatsia japonica* (Lopez Carbonell et al., 1994).

Ethylene and ABA may also influence morphological development in different species (Chopra and Kapur, 1988; Vreugdenhil and van Dijk, 1989a,b; Abeles et al., 1992; Smart et al., 1995). Therefore, the aim of this study was to verify the possible involvement of ethylene or ABA in the control of the developmental pattern of rhizome buds of *Kohleria eriantha* under the condition of low water availability.

MATERIAL AND METHODS

Rhizomes of *K. eriantha* were collected from plants cultivated in beds. Rhizomes were divided into 2 cm long sections, containing six modified leaves and one bud in each. The sections were placed in Petri dishes (9 cm diameter) lined with three layers of filter paper, of 6 µm porosity, that was moistened with 1 or 12 mL of water, and stored in polythene bags and kept in a growth chamber at 30ºC, in the light. The condition of 1 mL was considered as low water availability whereas 12 mL formed a film of water on the substrate surface and was regarded as high water availability. Rhizome sections were treated with solutions of ethrel, ABA, indole-3-acetic acid (IAA), 6-benzylamino-purine (6-BA), ACC, silver nitrate ($AgNO_3$) and aminoethoxyvinylglycine acid (AVG) (table 1). Sections were also kept on substrate with 12 mL of PEG (Polyethyleneglycol) solution (-6 MPa) prepared according to Michel and Kaufamann (1973), and treated with a droplet (50 µL) of $AgNO_3$ at 500 $mg.L^{-1}$.

Ethylene levels were estimated in rhizome sections on substrate with 1 and 12 mL of water. Flasks containing rhizomes sections were sealed with septa after a small glass tube containing three KOH pellets (0.28 g) had been introduced to reduce the CO_2 level (Tan and Thimann, 1989). Gas samples, 1 mL, were collected 24 and 48 h after the beginning of the experiment, and were injected into a "Varian 2440" gas chromatograph, equipped with a flame ionization detector and a 6 x ¼ in "Pyrex" column. The column solid phase consisted of Poropak T of 80/100 mesh and N_2 was used as the mobile phase. Temperatures for the equipment were: column, 100ºC; injector, 140ºC and detector 190ºC. Ethylene was quantified by comparison of the heights of the sample and standard peaks.

The extraction of ABA followed the method described by Saunders (1978), with modifications. Extracts of ABA

Table 1. Treatments applied to rhizome sections of *K. eriantha*.

Substance	Concentration	Number and interval of applications:	Solution volume (µL)	Water volume on substrate (mL)
Ethrel	10^{-3} M	5 – every 2 days	20	12
ABA	10^{-3} M	5 – every 2 days	25	12
6-BA	10^{-3} M	10 – every day	25	1, 12
IAA	10^{-3} M	10 – every day	25	12
IAA + 6-BA	10^{-3} M	10 – every day	25	1
ACC	10^{-3} M	10 – every day	20	12
$AgNO_3$	1,10,50,100, 200, 500 $mg.L^{-1}$	1 – every day	50	1
AVG	10^{-3} M	2 – every day	15	1

were obtained from rhizome sections (5 g) kept on substrate with 1 or 12 mL of water for 10 days. The tissue was macerated in 80 % methanol which was removed under reduced pressure at 35ºC. The remaining aqueous solution was acidified with 1 N HCl to pH 3 and partioned three times against 20 mL ethyl acetate. The organic fraction was obtained and partioned three times against 20 mL $NaHCO_3$ (5 %). The aqueous fraction was acidified to pH 3 with HCl and extracted three times with an equal volume of ethyl acetate to obtain the acid fraction. To purify the acid fraction, the ethyl acetate was removed under reduced pressure (35ºC), and the residue was resuspended in 2 mL of methanol. The resulting solution was applied to a sep pak C18 cartridge and eluated with 8 mL of methanol. The filtrate was dried under reduced pressure (35ºC) and resuspended in a solution of 1 mL of methanol and 0.5 % acetic acid (2:3 v/v). To separate ABA, the purified extract was subjected to high performance liquid chromatography (HPLC), using a C18 ODS-Hypersil (5 μM, 250 x 5 mm) column eluted at a flow rate of 1 $mL.min^{-1}$ with a gradient of 0.5 % acetic acid and methanol, increasing from 40 to 85 % methanol over 30 min, and monitored at 260 nm (Jensen et al., 1986, with modifications). Eluates from the acid fractions corresponding to the retention time of the standard ABA isomers were collected, dried under reduced pressure (35ºC) and methylated with 10 mL of diazomethane solution prepared according to Schlenk and Gellerman (1960). The methylated extract was dried under reduced pressure (35ºC) and resuspended in 500 μL of methanol, and then concentrated in nitrogen to 200 μL. An aliquot of 1 μL from each extract was injected into a gas chromatograph coupled to a mass spectrometer (HP 5988A), using a "HP-1 Fused Silica Capillary, 25 m x 0.2 mm" column and the same procedure being used for the standard ABA. The chromatographic conditions used were: initial temperature of the oven 50ºC and final temperature 230ºC, heating rate 30ºC per minute, injector 230ºC, detector 280ºC, and scan range: 40-400 amu (atomic mass unit).

