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EFFECTS OF PACLOBUTRAZOL (PBZ) ON FLORAL INDUCTION AND ASSOCIATED HORMONAL AND METABOLIC CHANGES OF BIENNIALLY BEARING MANGO (*Mangifera indica* L.) CULTIVARS DURING OFF YEAR

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ABSTARCT

The physiology of floral induction in mango is still controversial and thus further work is needed for a better understanding of reproductive physiology of this important fruit tree. The objectives of this study were to investigate the role of a gibberellins biosynthesis inhibitor, paclobutrazol on floral induction of biennially bearing mango during off year and to examine the possible correlated hormonal and non-structural carbohydrate changes. Three distinctly biennially bearing mango cultivars were tested for two years under North Sudan climates. Results indicated the advantage of paclobutrazol on inducing flowering of the biennially bearing mango cultivars, Miska, Mahmoudi, and Totocombo during off year. Similar trends of hormonal changes were observed during the floral induction period on the tested cultivars. More-specifically, the levels of cytokinins (zeatin (z) + zeatin riboside (zr) and isopentenyl Adenosine (i-Ado) + isopentenyl Adenine (i-Ade)), and to a less extent the levels of abscisic acid (ABA) generally showed trends of increase during the floral induction period, while those of gibberellins (GA_{1+3+20}) and auxin (IAA) were decreased during the same period. Starch levels in most of the cases were increased by the paclobutrazol treatment. Moreover, sucrose levels were generally increased during the floral induction period. To close, possible roles for some of the tested hormones and non-structural carbohydrates on mango flowering are probably implicated.

Keywords: paclobutrazol, floral induction, biennially bearing mango, ABA, cytokinins (z+zr and i-Ado+i-Ade), gibberellins (GA_{1+3+20}), IAA, starch, sucrose.

INTRODUCTION

World production of mango has increased by more than 60% during the period 1983-2007 (FAO, 2008). Although the area grown by mango is almost doubled during the same period, the yield per unit area has decreased by 14% (FAO, 2008). A better understanding of the nature of flowering induction in mango is necessary not only for yield sustainability but also for yield increase.

Mangoes are generally induced to flower during October-December in the northern hemisphere and during June-August in the Southern hemisphere. However, irregularity of flowering in mango, which varied in the time and intensity of flowering from year to year to almost complete biennial (alternate) flowering habit, is not an uncommon phenomenon. Accordingly, the un-raveling of the nature of flower triggering and signaling elements is of utmost significance if further improvement in mango fruit trees production is to be achieved. Advances in understanding of the molecular genetic of floral induction in annuals, e.g. Arabidopsis (Mitsutomo *et al*., 2005) and temperate trees, e.g. Aspen trees (Bohleius *et al*., 2006) and grapevine (Boss *et al*., 2006) will definitely shade some light on our understanding of flowering of tropical fruit trees. Different models were proposed to explain the phenomenon of floral induction in general (Sachs, 1977; Bernier, 1988; Davenport and Nunez-Elisea, 1997; Davenport, 2000; Kulkarni, 2004; Davenport *et al*., 2006 and Davenport, 2007). Nevertheless, a single model that can fully explain the floral induction process for even a single species is yet to be proved, the fact that reflects the intricate nature of floral induction in general and of fruit trees in particular (Wilkie *et al*., 2008).

Although low temperature (around 15°C) is considered as the most important flower trigging element in mango still biennially bearing mango cultivars usually do not flower during off year even under low temperature conditions. In such circumstances, growth retarding chemicals, e.g. triazoles group (paclobutrazol, PBZ), that can stimulate or mimic the effects of the environmental factors in checking vegetative growth are some times used to correct such a situation (Nartvaranant *et al.*, 2000). However, it seemed that there were few or no trials that correlate the effect PBZ on mango flowering with metabolic and hormonal change under tropical conditions. More specifically, little is known about the tree internal factors such as plant phytohormones and metabolic (assimilates) that are probably related to floral induction response in tropical fruit trees in general and mango in particular. Accordingly, the current study was conducted with a broad objective of investigating the possible role of plant growth retardants, paclobutrazol (PBZ) on floral induction of biennially bearing mango cultivars on off year. The specific objectives of this study were: to test the effect of paclobutrazol on floral induction of biennially bearing mango cultivars during off year under Sudan conditions (Arid tropics); to determine the possible

hormonal and metabolic factors that are associated with floral induction of mango under Sudan climates: and to explore the nature of a model for floral induction of mango under Sudan climates.

MATERIALS AND METHODS

In this experiment the effects of PBZ on flowering of biennially bearing mango cultivars (cvs.) were studied using three cultivars, viz., Mahmoudi (dwarf size tree stature), Miska (large size tree stature) and Totocombo (intermediate size tree stature) grown at the mango collection orchard, Horticultural Research Department, Hudieba Research Station, Agricultural Research Corporation, Sudan (Lat. 17° 34', Long. 33° 56' and 355m a. s. l). The soils of the experimental site are classified as Bauga series having dark brown, moderately well-drained with columnar structure, shallow calcic sandy loam and strongly alkaline subsoil (pH>8.0). The experiment was conducted during normal mango flowering season in north Sudan (December-January = dry winter season months) for two years, 2002 and 2003. Cultivars, Mahmoudi and Miska were used in the year, 2002, while Totocombo and Miska were used in the second year, 2003. The three tested cultivars have a distinct alternate bearing habit and no flowering takes place during off year. It was a 15 years record for individual trees from each cultivar. Only trees from a cultivar that are expected to be on off year were used. The experimental unit, consisted of three trees, were randomly either treated with PBZ or left un-treated in a randomized complete block design with three replications. Paclobutrazol (25% active ingredient) was applied as soil drenching at the rate of 2.5 g a.i/m^2 followed by application of sufficient irrigation water and irrigation was withhold thereafter for three weeks .

Sampling of plant tissues, leaves and buds, for the first year started one month after PBZ application, November17, 2001, to allow for the uptake of PBZ by the tree (since PBZ need three months to induce flowering in mango as reported else where, Nartvaranant *et. al*., 2000). Moreover, coincidence of beginning of sampling with the postulated floral induction period was another reason for delayed sampling. However, for the second year, sampling started at PBZ application time, January 15, 2003. The late application of PBZ in the second year experiment was to assure that the tested trees were on off year.

For hormone analyses, leaf and bud samples were collected from mature shoots, 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, auxin and abscisic acid (Weiler, 1981; 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), gibberellins (GA_{1+3+20}), and Abscisic Acid (ABA) 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 extraction 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 0.1M 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) regarding hormone elution. Aliquots of the purified hormones from each sample 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 anti-bodies followed by precipitation of the binded hormones by ammonium sulphate, ending with addition of scintillation solution, the concentrations of different hormone samples were measured using Scintillation counter.

For carbohydrates determination fresh leaf samples were collected and immediately oven dried at 75^{o}C for at least 48 hours. Sucrose and reducing sugars were quantified according to Blakeney and Mutton (1980) using the PAHBAH method. Starch quantification was done using the Anthrone reagent method (Yemm and Willis, 1954), except of using of α-amylase enzyme for starch digestion.

