8-2017

Physiological Parameters, Isoenzymes and Hormonal Profiling In Different Varieties of Date Palm in United Arab Emirates

Saeed Rashed Hamad Al Shamsi

United Arab Emirates University

College of Food and Agriculture

Department of Aridland Agriculture

PHYSIOLOGICAL PARAMETERS, ISOENZYMES AND HORMONAL PROFILING IN DIFFERENT VARIETIES OF DATE PALM IN UNITED ARAB EMIRATES

Saeed Rashed Hamad Al Shamsi

This thesis is submitted in partial fulfilment of the requirements for the degree of Master of Science in Horticulture

Under the Supervision of Dr. Abdul Jaleel Cheruth

August 2017

Declaration of Original Work

I, Saeed Rashed Hamad Al Shamsi, the undersigned, a graduate student at the United Arab Emirates University (UAEU), and the author of this thesis entitled "*Physiological Parameters, Isoenzymes and Hormonal Profiling in Different Varieties of Date Palm in United Arab Emirates*", hereby, solemnly declare that this thesis is my own original research work that has been done and prepared by me under the supervision of Dr. Abdul Jaleel Cheruth, in the College of Food and Agriculture at UAEU. This work has not previously been presented or published, or formed the basis for the award of any academic degree, diploma or a similar title at this or any other university. Any materials borrowed from other sources (whether published or unpublished) and relied upon or included in my thesis have been properly cited and acknowledged in accordance with appropriate academic conventions. I further declare that there is no potential conflict of interest with respect to the research, data collection, authorship, presentation and/or publication of this thesis.

Student's Signature: _____ Date: 10/10/2014

Approval of the Master Thesis

This Master Thesis is approved by the following Examining Committee Members:

Advisory Committee:

1) Advisor (Committee Chair): Dr. Abdul Jaleel Cheruth
   Title: Associate Professor
   Department of Aridland Agriculture
   College of Food and Agriculture, UAEU, UAE
   Signature _____ Date 10/10/2017

2) Member: Dr. Mohammad Salem Al-Yafei
   Title: Associate Professor
   Department of Aridland Agriculture
   College of Food and Agriculture, UAEU, UAE
   Signature _____ Date 10-10-2017

3) Member: Dr. Shyam S. Kurup
   Title: Associate Professor
   Department of Aridland Agriculture
   College of Food and Agriculture, UAEU, UAE
   Signature _____ Date 16/10/2017

4) Member (External Examiner): Dr. Amad M. Al-Azzawi
   Title: Associate Professor
   Department of Pharmaceutical Chemistry
   Institution: RAKMHSU
   Signature _____ Date 24/8/2017

This Master Thesis is accepted by:

Dean of the College of Food and Agriculture: Professor Bhanu P. Chowdhary

Signature Bhanu P. Chowdhary Date 11/10/2017

For Dean of the College of Graduate Studies: Professor Nagi T. Wakim

Signature Ali Hassan Date 12/10/2017

Copy 2 of 10

Abstract

The present investigation was carried out to explore the physiological changes in early, mid and late varieties of date palms in United Arab Emirates. The concentrations of biochemicals and antioxidant compounds, activities of antioxidant enzymes with their isoenzyme profiles, endogenous phytohormone contents were studied from preflowering, flowering, and post-flowering stages. Two varieties each from early (Shaham, Khanezi), mid (Barhee, Nabthasaif) and late (Khasab, Fardh) flowering types were used in the study. The protein content was higher in early varieties in preflowering stage, but lower in other two varieties. The phenol showed an opposite trend to protein. Ascorbic acid, reduced glutathione and tocopherol showed significant variation in different varieties. Similarly, the antioxidant enzyme ascorbate peroxidase was higher in preflowering stage in all varieties. Superoxide dismutase, polyphenol oxidase and catalase activities were highest in Bharhee for all the stages. Peroxidase activity (POX) was highest in Fardh variety of date palm whereas Khanezi exhibited the lowest. Several isoperoxidases bands were observed in gel electrophoresis at the time of flowering. The phytohormones varied within varieties. The levels of gibberellic acid, indole acetic acid, zeatin, and abscisic acid contents were positively influenced in pre-flowering and flowering stages due to the flower development process. The hormonal contents showed a transient rise in the preflowering stage and decreased during the flowering time and elevated in the post-flowering stage. This study provided an insight into the possible roles of biochemicals, antioxidants with isoenzymes and endogenous hormones and their interactions in the regulation of flower development in different date palm varieties.

Keywords: Antioxidant, biochemical, date palm, hormones, isoenzyme.

Title and Abstract (in Arabic)

المؤشرات الفسيولوجية، ونظائر الإنزيمات، والتحليل الهرموني في مختلف أصناف شجرة النخيل بدولة الإمارات العربية المتحدة

الملخص

تهدف هذه الدراسة إلى اكتشاف المتغيرات الفسيولوجية التي تطرأ على مختلف أصناف شجرة النخيل ذات الإثمار المبكر و المتوسط و المتأخر بدولة الإمارات العربية المتحدة ، و من خلال هذا البحث تمت دراسة مستويات تركيز الكيماويات الحيوية و المركبات المضادة للأكسدة ، بالإضافة إلى نشاط الإنزيمات المضادة للأكسدة مع تحليل نظائرها ، و المحتوى من الهرمونات النباتية خلال مراحل ما قبل الإزهار و بعد الإزهار ، حيث تم استخدام صنفي (شهام و خنيزي) في دراسة الأصناف ذات الإثمار المُبكر ، و صنفي (برحي و نبتة سيف) في دراسة الأصناف ذات الإثمار المتوسط ، و صنفي (خصاب و فرض) في دراسة الأصناف ذات الإثمار المتأخر ، لوحظ في هذه الدراسة بأن المحتوى البروتيني و الفينول متفاوت في مختلف المراحل بجميع أصناف النخيل ، و أظهر حمض الأسكوربيك و الجلوتاثيون المختزل و التوكوفيرول تفاوت كبير في مختلف الأصناف ، و على نحوٍ مماثل لوحظ بأن الإنزيمات المضادة للأكسدة و الديسموتاز الفائق (SOD) و نشاط إنزيم الكتالاز (CAT) هُم الأعلى في صنف البرحي بجميع مراحل الإزهار ، و أظهرت الدراسة بأن صنف الفرض سجل أعلى مستوى نشاط للبيروكسيدات (POX) ، بينما كان صنف الخنيزي هو أقلها نشاطًا ، علمًا بأن نظائر الإنزيمات تغيرت بشكل بارز وفقًا لتوقيت تحفيز الإزهار ، لاحظنا عدة نظائر لإنزيمات البيروكسيدات أثناء عمليات الفصل الكهربائي الهلامي ، و قد تنوعت الهرمونات النباتية في جميع الأصناف ، و تفاوتت مستويات المحتوى من حمض الجبريليك و الإندول حمض الخليك و الزياتين و حمض الأبسيسيك في مراحل ما قبل و بعد الإزهار وفقًا لعمليات الإزهار ، و اكتشفنا بأن أعلى تركيز هرموني يكون خلال مرحلة ما قبل الإزهار ، و من ثم يبدأ التركيز بالتناقص التدريجي خلال فترة الإزهار ، و بعد ذلك يعود للارتفاع في مرحلة ما بعد الإزهار ، تُقدم هذه الدراسة توضيحًا عن الأدوار المحتملة لمضادات الأكسدة في أثناء نشاط الإنزيمات المضادة للأكسدة ، و في خصائص الهرمونات الذاتية ، و في نظائر الإنزيمات ، و في التغيرات الكيميائية الحيوية المتعلقة بمجال تنظيم نمو أزهار النخيل على مختلف أصنافه.

