ISSN : 1812-5379 (Print)
ISSN : 1812-5417 (Online)
http://ansijournals.com/ja

JOURNAL OF AGRONOMY

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Journal of Agronomy 3 (3): 208-214, 2004
ISSN 1680-8207

Effect of Promalin on Growth and Development of Kale (*Brassica oleracea* L. Var. Acephala DC)

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Abstract: A net greenhouse experiments were carried out to evaluate the effect of promalin on growth and development of kale. Promalin at 25, 50 and 75 mg L^{-1} significantly increased total leaf chlorophyll content, chlorophyll a and b contents, the percentage of chlorophyll a and b to chlorophyll, total leaf area, plant height, leaf number per plant, leaf size (leaf length, leaf blade diameter, leaf blade length and petiole length), plant dry matter and fresh leaf yield. Promalin at 50 or 75 mg L^{-1} increased stem and root dry matter accumulation and partitioning to the stem. Promalin at 25, 50 or 75 mg L^{-1} decreased significantly the leaf water content and significantly increased vegetative, root growth and yield of kale plants. Promalin also induced the growth of a leaf petiole from the mid-rib leading to a leaflet from the leaf mid-rib. Promalin at 50 mg L^{-1} has the potential to be used to improve the growth, development and yield of kale.

Key words: Promalin, leaf chlorophyll content, induced leaf growth, leaf size, yield of kale

INTRODUCTION

Kale is a biennial crop, closely related to cabbage but grown as an annual for its edible leaves. It is a hardy, cool-season green of the cabbage family. Kale tolerates summer heat, but grows well in winter in Botswana. Its main season of availability is winter through spring in Botswana. It is rich in dietary fibre, calcium, iron, vitamin A and C[1]. It can be cooked or processed in a number of ways like stews, soups, creamed vegetable but the best way is to boil the leaves or brace with onions, spices and parsley.

Due to fast growth in human population, there is need for the supply of right foot stuffs in the developing countries. In order to meet the rising demand for food, emphasis has to be put on improving yields per hectare which can be achieved through plant breeding, biotechnology and application of plant growth regulators to positively modify plant growth to economic advantage[2]. Plant growth regulators are at the present time used in agriculture for various purposes such as delaying or promoting ripening, induction of rooting, promotion of abscission, weed control, size control and many other responses[2]. Plant growth regulators can be used to modify photosynthetic efficiency and assimilate partitioning in crops thereby increasing crop yield[3]. The objective of this study was to evaluate the effect of promalin on growth and development of kale (*Brassica oleracea* L. Var. Acephala DC).

MATERIALS AND METHODS

Location and site: Greenhouse house experiments were conducted during the periods of May to October 2002 and April to October 2003, at the Botswana College of Agriculture, Farm at Sebele (24°33'S, 25°54'E, 994 m above sea level). The climate in Sebele is semi-arid with an average annual rainfall (30 year mean) of 538 mm[4]. Most rain falls in summer, which generally starts in late October and continues to March/April. Prolonged dry spells during rainy seasons are common and rainfall tends to be localized[5]. The soils are shallow, ferruginous tropical soils, mainly consisting of medium to coarse grain sands and sandy loams with a low water holding capacity and subject to crusting after heavy rains. The soils are deficient in phosphorus, have low levels of mineral nitrogen and low organic matter[5].

Experimental design: The experiments were carried out in a greenhouse (green netting with 40% shading and an average solar irradiance of 600 J m^{-2} S^{-1} at noon). The experimental plots were concrete benches measuring 1.28x3.5 m (with slanted floors for drainage, 0.5 m deep and a drainage hole at the end of each concrete bench).

