

# Growth-promoting effects of a seaweed concentrate at various pH and water hardness conditions

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Kelpak® – a liquid seaweed concentrate made from the kelp *Ecklonia maxima* (Osbeck) Papenfuss – is used as a natural biostimulant to promote rooting and improve yield in crops. Plant–soil environmental conditions and the chemistry of water used for irrigation may affect the efficiency of Kelpak. The effect of pH (pH 4.5, 6.5 and 8.5) and water hardness (200 mg/L and 400 mg/L Ca<sup>2+</sup>) on the growth-promoting ability of Kelpak was assessed using the mungbean rooting bioassay and in a pot trial with Swiss chard. Kelpak promoted rooting in all the treatments in the mungbean bioassay with maximum rooting generally achieved with 20% Kelpak. With 20% Kelpak, the addition of 200 mg/L and 400 mg/L Ca<sup>2+</sup> decreased rooting at pH 4.5, increased rooting at pH 6.5 and did not affect rooting at pH 8.5. A similar trend was observed in the pot trial with Swiss chard: leaf and root (fresh weight) and pigment content (chl *a*, chl *b* and carotenoids) improved with the addition of 200 mg/L Ca<sup>2+</sup> + 5% Kelpak at pH 6.5 or pH 8.5, while Kelpak was able to partially mask the negative effect of 200 mg/L Ca<sup>2+</sup> at pH 4.5. These results suggest that while Kelpak is most effective in neutral pHs, it can be used to promote plant growth in a wide range of pH and water hardness conditions.

## Introduction

Since the 1960s, seaweeds have been processed and sold in powder or liquid forms. Seaweed concentrates (SWCs) are now gaining increased favour as natural, organic plant biostimulants because of the detrimental effects of high fertiliser consumption.<sup>1</sup> These organic supplements can produce comparable yields to conventional fertilisers.<sup>2</sup> SWCs have many beneficial effects on plant growth with the most notable being the promotion of rooting and improved growth and yield. Other positive responses include increased nutrient uptake and mobilisation, enhanced chlorophyll content, delayed senescence, improved shelf life of the fruit, increased resistance to frost, insect and pathogen attack, and improved resistance to abiotic stress such as drought and salt stress.<sup>3-6</sup> SWCs also improve other biochemical constituents such as carotenoid, soluble sugar and protein concentrations.<sup>7</sup> As these beneficial effects are achieved with small doses of SWC, the active constituents are thought to be plant hormones, betaines, micronutrients, amino acids and vitamins that are all effective at low concentrations.<sup>1,3,4,6</sup> The active constituents in SWCs differ depending on the seaweed species used as well as the method of extraction.<sup>5,6</sup>

The kelp *Ecklonia maxima* (Osbeck) Papenfuss, which is harvested from the west coast of South Africa, is processed by a cell burst method to produce a liquid SWC marketed as Kelpak®. Unlike other methods of SWC preparation, the cell burst method does not involve heat and chemicals and does not include a dehydration step – all of which could potentially denature the various active constituents in the SWC.<sup>6</sup> The beneficial effects of Kelpak have been extensively reported for a wide range of plants including vegetables, ornamentals, trees and monocotyledonous crops as well as for cuttings, for which improved rooting has been documented.<sup>4</sup> Biological activity of Kelpak has previously been confirmed using the mungbean rooting bioassay and the soybean callus cell division bioassay.<sup>8</sup> Auxins and cytokinins have been positively identified in Kelpak with auxins occurring in higher concentrations than cytokinins. Indole-3-acetic acid (IAA) and seven indole conjugates were identified with a total auxin concentration of 33.91 pmol/mL Kelpak. Ten isoprenoid (*trans*-Zeatin, *cis*-Zeatin, isopentyladenine and dihydrozeatin) and seven aromatic (benzyladenine and topolins) cytokinin conjugates were identified with a total cytokinin concentration of 4.88 pmol/mL Kelpak.<sup>8</sup> In addition, two polyamines (putrescine and spermine) have recently been identified in Kelpak.<sup>9</sup> Auxin acts as a signal for cell division, elongation and differentiation<sup>10</sup> and plays a central role in promoting adventitious rooting (required for root initiation), morphogenesis and continued root viability as well as in root hair formation.<sup>11</sup> Thus, the mungbean rooting bioassay was selected for the present study to test the root-promoting ability of Kelpak under various conditions. In a previous pot trial, Swiss chard showed improved growth and a higher chlorophyll content when Kelpak was applied either as a soil drench or to the foliage,<sup>12</sup> and was thus also selected for the present study.