RESULTS

Flowering percentage in the PBZ-treated trees were 50%, and 100% at 60, and 90 days, respectively, after PBZ application for all tested cultivars, while the control trees did not flower (Table-1).

Table-1. Effects of paclobutrazol (PBZ) on percentage flowering of biennially bearing mango cultivars, Miska, Mahmoudi and Totocombo during years 2002 and 2003.

Cultivar	**Flowering percentage (Year 2002)**			
	60 days after PBZ treatment		90 days after PBZ treatment	
	PBZ treated	Control	PBZ treated	Control
Miska	50%	0.0%	100%	0.0%
Mahmoudi	50%	0.0%	100%	0.0%
	Flowering percentage (Year 2003)			
	60 days after PBZ treatment		90 days after PBZ treatment	
	PBZ treated	Control	PBZ treated	Control
Totocombo	50%	0.0%	100%	0.0%
Miska	50%	0.0%	100%	0.0%

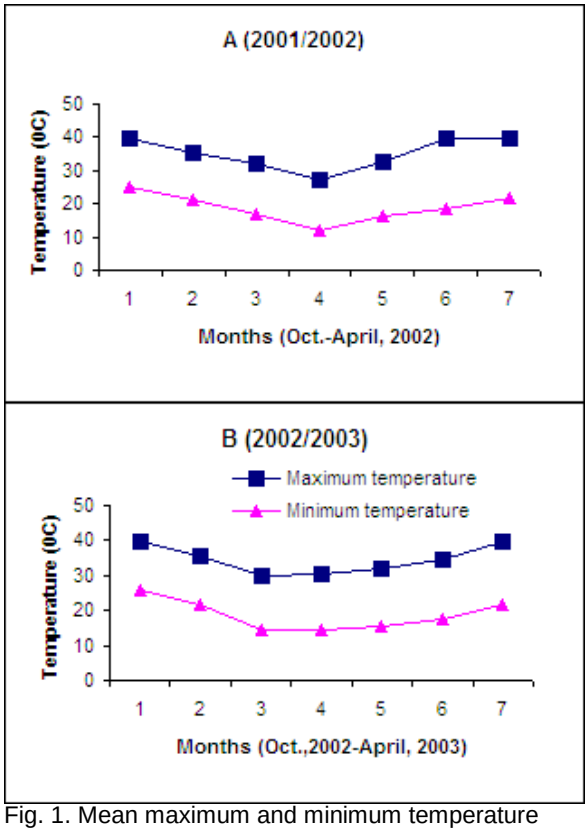

Fig. 1. Mean maximum and minimum temperature during winter seasons, 2001/2002 and 2002/2003 at the experimental site.

The minimum temperature was below 20°C for the months, December to March during both testing years, 2002 and 2003 (Figure-1).

To start with, the recovery of the internal standard used was above 85%. Paclobutrazol generally increased cytokinins levels in both buds and leaves of the PBZ-treated trees as compared to the control trees. For instance, zeatin and zeatin ribosides (z+zr) and isopentenyl adenosine and isopentenyl adenine (i-Ado+i-Ade) levels in the leaves of the PBZ-treated trees of the biennially bearing mango cultivar 'Miska' were increased by PBZ treatment (Figures 2A and 2B). For buds, much higher positive differences in favor of the PBZ-treated trees for both z+zr and i-Ado+i-Ade contents were observed (Figures 2C and 2D), with the highest differences occurred at the second sampling date for i-Ado+i-Ade and the third sampling date for z+zr.

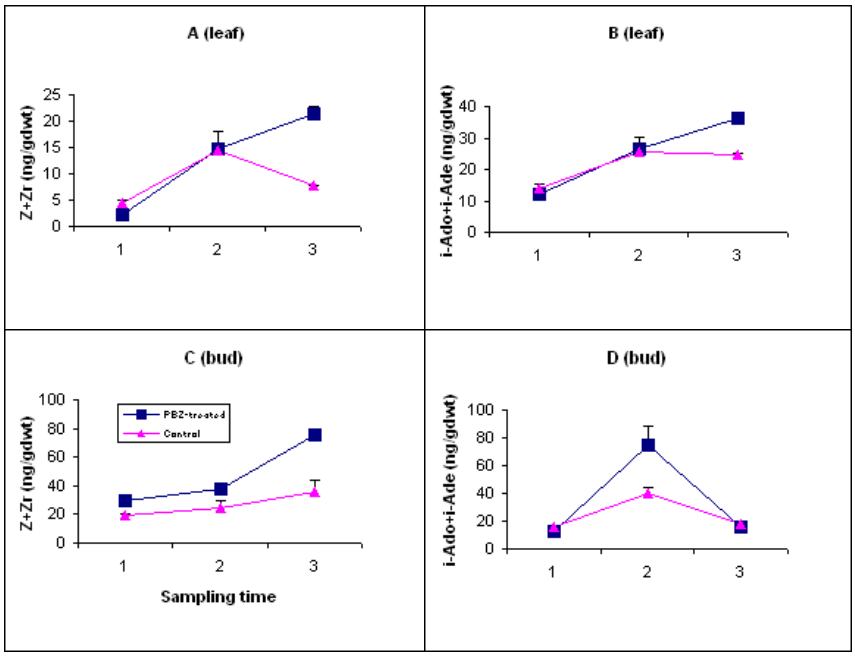

Fig. 2. Effects of paclobutrazol (PBZ) on leaf (A,B) and bud (C,D) zeatin and zeatin riboside (z+zr) and isopentenyl adenosine and isopentenyl adenine (i-Ado+i-Ade) levels of biennially bearing mango cultivar, Miska during off year. Sampling dates were 30, 40 and 60 days after PBZ application. Bar= Standard error. ng/gdwt=nanogram/gram dry weight.

For cv. Mahmoudi, leaves z+zr and i-Ado+i-Ade levels in PBZ treated trees were generally above those of the control trees (Figures 3A and 3B). For the buds, z+zr and i-Ado+i-Ade contents exhibited distinguishably higher trends of increase in PBZ-treated trees as compared to those of the control trees' (Figures 3C and 3D). In general, our data showed that the magnitudes of differences between the PBZ-treated and the control trees were higher in the bud samples as compared to the leaf samples (Figures 2A-3D).

