

Effects of chlorocholine chloride and paclobutrazol on cassava (*Manihot esculenta* Crantz cv. Rocha) plant growth and tuberous root quality

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SUMMARY

The effects of chlorocholine chloride (CCC) and paclobutrazol (PBZ) foliar application on shoot and root parameters of cassava field-grown plants were studied (0, 45 and 90 mg active ingredient per plant). CCC and PBZ reduced total plant and first branch height, aerial fresh mass and tuberous root number. PBZ delayed branching and significantly decreased tuberous root fresh mass, while CCC caused no modifications in these parameters. In addition, CCC and PBZ treatments did not modify tuberous root diameter, while PBZ reduced tuberous root length significantly. Starch content was increased by both growth regulators at the lower dose, whereas dry matter content was increased only by CCC. In conclusion, CCC suppresses excessive vegetative growth, favours quality attributes and does not alter yield, hence improving harvest index. Although PBZ at a low dose increases the starch content and harvest index, its effects on other parameters are undesirable.

Keywords: chlorocholine chloride, *Manihot esculenta*, paclobutrazol, starch, yield

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RESUMEN

Se estudiaron los efectos de la aplicación foliar del cloruro de cloro colina (CCC) y del paclobutrazol (PBZ) sobre parámetros de crecimiento aéreo y radical de plantas de mandioca cultivadas a campo (0,45 y 90 mg de principio activo por planta). El CCC y el PBZ redujeron la altura total de las plantas y la altura de la primera ramificación, el peso fresco de los órganos aéreos y el número de raíces tuberosas por planta. El PBZ retrasó la ocurrencia de la ramificación y causó reducciones significativas del peso fresco de las raíces tuberosas, mientras que el CCC no afectó dichos parámetros. Los tratamientos con CCC y PBZ no modificaron el diámetro de las raíces tuberosas, aunque el PBZ redujo significativamente su longitud. El contenido de almidón de raíces tuberosas se incrementó por la aplicación de ambos reguladores del

crecimiento en su dosis mínima; sin embargo, el contenido de materia seca de estas sólo fue aumentado por el tratamiento con CCC. En conclusión, el CCC evita el crecimiento vegetativo excesivo, favorece los atributos de calidad de raíces tuberosas sin afectar el rendimiento y mejora el índice de cosecha. Si bien el PBZ en bajas concentraciones incrementa el contenido de almidón de las raíces tuberosas y el índice de cosecha, sus efectos sobre otros parámetros son indeseables.

Palabras clave: almidón, cloruro de cloro colina, *Manihot esculenta*, paclobutrazol, rendimiento

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INTRODUCTION

Cassava (*Manihot esculenta* Crantz) is a perennial shrub of the Euphorbiaceae family, cultivated mainly for its fleshy tuberous roots, and is one of the most important sources of food energy and industrial raw materials in tropical and subtropical regions (Santisopasri *et al.*, 2001). Cassava is often grown under low-input/low-output production systems, resulting in low yields; therefore, new alternative practices aimed to improve yield and quality are required.

Plant growth retardants, such as chlorocholine chloride (CCC: 2-chloroethyl trimethyl ammonium chloride) and paclobutrazol (PBZ: [(2R, 3R + 2S, 3S)-1-(4-chloro-phenyl) 4,4-dimethyl-2-(1,2,4-triazol-1-yl)-pentan-3-ol]), which are traditionally used in crop field management with many purposes, often have a beneficial effect on quantity and quality of harvestable products (Šimko, 1994; Tsegaw & Hammes, 2004, 2005).

CCC induces a reduction in aerial organ growth, and increases yield and tuber dry matter content in potato plants (Radwan *et al.*, 1971; Wang *et al.*, 2010). PBZ improves potato tuber yield by partitioning more assimilates to the tubers and increases starch accumulation in the tuber tissue (Tsegaw & Hammes, 2005). It has also been reported that PBZ treatment significantly increases tuber fresh mass of potato, dry matter content, and specific gravity, regardless of the method of application (spraying or drenching) (Tsegaw & Hammes, 2004). Other triazole compounds such as Triadimefon (TDM) and Hexaconazole (HEX) enhance tuberous root yield and starch content throughout the growing season in cassava plants (Gomathinayagam *et al.*, 2007).

