CHAPTER 5

RESPONSE OF POTATO GROWN IN A HOT TROPICAL LOWLAND TO PACLOBUTRAZOL. I: SHOOT ATTRIBUTES, PRODUCTION AND ALLOCATION OF ASSIMILATES

5.1 ABSTRACT

The growth response of potato to PBZ under the hot tropical condition of eastern Ethiopia was investigated in two field experiments. A month after planting PBZ was applied as a foliar spray or soil drench at rates of 0, 2, 3, and 4 kg a. i. PBZ per ha. Regardless of the method of application, PBZ increased chlorophyll *a* and *b* content and net rate of photosynthesis, but reduced shoot growth, plant height, stomatal conductance and the rate of transpiration. PBZ delayed the onset of leaf senescence and increased the partitioning of assimilates to the tubers while reducing assimilate supply to the leaves, stems, roots and stolons. PBZ improved the productivity of potatoes grown in the hot tropical lowlands by reducing shoot growth, increasing leaf chlorophyll content, enhancing the rate of photosynthesis, improving water use efficiency, and increased partitioning of dry matter to the tubers.

Keywords: Assimilation; chlorophyll content; photosynthesis; senescence; stomatal conductance; transpiration

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5.2 INTRODUCTION

Lowland tropical regions are characterized by high temperatures that limit successful potato cultivation (Midmore, 1984). In Ethiopia about 35 % of the available agricultural land is situated in semi-arid regions of the country, where high temperatures throughout the year limit potato production.

Leach *et al.* (1982) developed a detailed carbon budget for potato indicating that plant growth rate is strongly related to net photosynthesis and dark respiration. In the tropics, of the gross carbon fixed up to 50% may be utilized for respiration (Burton, 1972). Respiration increases with temperature and it is estimated to roughly double for each 10 ºC increase between 10 ºC and 35 ºC (Sale, 1973). Above 30 ºC the rate of net photosynthesis declines rapidly (Leach *et al.,* 1982; Thornton *et al.* 1996). Hence, reduced assimilate production due to decreased photosynthesis and increased respiration are important factors limiting potato productivity in hot tropical lowlands.

The most noticeable morphological features of potatoes grown under high temperatures are taller plants with longer internodes, increased leaf and stem growth, decreased leaf: stem ratio, and shorter and narrower leaves with smaller leaflets (Menzel, 1985; Manrique, 1989; Struik *et al.,* 1989). Although there are genetic differences (Manrique, 1989; Hammes & De Jager, 1990), high temperatures decrease the partitioning of assimilate to the tubers and increase partitioning to other parts of the plant (Wolf *et al.,* 1990; Vandam *et al.,* 1996). Under long photoperiods, high temperatures may shift partitioning of assimilates to the shoots thereby delaying leaf senescence (Struik *et al.,* 1989); but under short photoperiods, high temperatures favour rapid growth and development and shortens the growing period (Vander

Zaag *et al.,* 1990). Higher temperatures favour the production of high levels of GA-like compounds in potato plants (Menzel, 1983).

PBZ is a triazole plant growth regulator known to interfere with *ent*-kaurene oxidase activity in the *ent*-kaurene oxidation pathway (Rademacher, 1997). Interference with the different isoforms of this enzyme could lead to inhibition of GA biosynthesis and prevention of abscisic acid (ABA) catabolism. In addition, PBZ induces various plant responses such as shoot growth reduction (Terri & Millie, 2000; Sebastian *et al.,* 2002), enhanced chlorophyll synthesis (Sebastian *et al.,* 2002), delayed leaf senescence (Davis & Curry, 1991), improved water use by reducing the rate of transpiration (Ritchie *et al.,* 1991; Sankhla *et al.,* 1992; Eliasson *et al.,* 1994) and increased assimilate partitioning to the underground parts (Balamani & Poovaiah, 1985; Davis & Curry, 1991; Bandara & Tanino, 1995).