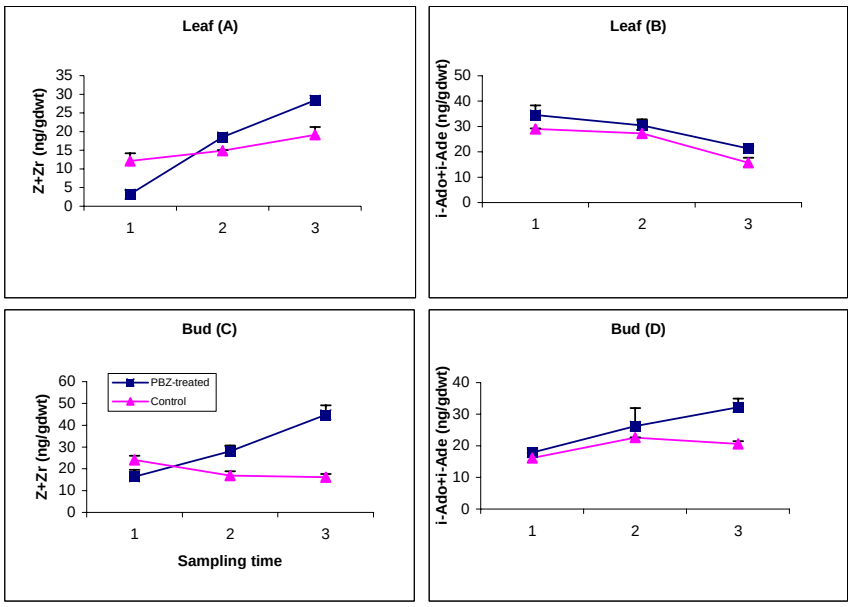

Fig.3. Effects of paclobutazol (PBZ) on leaf (A,B) and bud (C,D) levels of zeatin and zeatin riboside (z+zr) and isopentenyl adenosine and isopentenyl adenine (i-Ado+i-Ade) levels of alternate bearing mango cultivar, Mahmoudi during off year. Sampling were taken 30, 40 and 60 after PBZ application Bar= Standard error.

Paclobutrazol generally decreased the levels of gibberellins for both cvs. Miska and Mahmoudi (Figures 4A-4D). For instance, gibberellin concentrations in the leaves of the PBZ treated trees of both cvs. Miska and Mahmoudi exhibited pronounce reduction as compared to those of the control trees (Figures 4A and 4B). For the buds, although the differences in gibberellins concentrations between the PBZ-treated and the control trees of cv. Miska were small during the first two sampling dates, the difference was very high toward the third sampling date in favor of the control trees (Figure-4C). A much higher reduction in bud GA levels was measured on Mahmoudi's PBZ-treated trees as compared to those of Miska's (Figures 4D and 4C).

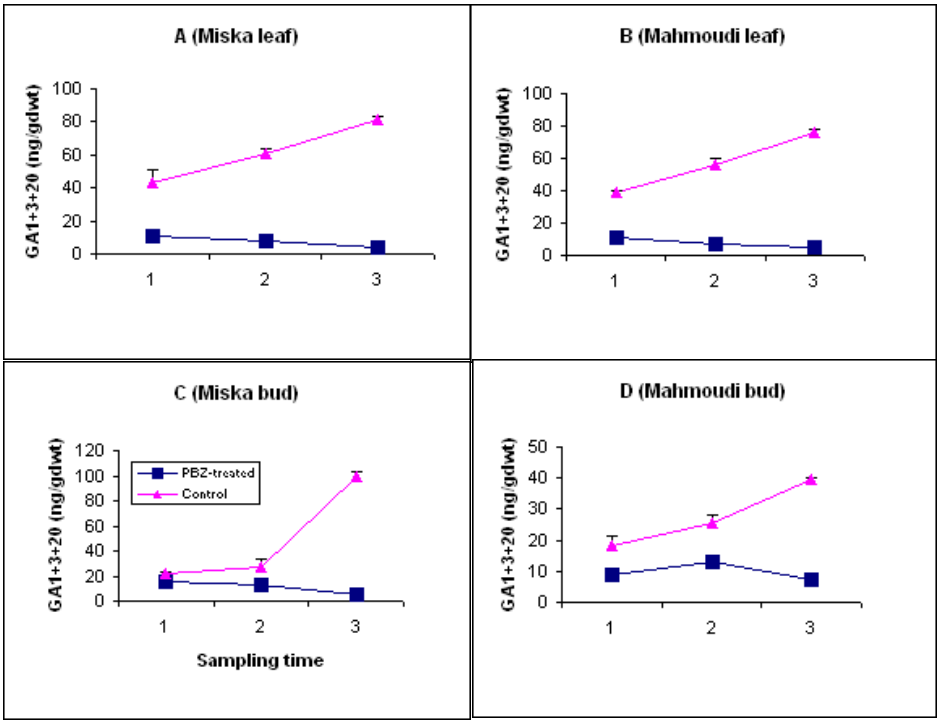

Fig. 4. Effects of paclobutrazol (PBZ) on leaf (A,B) and bud (C,D) gibberellins (GA1+3+20) content of the biennially bearing mango cultivars, Miska and Mahmoudi during off year. Sampling date were 30, 40 and 60 days after PBZ application. Bars=Standard error. ng/gdwt=nanogram/gram dry weight.

IAA levels in the leaves of the control trees of both cvs. Miska and Mahmoudi were generally higher than those of the PBZ treated trees (Figures 5A and 5b). Similarly, the levels of bud IAA of the control trees of both cvs. Miska and Mahmoudi were higher than those of the PBZ treated trees through out the sampling period (Figures 5C and 5D).

ABA levels in bud samples of both cvs, Miska and Mahmoudi were higher in the PBZ treated trees as compared to those of the control trees (Figures 6A and 6B). A more or less similar trend was observed for ABA levels in the leaves of the PBZ-treated trees of Miska cultivar (Figure-6C). However, for the mango cv. 'Mahmoudi', although the ABA levels in the PBZ-treated trees were below those of the control trees during the first two sampling dates, they surpassed those of the control trees towards the end of the sampling period (Figure-6D).

Starch levels in the PBZ treated trees of cvs. Miska and Mahmoudi measured during the first year 2002 were slightly above those of the control trees throughout the sampling period (Figures 7A and 7B) .Leaf reducing sugars contents of the PBZ-treated trees of cvs. Miska and Mahmoudi were generally above those of the control trees during the first year 2002, especially for cv. Mahmoudi (Figures 7C and 7D). Sucrose levels in the leaves of the PBZ-treated trees of cvs. Miska and Mahmoundi measured during the first year 2002 were above those of the control tree throughout the sampling period particularly for cv. Mahmoudi (Figures 7E and 7F).