In the present study, the effects of foliar application of CCC and PBZ on several biometric parameters of cassava field-grown plants were analyzed. Our purpose was to examine whether these plant growth retardants could regulate aerial growth as well as tuberous root yield, tuberous root dry matter and starch content of cassava plants.

MATERIALS AND METHODS

Plant material and experimental design

Cassava plants (cv. Rocha) were cultivated during the 2006-2007 growing season at the experimental farm of the Facultad de Ciencias Agrarias, Universidad Nacional del Nordeste (UNNE), Corrientes (27°28'S, 58°16'W), Argentina. The 'Rocha' cultivar is characterized by a decumbent growth habit, an average plant height of 2 m and a first branching height that exceeds 1 m (unpublished data). The soil of the experimental site is an alfic, mixed hyperthermic Udipsamment (Escobar *et al.*, 1994), and the climate is subtropical with an annual precipitation of 1500 mm and an average annual temperature of 21.5°C (Bruniard, 2000).

Each stem cutting was horizontally planted in a 1.0 x 1.0 m plot to a depth of 5 cm and the experiment was carried out without nutritional or water restrictions. The amounts of fertilizer needed to drive the crop without nutritional limitations were calculated considering the results obtained by chemical analysis of the experimental site soil (Table 1) and crop requirements reported by Howeler (1981).

The results of soil analysis showed that it had low fertility because of its low content of nitrogen,

Table 1 Chemical analysis of experimental site soil located in the farm of the Facultad de Ciencias Agrarias, Universidad Nacional del Nordeste (UNNE), Corrientes (27°28' S, 58°16' W), Argentina. OM: organic matter; P: phosphorus; Ca: calcium; Mg: magnesium; K: potassium.

Depth cm	pH	OM %	P ppm	Ca mEq.100 g ⁻¹	Mg mEq.100 g ⁻¹	K mEq.100 g ⁻¹
0-10	5.55	0.78	11.64	1.19	0.40	0.13
10-20	5.47	0.50	11.01	1.19	0.40	0.10

therefore the calculations were made for their input. Fertilization was done with ammonium nitrate (35% N) at 13 g of fertilizer per plant in two applications, at 45 days (6.5 g) and 75 days of planting (6.5 g). Phosphorus and potassium supply was not needed because their levels in soil (Table 1) were adequate for cassava crops according Howeler (1981).

Table 2 shows the annual distribution of rainfall in the experimental site (Corrientes, Argentina) during the years 2006 and 2007.

Forty days after planting (DAP), plants (20-cm-long and 9 expanded leaves) were treated with CCC or PBZ at rates of either 45 or 90 mg of active ingredient (a.i.) per plant at 9 AM in the morning when the dew had dried. Relative air humidity was 50% upon application. Both growth retardants were applied as a foliar spray using the commercial formulations Cotton Stop (750 g a.i. CCC L⁻¹) and Cultar (250 g a.i. PBZ L⁻¹). To this end, they were diluted in distilled water at 5000 mg L⁻¹, and 144 or 288 ml solution per plant were applied according to the rates, using an atomizer. Control plants were sprayed with distilled water of equal volume.

For this experiment we used a completely randomised block design, in which each block consisted of five treatments, with four replications. Twenty plants per treatment (four plants per replicate) were assessed.

Data recording and statistical analysis

Non-destructive measurements were taken on harvested plants at 100 and 220 DAP: plant height, first branching height, percentage of branching plants, number of nodes per main stem, and the average internode length.

Destructive measurements were recorded on harvested plants at 220 DAP: aerial fresh mass, tuberous root fresh mass per plant, number of

tuberous roots per plant, tuberous root length, tuberous root diameter, fibrous root fresh mass per plant, and harvest index. Tuberous root dry matter content and tuberous root starch content were evaluated in a representative root sample and determined according to Krochmal and Kilbride (1966).

The Contrast Test was used to verify whether the differences between control plants and each of the treatments were statistically significant (Montgomery, 1997). The data were analysed using the InfoStat software (InfoStat, 2009).

RESULTS

Effect of CCC and PBZ application on cassava shoot growth and branching

CCC application reduced plant height with respect to the control plants, but only a dosage of 90 mg pl⁻¹ caused a significant reduction in this parameter (Figure 1; Table 3). PBZ considerably reduced plant height, regardless of the application rates (Table 3). At 220 DAP, plants treated with CCC presented a lower first branching height than control plants, while PBZ diminished first branching height only when used at the higher rate (Table 3).

