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EFFECTS OF GROWTH RETARDANTS, PACLOBUTRAZOL (PBZ) AND PROHEXADIONE-CA ON FLORAL INDUCTION OF REGULAR BEARING MANGO (*Mangifera indica* L.) CULTIVARS DURING OFF-SEASON

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ABSTRACT

Off season (out of season) floral induction will extend the season of mango production and increase farmers' income. Accordingly, the aims of this study were to investigate the roles of two gibberellins biosynthesis inhibitors, paclobutrazol and prohexadione-Ca; on off season flowering of regular bearing mango cultivars as well as to examine the possible associated hormonal changes. Paclobutrazol experiment was conducted on two orchards while prohexadione-Ca experiment was conducted on one orchard; all are located under tropics of central Sudan (Latitude 14° 23', Longitude 29° 33'). Our results implicated the positive role of paclobutrazol on floral induction of the two regular bearing mango cultivars, Baladi AbuZaid and Baladi Burai during off season. Similarly, prohexadione-Ca was effective in advancing off season flowering of the regular bearing mango cultivar, Baladi Elriah. The levels of cytokinins (zeatin (z) + zeatin-riboside (zr) and isopentenyl Adenine (i-Ade) + isopentenyl Adenosine (i-Ado) were generally increased during the floral induction period, while those of gibberellins and auxin (IAA) were decreased during the same period. Our results might implicated the possibility of inducing out of season flowering and thus extending the season of mango harvest under central Sudan conditions.

Keywords: floral induction, mango cultivar, paclobutrazol, auxin (IAA), cytokinins (z+zr and i-Ade+i-Ado), gibberellins, off season.

INTRODUCTION

Mango is one of the most popular tropical and subtropical fruit trees in the world. Although the center of origin of mango (*Mangifera indica* L.) is still controversial, it is generally accepted that India is its center of origin, from which it spread to many tropical and subtropical areas of the world (Mukherjee, 1997). The Sudanese types are mainly Indian introductions, which entered Sudan through Egypt at the turn of the twentieth century (El Mardi *et al*., 1984).

In Sudan, selection on mango was practiced for regular bearing types since its first introduction into the country. Accordingly, most of today's grown cultivars are more or less of regular flowering nature. However, the need arise for extending the season of mango production, by inducing off season flowering, to meet the local demand, improve human nutritional balance and increase farmers' income. Moreover, with the presence of different ecological zones in a country like Sudan, the possibility of extending the season of mango production is there and deserved to be attempted.

Paclobutrazol (PBZ), triazole group, is the most commonly used growth retardant for floral induction in fruit trees. However, it seemed that there were few or no trials that correlated the effect of PBZ on mango flowering during off season with hormonal changes under tropical conditions. Prohexadione-Ca, which is a gibberellins biosynthesis inhibitor and a growth retardant, with a short residual effect, is potentially another option of flower induction treatments, although it is less widely recognized as such (Owen and Stover, 1997; Greene, 1997).

Not much is known about the internal factors that underline the floral induction response in fruit trees in general and mango in particular. More specifically, little is known about the hormonal factors that are associated with floral induction response in mango particularly under tropical conditions. Discerning the nature of the internal factors which are involve in the process of floral induction is indispensable if further improvement in mango production is to be attained.

The current study was mainly directed towards investigating the possible role of two plant growth retardants, viz. PBZ and prohexadione-Ca on floral induction of mango during off-season (out of season). The specific objectives of this study were; to test the effect of PBZ and prohexadione-Ca, on floral induction of regular bearing mango cultivars during off season under Sudan conditions (Arid tropics) and to determine the possible hormonal factors which are associated with floral induction in mango under Central Sudan climates.

MATERIALS AND METHODS

Experiment I

This experiment was conducted at two orchards along the eastern bank of the Blue Nile River, Wad Medani (Latitude 14° 23', Longitude 29° 33' and Altitude 405m), Sudan, with the objective of inducing out of season flowering on regular bearing mango cultivars. The soils at the experimental sites were medium textured clay loam to silty clay loam, slightly alkaline and normally recognized as good fertile soils. Since the local (Baladi) cultivar, originally known as Kitchener, is a ploy embryonic cultivar propagated by seeds, each cultivar was named after the orchard's owner, viz., Baladi AbuZaid and Baladi Burai. It is worth mentioning that normally only seedlings of asexual embryos are selected and used in mango propagation.