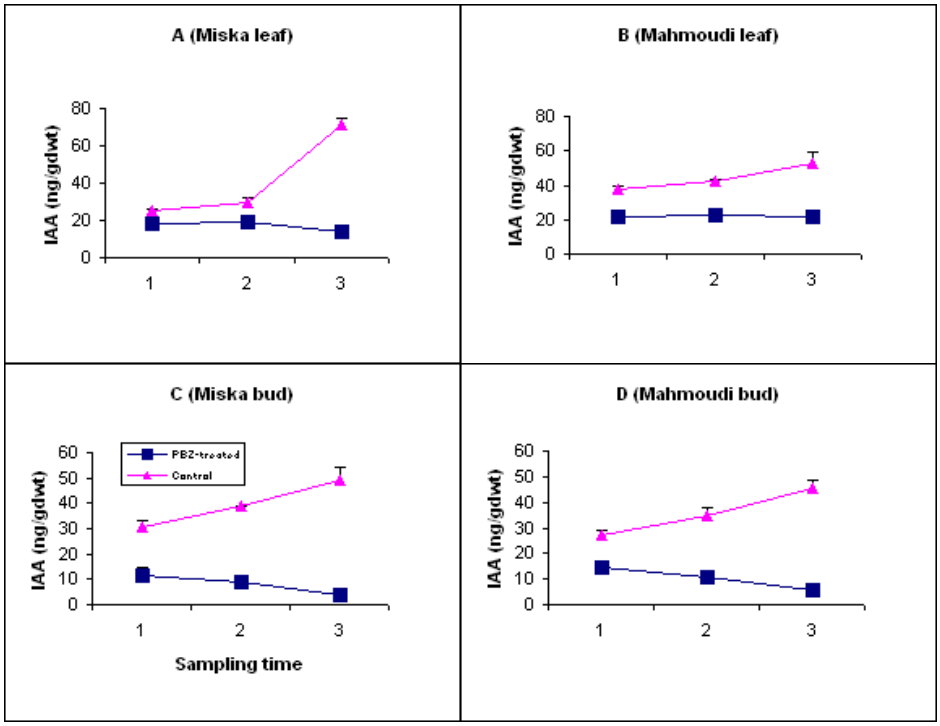

Fig. 5. Effects of paclobutrazol (PBZ) on leaf (A,B) and bud (C,D) auxin (IAA) levels of biennially bearing mango cultivars, Miska and Mahmoudi during off year. Sampling dates were 30, 40 and 60 days after PBZ application. Bars=Standard error. ng/gdwt=nanogram/gram dry weight.

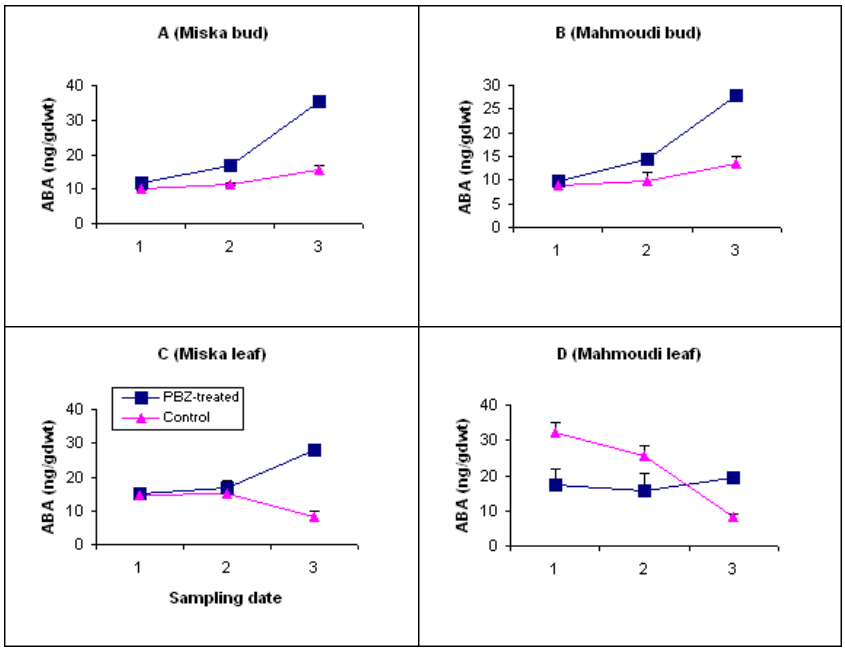

Fig.6. Effect of paclobutrazol (PBZ) on bud (A,B) and leaf (C,D) abscisic acid (ABA) content of biennially bearing mango cultivars, Miska and Mahmoudi during off year. Sampling dates were 30, 40 and 60 days after PBZ application. Bars=Standard error. ng/gdwt=nanogram/gram dry weight.

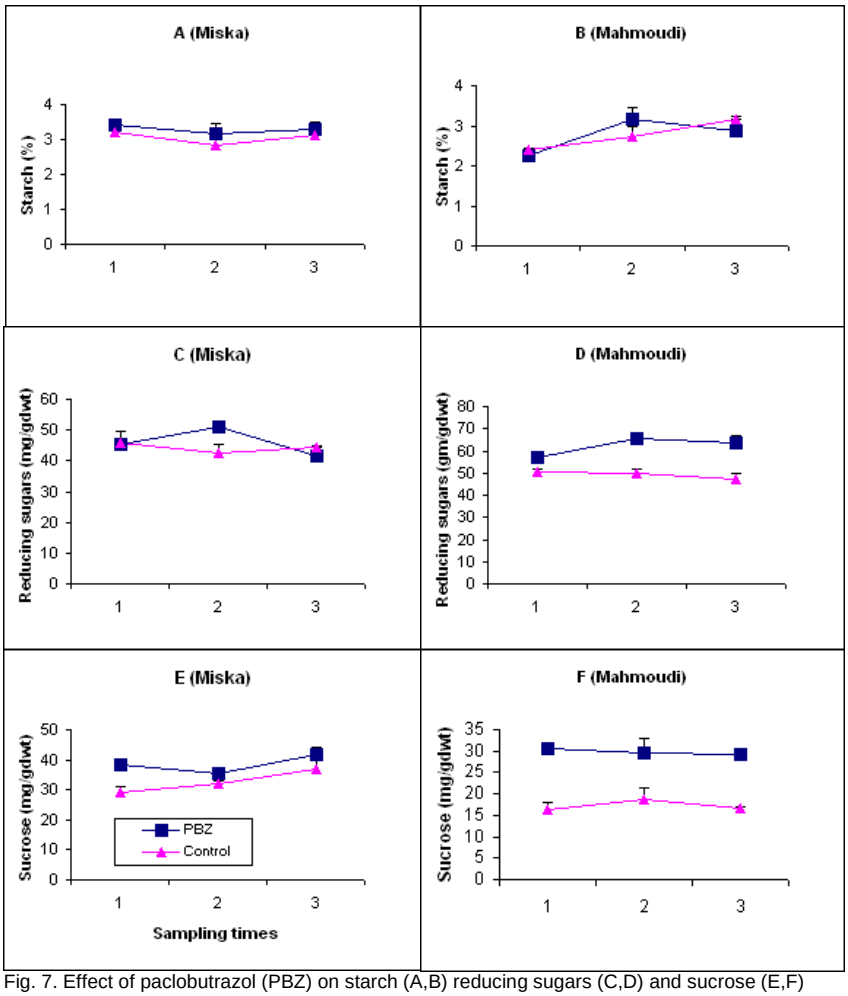

Fig. 7. Effect of paclobutrazol (PBZ) on starch (A,B) reducing sugars (C,D) and sucrose (E,F) levels of mango cultivars, Miska and Mahmoudi during off year 2001/2002. Bar=Standard error.