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The growing media was river sand. The concrete benches were equipped with drip irrigation. The experiment was laid down as a Completely Randomized Design with three replicates. The treatments were 0, 25, 50 and 75 mg L^{-1} promalin. Promalin is a liquid concentrate containing benzyladenine (cytokinin) and gibberellins (GA_{4+7}) in a ratio of 1:1 (1.8% a.i (w/w) benzyladenine and 1.8% a.i (w/w) GA_{4+7}, Abbott Laboratories, North Chicago, USA). All the two trials used whole kale plants, the cultivar `thousand headed'. Whole plants were sprayed with either promalin or distilled water to run-off using a pressurized knapsack sprayer. Kale plants were sprayed with either promalin or distilled water two weeks after transplanting.

Crop husbandry: Seeds of kale, cultivar `thousand headed' were sown in five polystyrene trays each holding 120 seedlings in the greenhouse. The seedlings were raised in a germination media consisting of 1 part vermiculite: 1 part loam soil: 1 part farmyard manure. The germination media was mixed with 30 g of NPK-fertilizer 2:3:2. The seedlings were watered twice a week. One week prior to transplanting, water was withdrawn to harden the seedlings. The seedlings were transplanted to the concrete benches after developing three true leaves. Before transplanting, the growing media in concrete benches was treated with nemacur for the control of nematodes.

During transplanting NPK-fertilizer 2:3:2 was applied at the rate of 42 kg ha^{-1}. After crop establishment, 8 g of calcium ammonium nitrate (26% N)/plant was top dressed every three weeks. The spacing was 0.3 m between plants and 0.4 m between rows. The plots were kept weed free by hand cultivation using a hoe. The insect pest *Brevicoryne brassicae* (cabbage aphids) were controlled with dimethoate at the rate of 30 mL/20 L of water.

Dependent variables determined: The dependent variables that were determined included; plant height at maturity, number of leaves per plant, leaf area, plant fresh weights, both shoot and roots dry weights, leaf length, blade and petiole length, diameter of blade, leaf water content and fresh leaf yield. The fresh and dry weights of plants were determined by destructive harvesting of five plants at random per replicate. The leaves, stems and roots of five plants per replicate were separated and put in already weighed brown paper bags. The fresh samples were weighed using Mettler PM 400 digital balance. The fresh weights were determined by subtracting weight of paper bag from weight of paper plus fresh sample. The plant samples (leaves, stems and roots) were oven dried at 66°C to constant weight. The leaf water content was determined by subtracting dry weights from their corresponding fresh weights. Fresh leaf yield was determined from cumulative leaf yield per harvest from five tagged plants per replicate. On average there were eight leaf harvests. During each harvest, fresh leaf weight was determined by weighing the fresh leaves using Mettler PM 400 digital balance. Leaf length, petiole and blade length and blade diameter were measured using a metre-ruler at every fresh leaf harvest (8 leaf harvests were done). The reported leaf length, petiole and blade length and blade diameter in cm is an average of 8 measurements. At each measurement 10 leaves per replicate were used. Leaf area was determined using 10 leaves per replicate. Twenty discs (2 discs/leaf) were made using a 17 mm diameter cork borer. The area of the leaf discs was calculated. The leaf discs and the corresponding leaves were put in separate paper bags and were dried at 66°C for 72 h. The dry weight of leaves and discs were determined. The leaf area was estimated using the following formula:

$$\text{Leaf area per plant } (cm^2) = \frac{\text{Total dry weight of leaves x area of sample discs}}{\text{Dry weight of sample discs}}$$

Total chlorophyll, chlorophyll a and chlorophyll b contents were determined from ten leaves per replicate (leaves per plant). Two discs (17 mm diameter) per leaf were cut using a cork borer. Twenty discs per replicate were extracted in 8 mL 0.1N HCl in methanol at 25°C in a dark room for 24 h. The absorbance of the extracts were measured using a spectrophotometer (UV-V13 RS Spectrophotometer Labo Med Inc). Chlorophyll a, chlorophyll b and total chlorophyll contents were estimated using the following formulae:

Chlorophyll a (mg cm^{-2}) = $12.7A_{663}$-$2.069A_{645}$
Chlorophyll b (mg cm^{-2}) = $22.9A_{645}$-$4.68A_{663}$
Total chlorophyll (mg cm^{-2}) = $24.88A_{653}$ [6].