CCC and PBZ had opposite effects on number of nodes per main stem: CCC tended to decrease while PBZ increased this parameter with respect to the control plants. PBZ treatment caused a drastic internode length shortening, whereas CCC caused no significant alteration in this parameter (Figure 2; Table 3).

CCC application did not vary percentage of branching plants significantly; in contrast, PBZ delayed the branching phenomenon at 100 DAP, an effect that was reverted 120 days later (Table 3).

Table 2 Annual distribution of rainfall (mm) in the experimental site located in Corrientes (27°28' S, 58°16' W), Argentina during 2006 and 2007.

Year	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Total
2006	94.3	18.5	205.0	157.3	47.7	96.2	9.5	20.5	36.3	198.2	127.2	144.2	1154.9
2007	174.5	95.5	153.4	117.4	11.2	18.0	4.5	1.3	155.8	134.0	135.9	178.3	1179.8

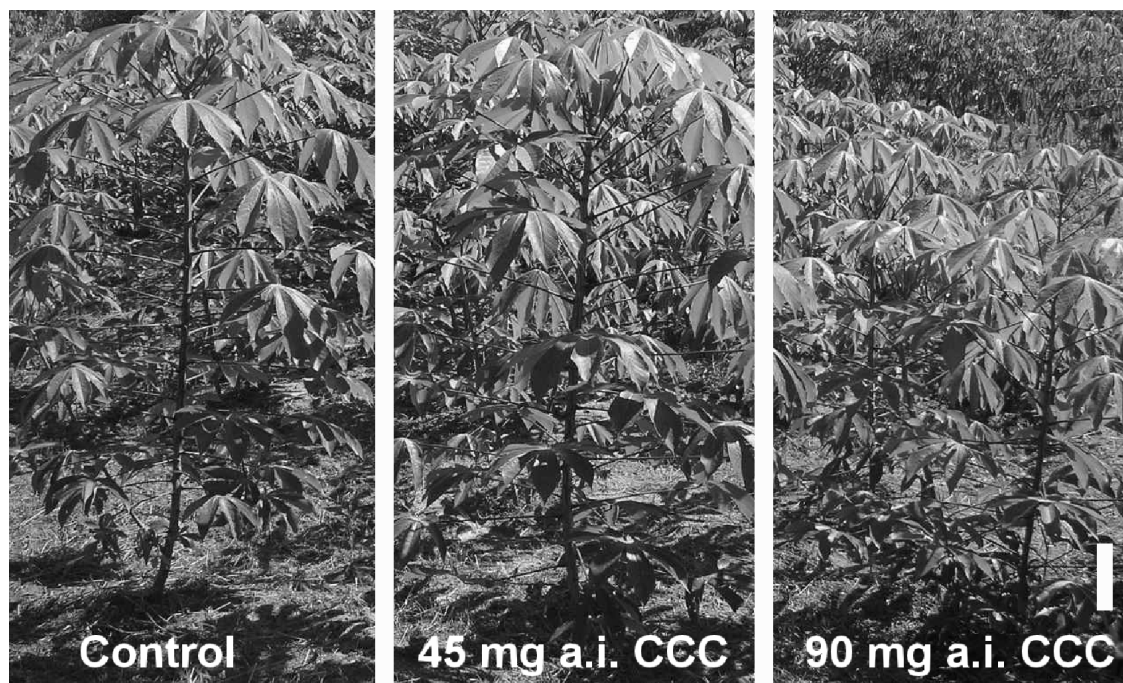


Figure 1 Effect of chlorocholine chloride (CCC) on the cassava plant height at 220 days after planting. Control plants; plants treated with 45 mg active ingredient CCC (45 mg a.i. CCC); plants treated with 90 mg active ingredient CCC (90 mg a.i. CCC). Bar = 10 cm.

Table 3 Effects of chlorocholine chloride (CCC) and paclobutrazol (PBZ) foliar application on shoot growth and branching cassava field grown plants. Plant height (PH), first branching height (FBH), number of nodes per main stem (NNS), average internode length (IL), and aerial fresh mass (AFM) were measured at 220 days after planting (DAP). Percentage of branching plants (PBP) was recorded at 100 and 220 DAP. Corrientes, Argentina (2006/2007).