Each experimental unit consisted of 5 trees. The experimental units, either treated with PBZ or left untreated, were arranged in a randomized complete block design with three replications. Paclobutrazol (10% a.i. (active ingredient)) was applied as soil drenching at a rate of 2.5 g a.i/m^2 on July 17, 2003. It is worth mentioning that the normal (regular) mango flowering season under central Sudan climates is November.-January. Data on maximum and minimum temperatures, rainfall and relative humidity were collected.

Leaf and bud samples were collected for hormone analyses on weekly basis starting from the time of PBZ application and continued for eight weeks. Samples were collected from mature shoots on all directions and at different heights of each tree. For hormones analyses, leaf and bud samples were collected, immediately immersed in liquid nitrogen and then stored at -20^{o}C. Samples were then freeze-dried before analysis. Samples were analyzed for cytokinins, gibberellins and auxin (Weiler, 1981 and Bohner and Bangerth, 1998).

Radio-Immnuno-Assay (RIA-^{3}H hormone, serum and antibody) was used for determination of cytokinins (zeatin+zeatin ribosides and isopentenyl Adenosine + isopentenyl Adenine), Auxin (IAA) and gibberellins (GA_{1+3+20}) according to Bohner and Bangerth (1998) 0.3-0.5 g of the homogenized freeze-dried samples was extracted in 80% methanol and internal standard of 2400dpm 1-^{14}C-IAA was added to samples at extract filtration stage. The extracted samples were purified by passing through a pre-conditioned column. The pre-conditioned column is a combination of Polyvinylpyrrolidone (PVP; Sigma Chemical Co., Deisenhofen, Germany) and DEAE-Sephadex-A25 (Amersham Bioscienes AB, Uppsala, Sweden). The column was conditioned by 15 ml 0.1 and 20 ml 0.01M ammonium acetate at pH of 8.5 and 7.5, respectively. C_{18} Sep-Pak cartridge (Waters, Eschborn, Germany) was adjusted to the pre-conditioned column for hormones trapping before elution using different elute solutions depending on the hormone to be eluted. The column was modified according to Bertling and Bangerth (1995). Aliquots of the purified hormones were placed into small vials in triplicates and evaporated in a vacuum concentrator. The dry purified hormone samples of the acidic hormones were methylated with few drops of diazomethane (around 50µl) while those of cytokinins were not. Following different steps of samples preparation, including addition of buffer, serum, labeled hormones and antibodies and precipitation of the binded hormones by ammonium sulphate (50% and 90%), ending with addition of scintillation solution, the concentrations of different hormone samples were measured using Scintillation counter.

Experiment II

Similar to experiment I, experiment II was conducted on a local regular bearing mango cultivar, Baladi Elriah. Soil type and weather data of the experimental site were similar to those of the paclobutrazol experiments mentioned above.

The prohexadione-Ca (15% a.i.) was applied twice as foliar spray at the rate of 0.5 g a.i. /L each, with two weeks time elapsed between the two applications. The first dose of prohexadione-Ca was applied on July 18, 2003. The experimental unit consisted of 5 trees. The experimental units, either treated with prohexadione-Ca or left untreated, were assigned to a randomized complete block design with three replications. Leaf and bud samples were collected in liquid nitrogen for hormonal analysis. Sample collection procedures were similar to that of experiment I. For details of the sample collection procedures for hormone analysis refer to experiment I.

RESULTS

Experiment I

Since mango flushing in tropics does not occur simultaneously, the percentage flowering were observed three times during the experimental period (Table-1). Paclobutrazol advanced off season (out of season) flowering of regular bearing mango cultivars, Baladi AbuZaid (26.7%) and Baladi Burai (30.7%) by almost 60 and 70 days, respectively, as compared to the control. Moreover, the control trees did not started flowering even when more than 60% of the PBZ treated trees from both tested cultivars had already flowered (Table-1). Although the minimum temperature did not fall below 20°C up to November, 2003, the PBZ treated started flowering early October, 2003 (Figure-1 and Table-1).

Table-1. Effect of paclobutrazol (PBZ) on flowering percentages of regular bearing mango cultivars during off season (autumn, 2003).