The uptake of any compound depends on the plant species as well as plant–soil environmental conditions. Soil pH is an important factor determining the redox potential and concentration of soluble ions in the soil.<sup>13</sup> However, soil pH is not constant. Plant roots are able to alter the pH of the rhizosphere through the cation–anion exchange balance of plants to regulate cellular pH, organic anion release, root exudation and respiration and redox-coupled processes.<sup>14</sup> Anthropogenic activities such as the excessive use of fertilisers, pollution from industries, acid mine drainage from mining activities and increased air pollution causing acid rain have all contributed to acidification of agricultural soils.<sup>15,16</sup>

The chemical nature of water differs as a result of factors such as the chemical composition of the underlying rocks and soil, as well as the length of time that the water was trapped underground.<sup>17</sup> Water containing high levels of calcium and magnesium metal cations, as well as other dissolved compounds such as bicarbonates and sulphates, is referred to as 'hard water'.<sup>18</sup> Water hardness increases as these cations increase and is categorised as soft (less than 17 mg/L Ca<sup>2+</sup>), slightly hard (17–60 mg/L Ca<sup>2+</sup>), moderately hard (60–120 mg/L Ca<sup>2+</sup>), hard (120–180 mg/L Ca<sup>2+</sup>) and very hard (180 mg/L Ca<sup>2+</sup> or more).<sup>16</sup> Water hardness is variable and, in certain conditions, can reach very high concentrations, e.g. a year-long study of a spring system in South Africa showed water hardness ranging from 92–122 mg/L CaCO<sub>3</sub> in winter, 99–229 mg/L CaCO<sub>3</sub> in spring and 171–328 mg/L CaCO<sub>3</sub> in summer. Similarly, the river into which the springs fed was soft in spring (31 mg/L CaCO<sub>3</sub>) and very hard

in summer (260 mg/L CaCO<sub>3</sub>).<sup>17</sup> In a year-long study carried out in an area of intense gold mining in South Africa, the pH of the surface and groundwater was mainly acidic, fluctuating between a pH of 3.1 and 7.9, while the Ca<sup>2+</sup> concentration varied between 34 mg/L and 619 mg/L. As a result of groundwater seepage, this polluted acidified water was discharged into nearby rivers and so affected the water table over a large area.<sup>19</sup> This water could potentially be used for irrigating crops.

The effectiveness of exogenous treatments in promoting plant growth depends on both endogenous plant factors and plant–soil environmental factors, such as soil pH and nutrient composition, as these factors will influence uptake and translocation of other compounds.<sup>20</sup> Variable success has been reported when using SWCs as natural biostimulants. This variability is attributed to the time of harvest of the seaweeds used in the SWC, the method of SWC preparation as well as the growth phase of the plant at the time of SWC application.<sup>4</sup> Additional factors that need to be considered are the plant–soil environmental conditions and the chemical nature of the water used for irrigation as pH and water hardness can also influence the uptake of the active constituents of the SWC. Our aim in this study was to test the effectiveness of the SWC Kelpak in promoting rooting in mungbean cuttings and improving growth of Swiss chard in a pot trial under a range of pHs and in water with a high Ca<sup>2+</sup> content.

## Materials and methods

### Mungbean rooting bioassay

Mungbean (*Vigna mungo* L.) seeds were surface decontaminated in 3.5% NaOCl for 20 min, rinsed thoroughly in running tap water and then soaked for 6 h in tap water. The seeds were planted in moist vermiculite and allowed to germinate in a growth chamber at 26±1 °C with a light intensity of 160 μmol/m<sup>2</sup>/s and a 16:8 h light:dark photoperiod. After 10 days, 12-cm uniform hypocotyl cuttings with two primary leaves were prepared from the seedlings and used in the bioassay.<sup>21,22</sup>

Buffered distilled water solutions were prepared at pHs of 4.5 and 6.5 with 2 mM 2-morpholinoethanesulphonic acid (MES) and at a pH of 8.5 with 2 mM tris-(hydroxymethyl)aminomethane (TRIS) and adjusted with 1 N HCl and 1 N NaOH. In addition, pH–Ca treatments were prepared with calcium (CaCl<sub>2</sub>·2H<sub>2</sub>O) at concentrations of 200 mg/L and 400 mg/L in the buffered distilled water solutions. These buffered solutions were used to dilute the Kelpak® (Kelp Products (Pty) Ltd, Simon's Town, South Africa) to 1%, 2%, 5%, 10%, 20% and 50% Kelpak solutions. The various buffered distilled water and 200 mg/L and 400 mg/L Ca<sup>2+</sup> solutions at the three pHs served as the controls in the bioassay. In addition, a dilution series of 10<sup>-7</sup>–10<sup>-3</sup> M indole-3-butyric acid (IBA) in distilled water at pH 6.5 served as a positive auxin standard for comparative purposes. In a separate bioassay, a dilution series of 10<sup>-7</sup>–10<sup>-3</sup> M IBA buffered at pH 4.5, 6.5 and 8.5 was tested to determine the effect of pH on IBA-stimulated rooting.

