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Full Length Research Paper

Role and significance of total phenols during rooting of *Protea cynaroides* L. cuttings

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Phenolic compounds, which are known to regulate root formation, are found abundantly in difficult-to-root *Protea cynaroides* stem cuttings. In this study, analysis of total phenol content was carried out on blanched and unblanched cuttings to observe its fluctuation throughout the entire rooting period (120 days) and establish its relationship with root formation. Results showed that blanching significantly increased the total phenol content in the basal ends of the cuttings. The high total phenol content was associated with significantly higher rooting percentage and increased the number of roots formed. Blanching reduced the time needed for the cuttings to root sufficiently to be transplanted to the field by 30 days. Analyses of different parts of cuttings throughout the entire rooting period showed continuous increase in total phenols at the basal end, while decrease in total phenols was observed in the leaves.

Keywords: Etiolation, king protea, phenolic compounds, Proteaceae, root formation

INTRODUCTION

Protea cynaroides L. (King Protea) is an important cut flower in the floriculture industry. At present, the production areas are expanding in Europe, with new plantations being established in Portugal and Spain (Leonardt, 2008). *P. cynaroides* plants show great variation in nature with many different sizes, colours and flowering times (Vogts, 1982). Due to the genetic variability of seeds, vegetative propagation is the preferred method used by growers to obtain and maintain genetic uniformity in the commercial production of *P. cynaroides* cut flowers. However, *P. cynaroides* is a woody plant, which typically has a poor physiological capacity for adventitious root formation and is notoriously known as a difficult-to-root ornamental plant. Using conventional vegetative propagation methods, *P. cynaroides* cuttings usually take six months to root with low rooting percentage. The application of commercially available rooting hormones does not improve its rooting. It is known that, starch content is important during root formation. The analysis of starch accumulation in *P. cynaroides* cuttings during rooting has been conducted (Wu et al., 2006). Results showed that an increase in the accumulation of starch in stem cuttings improved root formation. Furthermore, 3,4-dihydroxybenzoic acid was found in *P. cynaroides* stems and shown to stimulate root formation in micropropagated explants (Wu et al., 2007). Plants of the Proteaceae family are known to contain large amounts of phenolic compounds, however, currently, no study has been done on the role of total phenol content in *P. cynaroides* stem cuttings during root formation. The aim of this study was to analyze fluctuations in total phenol concentration of different parts of blanched and unblanched *P. cynaroides* stem cuttings throughout the entire rooting process and to establish their relationship with root formation.

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Abbreviations: ELISA, Enzyme-linked immunosorbent assay; **IAA,** indole-3-acetic acid.

MATERIALS AND METHODS

P. cynaroides stem cuttings that were used in this study were collected from mother plants grown in an open field in the summer rainfall region (Gauteng) of South Africa. Terminal semi-hardwood stems (15 cm in length) of the current year's growth, which were

Table 1. Rooting percentage, mean root dry mass and mean number of roots according to root length categories of *P. cynaroides* cuttings after 90 days in the mist bed.

Parameter	Control	Blanched
[1]Rooting %	60 [b]	100 [a]
[2]Mean root dry mass (mg)	96.7 ±16.5 [b]	159.8 ±17.9 [a]
[2]Mean number of roots categorized by root length		
Group 1 (1 - 10 mm)	6.6 ±2.6 [b]	11.6 ±3.4 [a]
Group 2 (11 - 20 mm)	4.8 ±2.2 [b]	10.8 ±2.8 [a]
Group 3 (21 - 30 mm)	5.8 ±3.6 [b]	11.6 ±0.5 [a]
Group 4 (31 - 40 mm)	4.0 ±0.7 [a]	4.2 ±1.1 [a]
Group 5 (41 - 50 mm)	4.4 ±2.3 [b]	9.6 ±3.8 [a]
Group 6 (>51 mm)	3.0 ±1.4 [b]	5.8 ±2.5 [a]

[1]Different letters in the same row indicate significant differences at $P \leq 0.05$ based on chi-square; [2]different letters in the same row indicate significant differences at $P < 0.001$, based on Tukey's studentized test.

either blanched for 30 days or untreated (control) were used as cuttings. The blanching treatment applied to the stems was done according to Wu et al. (2006). The rooting medium consisted of a peat moss and polystyrene ball (1:1 v:v) mixture. The cuttings were rooted under intermittent mist, which irrigated every 20 min for 1 min. The air temperature of the mist bed, which was constructed inside a white polyethylene structure, was maintained at 26°C±2, with no bottom heating.

