The efficacy of γ-aminobutyric acid (GABA) as a postharvest treatment in *Protea*, to enhance vase life and maintain quality

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Abstract

Protea **cut flowers are commercially important and are often transported to international markets for extended periods of time (e.g., one to three weeks via shipping). These periods have a significant effect on the postharvest quality and vase life of these products. Low temperature storage is often used as a postharvest treatment to delay the inflorescences from opening and to maintain quality. During the warm, dry season in the Western Cape, the bracts of the inflorescence of some** ***Protea*** **cultivars are prone to curl inward and appear desiccated, affecting quality and vase life. In addition, preharvest conditions such as heat stress contribute to increased levels of leaf blackening, a common postharvest disorder in** ***Protea*****. γ-Aminobutyric acid (GABA) is believed to accumulate in plants and horticultural crops during abiotic stress such as heat, drought and low oxygen, and enhances resistance to these stresses. GABA has been widely used for other cut flowers to alleviate chilling injury, spate browning and retard electrolyte leakage. As such, we tested the efficacy of GABA in two** ***Protea*** **cultivars, 'Sylvia' and 'Pink Ice'. GABA was applied in a range of concentrations as a 2-3 h pulsing treatment. Glucose was included as a treatment as it is used as a postharvest treatment in the** ***Protea*** **industry and water was used as the control. Stems were evaluated over an 11-day period and scored for flower quality and leaf blackening. Results show that the incidence of leaf blackening decreases with increasing concentrations of GABA in 'Sylvia' although the effect of the glucose is more significant to control blackening. In 'Pink Ice' the level of blackening in glucose and 20 mM GABA-pulsed stems were the same by day 11. A better flower quality was observed for both cultivars, in stems pulsed with GABA. GABA can therefore be considered as a potential technology for enhancing tolerance of high temperatures and hot, dry conditions, and reduce leaf blackening in** ***Protea*****.**

Keywords: anti-chilling, cut flower quality, leaf blackening, *Proteaceae*, stem pulsing

INTRODUCTION

Protea cut flowers have become internationally popular and in demand but many species and cultivars are susceptible to leaf blackening, an almost exclusively postharvest disorder in which the leaves become discoloured by brown to black areas (van Doorn, 2001). Leaf blackening severely reduces the entire product quality, and also restricts vase life. The disorder remains the single most important reason for entire consignment rejection by importers. To avoid this risk, *Protea* stems are often exported, as per customer request, as leafless stems, to be used in bouquets, where the bare stems can be concealed by indigenous Cape Flora foliage. However, if *Protea* leaves can be retained on cut stems the costs associated with removing foliage can be eliminated, whilst the benefit of intact foliage as nutritional and energy support to the flower can be maximized.

The extent and rate of blackening vary widely among species and cultivars (McConchie and Lang, 1993). The exact cause of leaf blackening is not clear, many factors are thought to

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contribute toward the disorder, including water stress, carbohydrate stress, light, etc. (Jones et al., 1995; van Doorn, 2001). To control the levels of leaf blackening, producers often pulse stems with sugars soon after harvesting, girdle stems right below the inflorescence, cool flowers at low temperatures or store flowers under low oxygen and high carbon dioxide conditions. The onset and incidence rate of leaf blackening has been found to accelerate with an increase in temperature (Ferreira, 1986). Increasing temperature and respiration has also been linked to membrane disruption and the onset of leaf blackening. On the other hand, low temperature may give rise to chilling injury. Although, chilling injury has not been documented in *Protea* (Stephens et al., 2003; van Doorn, 2001), dry conditions, which are exacerbated in the Western Cape, together with low temperatures following harvest and extended storage during transport to foreign markets, could all induce a form of stress.

γ-Aminobutyric acid (GABA) is believed to accumulate in plants and horticultural crops during abiotic stress such as heat, drought and low oxygen and enhances resistance to these stresses. GABA has been widely used for other cut flowers to alleviate chilling injury, spate browning and retard electrolyte leakage (Wang et al., 2014; Aghdam et al., 2016). The efficacy of GABA as a postharvest treatment in *Protea* to enhance tolerance of high temperatures and hot, dry conditions, which in turn may reduce blackening, was investigated.