Greenhouse experiments on the effect of PBZ on potato growth suggested that it enhances the productivity under non-inductive conditions (Chapter 3). It is proposed that PBZ reduces GA biosynthesis in potatoes, and should improve productivity in the lowland tropics and improves productivity. This paper reports the effect of foliar and root applied PBZ on shoot growth, chlorophyll content, stomatal conductance, rate of transpiration, photosynthetic efficiency as well as biomass production and partitioning in potato grown under hot tropical conditions in the lowland of eastern Ethiopia. As a follow up from the same experiments, growth analyses and tuber attributes are presented in Chapter 6 and Chapter 7, respectively.

5.3 MATERIALS AND METHODS

5.3.1 Site description

Two similar field experiments were conducted under irrigation from January to July 2003 at Tony Farm, research farm of Alemaya University, Ethiopia. The site is located at 41^{o} 50.4' E longitude, 09^{o} 36' N latitude, at an altitude of 1176 m.a.s.l. in the semi-arid tropical belt of eastern Ethiopia. During the growing period the total precipitation was 230 mm and the mean monthly minimum and maximum temperatures were 18 ºC (ranging from 15.4 to 21.3 ºC) and 31 ºC (ranging from 28.0 to 34.4 ºC), respectively. The mean relative humidity was 50%, varying from 20 to 81%. The soil was a well-drained deep clay loam with 2.36% organic matter, 1.36% organic carbon, 0.12% total nitrogen, 14.15 ppm phosphorus, 1.08 meq100 g^{-1} exchangeable potassium, 0.533 $mMhoscm^{-1}$ electric conductivity and a pH of 8.6.

5.3.2 Plant culture

Treatments were laid down as two-factor (rate and method of application) factorial experiments arranged in randomised complete block designs with three replications. In each plot (5.25 m x 2.1 m) forty-nine medium sized, well sprouted tubers of cultivar 'Zemen' were planted at a spacing of 75 x 30 cm. Phosphorus was applied as diammonium phosphate at planting time at a rate of 150 kg P ha^{-1} and nitrogen was side dressed after full plant emergence at a rate of 100 kg N ha^{-1} in the form of urea. The plots were furrow irrigated regularly to maintain adequate moisture in the soil. Standard cultural practices for regional potato production were applied (Teriessa, 1997) and no pests or diseases of importance were observed.

5.3.3 Treatments

Thirty days after planting (early stolon initiation) the plants were treated with PBZ at rates of 0, 2, 3, and 4 kg active ingredient (a.i.) PBZ ha^{-1} as a foliar application or soil drench using the Cultar formulation (250 g a.i. PBZ per liter, Zeneca Agrochemicals SA (PTY.) LTD., South Africa). To prepare the aqueous solutions PBZ was diluted in distilled water (250 ml $plot^{-1}$). For the foliar treatment, the solution was applied to each plant as a fine foliar spray using an atomizer. While applying the foliar treatment, the soil was covered with a plastic sheet to avoid PBZ seepage to the ground. The drench solution was applied to the soil in a ring around the base of each plant. The control plants were treated with distilled water at equivalent volumes.

5.3.4 Data recorded

Two weeks after treatment application stomatal conductance, rate of transpiration and photosynthesis were measured using a portable LCA4 photosynthesis system (ADC Bio Scientific Ltd., UK) and leaf chlorophyll content was determined. The measurements were made on three randomly selected plants using the terminal leaflets of the 2^{nd}, 3^{rd} and 4^{th}, fully expanded younger leaves. To determine the concentrations of chlorophyll *a* and *b*, spectrophotometer (Pharmacia LKB, Ultrospec III) readings of the density of 80% acetone chlorophyll extracts were taken at 663 and 645 nm, and their respective values were assessed using the specific absorption coefficients given by MacKinney (1941).