All the treatments had four replicates, each with ten rhizome sections, except for the quantification of ethylene, where each replicate consisted of seven sections. The experiments were conducted in a factorial type experimental design. The data were subjected to ANOVA, using the "F" test and comparison of means by the Tukey test, both at the 5 % level (Pimentel, 1984).

RESULTS

Rhizome sections of *K. eriantha* produced additional rhizomes (figure 1A) or developed aerial shoots (figure 1B) when kept on substrate with 1 and 12 mL of water, respectively. Sections treated with ethrel (10^{-3} M) and kept on substrate with 12 mL of water also developed a high percentage of rhizomes (figure 2A), similar to the response produced by the control on 1 mL of water. Control sections on 12 mL of water developed aerial shoots (figure 2B).

In figure 2 it can also be observed that the use of increasing doses of $AgNO_3$ caused a gradual reduction in the development of rhizomes, and favored the formation of aerial shoots. Concentrations of 50, 100, 200 and 500 $mg.L^{-1}$ of $AgNO_3$ inhibited the development of additional rhizomes on sections with 1 mL of water (figure 2C). Indeed, $AgNO_3$ treated rhizome buds started to develop aerial shoots to a level similar to that of the control on 12 mL of water (figure 2D).

The use of IAA, 6-BA and IAA + 6-BA, aimed at promoting the production of ethylene indirectly, did not

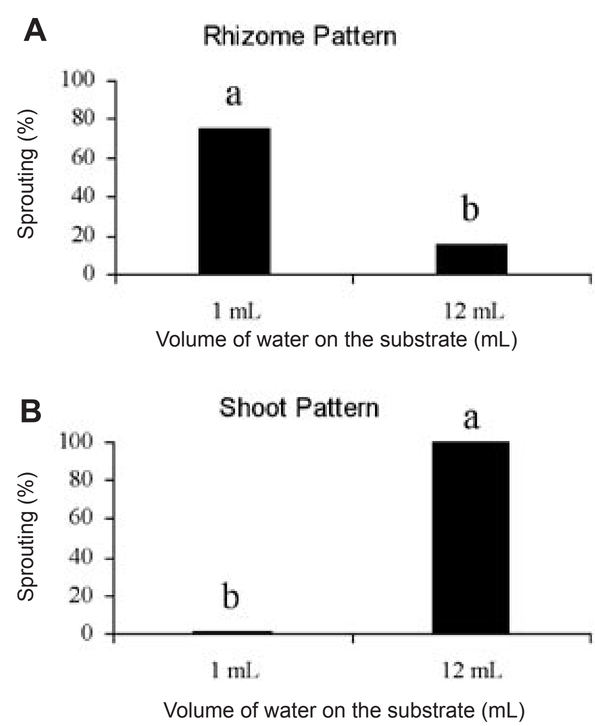

Figure 1. Effect of 1 and 12 mL of water in the substrate on the pattern of sprouting development of rhizome sections of *K. eriantha* kept under continuous light at 30ºC, on day 20 of the experiment. A: Rizhome pattern; B: Shoot pattern. Different letters in each figure indicate significant differences by the Tukey test at the 5 % level.

cause any alteration to the morphological structure of the rhizome sections kept on substrate with 12 mL of water (data not shown). Treatment with ACC (10^{-3} M) also did not have any effect on the pattern of development of rhizome sections on substrate with 12 mL of water (data not shown).