مفاهيم البحث الرئيسية: مضادات الأكسدة ، الكيماويات الحيوية ، نظائر الإنزيمات ، شجرة النخيل ، بيروكسيدات ، هرمونات.

Acknowledgements

I have great pleasure in communicating my profound feeling of significant appreciation to my research supervisor Dr. Abdul Jaleel Cheruth, Associate Professor, Department of Aridland Agriculture, for his important direction, consistent encouragement and periodical examination of the work at each stage, which paved the way for the genesis of this successful thesis. I would also like to thank my other committee members, Dr. Shyam S. Kurup and Dr. Mohammad Salem Al-Yafei, Associate Professors in the Department of Aridland Agriculture.

I want to express my gratefulness to the Dean of College of Food and Agriculture, Department Chair and all faculty and staff members of the Department of Aridland Agriculture, College of Food and Agriculture at UAEU for the help and the joint effort during my graduate studies.

I significantly value the genuine help of Mr. Abou Messallam Azab (Lab Specialist, Horticulture Lab), and Eng. Arshed M. Eldaly (Agriculture Engineer, Al-Foah Farm). I would like to recognize the Research Assistant Mr. Gabriel A. Rabert for his kind help in sample collection, data calculation and compilation.

Without the consistent encouragement and support from my mother and my relatives, I would not have possessed the capacity to embrace and finish this work.

Most importantly, I bow before the Almighty for showering me with His endowments and empowering me to satisfy this attempt.

Dedication

To my beloved Mother and family

Table of Contents

List of Tables

List of Figures

Chapter 1: Introduction

Date palm (*Phoenix dactylifera* L.) is one among the most important plants in the arid and semi-dry lands that can tolerate the harsh environment in desert ecosystem. Date palm trees are important food plants in parts of the Arabian Peninsula, North Africa, and the Middle East including many states of the Arabian Gulf Cooperation Countries (GCC). It has been set up as a sustainable food crop of Arabian countries including the traditional nomads (Khan et al., 2016). Dates constitute a critical piece of a balanced diet as they are regular natural supplements required by human and animals. These supplements include but not limited to protein, sugars, minerals and dietary fiber (Sadiq et al., 2013).

The world production of dates has expanded 2.9 times more than 40 years, while the total populace has doubled. The aggregate world export of dates expanded by 1.71% more than 40 years (Al-Maasllem, 1996). From multiple points of view, dates might be considered as a practically perfect nourishment, giving an extensive variety of fundamental supplements and potential medical advantages. The nourishing and biochemical parts of date organic products were accounted by numerous researchers (Ashraf and Hamidi-Esfahani, 2011; Shabani et al., 2016). Everywhere on these hot deserts, a date palm desert garden still is an appreciated sight, where water and shade are accessible (Jaradat and Zaid, 2004). Dates can be effectively developed in territories having long hot summer and mild temperature during flowering (February to April) and natural fruit maturing (May to August) ought to be 25 to 29ºC. Contingent upon the period of maturing, the cultivars are named early, mid and late cultivars (Kurup et al., 2009; Cheruth et al., 2015).

The temperature for plant flowering depends on the variety of plants and mainly it is measured from the onset of initiation until flower set, similarly the fruiting period is from fruit set until maturation. In date palm this period is about 120 to 200 days. And, it depends on the variety and environmental conditions (Zaid and de Wet, 2002). The nutritious and biochemical parts of date products were studied by many researchers (Allaith, 2008). An antioxidant, which can extinguish receptive free radicals, can keep the oxidation of different particles and may, in this way, it can prevent degenerative diseases (Lobo et al., 2010) and dates have been proved to be antioxidant food (Vayalil, 2002). Moreover, dates have been accounted for an assortment of polyphenols, including phenolic acids, hydroxycinnamates, flavonoid glycosides, and proanthocyanidins (Hong et al., 2006) which make it an intriguing natural product from a pharmaceutical and therapeutic perspective.

Date products contains phenolics and consequently have a great degree of antioxidant capacity (Eberhardt et al., 2000). In recent years, the interest in phytochemical content and antioxidant capacity of date palms is increasing. Among the antioxidants, phenolic compounds play important role in protection against disease and pests (Silva et al., 2006). Similarly, the Vitamin C makes up an antioxidant in mammalian cells. Vitamin C (ascorbic acid) is viewed as the most vital cell reinforcement in plasma and structures the primary line of guard against plasma lipid peroxidation (Young and Woodside, 2001). Dates contain no less than six vitamins including a little measure of vitamin C, and vitamins B1 (thiamine), B2 (riboflavin), nicotinic acid (niacin) and vitamin A (Al-Shahib and Marshall, 2003). Recent reviews show that the aqueous extracts of dates have strong antioxidant action and good sources of sugars, minerals and fibers (Mansour et al., 2005).