Directly after treatment application and two, four, six, and eight weeks after treatment, three randomly selected plants were harvested from each plot. Samples were separated into leaves, stems, tubers, and roots and stolons. Leaf area of photosynthetically active green leaves was measured with a portable CI-202 leaf area meter (CID Inc., Vancouver, Washington state, USA). Plant tissue were oven dried at 72 °C to a constant mass. Dry matter partitioning was determined from the dry mass of individual plant components as a percentage of the total plant dry mass. Plant height was measured from the base of the stem to shoot apex. Days to physiological plant maturity were recorded when 50% of the leaves turned yellow.

5.3.5 Statistical analysis

Analyses of variance were carried out using MSTAT-C statistical software (MSTAT-C, 1991). Means were compared using least significant differences (LSD) test at 1% probability level. Correlations between parameters were computed when applicable. Combined analysis of variance of the two experiments revealed that there was no significant treatment by experiment interaction. Hence, pooled data are presented for discussion.

5.4 RESULTS

There were no significant differences between the foliar spray and soil application with respect to chlorophyll content, stomatal conductance, rate of transpiration, and plant height. Means pooled over methods of application showed that PBZ treatments reduced total leaf area (Figure 5.1). PBZ treatment resulted in a significant height reduction and application of 3 or 4 kg a.i. PBZ ha^{-1} resulted in a mean reduction of 63% in stem length (Table 5.1).

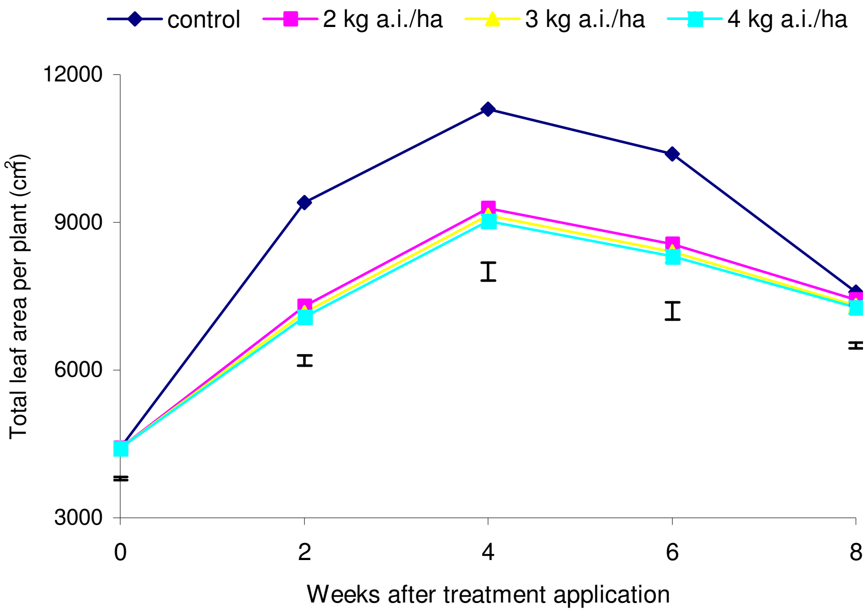

Figure 5.1. Total leaf area of potato plants grown under hot tropical lowland conditions as influenced by rates of PBZ application. The vertical bars represent least significant differences at P < 0.01

The concentrations of chlorophyll *a* and *b* in leaf tissue were significantly increased with PBZ treatments (Table 5.1). Compared to the control, application of 3 or 4 kg a.i. PBZ ha^{-1} increased the chlorophyll *a* content of leaf tissue by an average of 65%. In the same manner, regardless of the concentrations, PBZ treatment increased the chlorophyll *b* content by an average of 55% compared to the control. Total leaf area negatively correlated with chlorophyll *a* (r = - 0.93**) and chlorophyll *b* (r = - 0.97**) content.

Irrespective of the rate of application, PBZ treatment greatly reduced leaf stomatal conductance and rate of transpiration (Table 5.1). The lowest stomatal conductance (0.16 mol m^{-2} s^{-1}) and rate of transpiration (3.78 mol m^{-2} s^{-1}) values were recorded for plants that received 4 kg a.i. PBZ ha^{-1}. In contrast, PBZ treatment enhanced the rate of leaf net photosynthesis, with the highest value observed in plants treated with 3 or 4 kg a.i. PBZ ha^{-1}.