For starch content, an almost opposite trend to that of the first year 2002 was observed during the second year 2003 on leaves of cv. Miska (Figure-8B). There was a trend of general increase in starch levels (%) in the leaves of the PBZ treated trees of the biennially bearing mango cultivar, Totocombo as compared to the control trees, especially towards the fourth sampling date and up to the end of the sampling period (Figure-8A). However, an almost opposite trend to that of the first year 2002 was observed during the second year 2003 for cvs. Miska and Toto combo (Figures 8C and 8D).

Similar to the first year, 2002, results, sucrose concentrations in the leaves of the PBZ-treated trees of cv. Miska during the second year 2003 were generally higher than those of the control trees, with two peaks for the PBZ treated trees, one was towards the second sampling date and the other was towards the fifth sampling date up to the end of sampling (Figure-8E). Sucrose levels in the leaves of the PBZ-treated trees of cv. Toto combo were generally higher than those of the control trees especially towards the third and the fourth sampling dates (Figure-8F).

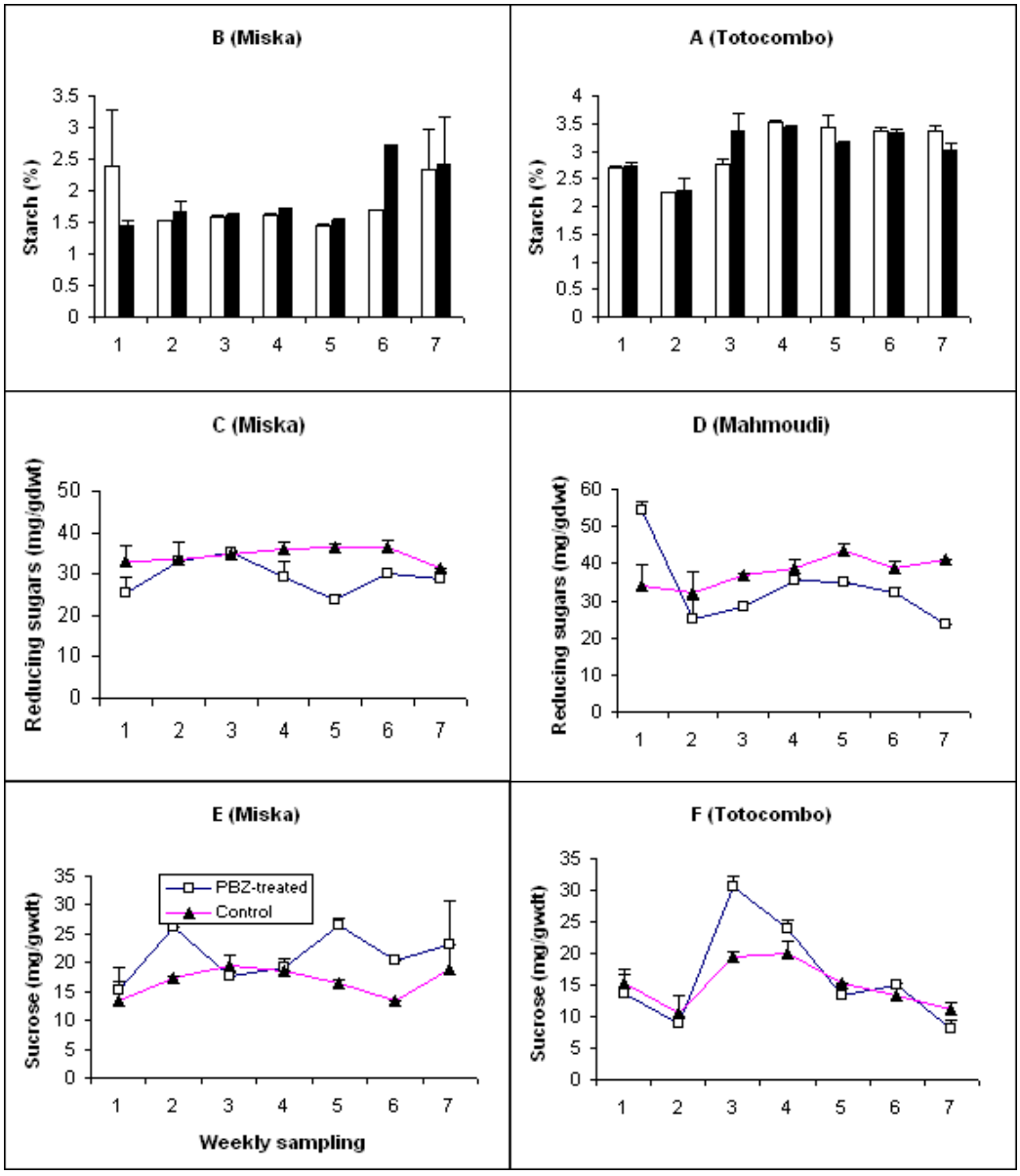

Fig. 8. Effect of paclobutrazol (PBZ) on starch (A,B), reducing sugars (C,D) and sucrose (E,F) levels of mango cultivars, Miska and Totocombo during off year, 2002/2003. Bars=Standard error.

DISCUSSIONS

Since the growth retardant, paclobutrazol (PBZ), resulted in 100% flowering in the off-year of the biennially bearing mango cultivars (Table-1), the resulting changes in hormonal concentrations in the PBZ treated trees most probably had correlations with the observed flowering phenomenon. It is worth mentioning that the recovery of the internal standard used in hormonal analyses was above 85%, a result indicating that the measured differences in hormonal concentrations were reliable. These results are corroborated by the observation that although the minimum temperatures were below 20^{o}C (the flowering temperature for regular bearing cultivars) during the period December.-March for both testing years (Figures 1A and 1B), the control trees did not flower and instead resumed vegetative flushing. It seemed that, unlike in regular bearing mango cultivars, flowering of alternate bearing cultivars occurs on older resting shoots and is probably controlled by genetic factors. This might implicated the presence of relatively high levels of shoot gibberellins, a possible floral induction inhibitor (Davenport, 2000, 2007), in these biennially bearing mango cultivars. Similar results on the positive effects of PBZ on mango flowering were reported in many tropical and subtropical regions of the world (Abdel Rahim *et al*., 2008, Kulkarni, 1988; Winston, 1992; Kurian and Iyer, 1993; Goguey, 1990; Rowley, 1990; Burondkar and Gunjate, 1993; Burondkar, 1991; Salomon and Reuveni, 1994; Salazar-Garcia and Vazquez-valdivia, 1997; Blaikie *et al*., 2004; Gonzalez and Blaikie, 2003; Perez-Barraza *et*

al., 2000; Nartvaranant *et al.*, 2000; Jose and Reboucas, 2000; and Maiti *et al.*, 1972).