Most plant-derived products are low in levels of vitamin E activity, and due to the extensive use of plant foods, they provide consistent source of this vitamin (Eitenmiller and Lee, 2004). Many reviews have shown that tocotrienols display predominant antioxidative and anticancer properties (Aggarwal et al., 2010). Palms have been a product of interest in recent years for applications in different aspects, for example, nutraceuticals, pharmaceuticals and cosmeceuticals. Reduced Glutathione (GSH) is viewed as the real water solvent antioxidant in photosynthetic and non-photosynthetic tissues. It additionally upgrades cell division and expanding the integrity of cell structure (Mullineaux and Rausch, 2005). GSH assumes a vital part in numerous natural processes, for example, intracellular reduction–oxidation metabolic cycles, transportation, protein union, catabolism, and digestion (Zheng et al., 2017). To moderate the oxidative harm created by ROS, plants have built up a complex cell reinforcement barrier framework that incorporates enzymatic and nonenzymatic antioxidant agents.

Induction of ROS scavenging catalysts, for example, superoxide dismutase (SOD), catalase (CAT), polyphenol oxidase (PPO) and ascorbate peroxidase (APX) is the most widely recognized system that secure plants against oxidative harm (Sharma et al., 2012; Sofo et al., 2015). SOD repressed ethylene generation by restricting hydroxyl radical formation. Superoxide and hydroxyl radicals are exceptionally reactive and would be relied upon to respond maliciously with natural macromolecules and SOD clearly secures the cell against reactive oxygen danger (Caughey, 2012).

SOD and PPO are mainly intended to prevent oxidative damages, and have been attributed to reduction in quality of plant foods during the ripening and post-

harvest (Hornero-Méndez et al., 2002). Peroxidases are in some cases used for biosynthesis and degradation of lignin in cell walls of plants. But in most of the occasions, they are meant for defense against pathogens and environmental stresses. However, the specific function of plant peroxidases is still unclear (Cosio and Dunand, 2009). PPO are copper metaloproteins that add to plant cell resistance by catalyzing the oxidation of phenolics and their change into antimicrobial quinones. These mixes are profoundly responsive, altering and cross-connecting a variety of cell constituents (Tran and Constabel, 2011). APX is a standout amongst the significant antioxidant compounds of plants that detoxify hydrogen peroxide utilizing ascorbate for reduction. This enzyme decreases H_2O_2 to water by ascorbate as particular electron donor (Hasanuzzaman et al., 2017). Plants produce H_2O_2 in metabolic process and cause damage of cell oxidation function, while CAT eliminates H_2O_2 and plays a key role in elimination of O_2^- (Tan et al., 2006).

Auxins, cytokinins, Zeatin, IAA, gibberellins, ABA and ethylene are the natural plant hormones of which auxins and cytokinins direct cell division and differentiation of explants in tissue culture frameworks. ABA is also employed to induce somatic embryos to enter a quiescent state and synchronize maturation of somatic zygotes in plant tissue culture systems. ABA can also act as anti-transpiration agent during acclimatization of tissue cultured plantlets (Rai et al., 2011). Indole acetic acid (IAA) is a characteristic auxin which is likewise combined in numerous types of non-seeded plants, numerous microscopic organisms, fungi and algae. The amino acid tryptophan is generally viewed as the antecedent for the biosynthesis of auxin in plants (Sergeeva et al., 2002). Zeatin neutralizes free radicals that promote aging. It boosts the activity of antioxidant enzymes, acting “synergistically with other inner antiaging molecules, orchestrating a stronger

offensive against senescence", or aging. It was found to delay biochemical damage to skin cells, buoying their resistance to environmental stresses. Gibberellins were found to enhance the stem length in higher plants due to cell elongation in the internodes and to stimulate the cell division, and increase volume of individual cells (Stefanini et al., 2002). These effects lead to stem elongation, internode length, leaf area and growth of dry mass, although plant species might react to certain GAs (Doaigey et al., 2013).

Plant hormones direct the greater part of the life cycle occasions in plants, for example, cell division, seed germination, seed and bud dormancy, flowering, fruit set and maturing (Miransari and Smith, 2014). Hormones works in plants at a low concentration, together with numerous other compound blends (Nakurte et al., 2012). The hormones in plants, particular chemical substances produced by plants, are the main inner aspects controlling growth and development (Garay Arroyo et al., 2012).

There are three distinct varieties in date palms in UAE, which are early, mid and late flowering palms. In these three different date palm genotypes if the exact process of flower induction is known, it will be highly beneficial to initiate the production of early bearing varieties of date palm in future. Indeed, even today, with all the knowledge of molecular biology, the exact processes involved in inducing flowers are not known. The different physiological factors responsible for flowering behavior of date varieties are to be explored in order to begin the process of revealing the flower induction mechanism. It is very significant to comprehend the character of enzymes and hormones in flower induction process in date palm. Cheruth et al. (2015) studied the variation of antioxidant status in relation to early, mid, and late varieties of date palm (*Phoenix dactylifera*) from United Arab Emirates. Analysis of

phytohormones and isoenzyme status in connection to flowering in early, mid, and late varieties of date palm is a unique aspect. The expected outcome of the experiment is to identify the different physiological factors responsible in relation to flowering behavior of date palm varieties which in turn will be highly significant to initiate the production of early bearing varieties of date palm in future.

The objectives of this study were to assess the biochemical changes, non-enzymatic and enzymatic antioxidants, endogenous hormonal profile and isoenzyme characterization in six date palm varieties of the United Arab Emirates, namely, Shaham, Khanezi, (early flowering) Barhee, Nabthasaif, (mid flowering) Khasab, and Fardh (late flowering) in order to identify the antioxidant contents and different physiological factors responsible for flowering.

Chapter 2: Literature Review

Date palm (*Phoenix dactylifera* L.), a diploid (2n = 2x = 36), perennial and monocotyledonous plant, is considered as one of the most important fruit trees in regions like Arabian Peninsula, North Africa, and the Middle East (Barrow, 1998). This tree belongs to Palmaceae (Chao and Krueger, 2007). *P. dactylifera* is a strict dioecious evergreen tree equipped for living more than 100 beneficial years. It is one of the most cultivated trees as well as of socio-economic significance (Mahmoudi et al., 2008).

Date palm is mainly grown in arid and semi-dry regions, the temperature necessities are vital for deciding development, flowering and fruit development (Zaid and de Wet, 2002). Flower bud initiation and time of fruit set in date palm are important factors determining the commercial production of date fruits (Fki et al., 2011). Accordingly, there are three flowering forms in date palms, based on the early, mid, and late season varieties (Kurup et al., 2009).