The test solutions (20 mL) were placed in a vial, with four vials for each solution. Five mungbean cuttings (12 cm in height) were placed in each vial (*n*=20) and left for 6 h at 26±1 °C with a light intensity of 160 μmol/m<sup>2</sup>/s. After this 6-h pulse treatment, the stems of the cuttings were rinsed in water to remove any residual solution and transferred to clean vials containing 20 mL tap water (pH 6.1). The cuttings were left for 10 days at 26±1 °C in a 16:8 h light:dark photoperiod and a light intensity of 160 μmol/m<sup>2</sup>/s. Water was added to the vials when necessary to maintain the original volume. The number of roots on each cutting was counted after 10 days.<sup>21,22</sup> The mean number of roots and the standard error were calculated for each solution after the bioassay was repeated (*n*=40).

### Pot trial with Swiss chard

A pot trial using Swiss chard (*Beta vulgaris* L. cv. Fordhook Giant; Ball Straathof (Pty) Ltd, Johannesburg, South Africa) was conducted in a greenhouse at the University of KwaZulu-Natal, Pietermaritzburg Campus from October to December 2011. Five seeds were sown individually in 5-L pots (250 mm wide x 210 mm high) containing potting mix media consisting of compost, bark, limestone ammonium nitrate and

NPK (nitrogen, phosphorus and potassium; 2:3:2) fertiliser in the ratio 15:3:0.5:0.5 (v/v). The pots were watered as required. After 2 weeks, the seedlings were thinned to one seedling per pot.

The control and treatment solutions were prepared by buffering distilled water at pHs of 4.5 and 6.5 with 2 mM MES and at a pH of 8.5 with 2 mM TRIS. The pH–Ca treatment solutions were prepared by adding 200 mg/L Ca<sup>2+</sup> (CaCl<sub>2</sub>·2H<sub>2</sub>O) to the three pH-adjusted water treatments. These pH-buffered water solutions and pH–Ca-treated solutions were used to prepare 5% Kelpak solutions. All the solutions were prepared the day before application. The pot trial consisted of 12 treatments with six replicates arranged in a complete random block design. Three soil drench applications of the treatment solutions (200 mL/pot) were carried out at 2-week intervals throughout the growing period, starting from the day of thinning. Between treatments, plants were watered every second day with the respective pH-buffered solution (control; 200 mL/pot). The temperature in the greenhouse was 25±2 °C with a midday photosynthetic photon flux density of 950±50 μmol/m<sup>2</sup>/s.

Plants were harvested 1 week after the last treatment. Roots were rinsed thoroughly with tap water to remove all traces of media. Vegetative growth parameters such as plant height, number of leaves, leaf area and the fresh and dry weights of the leaves and roots were recorded. Dry weights were measured after the plant material was oven dried at 50 °C for 7 days.

### Quantification of pigment content in Swiss chard grown in pot trial

Chlorophyll *a* (chl *a*), chlorophyll *b* (chl *b*) and carotenoid content were determined as previously described.<sup>23</sup> Samples (1 g fresh weight, FW) from the youngest leaves (1–2 leaves per plant depending on size) on the day of harvest of individual plants in each treatment (*n*=6) were homogenised in a mortar and pestle in 5 mL acetone with a small amount of acid-washed sand. The extract was centrifuged using a benchtop centrifuge (Hettich Universal, Tuttlingen, Germany) at 5000 rpm for 5 min at room temperature. The absorbance (*A*) of the supernatant was recorded at 470 nm, 645 nm and 662 nm (Varian Cary 50 spectrophotometer, Belrose, Australia). The pigment content expressed as μg/g FW, was estimated using the formulae:

$$\text{Chl } a = 11.24 A_{662} - 2.04 A_{645};$$

$$\text{Chl } b = 20.13 A_{645} - 4.19 A_{662};$$

$$\text{Total carotenoids} = (1000 A_{470} - 1.90 \text{ chl } a - 63.14 \text{ chl } b)/214$$

### Statistical analysis

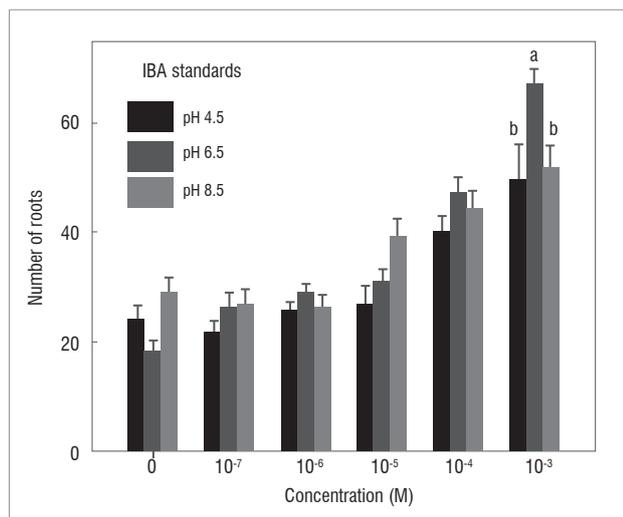
One-way analysis of variance was performed to determine significant differences between treatments. Duncan's Multiple Range Test was used to separate mean values and significant effects were accepted at *p*<0.05. For the mungbean assay, the results of 0%, 2% (linear relationship to Kelpak concentration) and 20% (maximum rooting) Kelpak solutions were analysed. Statistical computations were done using SPSS for Windows (version 15.0 SPSS®, Chicago, USA).