AVG not only inhibited the development of additional rhizomes on sections treated with 1 mL of water (figure 2E), but induced development of aerial shoots (figure 2F). Rhizome sections on substrate to which 12 mL of PEG (-6 MPa) solution was added attained as high a percentage of rhizomes as the control on 1 mL of water (figure 3A). On the other hand, sections on substrate with 12 mL of PEG (-6 MPa) with the addition of $AgNO_3$ developed aerial shoots (figure 3B). PEG is able to modify the osmotic potential and thus induce plant water deficit.

Rhizome sections on substrate with 12 mL of water and treated with ABA (10^{-3} M) developed a rhizogenous growth pattern, similar to the control on 1 mL of water (figure 3C). However only aerial shoots developed on sections with 12 mL of water and no ABA (figure 3D).

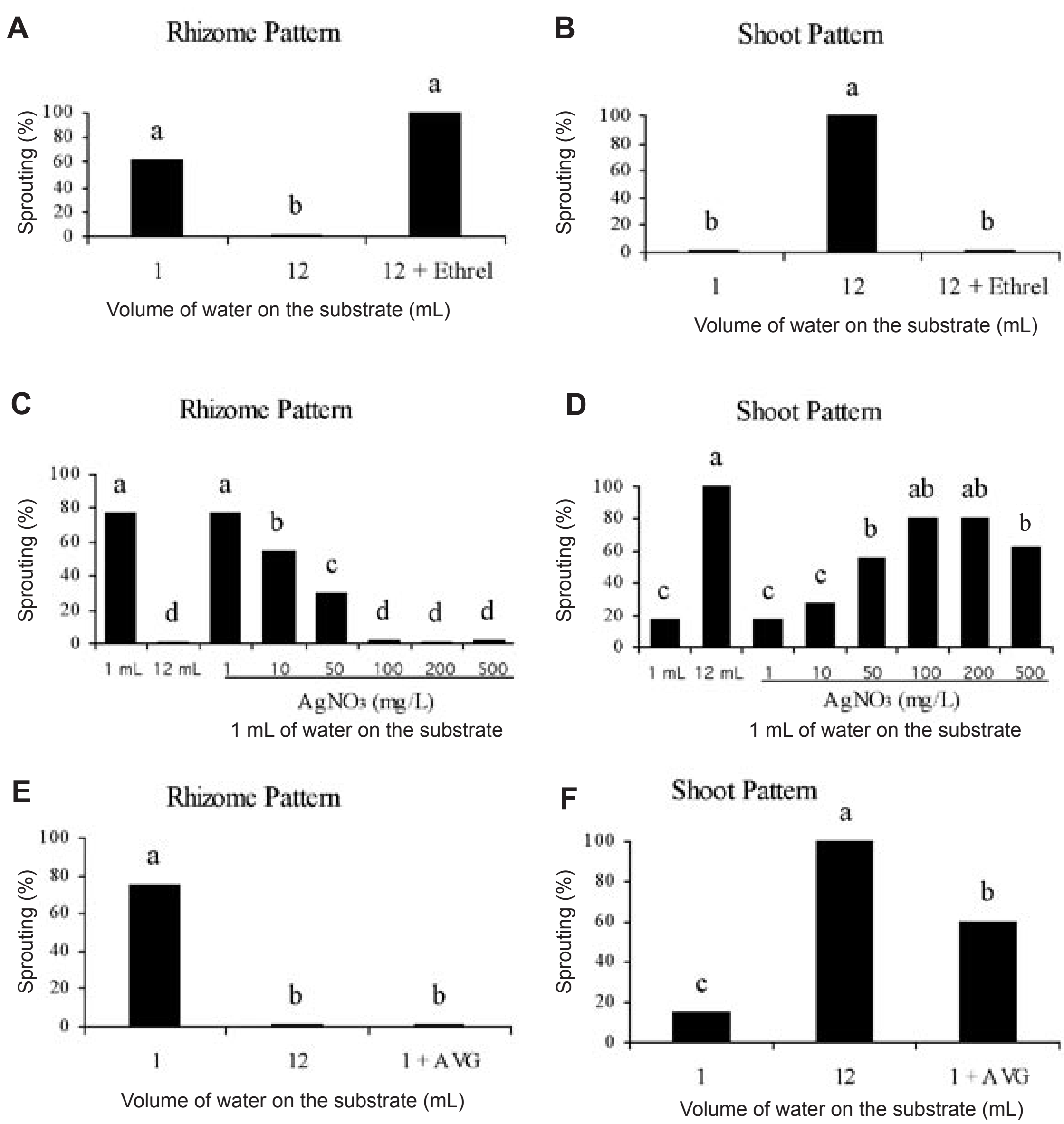

Figura 2. Effect of different substances on the pattern of sprouting development of rhizome sections of *K. eriantha* kept on substrate with 1 and 12 mL of water under continuous light at 30 ºC, on day 20 of the experiment. A,B: 10^{-3} M Ethrel (20 µL, applied every 2 days for 10 days); C,D: $AgNO_3$ (50 µL, applied once); E,F: 10^{-3}M AVG (15 µL applied on day 1 and 3 of the experiment). Different letters in each figure indicate significant differences by the Tukey test at the 5 % level.

Production of ethylene was found in sections kept on substrate with either 1 or 12 mL of water at both time points, however the concentration was higher in sections on substrate with low water availability (figure 4).

A gas chromatograph coupled to a mass spectrometer (GC/MS) was used to characterize the possible involvement of ABA in the control of development of the rhizome pattern. Acid fractions of extracts from rhizome sections kept on 1 or 12 mL of water were used. The retention time of methylated cis and trans standard ABA isomers was determined by means of GC-MS at 26,2 and 27,8 min. Figures 5A, B and C show the characteristic signals of its isomers and their respective mass spectra, with predominance of the ion at m/z 190. In figures 6 and 7 the ion at m/z 190 is monitored and detected in the chromatogram of the methylated acid fractions of the extracts from sections on substrate with 1 and 12 mL of water, figures 6A, B and C and 7A, B and C, respectively. Although the ion at m/z 190 was found in both samples, injection of equal sample volumes (1μL) and use of the same analysis conditions allow us conclude that its abundance was greater in the chromatogram of the acid fraction obtained from sections on substrate with 1 mL of water than that from sections on 12 mL.

DISCUSSION

This study points out the ability of ABA and ethylene to influence the morphological development of rhizome buds of *K. eriantha.* The meristem is the site of