Date palm flowering starts after a chilly period, when the temperature turns out to achieves a level known as the blossoming zero (0). This temperature level fluctuates as per varieties and to nearby climatic conditions (Zaid and de Wet, 2002). Date palm cultivation depends on its climatic requirements (Erskine et al., 2004). The successful cultivation of date palm requires long summer season with high day and night temperatures, a mild winter without frost, the absence of rain during flowering and fruit setting, and low relative humidity with sufficient sunshine (Jassim and Limoges, 2014).

The plant's transition from vegetative development to flowering is of fundamental significance in farming, agriculture, and plant rearing since flowering is the initial step of sexual propagation (Samuolienė et al., 2008). Kurup et al. (2009) evaluated four varieties of date palm based on morphological, physiological and marker assisted characterization for improved performance under progressive increase in salinity in terms of growth and flowering characteristics. Abohatem et al. (2011) revealed the tendency to increase peroxidase activity in female flowers than the males and female inflorescences showed two major acidic isoperoxidases in rachillae indicating high levels of peroxidase activity at flowering stage. Isoenzyme polymorphism studied in different clones of date palm showed variations in inflorescence formation and morphological responses (Azeqour et al., 2002). El Hadrami et al. (2011) studied the transition from vegetative phase to flowering and the effect of secondary metabolites in date palm and also established other benefits with special emphasis on metabolic pathways.

There are reports about the antioxidant characteristics of date fruit (Saleh et al., 2011) and variations in these parameters during fruit development and ripening (Awad et al., 2011). The enzymatic antioxidants and non-enzymatic antioxidants variations provided an insight into the possible roles of antioxidants and in the activities of antioxidant enzymes in the regulation of flower development in date palm varieties (Cheruth et al., 2015).

The antioxidant metabolism underlying the defense mechanism was the area of interest since long time in variety of crops (Zhu et al., 2009), but little attention is gained in terms of flowering related changes in antioxidant status. Notable variation in antioxidant properties was seen between various developmental phases of

Carthamus tinctorius blooms; the most elevated antioxidant action was seen at stage III (full blossoming) while phenolic composition was achieved its greatest at stage II (flower development) (Salem et al., 2011). There are reports about the antioxidant characteristics of date fruit (Saleh et al., 2011) and antioxidant capacity, antioxidant compounds in date cultivars during development and ripening (Awad et al., 2011). Studies have reported that palm date fruit might be a good source of secondary active components and has a potent ability to suppress free radicals (Mansouri et al., 2005).

It is very important to understand the role of enzymes and antioxidants in early flower induction in date palm. Apart from research conducted in the cultivation, production, physiology, stress tolerance, there are no reports in the basal mechanism underlying the physiology of flowering behavior of date palm. Flower formation is postulated to be enhanced with the activities of peroxidase, polyphenol oxidase.

Tocopherols are lipophilic cell antioxidants incorporated by all plants and they are the basic parts of biological membranes (Kiffin et al., 2006). α-tocopherol, found in green parts of plants scavenge lipid peroxy radicals through the purposeful activity of different cell antioxidants (Munne Bosch and Alegre, 2002). Combination of low-atomic weight cell antioxidants, for example, α-tocopherol, has been reported in stressed wheat plants (Sairam et al., 2002) and thereby shows its antioxidant and stress protecting abilities.

Vitamin C or ascorbic acid is viewed as the significant cell antioxidant in plasma and structures the principal line of protection against plasma lipid peroxidation (Chaudhari et al., 2012). The ascorbic acid content modifications noted in various junctures of growth stages like 30, 40 and 50 days before and after

flowering (Rabert et al., 2014). Gopal and Verma (2001) pointed out that glutathione - a cell antioxidant helped to withstand oxidative stress in transgenic lines of tobacco. The connection amongst genotypes and the outflow of specific GST qualities was found in wheat (*Triticum aestivum*) which facilitated in drought tolerance (Galle et al., 2009). The *Brassica juncea* plants (Asgher et al., 2014) has demonstrated that GSH creation and ethylene formation are connected with ozone resistance. Pandey and Shukla (2015) reported that the APX greatly increased in rice under various climatic conditions.

The H_2O_2 scavenging system is more actively involved in detoxification (Hameed et al., 2011). In addition, defense against active oxygen compounds plant antioxidant enzyme have other important cellular roles. Peroxidase enzyme can efficiently remove H_2O_2 both in the cytosol and chloroplast. Hence, increasing the activities of this enzyme in drought stress perhaps show the accumulation of H_2O_2 (Csiszar et al., 2005). Generally, the transition to flowering is correlated with peroxidase and polyphenol oxidase.

The hormones, specific synthetic substances synthesized by plants, are the principle internal viewpoints controlling growth and development (Hartmann et al., 1997). Plant Hormones control the greater part of the life cycle events in plants, for example, cell division, seed germination, seed and bud dormancy, flowering, fruit set and maturing (Eris, 1995). Hormones result in plants at a low level, together with numerous other compounds.

The polymorphism isozyme has been effectively used for signifying inter and intragenic variations in different species and cultivars (Angelov and Ivanova, 2012; Johnson et al., 2010). Polyphenol oxidase (PPO) is an enzyme appeared to exist in

various and interconvertible structures and is generally dispersed in plant kingdom (Kumar et al., 2013). It is outstanding that the compound assumes an essential part in the browning in fruits and vegetables (Mayer and Harel, 1973). It has been proposed that the chemical may be related with numerous essential physiological capacities, for example, development and differentiation (Gordon and Paleg, 1961). However, there have been no in depth studies on PPO and POX Isoenzyme changes for assessing in date palm. In this statement effort has been made to study the diversity of polyphenol oxidase isozyme in date palm.

Chapter 3: Materials and Methods

3.1 Plants and sampling

Experimental trees were identified and marked separately from Al-Foah Experimental Station (270N and 220S latitude and 510W and 570E longitude) of College of Food and Agriculture, UAEU in Alain city in 160km Eastern Abu Dhabi, United Arab Emirates.

The hormonal profiling and isoenzyme analysis with antioxidant estimation were conducted in three different phases of growth and development; they are preflowering, flowering and postflowering periods.