## Results

### Mungbean rooting bioassay

IBA had a positive response on the rooting of mungbean cuttings with increasing IBA concentrations resulting in increased rooting. A significantly better rooting response was obtained with 10<sup>-3</sup> M IBA applied at pH 6.5 than at pH 4.5 and pH 8.5 (Figure 1). Thus, IBA at pH 6.5 was used in the subsequent mungbean bioassays as the positive control.

All treatments of Kelpak applied as a 6-h pulse treatment, regardless of pH (pH 4.5, 6.5 and 8.5) or calcium concentration (200 mg/L and 400 mg/L), had a positive effect on rooting in mungbean cuttings, with the number of roots increasing with increasing Kelpak strength up to 20% Kelpak. In some treatments, the highest Kelpak concentration (50% Kelpak) slightly inhibited rooting compared with the 20% Kelpak treatment (Figure 2a–c). A similar positive response was obtained with the IBA standard at pH 6.5 (Figure 2d).

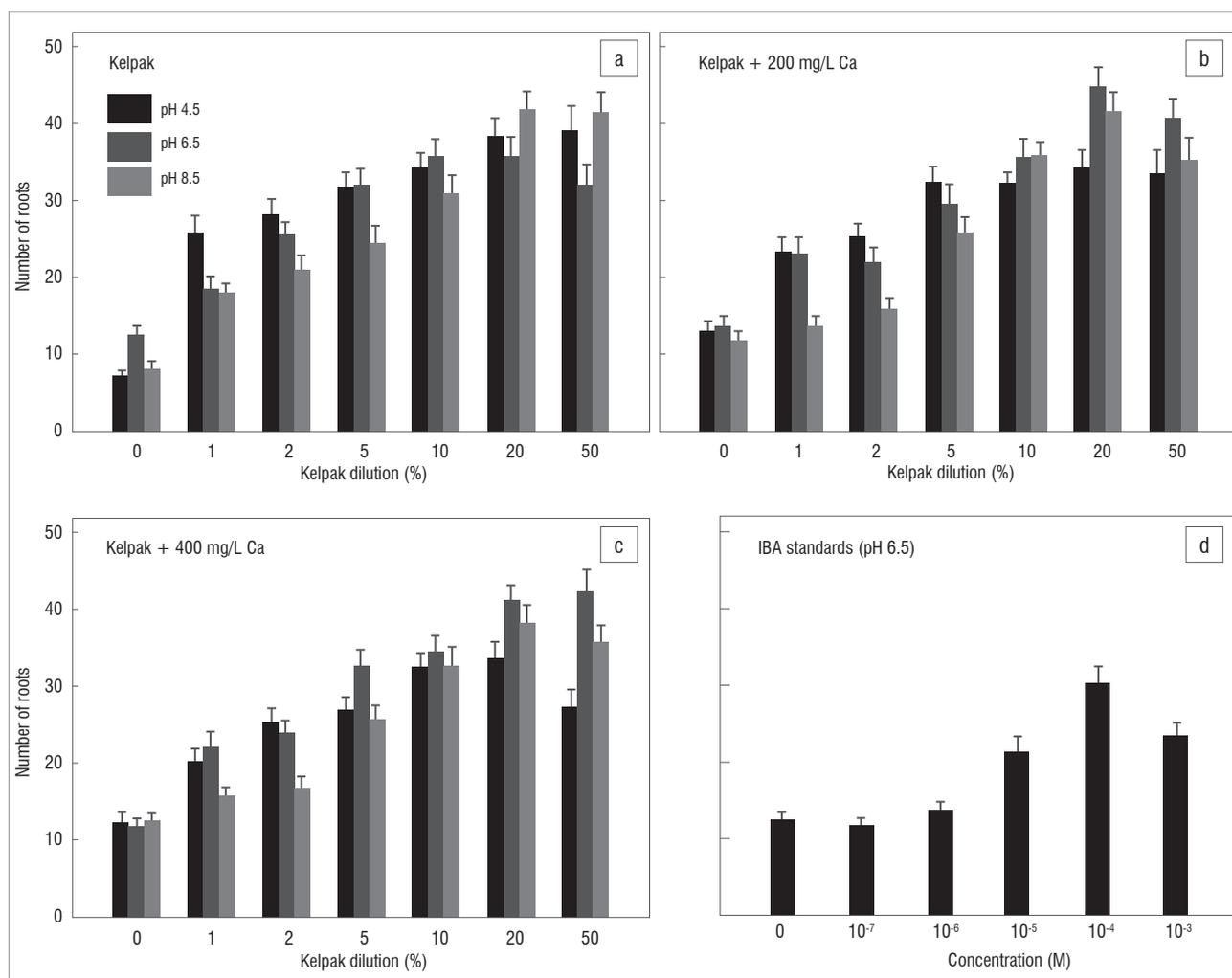


**Figure 1:** Effect of pH on the root-promoting ability of indole-3-butyric acid (IBA) tested at various concentrations ( $10^{-7}$ – $10^{-3}$  M IBA) in the mungbean rooting bioassay (mean  $\pm$  SE;  $n=20$ ). Different letters for the  $10^{-3}$  M results indicate significant differences ( $p < 0.05$ ) based on Duncan's Multiple Range Test.

When no calcium was added, rooting was significantly lower in acidic (pH 4.5) and alkaline (pH 8.5) conditions compared with that in more

neutral conditions (pH 6.5) in the control cuttings (0% Kelpak; Table 1). This pH effect was alleviated when Kelpak in low concentration (2%) was added at pHs 4.5 and 8.5, with this treatment showing statistically similar root-promoting activity as at pH 6.5 (Figure 2a; Table 1). No significant differences in rooting were observed among the 20% Kelpak treatments at the three pH values tested. Rooting was significantly higher with 20% Kelpak at pHs 4.5 and 8.5 than after treatment with  $10^{-4}$  M IBA (Figure 2a and 2d; Table 1).

Under acidic conditions, addition of  $Ca^{2+}$  significantly improved rooting while addition of Kelpak had a slight, but not significant, inhibitory effect on the root-promoting activity of 20% Kelpak (Figure 2b and 2c; Table 1). At a more neutral pH (pH 6.5), addition of 20% Kelpak + 200 mg/L  $Ca^{2+}$  significantly improved rooting (Figure 2b; Table 1) but addition of 