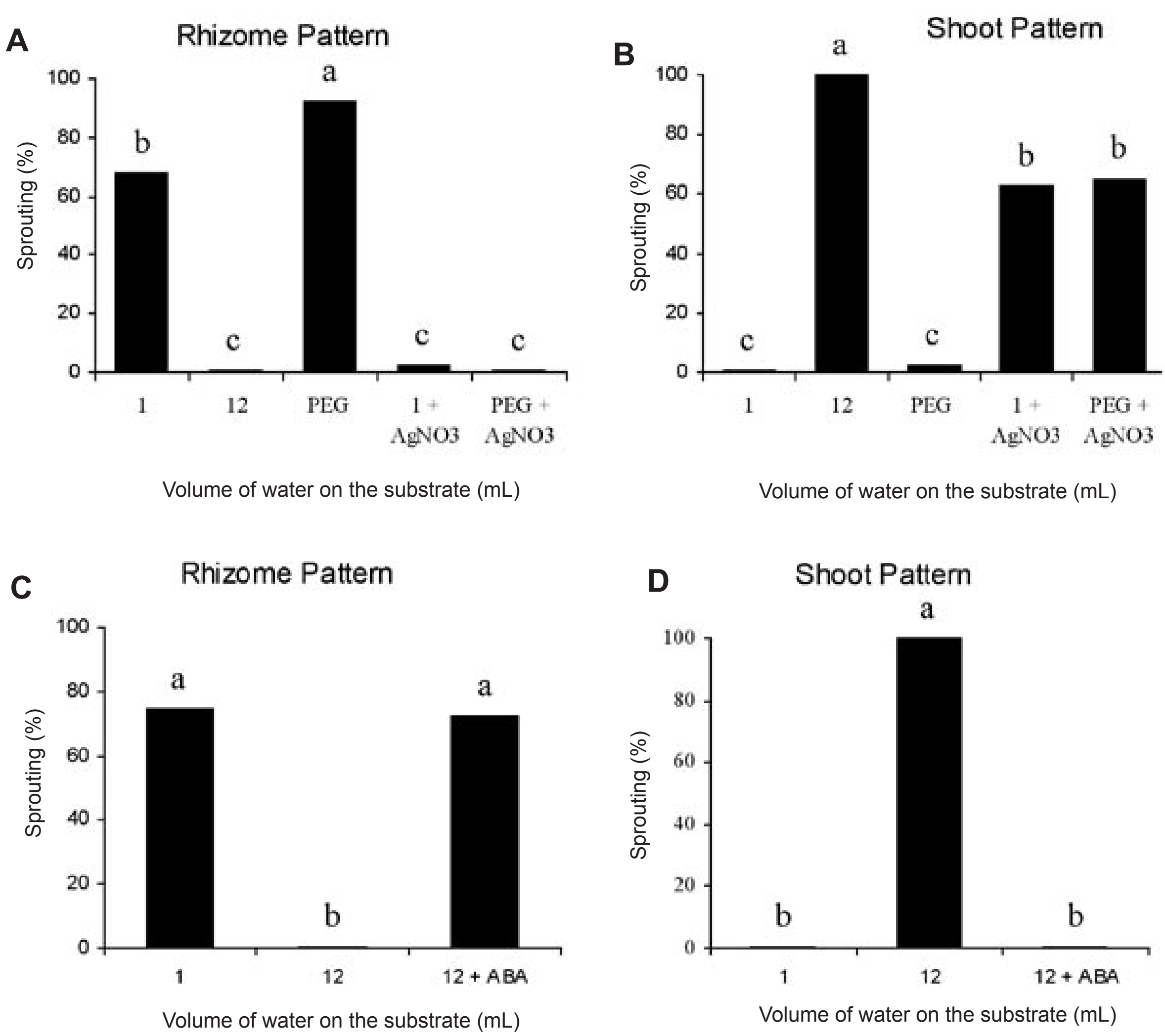

Figura 3. Effect of $AgNO_3$ (500 mg.L^{-1}) used as a droplet (50 μL) (A,B**)** and ABA (10^{-3} M) (25 μL applied every 2 days for 5 days) **(**C,D**)** on the pattern of sprouting development of rhizome sections of *K. eriantha* kept on substrate with 12 mL of PEG (-6 MPa) solution and 12 mL of water, respectively, under continuous light at 30ºC, on day 20 of the experiment. Different letters in each figure indicate significant differences by the Tukey test at the 5 % level.

morphogenetic processes and intense biochemical activity that precedes the morphological alterations, leading to cell division, differentiation and specialization (Cutter, 1986). Determination of the sprouting pattern in *K. eriantha* was rapid, with the sections attaining a high percentage of sprouting by day 4 of the experiment (Almeida and Pereira, 2002), and by day 6 the morphological pattern was visible and defined (data not shown). Thus, the bud meristem of *K. eriantha* quickly showed competence and determination to develop into one of the two sprouting patterns, following its exposure to different environmental conditions.

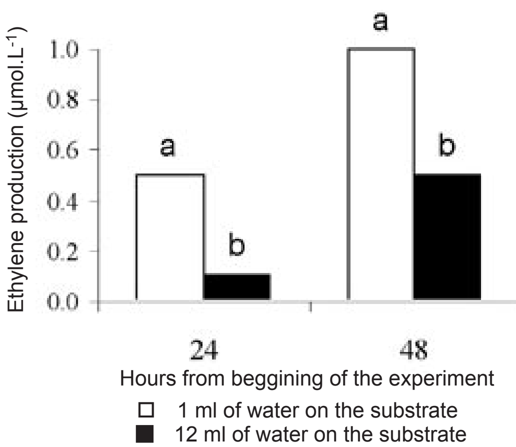

Figura 4. Ethylene released by rhizome sections of *K. eriantha* kept on substrate with 1 and 12 mL of water under continuous light at 30°C. Different letters on the observation day indicate significant differences by the Tukey test at the 5 % level.