The noticeable increases in cytokinins, z+zr and i-Ado+i-Ade, in buds and leaves of PBZ treated biennially bearing cultivars used in this study (Figures 2A-3D) suggest a possible association between cytokinins and flowering and thus a possible role for cytokinins, especially z+zr on floral induction. Although the direct role of PBZ is on inhibition of the oxidation step of ent-kaurene → ent-kaurenal → ent-kaurenoic acid and thus inhibition of gibberellin's (GAs) biosynthesis, it has a secondary role of promoting the synthesis of cytokinins. This was probably achieved through the diversion of reactions into the biosynthetic pathway of cytokinins, since they have the same intermediate precursor (Fletcher and Gilley, 2000; Fletcher and Arnold, 1986). Similar results that supported a possible role for cytokinins, which are synthesized mainly in roots (Van Staden and Davey, 1979; Forsyth and Staden, 1981; Carmi and Van Staden, 1983), leaves and stems (Carmi and Van Staden, 1983; Chen *et al*., 1985), on floral induction were reported in mango (Abdel Rahim *et al*., 2008, Chen, 1987; Naphrom *et al*., 2004), lychee (Chen 1990; 1999), olive (Baktir *et al*., 2004; Ulger *et al*., 2004), pecan (Wood, 1983), *Cymbidium ensifolium* var. misericors in vitro (Chang and Chang, 2003), *Sinapis alba* (Lejeune *et al*.,1994; Lejeune *et al.,* 1988; Havelange *et al*.,1986) and rice (Izumi *et al*., 1988). Cytokinins, probably accumulate during growth check period, are necessary for cell division process during floral induction.

Our results showed that gibberellins (GAs) concentrations in biennially bearing mango cultivars were reduced by PBZ treatment (Figures 4A-4D). These results were in consistence with the well documented effects of the growth retardant, PBZ, on inhibition of GAs biosynthesis (Graebe, 1987; Rademacher, 1995; Fletcher and Gilley, 2000). The widely accepted floral inhibiting role of GA in many fruit trees is not only supported by the results that GAs-biosynthesis inhibitors cause early floral bud break but also by the delay of floral bud initiation (Turnbull, 1996; Davenport *et al.*, 2001; Nunez-Elisea and Davenport, 1998; Tomer, 1984) or complete conversion to vegetative shoots (Kachru *et al*., 1971) following the exogenous application of gibberellic acid. The results on the effect of PBZ on GAs levels reported here are in agreement with those reported by Abdel Rahim *et al*., (2008), Pal and Ram (1978) and Chen (1987) in mango, Chen (1990) in lychee, Baktir *et al*., (2004) and Ulger *et al*., (2004) in olive, Izumi *et al*., (1984) in rice, Wood (1983) in pecan and Saidha *et al.* (1983) and Koshita *et al*., (1999) in citrus. Since floral induction is preceded by a check in vegetative growth and thus temporal cessation of shoot elongation, reduction in gibberellins biosynthesis is an expected feed back. This might infer that any environmental cue or treatment that can trigger a check in vegetative growth probably have some role to play with initiation of floral induction process.

Auxin levels were reduced in both leaves and buds of the PBZ treated trees of biennially bearing mango cultivars as compared to those of the control trees (Figures 5A-5D). However, a direct role of IAA on floral induction is yet to be established. Nevertheless, low levels of IAA were found in the exudates of shoot tips during early flowering (Chen, 1987) as well as in terminal buds of mango (Naphrom *et al*., 2004) during low floral inductive temperatures as compared to the controls. Similar results were reported in olive (Baktir *et al*., 2004; Ulger *et al*., 2004) and citrus (Koshita *et al*., 1999). On the other hand, no obvious differences in IAA concentrations could be detected between the floral and the vegetative buds in lychee (Chen, 1990) or between uniconazole, a growth retardant, treated and non-treated rice plants (Izumi *et al*., 1988). The reduction in auxin levels during floral induction period can be correlated with those of gibberellins since they act synergistically in cell and shoot elongation (Ross *et al*., 2003).

The concentrations of ABA were higher in bud samples of the PBZ treated trees as compared to control trees of the biennially bearing mango cultivars (Figures 6A-6D). These results were in agreement with the well documented role of triazole growth retardants, mainly PBZ, on promotion of ABA biosynthesis through their intervention in the isoprenoid pathway (Graebe, 1987; Fletcher and Grilley, 2000). Similar results were published elsewhere on mango (Chen, 1987; Pongomboon *et al*., 1997; Naphrom *et al*., 2004, Abdel Rahim *et al*., 2008) lychee (Chen, 1990) olive (Ulger *et al*., 2004; Baktir *et al*., 2004) citrus (Koshita *et al*., 1999). On the other hand, ABA levels did not change during floral induction and before bud break in pecan (Wood, 1983) or following uniconazole-P, a growth retardant, treatment in rice (Izumi *et al*., 1988). High ABA levels are probably associated with bud dormancy and the increase in ABA levels during the growth check period (floral induction period) was expected as flowering in mango occurs on resting buds.

It is worth noting that although hormonal quantifications could not be made for PBZ experiments on biennially mango cultivars conducted during the second year 2003, all the PBZ-treated trees of biennially bearing mango cultivars, Miska and Totocombo, showed 100% flowering 90 days after PBZ application while the control trees from both cultivars did not flower (Table-1) but resumed vegetative flushing. Accordingly, the flowering results of the year 2003 confirmed the results of the first testing year 2002 season and thus the positive effect of PBZ on flowering of biennially bearing mango cultivars during off year.

Starch concentrations were generally increased in most occasions by PBZ treatment of biennially bearing mango cultivars, except in one occasion (Figures 7A-7B and 8A-8B). However, our results could not fully support a claim for a direct role of starch on floral induction of mango. Similar results which probably exclude a direct role for starch on flowering were published by Whiley *et al*., (1989) in mango and Hoch *et al*., (2003) in temperate forest trees. Moreover, many authors reported that carbohydrates (CH_2O) accumulation and utilization is a seasonal process characterizing the plant developmental

processes and that carbohydrates levels decrease during flowering, fruit set as well as during active vegetative growth (Phavaphuntanon *et al*., 2000; Davies *et al*., 2000) in mango (Menzel *et al*., 1995) in lychee (Scholefield *et al*., 1985) in avocado (Bates *et al*., 2002) in grapevine (Stutte and Martin, 1986) in olive (Ulger *et al*., 2004) in young poplars (*Populus trichocarpa X Popoulus deltoids*, cv. Raspalje) (Bonicel *et al*., 1987) and in woody plants in general (Loeschere *et al*., 1990). Contrarily, a direct role for CH_2O on flowering in citrus was proposed by Goldschmidt *et al*., (1985) and Goldschmidt and Golomb (1982). Moreover, positive correlations between flowering and CH_2O were reported in mango by Suryanarayana *et al*., (1978) and Pongsomboon *et al*., (1997). However, both studies could not establish a possible causal relationship between flowering and CH_2O levels in mango. On the other hand, starch levels were reported to decrease during floral induction in pineapple (Madhusudanan and Nandakumar, 1983). To close, the contradicting results on the possible role for starch on flowering of fruit trees in general and mango in particular probably urge the need for standardization of sampling techniques and experimentation methods before any generalization on the role of starch on floral induction could be made. Nevertheless, the use of other more relevant tree tissues like root and wood samples might give a better view on the correlation of starch reserves with the tree developmental stage of growth as was reported elsewhere (Davies *et al*., 2000; Menzel *et al*., 1995; Bates *et al*., 2002).