Three varieties from each of the 3 categories were identified with three replicates. In each individual variety, three plants were located and marked for analysis. Normal date palm cultivation practices and agriculture procedures were continued for all the plants under study.

The varieties used for the study are given below:

1. Early season: Shaham, Khanezi.
2. Mid season: Barhee, Nabthasaif.
3. Late season: Khasab, Fardh.

These date palm varieties are in the same age group and were in a healthy condition with stabilized production.

3.2 Biochemical analysis

3.2.1 Protein

Protein was estimated following the method of Bradford (1976).

Extraction

Five hundred milligrams of fresh plant material were mixed in a mortar and pestle with 10 ml of 20 per cent trichloro acetic acid (TCA). The homogenate was centrifuged for 15 minutes at 800 g. The supernatant was discarded and to the pellet 5 ml of 0.1 N NaOH was added to solubilize the protein and the solution was centrifuged at 800 g for 15 minutes. The supernatant was made up to 10 ml with 0.1 N NaOH and used for the estimation of protein content.

Assay

Protein solution containing 10 to 100 µg protein in a volume of 0.1 ml was pipetted into 12 x 100 mm test tubes. Five milliliters of Bradford's reagent was added to the test tube and the contents were mixed by vortexing. The absorbance at 595 nm was measured after 2 minutes with 3 ml cuvette against a reagent blank, prepared by adding 0.1 ml of 0.1 N NaOH and 5 ml of Bradford's reagent standard curve was prepared using BSA V fraction was used to determine the protein content and the results were expressed in milligram per gram dry weight.

3.2.2 Total phenol

Total phenols were estimated by the method of Malick and Singh (1980).

Extraction

500 milligrams of fresh plant tissue was ground in a pestle and mortar with 10 ml of 80 per cent ethanol. The homogenate was centrifuged at 10,000 rpm for 20 min. The supernatant was evaporated to dryness. The residue was dissolved with 5 ml of distilled water and used as extract.

Estimation

To 2 ml of the extract, 0.5 ml of Folin-Ciocalteau reagent was added. After 3 min, 2 ml of 20% Na_2 CO_3 solution was mixed thoroughly. The mixture was kept in boiling water for exactly one min. and after cooling the absorbance was read at 650 nm. The total phenol was determined using a standard curve prepared with different concentration of gallic acid.

3.3 Antioxidants

3.3.1 Ascorbic acid

Ascorbic acid content was assayed as described by Omaye et al. (1979).

Extraction

One gram of fresh material was ground in a pestle and mortar with 5 ml of 10% TCA, the concentrate was centrifuged at 3500 rpm for 20 minutes. The pellet was re-separated twice with 10% TCA and supernatant was made to 10 ml and utilized for estimation.

Estimation

To 0.5 ml of extract, 1 ml of DTC reagent (2,4-Dinitrophenyl hydrazine-Thiourea-$CuSO_4$ reagent) was added and mixed thoroughly. The tubes were incubated at 37°C for 3 hours and to this 0.75 ml of ice cold 65 per cent H_2SO_4 was added. The tubes were then allowed to stand at 30°C for 30 minutes. The resulting colour was read at 520 nm in spectrophotometer (U-2001-Hitachi). The ascorbic acid content was determined using a-standard curve prepared with ascorbic acid and the results were expressed in milligrams per gram dry weight.

3.3.2 α-Tocopherol

α-Tocopherol activity was assayed as described by Baker et al. (1980).

Extraction

500 mg of fresh tissue was homogenized with 10 ml of a mixture of petroleum ether and ethanol (2:1.6 v/v) and the extract was centrifuged at 10,000 rpm for 20 minutes and the supernatant was used for estimation of α-tocopherol.

Estimation

To one ml of extract, 0.2 ml of 2 per cent 2,2-dipyridyl in ethanol was added and mixed thoroughly and kept in dark for 5 minutes. The resulting red colour was diluted with 4 ml of distilled water and mixed well. The resulting colour in the aqueous layer was measured at 520 nm. The α-tocopherol content was calculated using a standard graph made with known amount of α-tocopherol.

3.3.3 Reduced glutathione

Reduced glutathione activity was assayed as described by Griffith (1980).

Extraction

200 mg of plant material was ground in a pestle and mortar with 5 ml of 2 per cent metaphosphoric acid. The extract was centrifuged at 17,000 g for 10 minutes and the resultant supernatants were saved for estimation.

Estimation

To 0.9 ml of the extract, 0.6 ml of 10 per cent sodium citrate buffer was added to neutralize the extract. The assay mixture 1 ml containing 700 µ1 NADH (0.3 mM/L), 100 µ1 Dithionitrobenzoic acid (DTNB) (6.0 mM/L), 100 µ1 distilled water and 100 µ1 of neutralized extract. This mixture was stabilized at 25ºC for 4 minutes, and 10 µl of Glutathione Reductase (Sigma) was added to this and read at 412 nm against appropriate blank.

3.4 Antioxidant enzymes

3.4.1 Ascorbate peroxidase

Ascorbate peroxidase (APX) was extracted and estimated by the method of Asada and Takahashi (1987).

Extraction

500 mg of fresh plant tissue was ground in a pestle and mortar under liquid nitrogen and 10 ml of 50 mM potassium phosphate buffer (pH 7.0) containing 1 mM EDTA, 1% PVP and 1 mM ascorbic acid. The homogenate was filtered through

twofold layered cheesecloth and centrifuged at 15,000 rpm for 20 minutes at 4˚C. The supernatant was utilized as source of enzymes.

Estimation

One ml of reaction mixture contained 50 mM potassium phosphate buffer (pH 7.0), 0.5 mM ascorbic acid, 0.1 mM H_2O_2 and 200 µl of enzyme extract. The absorbance was read at 290 nm against the blank, correction was accomplished for the low, non-enzymatic oxidation of ascorbic acid by H_2O_2 (extinction coefficient 2.9 mM^{-1} cm^{-1}). The enzyme activity is expressed in µg per gram dry weight.

3.4.2 Superoxide dismutase

Crude enzyme extract was prepared, for the assay of superoxide dismutase (SOD) by the method of Hwang et al. (1999).

Extraction

One gram of fresh tissue was homogenized with 10 ml of ice-cold 50 mM sodium phosphate buffer containing 1 mM PMSF. The extract was filtered through a double-layered cheesecloth. The extract was centrifuged at 12,500 rpm for 20 minutes at 4°C. The supernatant was saved and made up to 10 ml with extraction buffer and used for estimation of the SOD enzyme activity. The enzyme protein was determined by Bradford (1976) method.