Where, A is the absorbance at 645, 653 and 663 nm, respectively and the coefficients are the molar extinction coefficients at the respective wavelengths mentioned above at 25°C.

Data analysis: Analysis of Variance was performed on the data collected using the general linear models (PROC GLM) procedure of the statistical analysis system program package. Multiple comparisons among means were performed using the Protected Least Significant Difference at p=0.05. Proc Univariate procedure was carried out on the residuals to support the assumptions of normality made by the researchers.

RESULTS

Application of promalin at the rate of 25, 50 or 75 mg L^{-1} significantly increased leaf chlorophyll content compared to plants sprayed with distilled water (Table 1). Kale plants sprayed with 50 and 75 mg L^{-1} promalin significantly increased chlorophyll a compared to the control plants (Table 1). However, there were no significant differences on leaf chlorophyll a content among plants sprayed with 25, 50 or 75 mg L^{-1} promalin (Table 1). There were no chlorophyll a differences between the control plants and the plants sprayed with 25 mg L^{-1} promalin.

Spraying kale plants with 25, 50 or 75 mg L^{-1} promalin significantly increased leaf chlorophyll b content compared to control plants (Table 1). There was no significant difference on leaf chlorophyll b content between kale plants sprayed with either 25 or 50 mg L^{-1} promalin (Table 1). However, kale plants sprayed with 75 mg L^{-1} promalin had significantly lower leaf chlorophyll b content than plants sprayed with either 25 or 50 mg L^{-1} promalin (Table 1). Application of 25, 50 or 75 mg L^{-1} promalin significantly increased total leaf chlorophyll content compared to control plants (Table 1). There were no significant differences among promalin concentrations with respect to increasing total leaf chlorophyll content (Table 1). In relative terms, promalin application significantly increases leaf chlorophyll b content, but decreased leaf chlorophyll a content in relation to total leaf chlorophyll (Table 1).

Kale plants sprayed with 25, 50 or 75 mg L^{-1} promalin had significantly higher leaf area than control plants (Table 2). Increase in leaf area increased with increase in promalin concentration. However, 25 or 50 mg L^{-1} promalin increased kale leaf area in a similar fashion, but plants sprayed with 75 mg L^{-1} promalin had significantly higher leaf area than plants sprayed with either 25 or 50 mg L^{-1} promalin (Table 2).

Application of 50 or 75 mg L^{-1} promalin significantly increased the plant leaf number compared to the control (Table 2). However, 25 mg L^{-1} promalin had a non-significant increase in leaf number (Table 2).

Promalin significantly increased kale leaf size (Fig. 1 and Table 2). All promalin concentrations used in

Table 1: Effect of promalin on leaf chlorophyll content of kale

Promalin (mg L^{-1})	Chlorophyll a (mg cm^{-2})	Chlorophyll b (mg cm^{-2})	Total chlorophyll (mg cm^{-2})	Ratio of chlorophyll a to total chlorophyll (%)	Ratio of chlorophyll b to total chlorophyll (%)
0	0.426b	0.448c	0.873b	48.93a	51.07b
25	0.662ab	1.371a	2.033a	32.37b	67.63a
50	0.702a	1.270a	1.972a	35.63b	64.37a
75	0.801a	1.021b	1.822a	44.00a	56.00b
Significance	*	****	***	****	****
LSD	0.252	0.201	0.391	5.241	5.241

*, ***, ****, Significant at p = 0.05, 0.001, 0.0001, respectively

Means separated by the Least Significant Difference at p = 0.05; means within columns followed by the same letter(s) are not significantly different