Although PBZ treatment increased leaf reducing sugars levels during the year 2002 (Figures 7C-D), it increased (Figure-8C) and decreased (Figure-8D) leaf reducing sugars contents during the year 2003. Accordingly, it seemed that reducing sugars behaved like starch. On the other hand, PBZ increased sucrose levels for all tested alternate bearing mango cultivars (Figures 7E-F and 8E-F). In photoperiodic floral responsive plants, both long day and short day, it was well established that plants increase sucrose content of leaf exudates before or at floral transition (Houssa *et al*., 1991 in *Xanthium stramsriu*; Lejeune *et al*., 1993; Havelange *et al*., 2000 in *Sinapis alba* plants; Corbesier *et al*., 1998 in wild type of *Arabidopsis thaliana*). Moreover, under tissue culture conditions sucrose promoted flowering in both *Brassica campestris L* cv. Ceres (Friend *et al*., 1984) and dark grown *Arabidopsis spp.* (Roldan *et al*., 1999). In perennials, the maximum sucrose concentrations were found during the winter season, which coincides with bud burst in young poplar (Bonicel *et al*., 1987) and during floral induction and initiation as compared to bud differentiation in olive (Ulger *et al*., 2004) and flower induction in mango (Abdel Rahim *et al*., 2008). Moreover, an indirect evidence for the role of sucrose in accelerating bud growth was manifested by the correlation between bud growth rate and acid invertase activity in pear, *Pyrus pyrifolia* (Ito *et al*., 2002). In short, although sucrose concentrations increased during floral transition, it did not suffice to trigger the complete sequence of floral evocation (Houssa *et al*., 1991; Ulger *et al*., 2004) and it was rather that hormonal roles were implicated (Roldan *et al*., 1999; Havelange *et al*., 2000).

Generally, the apparent increase in sucrose levels during the floral induction period in this study was probably a response for the strong sinks created by the dividing cells of the induced flower buds and thus the high energy requirement of the floral induction process. Another explanation was that the increase in sucrose levels during floral induction period was probably a result of reduced or checked vegetative growth and thus the absence of other potentially competitive actively growing sinks.

Based on the results of this study it can be concluded that paclobutrazol was necessary to substantiate the effect of low temperature (below 20^{o}C) on floral induction of alternate bearing mango cultivars during off-year. Moreover, cytokinins (Zeatin+Zeatin riboside and Isopentenyl Adenine + Isopentenyl Adenosine) and to some extent Abscisic acid seemed to be associated with floral induction, while gibberellins and probably auxin seemed to suppress floral induction in mango under tropical conditions of Sudan. Our results call for a floral induction model for mango that might come into function with the on set of low temperature alone or in combination with chemical growth retardant cue (depending on genetic background) leading to changes in hormonal and some carbohydrate levels and when these changes are associated with the presence of mature leaves and receptive buds the result is most probably induction of flowering (Figure-9). However, the proposed model needs to be validated by pruning, girdling and water stress experiments before any generalization could be made.

Floral induction
↑ ← mature leaves and receptive buds
Increased sucrose levels
Timely increase in nitrogen levels?
Possible increase in starch levels?
Increase in ethylene levels?
Increased abscisic acid levels
Increased cytokinin levels
Reduced or maintained auxin levels?
Reduced gibberellins levels
↑ ←Genetic factors (varietal differences)
Temporal check of vegetative growth
↑
Low temperature (<20° C)
Growth retardant

Figure-9. Proposed model for floral induction in mango under Sudan climate (tropics).

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REFERENCES

Abdel Rahim A.O.S., Elamin O.M and Bangerth F.K. 2008. Effects of paclobutrazol on floral induction and correlated phyto-hormonal changes in grafted seedlings of different mango (*Mangifera indica* L.) cultivars. Sudan J. Agric. Res. 11: 111-120.

Aron Y, Monselise S.P, Goren R and Costo J. 1985. Chemical control of vegetative growth in citrus trees by paclobutrazol. J. of Hort. Sci. 20(1): 96-98.

Baktir I., Ulger S., Kaynak L. 2004. Relationship of seasonal changes in endogenous plant hormones and alternate bearing of olive trees. Hort. Science. 39(5): 987-990.

Balley I.S.E., Haris M. and Whiley A.W. 2000. Effect of water stress on flowering and yield of 'Kensington Pride' mango (*Mangifera indica* L.). Acta Horticulturae. 509: 277-281.

Bernier G. 1988. The control of floral evocation and morphogenesis. Ann. Rev. Plant physiol. plant. Mol. Biol. 39: 175-219.

Bertling I. and Bangerth F. 1995. Changes in hormonal pattern of the new growth of *Sclerocarya birrea* after rejuvenation treatment with GA3 and heading back. Gartenbauwissenschaft. 60: 119-124.

Blaikie S.J., Kulkarni V.J. and Muller W.J. 2004. Effect of morphactin and paclobutrazol flowering treatments on shoot and root phenology in mango cv. Kensington Pride. Scientia Horticulturae. 101: 51-68.

Blakeney A.B. and L.L. Mutton. 1980. A simple colorimetric method for determination of sugars in fruits and vegetables. J. Sci. Food. Agric. 31: 889-897.

Bohner J. and Bangerth F. 1998. Effect of fruit set sequence and defoliation on cell and hormone levels of tomato fruits (*Lycopersicon esculentum* Mill) within a tree. Plant Growth Regulation. 7: 141-155.

Bohlenius H., Huang T., Charbonnel-Campaa L., Brunner A.M., Jansson S., Strauss S.H. and Nilsson O. 2006. CO/FT Regulatory Module Controls Timing of Flowering and Seasonal Growth Cessation in Tree Science. 312: 1040-1043.

Boss P.K., Sreekantan L. and Thomas M.R. 2006. A grape vine TFL1 homologue can delay flowering and alter floral development when over expressed in heterologous species. Functional Plant Biology. 33: 31-41.

Burondkar G. 1991. Regulation of shoot growth and flowering in Alphonso mango with paclobutrazol. Acta Horticulturae. 291: 79-83

Burondkar M.N and Gunjate R.T. 1993. Control of vegetative growth and induction of regular and early cropping in 'Alphonso' mango with paclobutrazol. Acta Horticulturae. 341: 206-215.

Carmi A. and Van Staden J. 1983. Role of roots in regulating the growth rate and cytokinin content in leaves. Plant physiology. 73: 76-78.

Chen W.S. 1987. Endogenous growth substances in relations to shoot growth and flower bud development of mango. J. Amer. Soc. Hort. Sci. 112(2): 360-363.

Chen W.S. 1990. Endogenous growth substances in the xylem and shoot tip diffusate of lychee in relation to flowering. Hort Science. 25(3): 314-315.