Estimation

The activity of SOD was assayed as described by Fridovich (1975). The reaction medium was prepared and to 3 ml reaction medium, 1 ml of enzyme extract was added. The reaction mixture contained 1.17 × 10-6 M riboflavin, 0.1 M

methionine, 2×10^{-5} potassium cyanide and 5.6×10^{-5} M nitroblue tetrasodium salt (NBT), dissolved in 0.05 M sodium phosphate buffer (pH 7.8). The mixture was illuminated in glass test tubes by two sets of Philips 40 W fluorescent tubes. Illumination started to initiate the reaction at 30°C for one hour. Those without illumination saved as blank and kept in dark. The absorbance was read at 560 nm in the spectrophotometer against blank. Superoxide dismutase activity was expressed in units. One unit is defined as the amount of change in the absorbance by 0.1 per hour per milligram protein under the assay condition (Cherry, 1963).

3.4.3 Catalase

Activity of catalase (CAT) was assayed as described by Chandlee and Scandalios (1984).

Extraction

500 mg of frozen material was homogenized in 5 ml of ice cold 50 mM sodium phosphate buffer (pH 7.5) containing in 1mM PMSF. The extract was centrifuged at 4°C for 20 minutes at 12,500 rpm. The supernatant was used for enzyme assay.

Assay

Catalase activity was measured using the method of Chandlee and Scandalios (1984) with modification. The assay mixture contained 2.6 ml of 50 ml of 50 mM potassium phosphate buffer (pH 7.0) 0.4 ml, 15 mM H_2O_2 and 0.04 ml of enzyme extract. The decomposition of H_2O_2 was followed by the decline in absorbance at 240 nm. The enzyme activity was expressed in units 1 mM of H_2O_2 reduction per minute per mg protein.

3.4.4 Polyphenol oxidase

The assay of Polyphenol oxidase (PPO) was carried out by the method of Kumar and Khan (1982). The assay blend for PPO contained 2 ml of 0.1 M phosphate buffer (pH 6.0), 1 ml of 0.1 M catechol and 0.5 ml of enzyme extract. This was incubated for 5 min at 25°C, after which the reaction was halted by mixing 1 ml of 2.5 N H_2SO_4. The absorbancy of the purpurogallin framed was read at 495 nm. To the blank 2.5 N H_2SO_4 was added of the zero time of a similar assay mixture. PPO activity is expressed in U mg^{-1} protein (U = Change in 0.1 absorbance min^{-1} mg^{-1} protein). For all the enzymatic calculation protein was estimated by the using Bradford (1976) method, utilizing bovine serum albumin (BSA, Sigma, USA) as the standard.

3.4.5 Peroxidase

Peroxidase (POX) activity was estimated by the method of Kumar and Khan (1982). Assay mixture of peroxidase contained 2 ml of 0.1 M phosphate buffer (pH 6.8), 1 ml of 0.01 M pyrogallol, 1 ml of 0.005 M H_2O_2 and 0.5 ml of enzyme extract. The solution was incubated for 5 min at 25°C after which the response was ended by including 1 ml of 2.5 N H_2SO_4. The measure of purpurogallin formed was determined by measuring the absorbance at 420 nm against a blank prepared by adding the extract after the addition of 2.5 N H_2SO_4 at zero time. The action was communicated in unit mg^{-1} protein. One unit is characterized as the adjustment in the absorbance by 0.1 min^{-1} mg^{-1} protein.

3.5 Isozymes profiling

Native Page

Protocol Native - PAGE

Isozyme profiling was done using 10 % acrylamide gel as described by Laemmli (1970) in a Bio-Rad Mini-PROTEAN Tetra System electrophoresis unit. The gel consisted of 10 % separating gel at the bottom, overlaid with 4 % of stacking gel (Section 2.2). The separating gel was made up of 4.0 mL distilled water, 3.33 mL of 30:0.8 % acrylamide: bis-acrylamide stock solution, 2.5 mL of 1.5 M Tris-HCl (pH 8.8), 100 µL of 10 % ammonium persulphate (APS), and 5 µL of N,N,N',N'-tetramethylethylenediamine (TEMED, Vivantis Ltd., UK) for gel polymerization. The separating gel was loaded into the gel cassette and air bubbles formed were removed by adding 10 µL isopropanol. The gel was allowed to polymerize for 30 minutes. The isopropanol was then removed by rinsing with distilled water.

Following that, stacking gel was prepared by mixing 3.4 mL distilled water, 830 µL of 30:0.8 % acrylamide: bis-acrylamide stock solution, 630 µL of 0.5 M Tris-HCl (pH 6.8), 50 µL of 10 % APS and 5 µL of TEMED. Ten-well comb was inserted immediately after the loading of stacking gel into the gel cassette and the gel was left undisturbed for 15 minutes for gel polymerization. Once the gel had polymerized, the comb was removed carefully without damaging the wells formed. The native PAGE was run at 40°C for approximately four hours until the tracking dye reached approximately 0.5 cm from the bottom of the gel.

Native-PAGE buffers and reagents

Monomer solution: Acrylamide: bis-acrylamide (30:0.8)

Acrylamide: bis-acrylamide stock solution was prepared by dissolving 30 g of acrylamide and 0.8 g of N'N'-bis methylene acrylamide in 50 mL of distilled water. The solution was made to 100 mL with distilled water and stored at 4ºC in dark.

Separating gel buffer: 1.5 M Tris-HCl, pH 8.8

Separating gel buffer was prepared by dissolving 18.15 g of Tris base in 50 mL distilled water. pH was adjusted to 8.8 with 1.0 M HCl. The buffer was made to 100 mL with distilled water.

Stacking gel buffer: 0.5 M Tris-HCl, pH 6.8

Stacking gel buffer was prepared by dissolving 6.05 g of Tris base in 50 mL distilled water and pH was adjusted to 6.8 with 1.0 M HCl. The volume of buffer was brought to 100 mL with distilled water.

10 % ammonium persulphate (APS): prepared fresh

10% APS was prepared by dissolving 10 g of APS in 100 mL distilled water.

Sample buffer

The sample buffer composed of 4.4 mL distilled water, 1.0 mL of 0.5 M Tris-HCl pH 6.8, 1.0 mL glycerol, and 0.2 mL of 0.5 % bromophenol blue.