Cultivar	**Date of flowering**									
	4.10.03		**15.10.03**		**1.11.03**		**15.11.03**		**15.12.03**	
	PBZ	Control	PBZ	Control	PBZ	Control	PBZ	Control	PBZ	Control
Baladi AbuZaid	26.7%	0%	40%	0%	60%	0%	66.7%	0%	80%	20%
Baladi Burai	0%	0%	30.7%	0%	46.20%	0%	61.6%	0%	100%	40%

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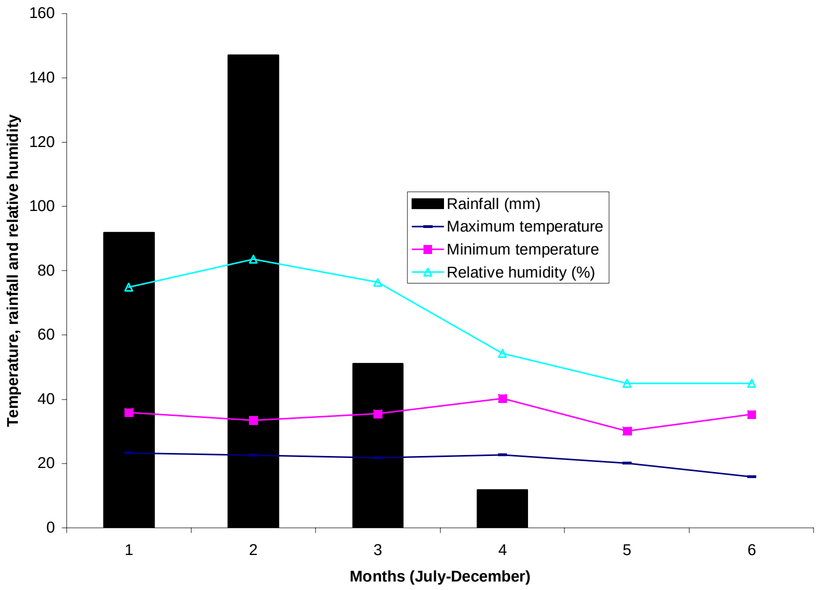

Fig. 1. Maximum and minimum temperatures, rainfall, and relative humidity during the period July-Dec., 2003 (off season).

The recovery of the internal standard used in analysis of hormones was above 85%. Paclobutrazol (PBZ) generally increased cytokinins concentrations in PBZ treated trees of both cvs. Baladi AbuZaid and Baladi Burai. For instance, bud z+zr and i-Ado+i-Ade concentrations in PBZ treated trees of cvs. Baladi AbuZaid and Baladi Buraa were higher than those of the control trees especially towards late sampling dates (Figures 2A-2D).

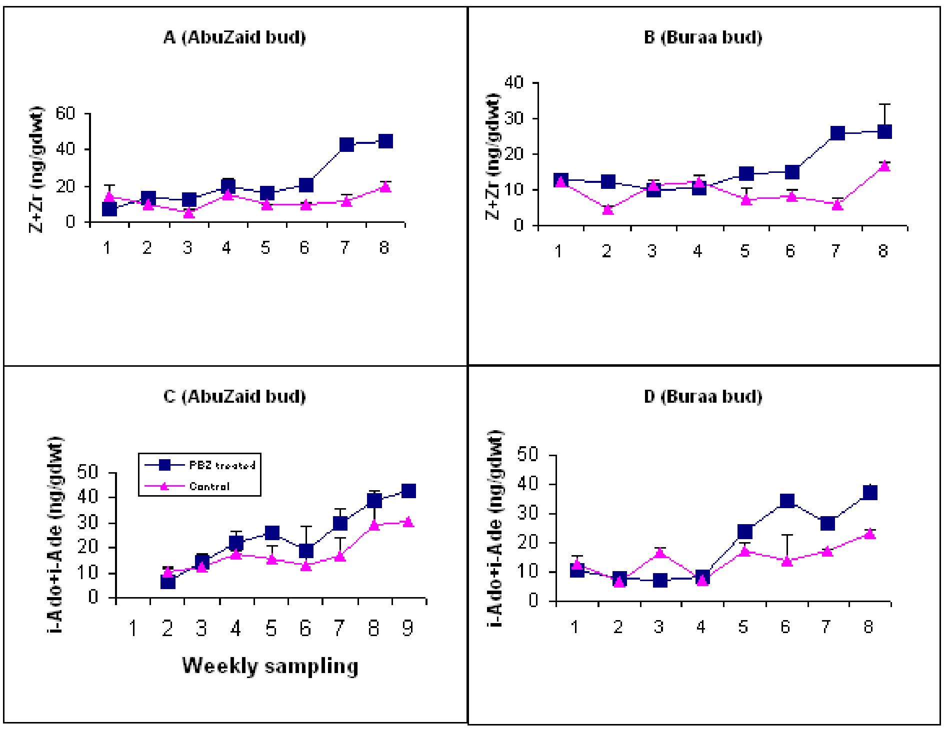

Fig. 2. Effect of paclobutrazol (PBZ) on bud (A,B) and leaf (C,D) zeatin and zeatin riboside (z+zr) and isopentenyl adenosine and isopentenyl adenine (i-Ado+i-Ade) contents of mango cultivars, AbuZaid and Buraa. Bars=Standard error. ng/gdwt= nanogram/gram dry weight.