20% Kelpak + 400 mg/L  $Ca^{2+}$  did not significantly improve rooting compared with the control at pH 6.5 (Figure 2c; Table 1). In more alkaline conditions (pH 8.5), addition of both 200 mg/L and 400 mg/L  $Ca^{2+}$  had a negative effect on the root-promoting activity of low concentrations of Kelpak, but higher Kelpak concentrations elicited a significantly positive rooting response, similar to those obtained at pHs 4.5 and 6.5 (Figure 2b and 2c, Table 1). Thus, application of low concentrations of Kelpak (2%) in the presence of  $Ca^{2+}$  produced significantly similar rooting to that at pH 4.5 and pH 6.5 while, in more alkaline conditions, higher Kelpak concentrations (20%) were required to promote significantly similar rooting in the presence of  $Ca^{2+}$  (Figure 2a–c; Table 1).



**Figure 2:** Effect of pH and water hardness ( $Ca^{2+}$  concentration) on the root-promoting ability of the seaweed concentrate Kelpak at 0–50% in the mungbean rooting bioassay (mean  $\pm$  SE;  $n=40$ ). Specific values for the various treatments with Kelpak® at 0%, 2% and 20% are given in Table 1.

**Table 1:** Effect of pH and water hardness (Ca<sup>2+</sup> concentration) on the root-promoting ability of 0%, 2% and 20% Kelpak® solutions applied to mungbean cuttings

pH	Water hardness	0% Kelpak	2% Kelpak	20% Kelpak	Indole-3-butyric acid
	(mg/L Ca <sup>2+</sup> )				(10 <sup>-4</sup> M)
4.5	0	7.05±0.86 <sup>b</sup>	27.97±2.11 <sup>a</sup>	38.18±2.56 <sup>abc</sup>	
	200	12.90±1.40 <sup>a</sup>	25.02±1.98 <sup>ab</sup>	34.18±2.36 <sup>bcd</sup>	
	400	12.33±1.17 <sup>a</sup>	25.25±1.93 <sup>ab</sup>	33.33±2.33 <sup>cd</sup>	
6.5	0	12.53±0.90 <sup>a</sup>	25.45±1.81 <sup>ab</sup>	35.80±2.43 <sup>bcd</sup>	30.30±2.23 <sup>d</sup>
	200	13.53±1.39 <sup>a</sup>	21.92±1.78 <sup>bc</sup>	44.59±2.78 <sup>a</sup>	
	400	11.63±1.14 <sup>a</sup>	23.70±1.84 <sup>ab</sup>	41.00±2.13 <sup>abc</sup>	
8.5	0	8.05±1.10 <sup>b</sup>	20.77±1.85 <sup>bcd</sup>	41.74±2.40 <sup>ab</sup>	
	200	11.68±1.24 <sup>a</sup>	15.65±1.53 <sup>d</sup>	41.33±2.80 <sup>ab</sup>	
	400	12.38±1.06 <sup>a</sup>	16.76±1.5 <sup>cd</sup>	38.03±2.55 <sup>abc</sup>	

Values shown are the mean±SE number of roots. Different letters indicate significant differences in each column (n=40; p<0.05); the last column was compared with 20% Kelpak. The full set of results is illustrated in Figure 2.