Treatments ^a	PH (cm)	FBH (cm)	NNS ^b	IL (cm)	PBP (%) ^b		AFM (g)
					at 100 DAP	at 220 DAP	
Control	212.1±5.7	119.2±3.2	47.3±1.7	2.5±0.1	90.9±3.1	100.0±0.0	1542.2±97.9
CCC 45 mg pl ⁻¹	197.8±6.6	107.8±3.3*	43.9±2.0	2.5±0.1	95.8±4.2	100.0±0.0	1082.2±108.9*
CCC 90 mg pl ⁻¹	187.5±4.9*	103.3±3.1*	39.2±1.3*	2.6±0.1	90.4±6.7	100.0±0.0	1109.1±143.8*
PBZ 45 mg pl ⁻¹	188.3±6.9*	117.8±3.1	52.1±2.5*	2.2±0.1*	52.4±5.8*	95.8±4.2	931.6±82.1*
PBZ 90 mg pl ⁻¹	188.6±4.4*	108.8±2.3*	57.3±1.7*	1.9±0.1*	22.6±6.2*	97.2±2.8	1104.4±102.4*

^a All numerical data are given as mean with standard errors (±SE). The significance of changes in the test responses was assessed using an analysis of variance (ANOVA) followed by a contrast test.

^b For these parameters contrast test was carried out from squared transformed data.

* Asterisk indicates statistical significance at $P < 0.05$.

On the other hand, CCC and PBZ reduced aerial fresh mass significantly (Table 3).

Effect of CCC and PBZ application on cassava root growth

PBZ significantly reduced fibrous root fresh mass, while CCC did not modify this parameter with respect to the control plants. Foliar spray with CCC did not cause changes in the yield (expressed as tuberous root fresh mass per plant), but significantly

decreased number of tuberous roots per plant. PBZ application caused a significant decrease in tuberous root fresh mass per plant, as well as a marked decrease in number of tuberous roots per plant (Table 4). Both CCC and PBZ increased harvest index (Table 4).

The lower dose of CCC increased tuberous root dry matter content, whereas PBZ did not cause differences compared to the control. Both plant growth retardants improved tuberous root starch content at the lower dose (Table 4). Both