VOL. 6, NO. 3, MARCH 2011 ISSN 1990-6145
ARPN Journal of Agricultural and Biological Science

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Moreover, the concentrations of z+zr and i-Ado+i-Ade in the leaves of the PBZ-treated trees of cvs. Baladi AbuZaid and Baladi Buraa were above those of the control trees, especially towards late sampling dates (Figures 3A-3D).

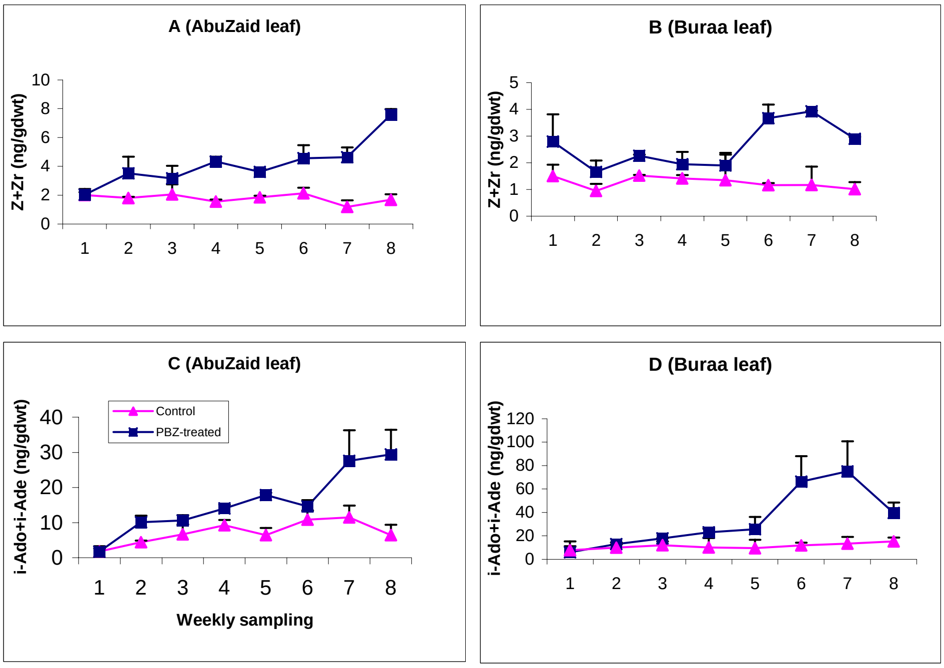

Fig. 3. Effect of paclob butrazol (PBZ) on leaf zeatin and zeatin riboside (A,B) and isopentenyl adenosine and isopentenyl adenine (C,D) contents of mango cultivars, AbuZaid and Buraa

Gibberellins levels in the buds of cvs. Baladi AbuZaid and Baladi Buraa were lower in PBZ-treated trees compared to non-treated trees (Figures 4A and 4B). Similarly, gibberellins levels in the leaves of the control trees of cvs. Baladi AbuZaid and Baladi Buraa were above those of the PBZ-treated trees throughout the sampling period and the differences increased towards late sampling dates (Figures 4C and 4D).