### Pot trial with Swiss chard

There were no significant differences in the growth parameters (leaf and root FW) among the three control treatments, indicating that pH alone did not affect the growth of Swiss chard (Figure 3). However, under acidic conditions, addition of 200 mg/L Ca<sup>2+</sup> or 5% Kelpak resulted in a slight decrease in leaf and root FW. When 5% Kelpak + 200 mg/L Ca<sup>2+</sup> was applied, the leaf and root FW were similar to those of control plants (Figure 3). At pH 6.5, addition of either 200 mg/L Ca<sup>2+</sup> or 5% Kelpak resulted in an increase in both leaf and root FW. Application of 5% Kelpak + 200 mg/L Ca<sup>2+</sup> had no effect on leaf FW (Figure 3a) or on root FW (Figure 3b) compared with the control at pH 6.5. At pH 8.5, addition of 200 mg/L Ca<sup>2+</sup> resulted in a slight increase in leaf FW but had no effect on the roots. Addition of 5% Kelpak improved both the leaf and root FW of Swiss chard. Addition of 5% Kelpak + 200 mg/L Ca<sup>2+</sup> resulted in significantly improved leaf FW (Figure 3a) but not root growth (Figure 3b). The data for plant height, stem weight, leaf area and root

length are not shown as there were no significant differences observed among treatments.

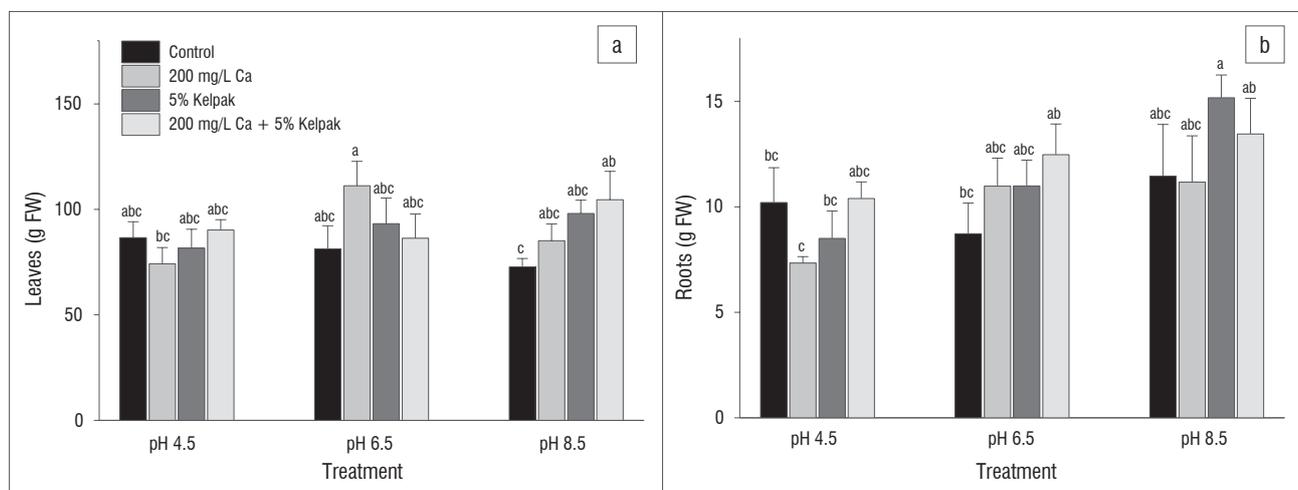
### Pigment content in Swiss chard

The largest fluctuations in pigment content were observed in chl *b* concentrations, with chl *a* and carotenoids following a similar trend. The pigment content was higher (significantly higher for chl *b*) in plants grown under alkaline conditions. While application of 5% Kelpak increased the pigment content under acidic conditions, 200 mg/L Ca<sup>2+</sup> resulted in lower pigment levels. Application of 5% Kelpak + 200 mg/L Ca<sup>2+</sup> could not overcome the negative effects of Ca<sup>2+</sup> at pH 4.5 (Figure 4). At a neutral pH, application of either 5% Kelpak or 200 mg/L Ca<sup>2+</sup> caused a significant decrease in the pigment content. However, when applied in combination, there was a significant improvement in chl *a* and chl *b* concentrations compared to the control (Figure 4). Under alkaline conditions, application of 200 mg/L Ca<sup>2+</sup> caused a significant decrease in all pigments. However, this negative effect was overcome with the addition of 5% Kelpak (Figure 4).