Table 5.1 Chlorophyll *a* and chlorophyll *b* concentrations, stomatal conductance (Gs), rate of transpiration (E), net photosynthesis (Pn) of leaf tissue, and potato plant height as influenced by rates of PBZ application

Rate (a.i. kg ha^{-1})	Chlorophyll *a* (mg g^{-1} FW)	Chlorophyll *b* (mg g^{-1} FW)	Gs (mol $m^{-2} s^{-1}$)	E (mol $m^{-2} s^{-1}$)	Pn (μmol $m^{-2} s^{-1}$)	Plant height (cm)
0 (control)	0.50c	0.15b	0.25a	5.00a	6.47b	77.92a
2	0.68b	0.22a	0.19b	3.97b	7.34ab	33.02b
3	0.81a	0.23a	0.18b	4.08b	8.40a	30.03bc
4	0.84a	0.25a	0.16b	3.78b	8.21a	27.63c
SEM	0.03	0.01	0.02	0.26	0.36	0.81

SEM: Standard error of the mean.

Means within the same column sharing the same letters are not significantly different ($P < 0.01$).

A significant interaction between application method and PBZ application rate was observed for days to physiological maturity (Table 5.2). Compared to the control, regardless of the concentrations, foliar spray of PBZ delayed the onset of senescence by an average of 17 days, while applying 3 or 4 kg a.i. PBZ ha^{-1} as a soil drench delayed the maturity by about 15 days.

Table 5.2 Days to physiological maturity for potato plants grown in a hot tropical lowland as influenced by PBZ application method and rate

Application method	PBZ rate (a.i. kg ha^{-1})			
	0 (control)	2	3	4
Foliar spray	83.00e	100.83a	100.83a	100.00ab
Soil drench	83.17e	97.33d	98.00cd	99.17bc
SEM	0.38			

SEM: standard error of the mean.

Means within columns and rows sharing the same letters are not significantly different ($P < 0.01$).

PBZ significantly affected total dry matter production and assimilate allocation to the different plant parts (Table 5.3). At all harvesting stages PBZ treatment greatly reduced the dry mass of the leaves, stems, and roots and stolons, and increased the tubers. At the first harvest, tubers were present on PBZ treated plants, while the control had not yet initiated tubers. At the second and third harvests, tubers represented about 31 and 36% of the total dry mass of PBZ treated plants, and only 14 and 22% in the case of untreated plants. Correspondingly, at the fourth harvest, the plants treated with 3 or 4 kg a.i. PBZ ha^{-1} had partitioned about 40% of the assimilates to the tubers, compared to 26% in the control. Foliar application of PBZ increased total biomass production more than the soil drench during the third and fourth harvesting periods.

Table 5.3 Total dry matter production (g) and distribution (%) amongst different parts of potato plants grown under a hot tropical condition, as influenced by rate and method of PBZ application