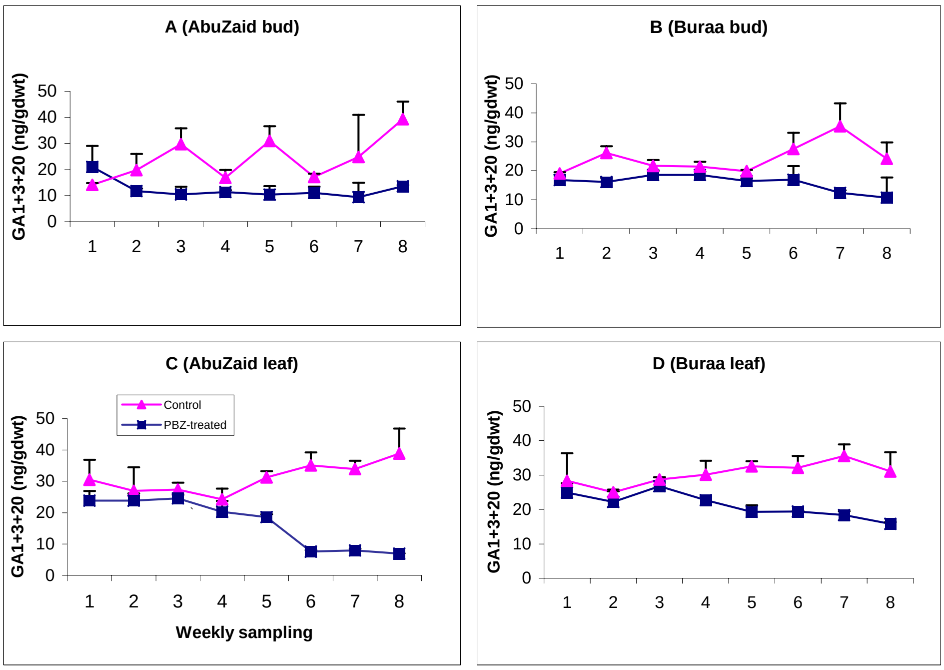

Fig. 4. Effect of paclob butrazol (PBZ) on bud (A,B) and leaf (C,D) gił ibberellins (GA1+3+20) contents of mango cultivars, AbuZaid and Buraa

VOL. 6, NO. 3, MARCH 2011 ISSN 1990-6145
ARPN Journal of Agricultural and Biological Science

Bud IAA levels in the control trees of cvs. Baladi AbuZaid and Baladi Buraa were generally more or less higher than those found in PBZ-treated trees (Figures 5A and 5B). Leaf auxin contents of the control trees of cv. Baladi AbuZaid were above those of the PBZ-treated trees throughout the sampling period, except at the first sampling week (Figure-5C). For cv. Baladi Burai, obvious increases in IAA levels in the leaves of the control trees were observed towards the fourth sampling week and on ward as compared to the PBZ-treated trees (Figure-5D).

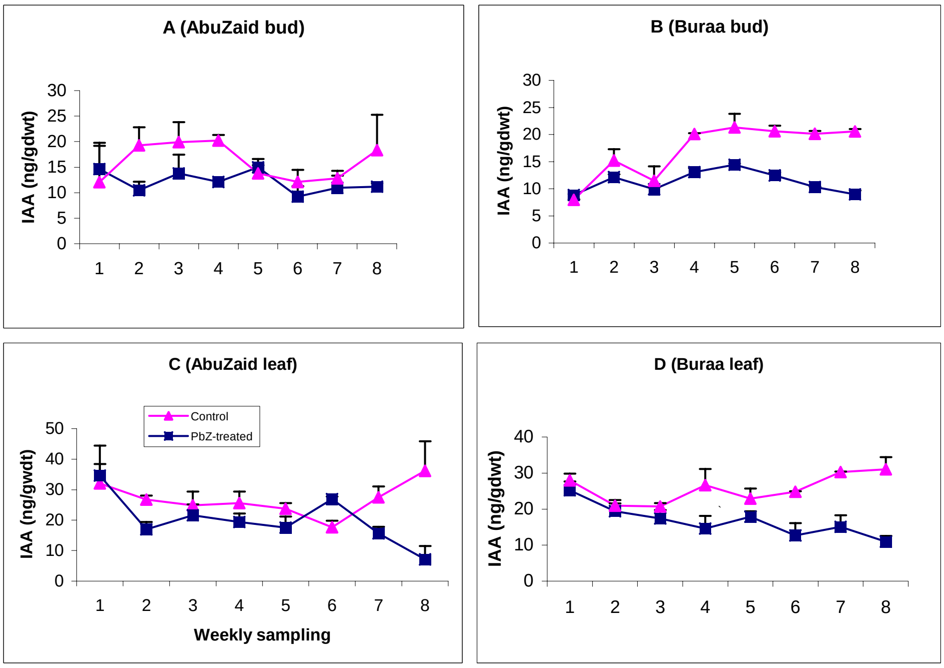

Fig. 5. Effect of paclobutrazol (PBZ) on bud (A,B) and leaf (C,D) auxin (IAA) con ontents of mango mango cultivars, AbuZaid and Buraa.