MATERIALS AND METHODS

Plant material and preparation

Flowering stems of 'Sylvia' and 'Pink Ice' were sourced from Fynbloem, Riviersonderend. The stems were picked at the soft-tip stage in November 2016 and May 2017. Upon arrival at the laboratory, at the Department of Horticultural Sciences, Stellenbosch University, the stems were standardized to a length of 40 cm and the leaves reduced to ±20 per stem.

Pulsing

Eight stems were pulsed with 10 mL of one of the pulsing solutions for approximately 2-3 h while an additional eight stems were placed in tap water (control) for the pulsing period. Pulsing solutions consisted of 5, 10 and 20 mM GABA, and 6% glucose, which serves as the industry standard. During the pulsing period, vials were covered with Parafilm to reduce the loss of the pulsing solution through evaporation. The stems were pulsed in an evaluation room of 20°C, light/ dark cycles of 10/12 h, and a relative humidity of 60%.

Evaluation and statistical analysis

After the pulsing period, the stems were placed in vases containing tap water and evaluated over an 11-day vase life period. Leaf blackening was scored by recording the percentage leaves exhibiting ≥10% blackening per stem throughout the vase life period (Ekman et al., 2008). Flower quality was scored on a scale of 1-5 whereby 5 = perfect quality, and 1 = poor quality and consumer rejection (Floral Solutions, 2006).

Analysis of variance (ANOVA) and repeated measures analysis (RANOVA) were obtained using Statistica version 13.2 (Statsoft Inc, 2017). Means were separated by Fisher's least significant differences (LSD) posthoc test, with a significance level at $P<0.05$.

RESULTS AND DISCUSSION

'Sylvia'

Time, treatment and the interaction between time and treatment had a significant effect on leaf blackening (Table 1). Leaf blackening developed on day 2 in 'Sylvia'. The highest incidence of blackening was observed in stems placed in water (control treatment), reaching between 80-100% between days 8 and 11. The lowest incidence was observed in stems pulsed with glucose (Figure 1). Blackening decreased with increasing concentrations of GABA (Figure 1). By day 11, the average leaf blackening score for glucose-pulsed stems was

54% and for 20 mM GABA-pulse stems it was 60%. Leaf blackening in 'Sylvia' was significantly less in stems that were pulsed with glucose, which was expected (Stephens et al., 2005; Windell, 2012). Levels of blackening decreased with higher concentrations of GABA in 'Sylvia' but the effect of glucose was more significant. This suggests that GABA has the potential to reduce leaf blackening.

Table 1. The F statistic and P value for the ANOVA tests and for the leaf blackening and flower quality.

Cultivar	Effect	% leaf blackening		Flower quality	
		F value	P value	F value	P value
Sylvia	Treatment	4.3	0.006	2.7	0.049
	Time	548	0.000	956.3	0.000
	Time*treatment	5.1	0.000	1.1	0.333
Pink Ice	Treatment	3.8	0.014	6.7	0.001
	Time	259.9	0.000	239.1	0.000
	Time*treatment	7.3	0.000	3.1	0.001

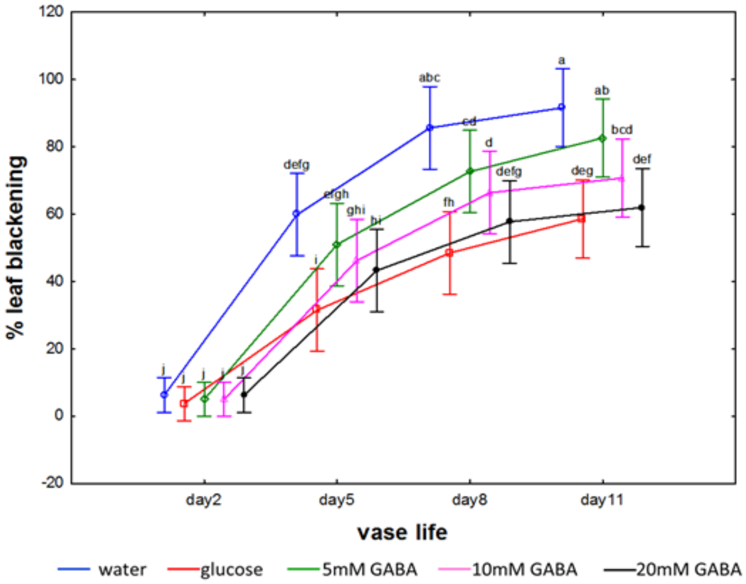

Figure 1. The variation in percentage (%) leaf blackening in *Protea* 'Sylvia' over an 11-day vase life period for stems pulsed with glucose and GABA. Vertical error bars indicate the Standard Error of the mean. Different letters indicate a significant difference at the P<0.05.