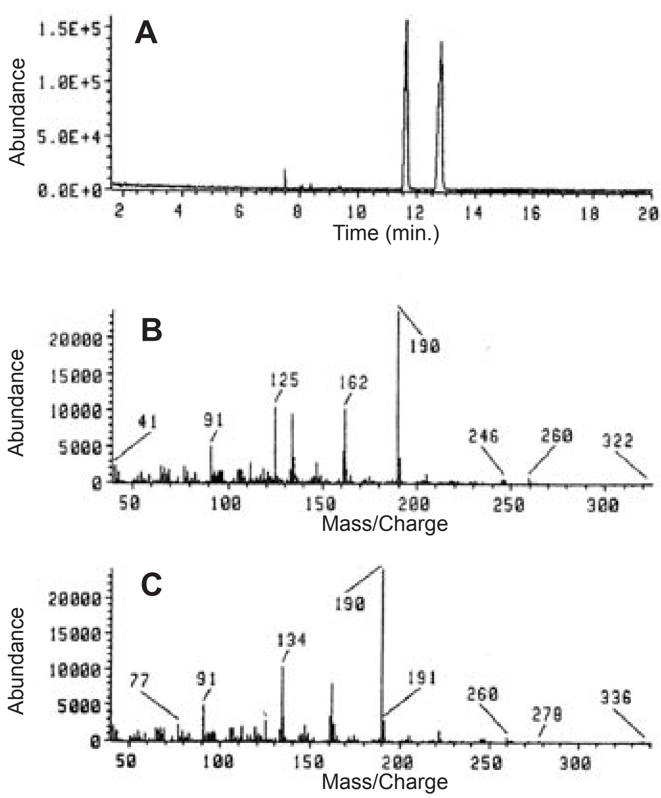

Figure 5. Mass spectrum of standard ABA (A), and mass spectrum of methylated cis- (B) and trans- (C) isomers of standard ABA.

Rhizome sections of *K. eriantha* also showed resistance to water loss, since they sprouted and developed additional rhizomes even under low water availability (1 mL). This corroborates observations by Kvist and Skog (1992) that *K. eriantha* rhizomes are resistant to water loss. According to Bewley et al. (1993) plants develop mechanisms to prevent water loss, and many escape drought by virtue of morphological and/or physiological attributes.

Involvement of ethylene and ABA in the control of development of rhizome pattern in *K. eriantha* under the condition of low water availability (1 mL) was verified indirectly and directly. Ethrel, a synthetic source of ethylene, induced the development of rhizogenous growth to a level seen under water limiting conditions even in the presence of sufficient water to induce shoot development. Treatment with ethylene inhibitors, $AgNO_3$ and AVG, prevented the occurrence of the rhizogenous growth pattern and induced

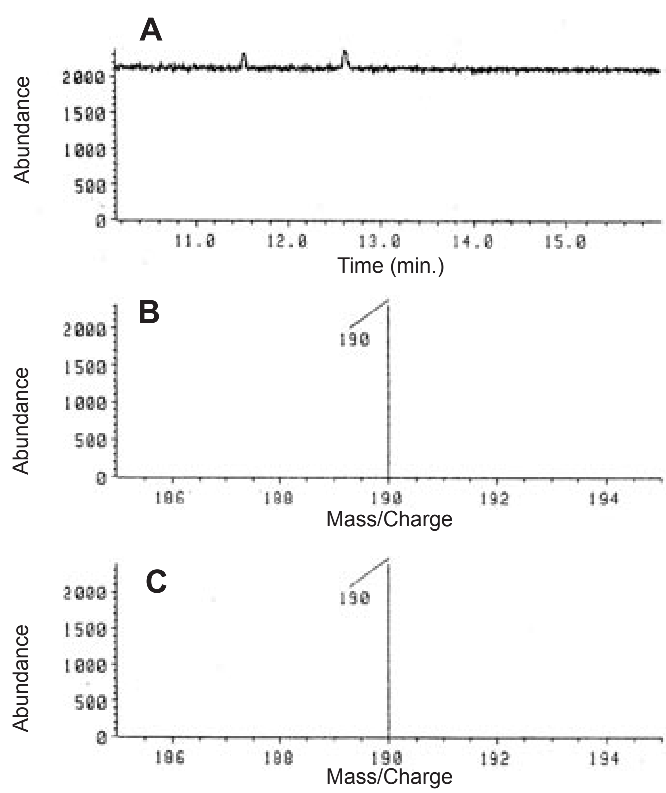

Figure 6. Mass spectrum using the selected ion at m/z 190 of ABA of the extract from rhizome sections kept on substrate with 1 mL of water (A), and mass spectrum of cis- (B) and trans- ABA (C) isomers found in the extract.