### Discussion

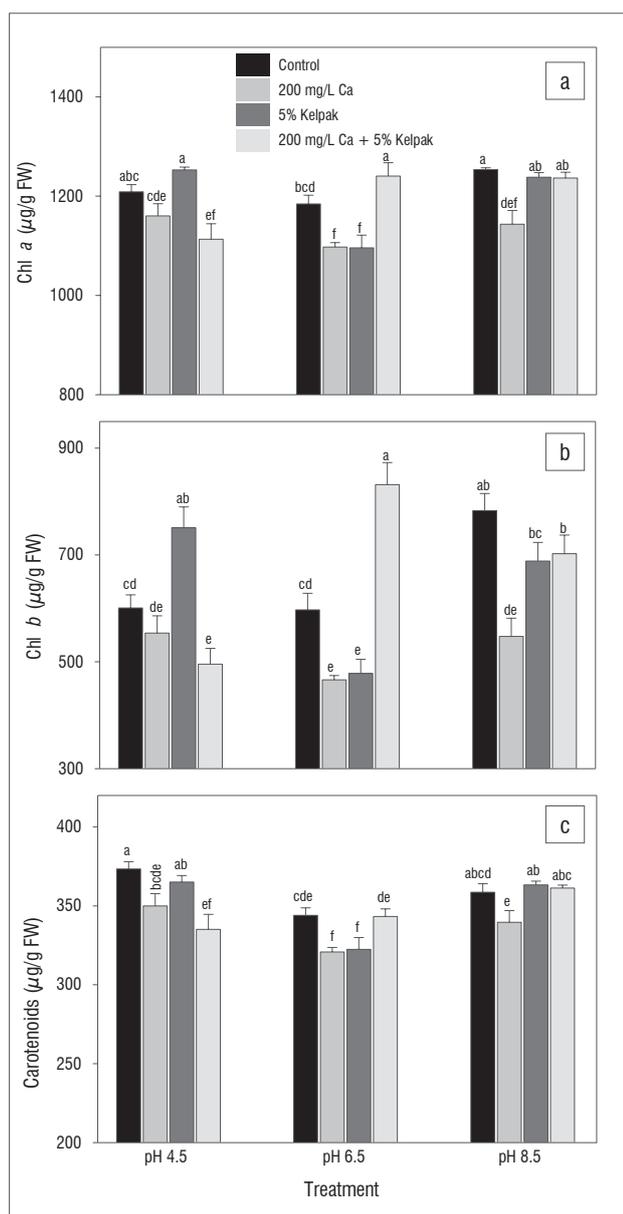
The plasma membrane plays a key function in uptake by plants with the electrochemical gradient being important in driving active uptake.<sup>24</sup> Many compounds enter cells via channels in the plasma membrane where there are various high-affinity transporters with different permeabilities for individual compounds that compete for uptake sites.<sup>25</sup> In acidified soils, there is net H<sup>+</sup> in the soil that causes an increase in the permeability of the plasma membrane as a result of a reduction in net H<sup>+</sup> release. Net H<sup>+</sup> release by H<sup>+</sup> ATPase is essential for nutrient uptake (as it drives the proton pump), for maintaining turgor and for cytoplasmic pH regulation.<sup>26</sup> Thus, a low soil pH favours anion uptake<sup>27</sup> and increases the concentration of soluble ions to the extent that certain ions may become toxic.<sup>28</sup> For example, in germinating *Pinus pinaster* seeds, pH influenced the uptake of certain mineral elements, with significantly lower uptake of most elements at the more acidic pHs tested.<sup>27</sup> More alkaline pHs also result in decreased uptake.<sup>25</sup> Of note is that, in this study, Kelpak significantly promoted rooting at pH 4.5 in the mungbean bioassay and was effective at masking the negative effects of Ca<sup>2+</sup> application at pH 4.5 in Swiss chard. As it is estimated that 30–50% of the world's potentially arable land is acidic,<sup>24,29</sup> it is of importance for agricultural use that Kelpak is still effective at promoting rooting at a low pH.

Many spray solutions used in agriculture have additives such as nutrients, surfactants and herbicides. When hard water is used in these spray solutions, Ca<sup>2+</sup> and Mg<sup>2+</sup> may be antagonistic, forming complexes with the target compounds so that they are less readily taken up through the plasma membrane.<sup>30,31</sup> In both greenhouse and field studies in which



**Figure 3:** Effect of pH, water hardness (Ca<sup>2+</sup> concentration) and 5% Kelpak on (a) leaf and (b) root (fresh weight) of Swiss chard grown in pots. Letters above bars indicate significant differences (mean±SE where n=6; p<0.05) obtained when data was analysed using Duncan's Multiple Range Test.

a range of solutions from 0 mg/L to 1000 mg/L  $\text{Ca}^{2+}$  was used, high concentrations ( $>250$  mg/L  $\text{Ca}^{2+}$ ) significantly reduced glyphosate activity in several weed species.<sup>30</sup> In the present study, high  $\text{Ca}^{2+}$  concentrations during the pulse treatment in the mungbean bioassay did not have a negative effect on the root-promoting activity of Kelpak at pH 4.5 and pH 6.5, while under alkaline conditions (pH 8.5), higher Kelpak concentrations promoted rooting. Similarly, in the pot trial, Kelpak promoted growth even when applied in combination with 200 mg/L  $\text{Ca}^{2+}$  at pH 6.5 and pH 8.5 and alleviated the negative effects of  $\text{Ca}^{2+}$  at pH 4.5. These results provide evidence that the active constituents of Kelpak are not forming complexes with the available cations and so can be effectively applied regardless of water hardness.