Both time and treatment had a significant effect on flower quality, but not the interaction between time and treatment (Table 1). Flower quality was excellent across all treatments on day 2 of the vase life evaluation. By day 5 it declined, with scores ranging between 3 and 3.6 (Figure 2). On day 5 and 8, flower quality was best in stems treated with GABA, but by day 11, stems treated with glucose and 5 and 10 mM GABA, were the same. On day 11, stems treated with 20 mM GABA exhibited the highest flower quality score (Figure 2). Stems treated with water (control treatment), had the lowest score throughout the vase life evaluation.

'Pink Ice'

Time, treatment and the interaction between time and treatment had a significant effect on leaf blackening in 'Pink Ice' (Table 1). Leaf blackening was observed by day 3 in

‘Pink Ice’, irrespective of treatment. Stems placed in water (control treatment), exhibited the highest levels of leaf blackening throughout the vase life period, reaching an average of 64% by day 11. For the other treatments, leaf blackening progressed slowly. Stems pulsed with glucose and GABA exhibited levels of blackening below 40% by day 11 (Figure 3). By day 11, the lowest incidence of blackening was observed for stems treated with glucose (33%) and 20 mM GABA (33%).

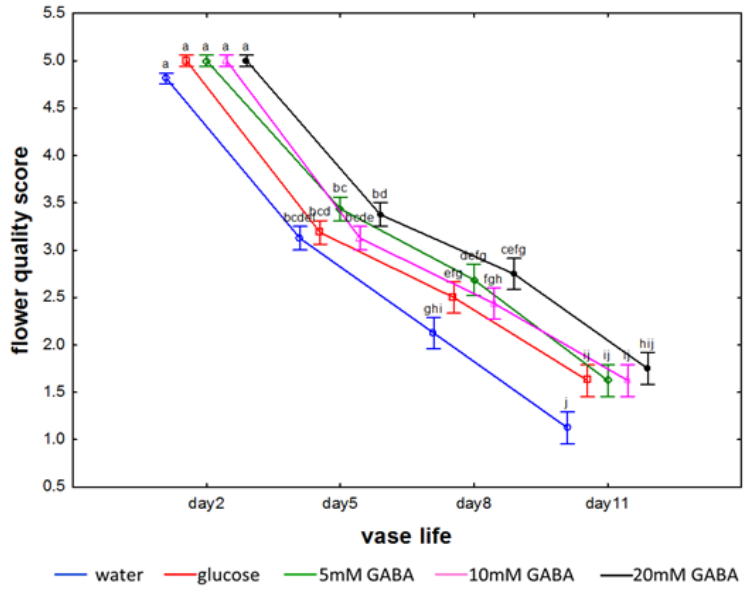

Figure 2. The variation in flower quality in *Protea* ‘Sylvia’ over an 11-day vase life period for stems pulsed with glucose and GABA. Vertical error bars indicate the Standard Error of the mean. Different letters indicate a significant difference at the P<0.05.