Treatment	Total (g)	Leaves (%)	Stems (%)	Roots & stolons (%)	Tubers (%)	Total (g)	Leaves (%)	Stems (%)	Roots & stolons (%)	Tubers (%)
	Harvest I					**Harvest II**				
Foliar spray	48.9a	43.3a	27.7a	10.1a	18.8a	92.5a	38.3a	24.5a	10.5a	26.7a
Soil drench	46.7a	44.1a	27.2a	10.1a	18.6a	89.0a	39.9a	23.6a	10.2a	27.2a
SEM	0.50	0.52	0.44	0.20	0.31	0.70	0.47	0.31	0.20	0.33
0 (control)	51.3a	53.6a	34.5a	11.9a	0.0c	99.0a	43.5a	29.2a	13.5a	13.7b
2	50.5a	42.4b	25.6b	9.3b	22.7b	90.3b	37.2b	22.7b	9.8b	30.2a
3	46.3b	38.7c	25.1b	9.3b	26.5a	88.6bc	36.7b	22.0b	9.3bc	31.9a
4	43.2c	40.2bc	25.0b	9.4b	25.4a	85.0c	37.0b	22.3b	8.7c	32.a
SEM	0.71	0.75	0.62	0.28	0.44	0.99	0.66	0.44	0.28	0.46
	Harvest III					**Harvest IV**				
Foliar spray	129.7a	34.1b	23.5a	9.6b	32.4a	151.9a	32.4a	23.2a	9.0b	35.1a
Soil drench	124.6b	35.5a	23.0a	9.0a	32.5a	146.9b	33.1b	22.5a	8.4a	36.0b
SEM	0.78	0.26	0.33	0.13	0.28	0.82	0.21	0.32	0.13	0.18
0 (control)	138.0a	39.8a	26.3a	11.9a	22.0b	162.2a	37.3a	25.8a	11.1a	25.8c
2	125.1b	33.3b	22.4a	8.8b	35.5a	146.3b	31.3b	22.1b	8.2b	38.4b
3	124.4b	33.4b	22.3b	8.4b	35.9a	146.3b	31.2b	21.9b	7.7b	39.0ab
4	121.2b	33.4b	22.0b	8.1b	36.5a	142.6b	31.2b	21.6b	7.6b	39.6a
SEM	1.10	0.37	0.47	0.19	0.40	1.16	0.30	0.46	0.18	0.35

Harvest I, II, III and IV done two, four, six and eight weeks after treatment application.

SEM: standard error of the mean.

Means of the same main effect within the same column sharing the same letters are not significantly different (P < 0.01).

5.5 DISCUSSION

PBZ is a potent synthetic plant growth regulator and at low concentrations induces physiological, anatomical and morphological changes in plants. The most striking growth response of potato to PBZ treatment was reduced shoot growth. Treated plants were short and compact due to the reduction in total leaf area and stem elongation. Davis & Curry (1991) reported that depending on the species and cultivar, PBZ reduced shoot growth mainly by reducing internode length. It is postulated that reduced GA synthesis in response to PBZ treatment may have resulted in a reduction in cell proliferation leading to a reduction in stem elongation and leaf expansion. In support of this postulation, Haughan *et al.* (1989) reported that the 2R configuration of PBZ greatly retarded cell proliferation in celery. PBZ effectively suppressed growth in a wide range of plant species and the treated plants tended to be darker, and more compact in appearance (Kamoutsis *et al.*, 1999; Terri & Millie, 2000; Sebastian *et al.,* 2002).

The foliage of PBZ treated potato plants typically exhibited a dark green colour compared to the control. This may be due to an increase in chlorophyll content of the leaves either as the result of enhanced chlorophyll synthesis and/or the presence of more chloroplasts per unit leaf area of treated leaves. The observed negative correlation between total leaf area and chlorophyll content indicate that the reduction in total leaf area in response to PBZ treatment contributed to the increased chlorophyll *a* and *b* content. Balamani & Poovaiah (1985) and Bandara & Tanino (1995) also reported an increased chlorophyll concentration in potato leaves in response to PBZ treatment. Increased chlorophyll synthesis due to PBZ treatment was reported in *Dianthus caryophyllus* (Sebastian *et al*., 2002). Investigations undertaken by Khalil (1995) on cereals showed the existence of more densely packed chloroplast per unit leaf area in response to PBZ treatment.

The higher chlorophyll content and delayed senescence in the treated potato leaves may be related to the influence of PBZ on endogenous cytokinins. It has been proposed that PBZ stimulates cytokinin synthesis which increases chloroplast differentiation and chlorophyll biosynthesis, and prevents chlorophyll degradation (Fletcher *et al.,* 1982). Investigations on rice (Izumi *et al.,* 1988), soybean (Grossman, 1992) and *Dianthus caryophyllus* (Sebastian *et al.,* 2002) showed that exogenous application of GA biosynthesis inhibitors increased cytokinin content of plant tissues. The onset of senescence was considerably delayed with the aid of triazoles in several plant species and treated leaves were retained longer than the untreated leaves (Davis & Curry, 1991; Binns, 1994).