The determination of total soluble phenols was carried out on samples prepared from stem cuttings taken after 0, 60, 90 and 120 days in the mist bed. The roots were removed from the cuttings, dried and weighed. Each cutting was then separated into four parts, which consisted of the basal end (20 mm), the middle and top ends (equally divided from the remainder of each cutting) and the leaves. After each part was freeze-dried and ground into fine powder, 0.05 g samples were weighed into separate test tubes. The procedure for the extraction and quantification of total phenolic compounds was adapted from Fourie (2004). The solvent used was methanol:acetone:water (7:7:1). One millilitre of the solvent was added to 0.05 g of powdered sample. It was then placed in an ultrasound waterbath for 3 min and then centrifuged (Kubota® 2010 centrifuge) for 30 s. The extraction procedure was repeated twice. The concentration of phenolic compounds was determined using the Folin-Ciocalteu reagent (Bray and Thorpe, 1954). A 96-well enzyme-linked immunosorbent assay (ELISA) plate was used for the reaction mixture. A dilution series (10 to 1000 µg/ml methanol) was used to prepare standard curves for ferulic acid and gallic acid for the quantification of phenolic content. The reaction mixture comprised of 175 µl distilled water + 5 µl standard or extract sample + 25 µl Folin-Ciocalteu reagent + 50 µl 20% (v/v) Na_2CO_3. The samples were then incubated at 40°C for 30 min. Afterwards, the absorbance was read at 690 nm using an ELISA reader (Multiskan ascent V1.24354-50973). The phenolic concentration was expressed as gallic acid equivalents per gram dry sample material.

A completely randomized design was used. A total of eighty cuttings were used for each treatment. For total phenol content analyses, twenty cuttings were randomly collected in each treatment at 0, 60, 90 and 120 days after planting. To determine root growth parameters at day 90, roots of the twenty cuttings collected after 90 days were used to measure rooting percentage, mean root dry mass and mean number of roots. Where appropriate, chi-square analysis and Tukey's studentized range test were applied to compare treatment means. All statistical analyses were done using the Statistical Analysis System (SAS) program (SAS Institute Inc., 1996).

RESULTS AND DISCUSSION

After 90 days, a significantly higher rooting percentage was observed in blanched cuttings (100%) than those that were unblanched (60%) (Table 1). In addition, the amounts of roots formed in blanched cuttings were significantly higher than in unblanched cuttings, as indicated by the mean root dry mass. Similar findings were reported by Howard et al. (1985), Sun and Bassuk (1991) and Wu et al. (2006). Furthermore, in terms of root length, blanched cuttings formed significantly more roots in all root length groups except Group 4 (31 to 40 mm), confirming that, blanching significantly improved formation and elongation of roots (Table 1). Figure 1 illustrates the changes of total phenol content in the different parts of the unblanched and blanched cuttings during a rooting period of 120 days. At the basal end of the cuttings (Figure 1a), where rooting took place, the total phenol content of both the control and blanched treatments increased steadily throughout the propagation period. However, the total phenol content of the blanched cuttings (42.09 mg/g) was already significantly higher than the control (23.68 mg/g) on day 0, when phenolic analysis was done immediately after the blanching treatment was completed, which clearly showed that, blanching caused an increase in the accumulation of total phenols in stems (Figure 1a). This is contrary to many study results, which often reported that phenolic compound concentrations are reduced by etiolation treatments (George, 1996; Goupy et al., 1990; Sharma et al., 1995; Sharma and Singh, 2002).