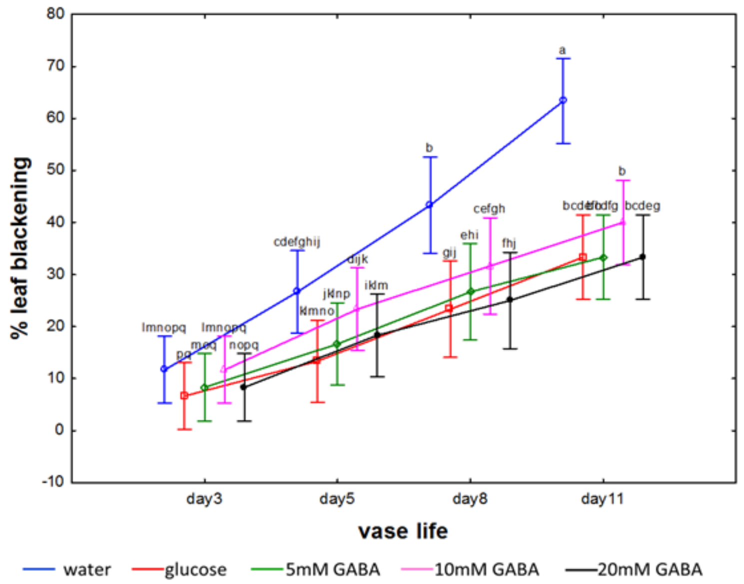

Figure 3. The variation in percentage (%) leaf blackening in *Protea* ‘Pink Ice’ over an 11-day vase life period for stems pulsed with glucose and GABA. Vertical error bars indicate the Standard Error of the mean. Different letters indicate a significant difference at the P<0.05.

Leaf blackening did not decrease with increasing concentrations of GABA in ‘Pink Ice’. Rather, similar levels of blackening was observed by the end of the vase life period, in stems pulsed with glucose, 5 and 20 mM GABA.

Time, treatment and the interaction between time and treatment had a significant effect on flower quality in ‘Pink Ice’ (Table 1). Flower quality deteriorated rapidly in stems that were placed in water, decreasing to an average score of 2.3 by day 8 (Figure 4). Higher scores were observed for stems pulsed with glucose and GABA. By day 11, the average flower score for stems pulsed with 5 and 20 mM GABA was 3.2 and 2.9 respectively, which was slightly higher than that of glucose- pulsed stems that had an average score of 2.4.

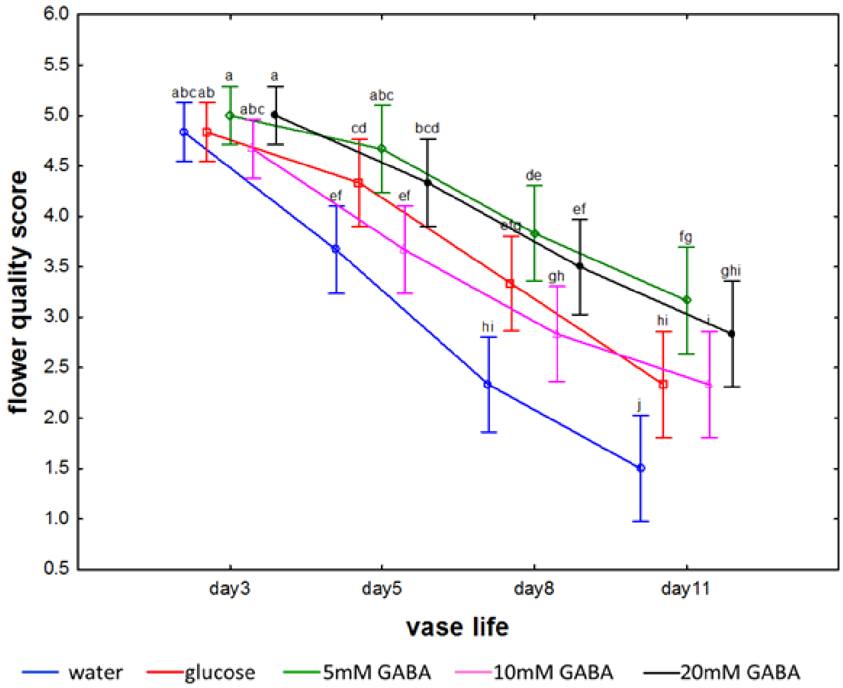

Figure 4. The variation in flower quality in *Protea* ‘Pink Ice’ over an 11-day vase life period for stems pulsed with glucose and GABA. Vertical error bars indicate the Standard Error of the mean. Different letters indicate a significant difference at the $P<0.05$.

CONCLUSIONS

The following conclusions can be drawn from the study:

- The effect of GABA is concentration dependent, which is an imperative factor for consideration for postharvest treatments.
- Further experiments for *Protea* with additional concentrations of GABA are warranted.

ACKNOWLEDGEMENTS

We thank the South African department of Science and Technology’s (DST) Post-harvest Innovation (PHI) Programme and Cape Flora SA for funding this research.

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