Experiment II

Prohexadione-Ca treatment advanced flowering by almost 40 days as compared to the control (Table-2). Moreover, when flowering percentage of the prohexadione-Ca treated trees of cv. Baladi Elriah was above 80% still the control trees did not flower.

Table-2. Effect of prohexadione-Ca (Prohex.) on percentage flowering during off-season of the regular bearing mango cultivar, Baladi Elriah (autumn, 2003).

Cultivar	Dates of flowering observations									
	4.10.03		**15.10.03**		**1.11.03**		**15.11.03**		**15.12.03**	
	Prohex.	Control	Prohex.	Control	Prohex.	Control	Prohex.	Control	Prohex.	Control
Baladi Elriah	30.7%	0%	68.6%	0%	84.6%	0%	92.3%	20%	100%	50%

Prohexadione-Ca induced noticeable increases in bud z+zr and i-Ado+i-Ade levels, especially towards the second and late sampling dates (Figures 6A and 6B). Moreover, the levels of z+zr and i-Ado+i-Ade in the leaves of the prohexadione-Ca treated trees were higher those of the control trees throughout the sampling period (Figures 6C and 6D).

VOL. 6, NO. 3, MARCH 2011 ISSN 1990-6145
ARPN Journal of Agricultural and Biological Science

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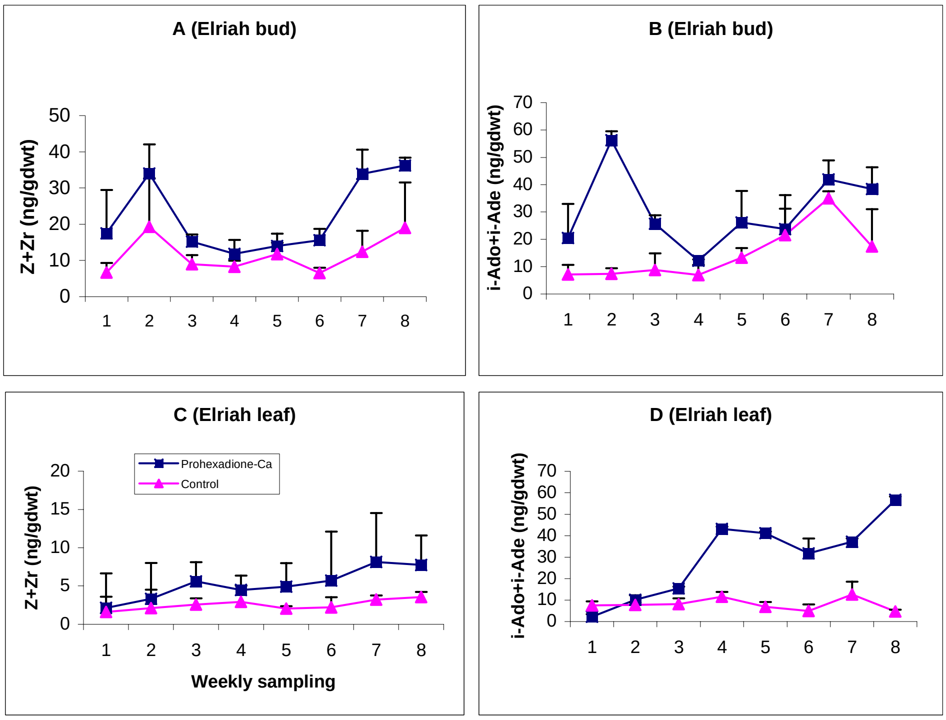

Fig. 6. Effect of prohexadione-Ca on bud (A,B) and leaf (C,D) zeatin and zeatin riboside (z+zr) and and isopentenyl adenosine and isopentenyl adenine (i-Ado+i-Ade) contents of mango cultivar Elriah during off season.

Prohexadione-Ca treatment created remarkable differences in buds and leaves GAs contents between treated and control trees of cv. Elraih (Figures 7A and 7B).

IAA levels in buds and leaves of cv. Elriah were generally reduced by prohexadione-Ca treatment (Figures 7C and 7D).