Furthermore, the increase in the total phenol content throughout the entire propagation period correlated with the rooting of the cuttings, as shown by the increase of mean root dry mass in Figure 1a. The total phenol content of the basal end of blanched cuttings was at its highest level (84.15 mg/g) on day 90, which is at the same time when considerable rooting had taken place. In

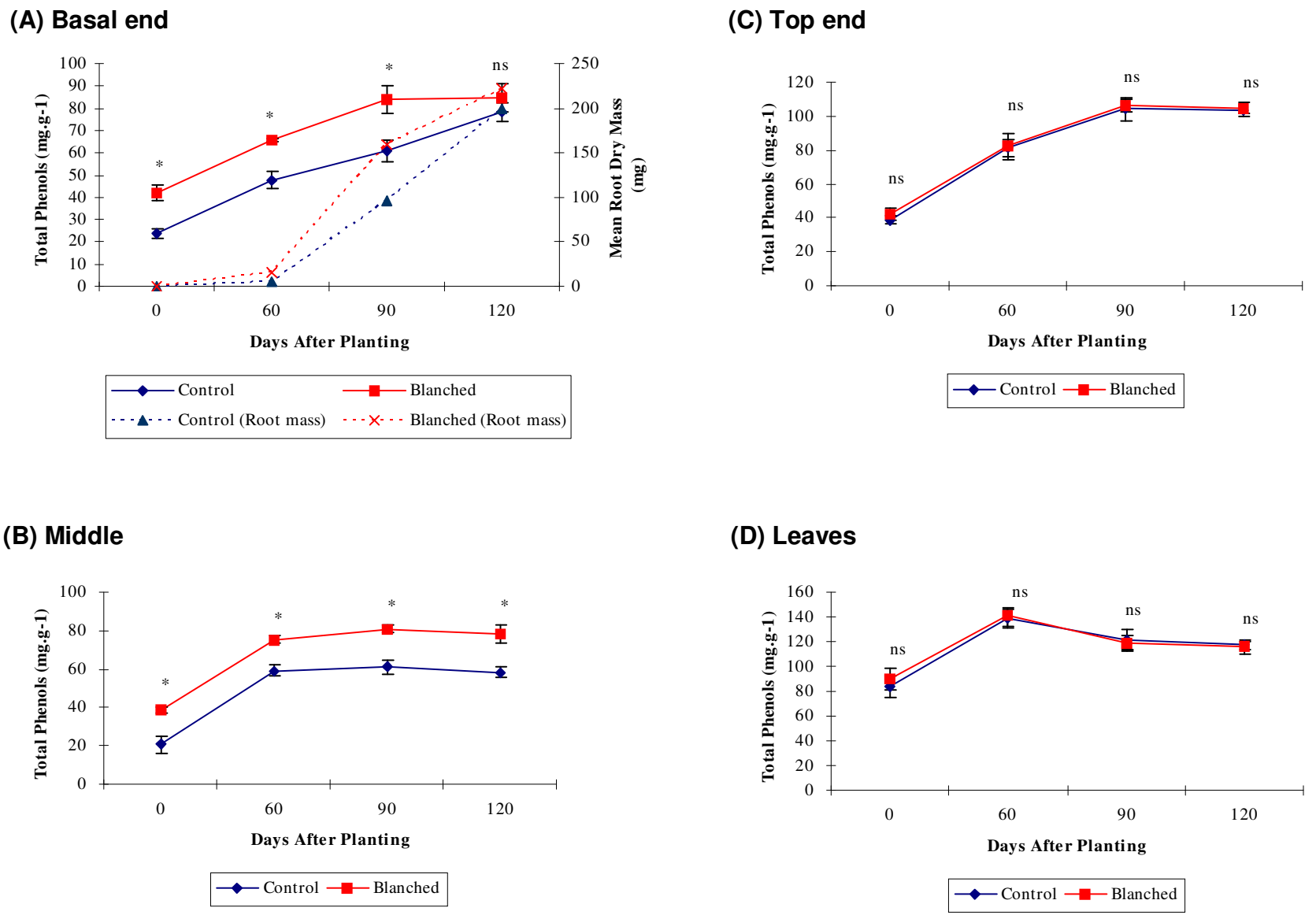

Figure 1. Fluctuations of total phenol content in different parts of *P. cynaroides* cuttings during a rooting period of 120 days. Means tested for significance at the same time period within each plant part based on Tukey's studentized test. *: significant ($P < 0.05$); ns: not significant.