Table 2: Effect of promalin on leaf characteristics and plant height of kale

Promalin (mg L^{-1})	Leaf area (cm^2)	Leaf number/plant	Leaf petiole length (cm)	Leaf blade length (cm)	Leaf blade diameter (cm)	Leaf length (cm)	Plant height (cm)
0	3535c	66.67b	15.37c	21.06c	17.56b	36.42c	54.20b
25	4479b	74.20ab	16.97b	24.31ab	19.69ab	41.28b	56.38b
50	5177b	84.33a	18.68a	25.68a	20.87a	44.36a	60.50b
75	6987a	82.67a	16.30b	23.29b	20.75a	39.62b	72.35a
Significance	***	**	*	**	**	**	**
LSD	904	15.38	0.89	2.21	2.73	2.4	11.35

*, **, ***, Significant at p = 0.05, 0.01, 0.001, respectively

Means separated by the Least Significant Difference at p = 0.05; means within columns followed by the same letter(s) are not significantly different

Table 3: Effect of promalin on dry matter accumulation and fresh leaf yield of kale

Promalin mg L^{-1}	Leaf water content (%)	Leaf dry matter content (%)	Leaf dry weight/ plant (g)	Stem dry weight/ Plant (g)	Root dry weight/ Plant (g)	Plant dry weight/ Plant (g)	Fresh leaf yield (tons ha^{-1})
0	80.45a	19.55d	13.90b	1.55c	12.55b	27.83c	43.31c
25	73.37c	26.63b	21.33a	2.58bc	13.72ab	37.63b	46.25b
50	70.53d	29.47a	25.95a	3.82ab	16.30a	46.07a	51.88a
75	75.94b	24.06c	25.55a	4.80a	14.80b	44.85a	45.19b
Significance	****	****	**	***	*	***	****
LSD	2.57	2.57	6.39	1.39	3.43	6.80	1.78

*, **, ***, ****, Significant at p = 0.05, 0.01, 0.001, 0.0001, respectively

Means separated using the Least Significant Difference at p = 0.05; means within columns followed by the same letter(s) are not significantly different

Fig. 1: Effect of promalin on leaf size of Kale

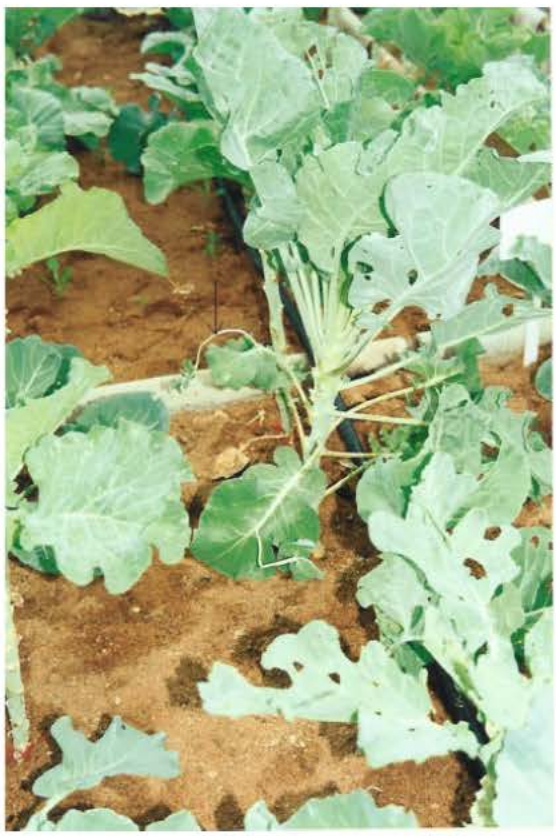

Fig. 2: Effect of promalin on mid-rib development. The arrow indicates that promatin induced-secondary mid rib development probably by induced cell division.