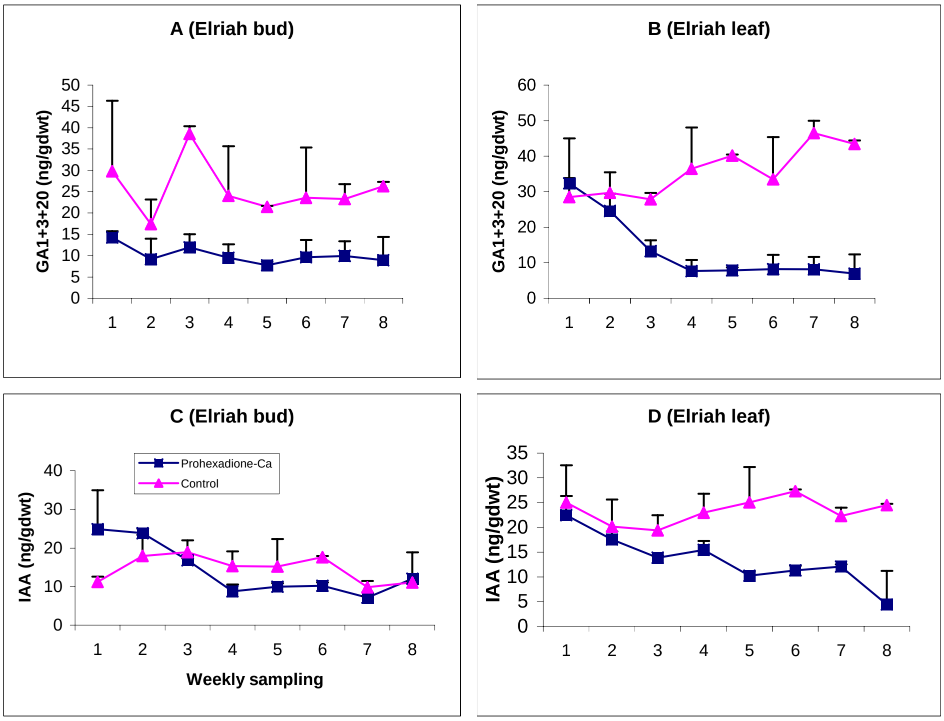

Fig. 7. Effect of prohexadione-Ca on bud and leaf gibberellins (A,B) and auxin (B,C) contents of mango cultivar Elriah during off season.

DISCUSSIONS

Experiment I

Paclobutrazol advanced flowering during off-season (out of season) of the regular bearing mango cultivars, Baladi AbuZaid and Baladi Burai by 60 and 70 days, respectively. Since flowering in the PBZ treated trees started at minimum temperature of above 20°C (October, 2003, Figure-1), PBZ seemed to substantiate the effect of marginal mango flowering temperature (slightly above or equal 20°C) resulting in early flowering. Similar results were reported elsewhere (Winston, 1992; Kurian and Iyer, 1993; Salazar-Garcia and Vazquez-Valdivia, 1997; and Perez-Barraza *et al*., 2000).

The trends of changes, which started less sharper but diverge later, for bud z+zr, i-Ado+i-Ade, GA_{1+3+20}, and IAA for both the PBZ experiments on cvs. Baladi Abu Zaid, and Baladi Burai, was similar to those found in the biennially bearing mango cultivars mentioned in the first manuscript (Figures 2A-5D). However, it seemed that the uptake and thus the action of PBZ were probably delayed by the heavy rains and high relative humidity during the rainy season of 2003. This was clearly demonstrated by the delayed reduction of gibberellins levels in the leaves of PBZ treated trees of both tested cvs. Baladi AbuZaid and Baladi Buraa by almost one month (Figures 4C and 4D). Moreover, evident variations in bud z+zr and i-Ado+i-Ade, IAA for both PBZ experiments on cvs., Baladi AbuZaid and Baladi Burai, were mostly observed towards late sampling dates (Figures 2A-3D, 5A, 5B). In short, the trends of changes in the above mentioned classes of hormones were similar to those found during floral induction of the PBZ experiments on biennially bearing mango cultivars in the first manuscript. Moreover, the high recovery percentage (above 85%) of the internal standard indicated that the measured differences in hormonal concentrations were reliable. For further discussion refer to first manuscript.

Experiment II

Prohexadione-Ca is a relatively new growth regulator, with a short residual effect lasting for only a few weeks (Evans *et al.*, 1997). Accordingly, unlike PBZ, its use for controlling plant vegetative growth and floral induction will alleviate the risk of the potential impact of residuals on underground water, environment, and human health. In this experiment, prohexadione-Ca advanced the flowering of the regular bearing mango cultivar by more than 30 days. These results are in agreement with those reported by Elfving *et al*., (2003) and Owen and Stover (1997; 1999).