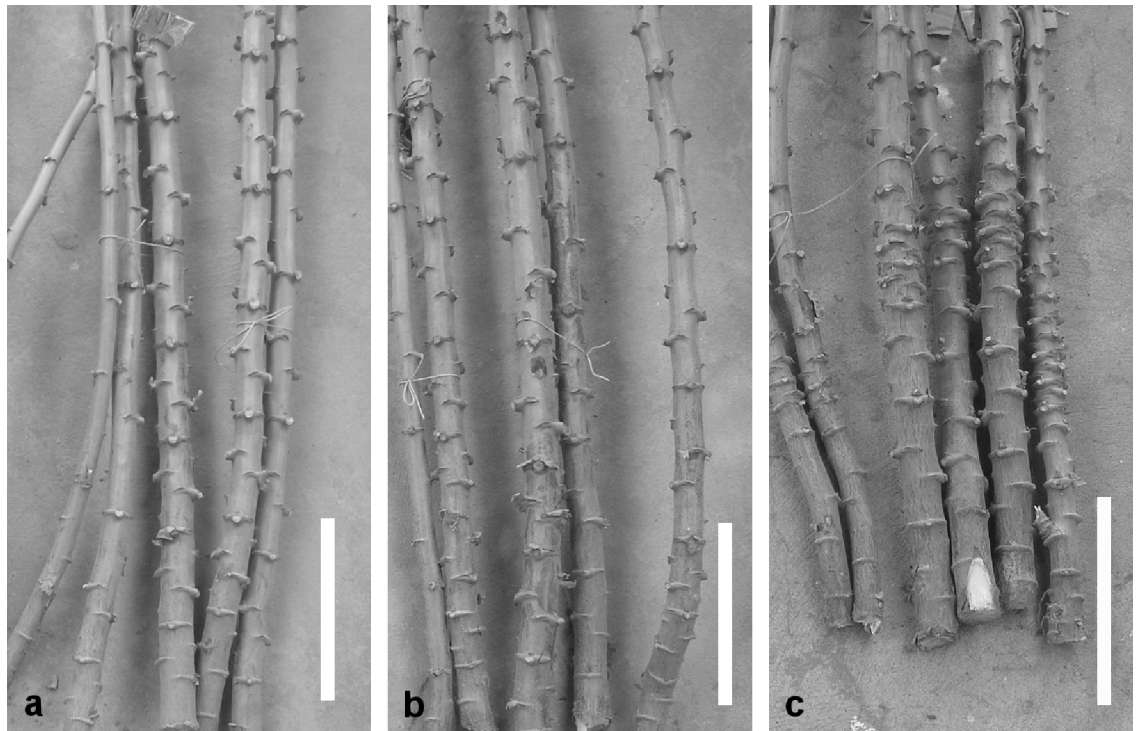


Figure 2 Effect of chlorocholine chloride (CCC) and paclobutrazol (PBZ) on the internode length of the main stem of cassava plants. a: control plants; b: CCC treated plants; c: PBZ treated plants. Bars = 17 cm.

Table 4 Effects of chlorocholine chloride (CCC) and paclobutrazol (PBZ) foliar application on radical growth of cassava field grown plants. Fibrous roots fresh mass per plant (FRFM), tuberous roots fresh mass per plant (TRFM), harvest index (HI), number of tuberous roots per plant (NTR), tuberous roots dry matter content (TRDM), tuberous roots starch content (TRSC), and tuberous roots length (TRL) were recorded at 220 days after planting. Corrientes, Argentina (2006/2007).

Treatments ^a	FRFM (g)	TRFM (Kg)	HI	NTR ^b	TRDM (%) ^b	TRSC (%) ^b	TRL (cm)
Control	8.1±0.9	3.8±0.2	0.70±0.01	13.8±1.0	31.0±0.3	29.6±0.3	32.0±1.2
CCC 45 mg pl ⁻¹	7.4±0.8	3.4±0.1	0.73±0.01*	9.9±0.8*	33.0±0.4*	31.3±0.3*	30.6±1.6
CCC 90 mg pl ⁻¹	7.5±0.9	3.5±0.4	0.71±0.01*	10.7±1.0*	29.1±0.6	28.1±0.6	29.3±1.5
PBZ 45 mg pl ⁻¹	4.4±0.7*	2.7±0.3*	0.71±0.01*	8.8±0.8*	32.6±0.9	31.6±0.8*	28.3±1.3*
PBZ 90 mg pl ⁻¹	3.9±0.7*	2.6±0.4*	0.71±0.01*	8.8±0.9*	30.3±0.3	29.4±0.3	27.9±0.7*

^a All numerical data are given as mean with standard errors (±SE). The significance of changes in the test responses was assessed using an analysis of variance (ANOVA) followed by a contrast test.

^b For these parameters contrast test was carried out from squared transformed data.

* Asterisk indicates statistical significance at $P < 0.05$.

CCC and PBZ treatments did not modify tuberous root diameter (ca. 4.7 cm; data not shown), while PBZ reduced significantly tuberous root length, regardless of the applied dose (Table 4).

DISCUSSION

Plant growth retardants, particularly onium compounds (e.g. CCC) and triazole compounds (e.g. PBZ), are able to increase the partitioning of assimilates to roots and thereby improve yield through the inhibition of gibberellin biosynthesis

or action (Fletcher *et al.*, 2000; Mansuroglu *et al.*, 2009). In addition, they can be used to control excessive vegetative growth (Rademacher, 2000; Ghosh *et al.*, 2010), and to increase quality attributes such as dry matter and starch content of the harvestable organ (Tsegaw & Hammes, 2005).

The results of this work show that CCC causes a retardation effect on cassava stem elongation and therefore a reduction in plant height, thus confirming pioneer studies (Radwan *et al.*, 1971). In cassava, PBZ application also was effective in reducing branch and plant height even at a low

concentration, as previously described for other species (Bañon *et al.*, 2002; Tsegaw & Hammes, 2004; Ghosh *et al.*, 2010). A reduction in the aerial dry mass and the partitioning of the dry matter to the canopy has also been observed in potato plants treated with PBZ and CCC (Wang *et al.*, 2009). In our work, CCC and PBZ also reduced aerial fresh mass.

The most striking growth response observed in different species treated with PBZ and CCC is shoot growth reduction mainly by shortening the internode length (Davis & Curry, 1991; Rossini Pinto *et al.*, 2005). In the present work, PBZ also reduced shoot growth by decreasing the internode length; however, CCC decreased it by decreasing the node number.