**Figure 4:** Effect of pH, water hardness ( $\text{Ca}^{2+}$  concentration) and 5% Kelpak on (a) chl a, (b) chl b and (c) carotenoid content of Swiss chard grown in pots. Letters above bars indicate significant differences (mean  $\pm$  SE where  $n=6$ ;  $p<0.05$ ) obtained when data was analysed using Duncan's Multiple Range Test.

Calcium plays an important role in uptake by plant cells as it is involved in maintaining the structural stability of the cell wall and the integrity of the cellular membrane.<sup>32</sup>  $\text{Ca}^{2+}$  is easily displaced from its binding

sites by other cations in low pH conditions.<sup>25</sup> The effect of a low pH on the integrity of the plasma membrane can be alleviated by application of  $\text{Ca}^{2+}$ .<sup>26</sup> In the present study, application of 200 mg/L  $\text{Ca}^{2+}$  under acidic conditions had a negative effect on the growth of Swiss chard. However, at more neutral and alkaline pHs, application of 200 mg/L  $\text{Ca}^{2+}$  significantly improved growth of Swiss chard. Improved functioning of the cell membranes would improve the uptake of the active constituents of Kelpak – hence the significant increase in leaf and root FW and pigment content when 5% Kelpak + 200 mg/L  $\text{Ca}^{2+}$  was applied at pH 6.5.

The relationship between calcium availability and root growth is not a simple direct dependence on the extracellular concentration of calcium. Plants maintain an equilibrium with their growth medium and thus external  $\text{Ca}^{2+}$  also affects cytosolic  $\text{Ca}^{2+}$  concentrations.<sup>33</sup> Although it occurs in much lower concentrations than those found in the cell wall and membranes, cytosolic  $\text{Ca}^{2+}$  is an important intracellular signalling agent and there are many calcium-dependent protein kinases in plant cells.<sup>32</sup> There is a well-established link between auxin and calcium with cytoplasmic  $\text{Ca}^{2+}$  playing a role in auxin transport and secretion.<sup>33</sup> Auxin moves both acropetally through the vascular tissue and basipetally to the outer cortex and epidermis in roots with specific influx and efflux carriers to facilitate the movement of the various auxins from cell to cell.<sup>11</sup> The auxin gradient is critical in regulating root meristem organisation and its activity.<sup>34</sup> Exogenous application of auxin lowers the cytosolic pH and increases  $\text{Ca}^{2+}$  concentrations. This change in pH can cause fluctuations in the cell membrane potential by increasing proton excretion.<sup>35</sup> One of the constituents of Kelpak is auxin, and both IAA and other indole amino acids have been positively identified.<sup>8</sup> Although IAA transport is linked to  $\text{Ca}^{2+}$ , the high  $\text{Ca}^{2+}$  concentrations tested in the present study did not have a negative impact on the root-promoting activity of Kelpak and, under neutral and alkaline conditions, even significantly enhanced rooting and growth in Swiss chard.

There is a broad relationship between crop productivity and photosynthesis.<sup>36</sup> Yields have been maximised by increasing light interception and, more recently, by manipulating photosynthetic metabolism.<sup>36</sup> In the present study, pigment content decreased with high  $\text{Ca}^{2+}$  concentrations while treatment with Kelpak was able to overcome this negative effect under neutral and alkaline conditions.

It is likely that the wide range of physiological responses evoked with SWC application is a result of several active constituents<sup>3,37</sup> and that soil–environmental factors and irrigation water could potentially affect their uptake. In practice, the recommended conventional soil application is 2–4 L Kelpak in 200–300 L water (1–2%) and foliar and ultra low volume (electrostatic machine) application is 2–4 L Kelpak in 25–60 L water (8–16%). Our results demonstrate that the root- and growth-promoting constituents in Kelpak can be effectively taken up and utilised by plants to improve growth and yield. While most effective in neutral pHs, Kelpak can be used under a wide range of pH and water hardness conditions that are likely to be encountered in the field.

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## Author's contributions

G.D.A. and W.A.S. designed the experiments. G.D.A., A.O.A. and M.M. performed all the experiments. W.A.S. wrote the paper with editorial contributions from G.D.A. and J.v.S.

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