The concentrations of z+zr and i-Ado+i-Ade in the buds of the prohexadione-Ca treated trees were increased and exhibited at least one sharp peak of increase as compared to those of the control trees following the application of prohexadione-Ca (Figures 6A and 6B). These results reflected the immediate uptake and absorption of prohexadione-Ca by the mango tree and thus the timely mode of action of the growth retardant on mango tree's hormonal balance. Moreover, these results are in agreement with other reported results on positive effect of prohexadione-Ca on plant cytokinins (Grossmann *et al*., 1991).

The levels of gibberellins were notably lower in buds of the prohexadione-Ca treated trees as compared to the control trees throughout the sampling period (Figure-A). These results are in agreement with the well documented effects of prohexadione-Ca on inhibition of gibberellins biosynthesis (Evans *et al*., 1997; Owens and Stover, 1997; Grossmann *et al*., 1991; Hedden *et al*., 1989). Although these results contradicted those reported by Naphrom *et al*., (2004), where prohexadione-Ca increased gibberellins concentrations, it is difficult to interpret such results for Naphrom *et al*., (2004) since no repeatable applications of prohexadione-Ca were carried out. Ishizue *et al*., (1992) reported that prohexadione-Ca retarded gibberellins biosynthesis in rice by inhibiting of 3β hydroxylation step. They further indicated that GA_1 and GA_{44} levels were decreased while GA_{20} and GA_{19} levels were increased after 5 and 20 days following Prohexadione-Ca treatment. However, the rate of increase in GA_{20} levels that was induced by a prohexadione-Ca dose of Img/m^2 was lower after 12 days compared to that measured after 5 days (Ishizue *et al*., 1992). This might implicated the reduction in the rate of GA_{20} biosynthesis as a result of accumulation and thus a feed back mechanism. Accordingly, long term monitoring of GA_{20} levels following prohexadione-Ca treatment might confirm this theory.

Indole-3-acetic acid levels of the prohexadione-Ca treated trees were generally below those of the control trees (Figure-7C). However, a positive effect of prohexadione-Ca on IAA levels in oilseed rape and pumpkin were reported by Grossmann *et al*., (1991). Accordingly, it is difficult to conclude a direct role for prohexadione-Ca on reduction of IAA levels of the treated trees. Nevertheless, these conflicting results could only be explained by the interactions between IAA and gibberellins reported by Ross *et al*., (2002; 2003).

In this experiment, the variations in hormonal concentrations not only appeared earlier as compared to those of the PBZ experiments on mango cultivars, Baladi AbuZaid and Baladi Burai but were also of higher magnitude and ratios and were comparable to those found in the biennially bearing mango experiments discussed in first manuscript. Reports on the effect of prohexadione-Ca on floral induction of crop plants in general and fruit trees in particular are meager. Nevertheless, the role of prohexadione-Ca, as a gibberellins biosynthesis inhibitor and a growth retardant is well documented by Ishizue *et al*., (1990), Ishizue *et al*., (1992), Greene (1997), Owens and Stover (1999), Unrath (1999), and Evans *et al*., (1999). Prohexadione-Ca promoted flowering of apple cultivar 'Macoun' (Owens and Stover, 1999), and sweet cherry in combination with ethylene (Elfving *et al*., 2003), with a three fold increase in flowering over the control. However, Naphrom *et al*., (2004) reported only a slight advantage for prohexadione-Ca on mango flowering over the control but not over the PBZ-treated mango trees. It is worth mentioning that prohexadione-Ca treatment for both apple (Owens and Stover, 1999) and mango (Naphrom *et al*., 2004) was applied only once. Since prohexadione-Ca has a short residual effect, with a half life span that lasts for only a few weeks (Evans *et al*., 1999), a single, non repeatable application was probably not enough to trigger and/or maintain the critical hormonal balance needed to stimulate floral induction.

CONCLUSIONS

Similar to the results of the biennially bearing mango cultivars in the first manuscript, cytokinins (z+zr and i-Ado+i-Ade) were increased, while gibberellins and auxin were decreased by the application of both paclobutrazol and prohexadione-Ca on regular bearing mango cultivars during off-season. Accordingly, these results might indicate possible roles for these classes of phytohormone on floral induction of mango. However, further work on the role of phytohormones on flowering of mango using different floral induction systems (pruning, girdling and water stress) is to be carried out before any generalization on the role of phytohormones on mango flowering could be made.

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