fact, the blanched cuttings had rooted sufficiently to be transplanted to the field after 90 days. Large amounts of rooting in untreated cuttings were only observed after 120 days when the phenol content reached its highest level (78.42 mg/g), which incidentally was similar to the levels obtained in blanched cuttings on day 90. This is in contrast with other studies, which showed that improvements in root formation are due to reductions of total phenols in etiolated plants (Sharma et al., 1995; Sivaci et al., 2007), while high total phenol content is generally associated with inhibition of root formation in woody plants (Curir et al., 1993). In the few cases (mostly in woody species) where etiolation treatments increased total phenol concentrations and stimulated rooting, such as those reported by Druart et al. (1982) and Gautam and Chauhan (1990), it has been hypothesized that, phenolic compounds protect the endogenous natural-occurring auxin - indole-3-acetic acid (IAA) from destruction by the enzyme IAA oxidase (Donoho et al., 1962; Fadl et al., 1979) or act as precursors to lignin formation for structural support (Haissig, 1986).

Regarding the changes of total phenol content in the basal end and the leaves of cuttings, a positive correlation was also evident. The highest amounts of total phenols in the entire cutting were found in the leaves, confirming that phenolic compounds are synthesized in the chloroplasts and transported to the vacuole for storage (Jähne et al., 1993; Mosjidis et al., 1989; Mueller and Beckman, 1974; Weissenböck et al., 1986). The total phenol content in the leaves of blanched cuttings decreased from its peak of 141.81 mg/g on day 60 to 118.64 mg/g on day 90 (Figure 1d), while at the same time period, the total phenol content in the basal end increased from 66.07 to 84.15 mg/g (Figure 1a). In relation to root formation during the same period, the mean root dry mass increased from 15.6 (day 60) to 159.8 mg (day 90) for blanched cuttings (Figure 1a). The fluctuations of total phenol concentrations in the leaves and basal ends of the unblanched cuttings during rooting were similar. Of particular importance is that, the results showed when the total phenol concentration was between 66.07 and 84.15 mg/g (day 60 to 90) in the basal end of blanched cuttings, large amounts of root formation took place. Considerable rooting also took place at a similar total phenol concentration range (60.84 to 78.42 mg/g) for the unblanched cuttings from day 90 to 120 (Figure 1a). This suggests that a minimum of 60.84 mg/g total phenols may be required to stimulate the formation of large amounts of roots in *P. cynaroides* cuttings. This finding is of practical significance to growers since it raises the possibility of inducing early rooting by applying phenolic compounds exogenously on the basal ends of cuttings to increase its endogenous phenol concentration or by using Brotomax®, which has been reported to increase total phenol concentrations in stems and leaves (Del Rio et al., 2003). The amounts of total phenols found in the top part of the cuttings in the unblanched and blanched treatments were very similar (Figure 1c). However, in the middle part of the cutting (Figure 1b), the total phenol content of the blanched cuttings was significantly higher than the control, which may be partly due to an increase in the accumulation of phenols in this area caused by the etiolation effect in the basal end below it.

In conclusion, by analyzing the total phenol content of *P. cynaroides* cuttings from when the plant materials were collected until they were well rooted, the relationship between total phenol content and root formation was established. In addition, through the phenolic analysis of different parts of the cuttings, a positive correlation among blanching, total phenol content and rooting was found. In contrast to many etiolation studies, blanching increased the total phenol content in *P. cynaroides* stems, particularly in the basal ends. The results of this study have contributed new knowledge regarding the role of total phenols during root formation in *P. cynaroides* cuttings.

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