development of the aerial shoots on substrate with low water availability (1 mL). Direct involvement of ethylene was shown by an increase in its production on sections grown under low water availability (1 mL) compared to that observed on sections grown with excess of water (12 mL). Detection of ethylene production by day two indicates that ethylene may play a direct role in regulating the development of rhizome buds of *K. eriantha* subjected to low water availability of water (1 mL).

Indirect involvement of ABA was verified by exogenous application of ABA which induced the development of the rhizome pattern even under high water availability and directly by comparisons of ABA levels from sections grown on substrates that induced or inhibited rhizome development. In the analysis, the most abundant components co-purified with the isomer standards of ABA and thus are likely ABA derivatives. Ions at m/z 190 have also been found in the purified extract of ABA from other species, in *Zea mays* (Rivier et al., 1977), *Malus domestica* (Subbaiah and Powell, 1987), *Citrus sinensis* (Cowan and Richardson, 1993) and *Larix decidua* (Label and Lelu, 1994). In *K. eriantha* the putative ABA ion at m/z 190 was more abundant in the sections kept on substrate with low water availability than that from sections with sufficient water. Therefore, this study shows that ABA is also likely to be involved in the control of development of the rhizome pattern of rhizome sections.

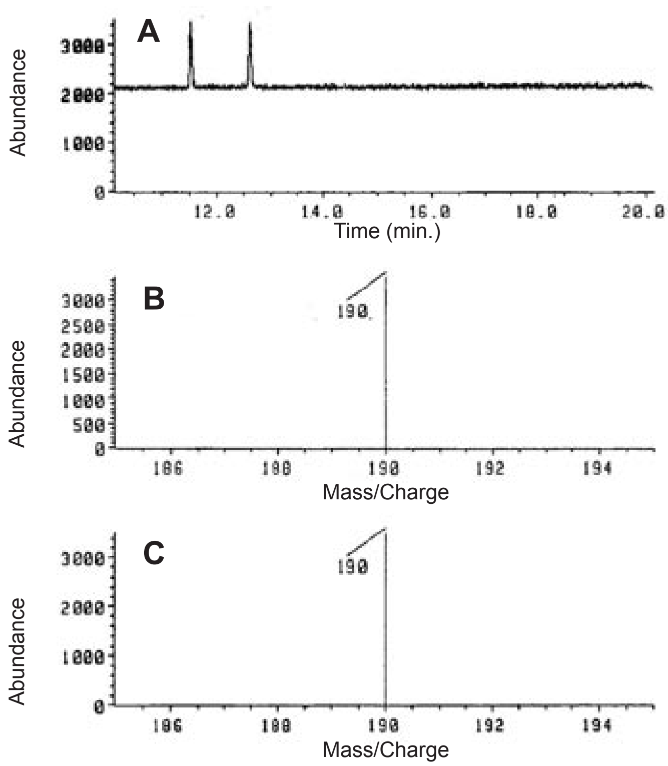

Figure 7. Mass spectrum using the selected ion at m/z 190 of ABA of the extract from rhizome sections kept on substrate with 12 mL of water (A), and mass spectrum of cis- (B) and trans-ABA (C) isomers found in the extract.

From the results obtained with ethylene and ABA, both indirectly and directly, it is suggested that these substances may play a significant role in the control of the developmental pattern of the rhizome sections of *K. eriantha* in response to water availability. Ethylene and ABA may control this process in different ways. Nevertheless, there are studies that indicate that ABA, under water stress, may promote the production of ethylene, as verified in *Gossypium hirsutum* (Cracker and Abeles, 1969), *Phaseolus vulgaris* (Jackson and Osborne, 1975), *C. sinensis* (Goren et al., 1979) and in *O. sativa* (Yeh et al., 1995). It is suggested, therefore, that this relation may also occur with regard to the rhizome bud development program *K. eriantha.*

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