PBZ treatments significantly reduced the rate of transpiration in potato leaves. This could be due to the partial closure of stomata in response to PBZ treatment as shown in the concomitant reduction in stomatal conductance. It is postulated that the reduction in stomatal conductance in response to PBZ treatment may have been mediated through its effect on the endogenous ABA content (Rademacher, 1997), as ABA is involved in regulating the opening and closing of stomata (Salisbury & Ross, 1992). Asare-Boamah *et al*. (1986) observed a reduction in the rate of transpiration, increased diffusive resistance and a transient rise in ABA levels in response to triazole treatment. This response may improve the drought tolerance of potato plants. PBZ treatment has been shown to reduce water loss and improve water use efficiency in grapevine, *Chrysanthemum*, and beetroot (Ritchie *et al.,* 1991; Smith *et al.,* 1992; Roberts & Mathews, 1995).

In contrast to its effect on stomatal conductance, PBZ increased photosynthetic efficiency. This response could be linked to the increase in chlorophyll concentration and earlier tuberization. Previous studies on carbon fixation and allocation in various crops showed that the source: sink balance influence the rate of photosynthesis in such a way that an increased sink demand increased the rate of photosynthesis and a decreased sink demand decreased photosynthesis (Geiger, 1976; Hall & Milthorpe, 1978; Peet & Kramer, 1980). A similar interaction has been observed in the potato. Nosberger & Humphries (1965) reported that removal of growing tubers reduced the rate of net photosynthesis, while tuber initiation increased the rate of net photosynthesis (Moorby, 1968; Dwelle *et al.,* 1981a). Similarly, Basu *et al*. (1999), from a tuber detachment experiment reported that within 6 hours of tuber removal, light saturated rates of net photosynthesis declined from 22 µmol m^{-2} s^{-1} to a value close to zero. Increased net photosynthesis in response to PBZ treatment has been reported in soybean (Sankhla *et al.,* 1985) and rape (Zhou & Xi, 1993). Reduced stomatal conductance did not lead to reduced net photosynthesis. This may be related to PBZ induced modification of the photosynthetic tissue (mesophyll) that may have allowed better diffusion of CO_2 to carboxylation sites. De Greef *et al.* (1979) reported that rate of photosynthesis increased as the mean cell size increased, because bigger mesophyll cells have larger surface to volume ratio. Microscopic observation showed that PBZ increased the size of epidermal, palisade and spongy mesophyll cells of potato leaves (Chapter 4).

PBZ affected the overall pattern of carbon fixation and assimilate partitioning to the different potato organs. Tubers were the dominant sinks that attracted the highest proportion of dry matter relative to the leaves, stems, roots and stolons. This dominance may be linked to low GA concentrations in tubers due to PBZ treatment, thus increasing tuber sink strength. This postulations is based on results by Menzel (1980) and Mares *et al.* (1981) who reported that exogenous GA_3 application inhibited tuber formation; decreased tuber sink strength and encouraged shoot and stolon growth. High temperatures decrease tuber growth rate, reduce the partitioning of assimilates to the tubers and increase assimilation to other parts of the plant probably associated with high GA levels (Menzel, 1980; Struik *et al.,* 1989; Vandam *et al.*, 1996).

5.6 CONCLUSION

The field trials indicated that PBZ treatment increased leaf chlorophyll content and enhanced the rate of net photosynthesis. PBZ potentially reduce water demand reducing leaf area, and stomatal conductance and the rate of leaf transpiration. PBZ also reduced shoot growth and increased partitioning of assimilates to the tubers. There was no difference between foliar spray and soil drench for most of the parameters considered.