Table 4: Effect of promalin on dry matter partitioning on kale

Promalin (mg L^{-1})	Leaf dry weight to total plant dry weight (%)	Stem dry weight to total plant dry weight (%)	Root dry weight to total plant dry weight (%)
0	50.10a	5.63c	44.27a
25	56.10a	6.73bc	37.17a
50	56.67a	8.27ab	35.07b
75	57.13a	10.30a	32.83b
Significance	ns	**	*
LSD	9.22	2.50	8.90

*, **, ns, Significant at p = 0.05, 0.01, nonsignificant, respectively
Means separated using the Least Significant Difference at p = 0.05; means within columns followed by the same letter(s) are not significantly different

the study increased leaf petiole length compared to control plants (Table 2). Kale plants sprayed with 50 mg L^{-1} promalin had significantly longer leaf petiole length than plants sprayed with either 25 or 75 mg L^{-1} promalin (Table 2). Kale plants sprayed with 25, 50 or 75 mg L^{-1} promalin had significantly larger leaf blades than control plants. There were no concentration differences between plants treated with 25 or 50 mg L^{-1} promalin, with respect to increasing leaf blade length (Table 2). There were also no concentration differences between plants treated with 25 or 75 mg L^{-1} promalin, with respect to increasing leaf blade length (Table 2). Application of 50 or 75 mg L^{-1} promalin significantly increased leaf blade diameter compared to control plants (Table 2). Application of 25 mg L^{-1} promalin had a non-significant increase in leaf blade diameter (Table 2). There were no significant promalin concentration differences in their ability to increase the leaf blade diameter (Table 2). Promalin at 25, 50 or 75 mg L^{-1} significantly increased kale leaf length and the response to increasing promalin concentration was quadratic (Table 2). There was no significant difference in leaf length between kale plants sprayed with either 25 or 75 mg L^{-1} promalin (Table 2).

Application of 75 mg L^{-1} promalin significantly increased kale plant height compared to plants sprayed with 0, 25 or 50 mg L^{-1} promalin (Table 2). However, plants sprayed with 25 or 50 m mg L^{-1} promalin were non-significantly taller than control plants (Table 2).

Application of promalin at the rate of 25, 50 or 75 mg L^{-1} significantly increased the leaf dry matter and reduced the leaf water content in comparison to control plants (Table 3). The increase in leaf dry matter and decrease in leaf water content was quadratic with increasing promalin concentration (Table 3). Kale plants treated with 75 mg L^{-1} promalin had significantly lower leaf dry matter and higher leaf water content than plants treated with 25 mg L^{-1} promalin (Table 3). Kale plants sprayed with 25, 50 or 75 mg L^{-1} promalin significantly increased leaf, stem, root and total plant dry weight and the response to increasing promalin concentration was quadratic (Table 3). There were no promalin concentration differences in their ability to increase leaf dry weight per plant (Table 3). Kale plants sprayed with 50 or 75 mg L^{-1} promalin had significantly higher stem dry weight per plant than plants sprayed with 0 or 25 mg L^{-1} promalin (Table 3).

Application of promalin at the rates of 25, 50 or 75 mg L^{-1} did not have any significance in the ratio of leaf dry weight- to- total plant dry weight compared to control plants (Table 4). However, there was a significant increase in the ratio of stem dry weight-to- total plant dry weight for plants sprayed with 50 or 75 mg L^{-1} promalin compared to control plants (Table 4). Plants sprayed with 25 mg L^{-1} promalin had a non-significant increase in stem dry weight-to-total plant dry weight than control plants (Table 4). Application of 50 or 75 mg L^{-1} promalin significantly decreased the root dry weight-to-total plant dry weight compared to kale plants sprayed with 0 or 25 mg L^{-1} promalin (Table 4). In general, kale plants partitioned more dry matter to the shoot (stem+leaves) compared to the roots (Table 4).

Kale plants sprayed with 25, 50 or 75 mg L^{-1} promalin had significantly higher fresh leaf yield than control plants and the increase in yield was quadratic with increasing promalin concentration (Table 3). There was no significant difference in yield of kale plants sprayed with either 25 or 75 mg L^{-1} promalin (Table 3).