Electrophoresis buffer

Electrophoresis buffer was prepared by dissolving 3.0 g of Tris Base (25 mM), and 14.4 g of glycine (192 mM) in 500 mL distilled water. The buffer was then made to 1 L with distilled water.

Gel storage solution

Gel storage solution was prepared by mixing 70 mL acetic acid with 50 mL methanol. Distilled water was then topped up to 1 L.

Preparation of resolving gel: 10.0 mL of 10 % gel

The resolving gel composed of 4.0 mL distilled water, 3.33 mL of 30:0.8 acrylamide: bis-acrylamide stock solution, 2.5 mL of 1.5 M Tris-HCl pH 8.80.1 mL of 10 % APS. Five (5) microlitres of TEMED was added at last for gel polymerization. Air bubbles formed in the gel cassette was removed using 10 μL isopropanol. The isopropanol was removed by rinsing by distilled water once the gel polymerized.

Preparation of stacking gel: 5.0 mL of 4.0 % gel

The stacking gel consisted of 3.4 mL distilled water, 0.83 mL of 30:0.8 acrylamide: bis-acrylamide stock solution, 0.63 mL of 0.5 M Tris-HCl pH 6.8, 0.05 mL of 10% APS. Five (5) microlitres of TEMED will be added at last for gel polymerization.

Peroxidase enzyme activity bands

Peroxidase enzyme activity bands from the different treatments was compared for any changes or polymorphism. After electrophoresis, the gels were immersed in the peroxidase activity bands staining solution according to Wetter and

Constabel (1982) in the dark at 25°C on an orbital shaker at 50 rpm for 45 minutes until their respective bands were fully developed and ready to be photographed. The gels were stored in 7% acetic acid.

Peroxidase activity bands staining solution

Peroxidase isoenzyme staining solution consists of 0.016 g o-dianisidine, which was dissolved in 2 mL of N, N-dimethylformamide and top up to 100 mL using 0.4 M sodium acetate buffer (pH 5). Total of 0.04 mL of hydorogen peroxide (30%) was added to the solution immediately before use.

Polyphenol oxidase activity bands and staining solution

For the identification of polyphenol oxidase isozymes on gel, staining solution was set up by dissolving 0.03 M catechol containing 0.05% p-phenylene diamine in phosphate citrate buffer (pH 6.0) (Vallejos, 1983). After electrophoresis, the gels were deliberately expelled from the device and precisely washed at 4°C with electrophoretic buffer and afterward the gels were incubated in the staining solution for couple of minutes till the clear bands showed up. Staining reaction was halted by washing the gel 3-5 times with d.H_2O and the gels were fixed with 7% acetic acid solution.

Data analysis

Relative mobility (Rm) values were calculated for each band based on the migration of the band relative to the front or tracking dye. The gels were scored as presence (+) or absence (-) of isozyme bands and their staining intensities i.e., faint, medium and intense. Depending upon the presence or absence of bands, similarity indices (SI) were calculated (Nei and Li, 1979). Cluster analysis UPGMA

(Unweighted pair group method with arithmetic averages) was performed on the similarity index by using statistical software SPSS for windows package (Version 10).

3.6 Hormone profiling

Chemicals and reagents

Hormone profiling was done according to Kelen et al. (2004). Analytical reagent grade chemicals were utilized, unless generally shown. Water, with a conductivity lower than 0.05 µS/cm, and acetonitrile (Merck) were of HPLC standard. Sodium hydroxide (Merck) and phosphoric acid (Merck) were utilized for pH change. Potassium hydrogen phthalate (dried at 110°C before utilize, Fluka) was used as the reference standard. Methanol, phosphate buffer parts, ethyl acetate, hydrochloric acid, diethyl ether and sodium sulfate were all of analytical purity (Merck) and were utilized for extraction of the plant hormones. ABA, IAA, Zeatin and GA_3 were all of standard purity (Sigma) and utilized without further purification. The stock standard solutions (1000 ppm) of these hormones for LC studies were set up by dissolving in the HPLC mobile phase. Working solutions were set up by diluting the stock solutions with the same HPLC mobile phase at fitting concentrations. Working solutions were arranged new upon the arrival of utilization. These solutions were filtered before injections.

Hormone extraction from leaf

Quantitative analysis of hormones was performed as described previously (Kelen et al., 2004). 10 g of fresh tissue sample which had been homogenized (motor and pestle) with liquid nitrogen in 70% methanol was stirred overnight at 4ºC. The extract was filtered through Whatman filter paper (No.1) and evaporated under

vaccum. The pH of the aqueous phase was adjusted to 8.5 using 0.1 M phosphate buffer. Later the aqueous phase was partitioned using ethyl acetate twice. The ethyl acetate phase was removed and the aqueous phase pH was adjusted to 2.5 using 1N hydrochloric acid (HCl). The hormones were partitioned into diethyl ether thrice. Any residual moisture content was removed by passing the diethyl ether phase through anhydrous sodium sulphate. The diethyl ether phase comprising the hormones was vacuum dried. The residue obtained was re-dissolved in 1 mL of methanol and stored at 4˚C for further analysis.

Apparatus

The chromatographic examination was performed on a Shimadzu Model LC. The chromatographic framework comprises of a Shimadzu Model LC 10 ADVP pump with an auto injector (SIL 10 AD VP) and diode array detector (SPDM 10 A DAD). This equipment has a column oven (CTO 10 AVP) and a degasser framework (DGU 14 A). The column utilized was a Luna C18, 250 mm x 4.6 I.D. stainless steel analytical column with 5 µm particle size (Phenomonex). The e.m.f. values used to evalute the pH of the mobile phase were measured with a Mettler Toledo MA 235 pH/particle analysis apparatus utilizing a Hanna HI 1332 Ag/AgCl combination pH electrode. All solutions were externally thermostated at 25ºC ± 0.1°C. The electrode was settled in fitting acetonitrile water mixtures before the e.m.f. estimations. In this study, pH estimations in acetonitrile-water parallel mixtures were performed by considering the operational definition of pH15−18.