Chen W.S. 1999. Changes in cytokinins before and during early flower bud differentiation in lychee (*Litchi chinensis* Sonn.). Plant physiology. 96: 1203-1206.

Cutting J.G.M. and Lyne M.C. 1993. Girdling and the reduction in shoot xylem sap concentrations of cytokinins and gibberellins in peach. J. Hort. Sci. 68(4): 619-626.

Davenport T.L. 2000. Processes influencing floral initiation and bloom: the role of phytohormones in the conceptual flowering model. Hort Technol. 10: 733-739.

Davenport T.L. 2007. Reproductive physiology of mango. Braz. J. Plant Physiol. 19(4): 363-376.

Davenport T.L. and Nunez-Elisea. 1997. Reproductive physiology. In The mango: Botany, production and uses. Edited by Litz, R.E. CABI publishing, CAB international, Wallingford, Oxon OX10 8DE, UK.

Davenport T.L. Ying Z., kulkarni V. and White T.L. 2006. Evidence for a translocatable florigenic promoter in mango. Scientia Horticulturae. 110: 150-159.

FAO. 2008. FAOSTATISTICS data. http:/www.faostatdata.agriculture.

Fernandez H., Fraga M.F., Bernard P. and Revilla M.A. 2003. Quantification of GA1, GA3, GA4, GA7, GA9, and GA20 in vegetative and male cone buds from juvenile and mature trees of *Pinus radiata*. Plant Growth Regulation. 40: 185-188.

Flectcher R.A. and Arnold V. 1986. Stimulation of cytokinins and chlorophyll synthesis in cumcumber cotyledons by triadimefon. Physiologia plantarum. 67: 695-701.

Fletcher R.A. and Gilley A. 2000. Triazole as plant growth regulators and stress protectants. Horticultural Review. 24: 55-138.

Forsyth C. and Van Staden J. 1981. The effect of root decapitation on lateral root formation and cytokinins production in *Pisum sativum*. Physiologia plantarum. 51: 375-379.

Goguey T. 1990. Effects of repeated application of cultar (paclobutrazol) on mango (*Mangifera indica* L.) var. 'Valencia'. Fruits. 45(6): 599-607.

Ito A., Hayama H. and Kashimura Y. 2002. Sugar metabolism in buds during flower bud formation: a comparison of two Japanese pear (*Pyrus pyrifolia* (Burm.) Nak) cultivars possessing different flowering habits. Scientia Horticulturae. 96: 163-175.

Jose A.R.S. and Reboucas T.N.H. 2000. Use of paclobutrazol in mango orchard in southwest region, Bahia State, Brazil. Acta Horticulturae. 509: 713-715.

Katz E., Ziv O., Venkatachalam R., Shlomo E., Halevy A.H. and Weiss D. 2003. Promotion of *Globularia sarcophylla* flowering by uniconazole, an inhibitor of gibberellin biosynthesis. Scientia Horticulturae. 98: 423-431.

Kulkarni V.J. 1988. Chemical control of tree vigor and promotion of flowering and fruiting in mango (*Mangifera indica* L) using paclobutrazol. J. Hort. Sci. 63: 557-566.

Kulkarni V.J. 2004. The tri-factor hypothesis of flowering in mango. Acta Horticulturae. 645: 61-70.

Kurian R.M. and Iyer C.P.A. 1993. Chemical regulation of tree size in mango (*Mangifera indica* L) cv. Alphonso II. Effect of growth retardants on flowering and fruit set. J. Hort. Sci. 68(3): 355-360.

Maiti S.C., Basu R.N. and Sen P.K. 1972. Chemical control of growth and flowering in *Mangifera indica* L. Acta Horticulturae. 24: 192-195.

Menzel C.M. and Simpson D.R. 1990. Effect of environment on growth and flowering of lychee (*Litchi chinensis* Sonn). Acta Horticulturae. 275: 161-166.

Mitsutomo Abe, Yasushi K, Sumiko Y, Yasufumi D., Ayako Y., Yoko I., Harutaka I., Michitaka N., Koji G. and Takashi A. 2005. FD, a bZIP protein mediating signals from the floral pathway integrator FT at the shoot apex. Science. 309: 1052-1056.

Nartvaranant P., Subhadrabandhu S. and Tongumpai P. 2000. Practical aspects in producing off-season mango in Thailand. Acta Horticulturae. 509: 661-668.

Rameshwar A. 1988. Mango flowering- Stress induced. Acta Horticulturae. 231: 433-439.

Ross J.J., O'Neill D.P. and Rathbone D.A. 2003. Auxin-gibberellin interactions in pea: Integrating the old with the new. J. Plant Growth Regulation. 22: 99-108.

Rowley A.J. 1990. Effect of cultar applied as a soil drench on Zill mango tree. Acta Horticulturae. 275: 211-215.

Sachs R.M. 1977. Nutrient diversion: A hypothesis to explain the chemical control of flowering. Hort Science. 12(3): 220-222.

Salazar-Garcia S. and Vazquez-Valdivia V. 1997. Physiological persistence of paclobutrazol on 'Tommy Atkins' mango (*Mangifera indica* L.) under rainfed conditions. J. Hort. Sci. 72(2): 339-349.

Salomon E. and Reuveni O. 1994. Effect of paclobutrazol treatment on the growth and the first flowering of intact and autografted seedlings of mango. Scientia Horticulturae. 60: 81-87.

Sanyal D. and Bangerth F. 1998. Stress induced ethylene evolution and its possible relationship to auxin-transport, cytokinins levels and flower bud induction in shoots of apple seedlings and bearing apple trees. Plant Growth Regulation. 24: 127-134.

Van Staden J. and Davey J.E. 1979. The synthesis, transport and metabolism of endogenous cytokinins. Plant Cell Environment. 2: 93-106.

Wang S.Y. and Steffens G.L. 1985. Effect of paclobutrazol on water stress induced ethylene biosynthesis and polyamine accumulation in apple seedlings leaves. Phytochemistry. 24(10): 2185-2190.

Weiler E.W. 1984. Immunoassay of plant growth regulators. Ann. Rev. Plant Physiol. 35: 85-95.

Wilkie J.D., Sedgley M. and Olesen T. 2008. Regulation of floral initiation in horticultural trees. Journal of Experimental Botany. 59(12): 3215-3228.

Wilkie J.D., Sedgely M. and olesen T. 2008. Regulation of floral initiation in horticultural trees. Journal of Experimental botany. 59(12): 3215-3228.

Winston E.C. 1992. Evaluation of Paclobutrazol on growth, flowering, and yield of mango cv. Kensington pride. Aust. J. Exp. Agric. 32: 97-104.

Yemm E.W. and Willis A.J. 1954. The estimation of carbohydrates in plant extracts by Anthrone. Biochemical J. 57: 508-514.

Yim K.O., Kwon Y.W. and Bayer D.E. 1997. Growth responses and allocation of assimilates of rice seedlings by paclobutrazol and gibberellin treatment. Journal of plant growth regulation. 16: 35-41.