It has also been demonstrated that regulation of branching can increase cassava yields until 75% (Lian & Cock, 1979). In our work, CCC and PBZ regulated the first branching height and PBZ also controlled branching time, which would allow adjusting crop density to achieve higher yields.

The beneficial effects of the plant growth retardants on the tuberous organ growth varied with the types and concentrations assayed (Šimko, 1993; Rayirath *et al.*, 2009). CCC stimulates potato tuber initiation (Leclerc *et al.*, 1994). On the other hand, high PBZ concentrations strongly reduce potato tuber mass and number (Šimko, 1994). In this work, CCC treatment did not modify tuberous root fresh mass, but significantly increased harvest index. PBZ also improved harvest index but significantly reduced tuberous root fresh mass and tuberous root number.

In addition, PBZ reduced cassava fibrous root formation like in potato nodal segments (Šimko, 1993). Probably, this effect could cause a lower nutrient uptake capacity and therefore a low ability to achieve good yields, as argued by Rieger and Scalabrelli (1990) studying *Prunus persica* trees treated with PBZ. CCC application did not reduce cassava fibrous root formation, apparently favouring tuberous root yield. Moreover, CCC application reduced aerial fresh mass, resulting in a higher harvest index compared to untreated cassava plants.

In potato, Tsegaw and Hammes (2004) reported that PBZ increases tuber fresh mass, dry matter content and specific gravity but reduces tuber number until 43% as compared to controls. Šimko (1994) also observed an increase in tuber yield in plants treated with PBZ. Changes in the net photosynthetic rate in potato plants induced by spraying CCC also increase crop productivity (Wang & Xiao, 2009). In this work, PBZ caused

a reduction in the tuberous root fresh mass and tuberous root number regardless of the dose, while CCC decreased tuberous root number but not tuberous root fresh mass.

Although it has been reported that PBZ causes root thickening (Tsegaw & Hammes, 2005), neither of the growth retardants used caused root thickening in cassava tuberous roots. Only PBZ treatment caused shortening of the tuberous roots.

Plant growth retardants are considered as tuberization promoters; in fact, the application of CCC and PBZ increases starch content in potato tubers (Tsegaw & Hammes, 2005; Sharma *et al.*, 1998). In this work, CCC and PBZ also improved the starch content of cassava tuberous roots when 45 mg a.i. per plant was applied; in addition, CCC increased dry matter content of the cassava tuberous roots at the same low dose. The export of the photoassimilates and the starch synthesis is reduced by the GA₃ application (Booth & Lovell 1972; Mares *et al.*, 1981), so the beneficial action of low concentrations of plant growth retardants on the tuberous root quality could be explained by its gibberellins antagonistic action, as argued by Fletcher *et al.* (2000) and Mansuroglu *et al.* (2009).

The application of others plant growth retardants such as TDM and HEX in cassava plants enhanced photosynthetic pigments, the non-enzymatic antioxidant content and the activity of antioxidant enzymes which demonstrates its utility as an alternative agricultural practice for improving crop productivity and profitability (Gomathinayagam *et al.*, 2007, 2008a, 2008b, 2009).

In summary, CCC is effective to control excessive vegetative growth, increase harvest index and improve tuberous root dry matter and starch content. In addition, PBZ at low doses allows increasing starch content, but its effects on others yield parameters are negative.

As cassava is an important source of industrial raw materials (Santisopasri *et al.*, 2001), foliar CCC application at low concentrations should be considered by farmers as an alternative to improve starch root content and hence crop profitability. Furthermore, plant size regulation could allow intercropping with other species for a longer period. On the other hand, it has been previously demonstrated in pioneering studies that CCC declines quickly in vegetative parts after treatments leaving no traces when applied at low doses and that it is inactivated by cooking (Jung & El-Fouly, 1969), properties that make CCC a promising growth regulator that offers a safe food product.

In potato, the most important tuber crop in the world, it has consistently demonstrated CCC and

other growth retardants applications increase tuber yield and quality under field conditions, by which it has been recommended as a good tool to optimize their productivity (Radwan *et al.*, 1971; Sharma *et al.*, 1998; Wang *et al.*, 2009; Wang *et al.*, 2010). However, to corroborate the beneficial effect of CCC spraying on the yield and quality of cassava tuberous roots are still needed further evaluations before such treatments can be practiced on a large-scale, routinely and safely.

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