DISCUSSION

Promalin is a plant growth regulator containing benzyladenine (cytokinin) and gibberellins (GA_{4+7}) in a 1:1 ratio. Promalin at 25, 50 or 75 mg L^{-1} significantly increased the total leaf chlorophyll content because promalin increased the synthesis of chlorophyll a and chlorophyll b contents of kale plants. Cytokinins have been reported to promote chloroplast development and chlorophyll synthesis by promoting grana formation and increasing RNA and protein synthesis for chlorophyll biosynthesis thereby increasing the rate of chlorophyll formation[7-9]. Benzyladenine has been reported to activate the synthesis of two proteins of the chloroplasts RUBP carboxylase and chlorophyll a/b complex[10]. Benzyladenine has also been shown to increase chloroplast DNA content of intact bean plants[11]. The relatively higher content of chlorophyll b than chlorophyll a induced by promalin in kale leaves suggests that cytokinins control the genes responsible for chlorophyll

synthesis probably via the transcriptional process. This could be a quantitative effect where benzyladenine and GA_{4+7} could accelerate the synthesis of all classes of RNA for chlorophyll a and/or b synthesis or their effect could be qualitative by the induction of specific enzymes responsible for the synthesis of either chlorophyll a or b.

All the promalin concentrations used in the present study increased leaf area. This was attributed to increased leaf number and size induced by benzyladenine and GA_{4+7}. Benzyladenine and GA_{4+7} in promalin, might have acted synergistically to increase leaf area, number, length, petiole length and blade length and diameter. Benzyladenine might have increased cell division in the apical meristems and leaf of kale plants leading to increased leaf number, induced mid-rib growth and blade diameter. Cytokinins have been shown to promote cell division[3,9,12]. Gibberellins are known to promote both cell division and elongation in several plants, thus explaining the increase in leaf number, length, petiole length and blade length observed in the current study[9,13]. Gibberellins promote cell division by stimulating cells in G_1 phase to enter the S phase and by shortening the S phase, resulting in faster cell division[13,14]. The increased leaf length, petiole length and blade length observed in the current study could be attributed to the GA_{4+7} effect on cell wall plasticity whose net result is elongation growth[9]. Studies in lettuce hypocotyls have indicated that the ultimate action of gibberellins in this tissue is to bring about increased wall plasticity or to maintain the cell walls in a plastic state for a longer time[9]. Hypocotyls treated with tritiated GA_1 accumulate significant amounts of labile cell wall linkages in a purified cell wall fraction and this process precedes growth response[15]. Several studies have demonstrated that gibberellins in treated tissue promote cell elongation and the cell elongation was paralleled by an increase in the synthesis of cell wall polymers[16-18]. Promalin treated plants were taller than the controls probably because benzyladenine and GA_{4+7} synergistically increased both cell division and/or elongation resulting in taller promalin-treated kale plants (Fig. 2).

The increase in leaf number due to promalin application can also be explained by the role of benzyladenine in promoting vegetative growth by overcoming apical dominance, a characteristic cytokinin correlative effect[3,19]. Promalin increased plant dry matter compared to control kale plants. This effect was due to benzyladenine because gibberellins are known to increase water content and not dry weight[9,20]. Benzyladenine increased kale plant dry weight probably because it increased loading and unloading of assimilates across the membrane boundaries of the vascular tissues of kale plants, leading to enhanced crop growth as evidenced by the large leaf area and dry matter portioning to the shoots[20-22]. The increase in plant dry weight and carbohydrate partitioning to the shoots (high shoot-to-root dry weight) due to promalin application can be attributed to the increased leaf chlorophyll content per unit leaf area and large leaf area of promalin treated kale plants, leading to high photosynthetic rate and carbohydrate assimilation. The increase in plant and shoot-to-root dry weights can also be explained by the role of benzyladenine in promoting carbohydrate metabolism and its ability to create new source-sink relationship[20,23,24]. Promalin increased kale leaf fresh weight ha^{-1} because of the increase in leaf number, area, chlorophyll content, leaf size, shoot dry matter and total plant dry matter accumulation induced by promalin. In conclusion, promalin positively modified the growth and development of kale, resulting in large leaf size, high leaf chlorophyll content, leaf number, dry matter accumulation and fresh leaf yield.

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