Chromatographic procedure

All through this study, the mobile phases utilized were acetonitrile-water (26:74; 30:70%; v/v). In these media, 30 mM phosphoric acid was changed in

accordance with various pH values with sodium hydroxide. The Luna C18 segment was equilibrated for every mobile phase condition with a time limit of 30 min. The column temperature was kept up at consistent 25± 0.1ºC. The separation was done by isocratic elution with a flow rate of 0.8 mL/min. An infusion volume of 10 µL was utilized for every examination. The standard solution of the individual acid was set up in the mobile phase and chromatographed independently to decide the retention time for each acid. The signal of the mixes was checked at 208, 265, 270 and 280 nm for gibberellic acid, abscisic acid, Zeatin and IAA individually. Capacity factors were calculated from k = (tR-to)/to, where 'to' was the hold-up time, and 'tR' was the maintenance time of every hormone for every mobile phase. In this equation the hold-up time, to, was established for every mobile phase composition using potassium bromide solution [Merck, 0.01% (w/v) in water, λmax= 200 nm]. The retention times and capacity factors of the solutes were determined from 3 different injections. Peak identification was based on retention time and spiking of the sample.

3.7 Statistical analysis

Statistical analysis was done using SPSS- 16.0 version for all the analyzed parameters. Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT). The values are mean ±SD for six samples in each group. p values $\leq$ 0.05 were considered as significant.

Chapter 4: Results

4.1 Biochemical contents

4.1.1 Protein and phenol content

The protein content was high in early flowering varieties in pre flowering stage (0.159 and 0.16 mg/g dw respectively in Shaham and Khanezi). It gradually decreased in flowering stage and then again started elevating in post flowering stage (Figure 1). In mid and late flowering varieties the protein concentration was low in preflowering stages.

The phenol contents were high during the post flowering stages in all the varieties of date palm (Figure 2). There was an increasing trend from preflowering to flowering and postflowering stages. In mid variety Barhee, there was no significant change in phenol contents among the flowering periods.

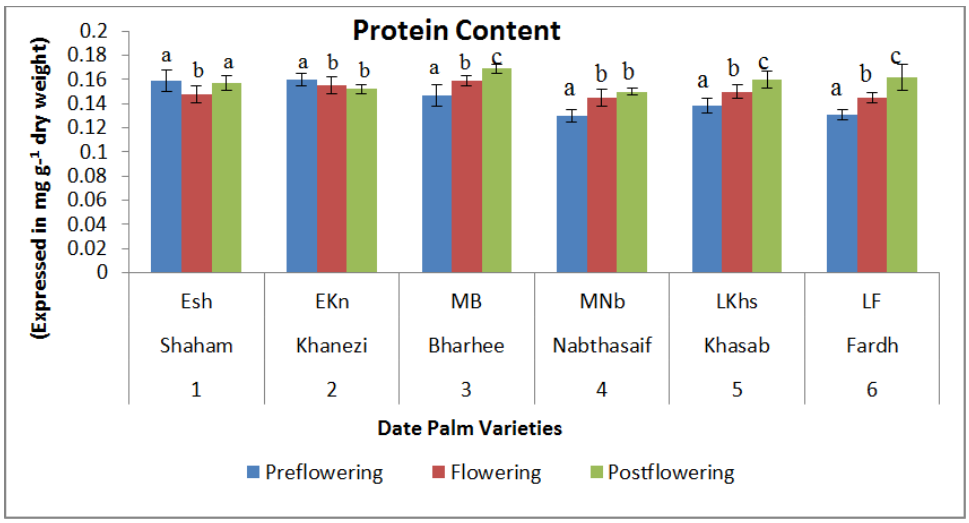

Figure 1: Variations in soluble protein content in date palm (*Phoenix dactylifera*) varieties during different flowering stages

Values are given as mean ±SD of six experiments in each group. Values, that are not sharing a common superscript (a,b,c) differ significantly at P≤0.05 (DMRT)

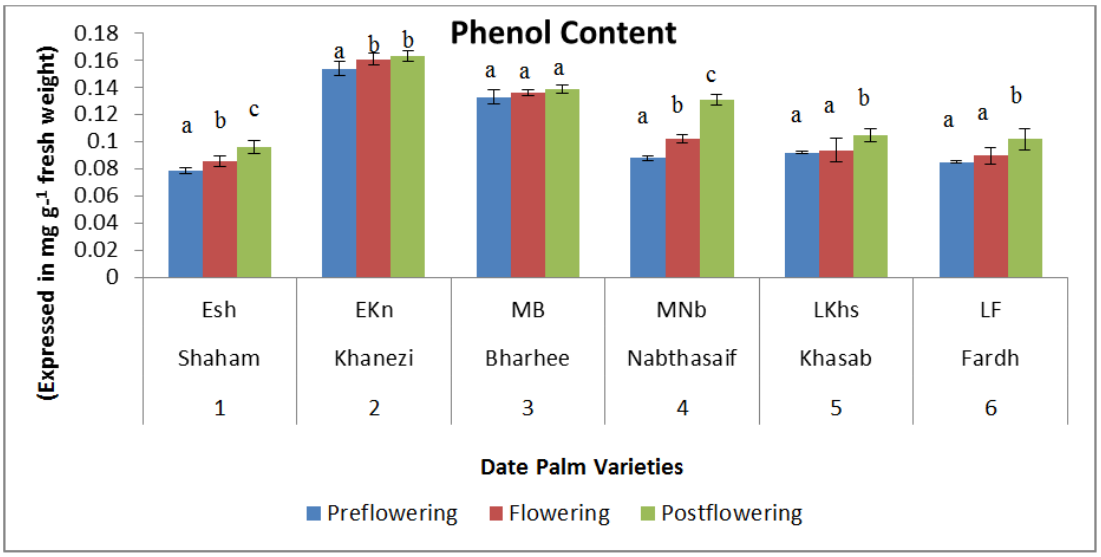

Figure 2: Variations in total phenol content in date palm (*Phoenix dactylifera*) varieties during different flowering stages

Values are given as mean ±SD of six experiments in each group. Values, that are not sharing a common superscript (a,b,c) differ significantly at P≤0.05 (DMRT)

4.2 Antioxidant compounds

4.2.1 Ascorbic acid content

In all the varieties studied, the ascorbic acid content was the lowest during pre-flowering stage. However, the content increased considerably during the flowering stage. There was only slight increase again in the post flowering period in all variety. Among the varieties studied, the ascorbic acid content was high in post flowering stage of Nabthasaif (2.397 mg/g DW), which is a mid-variety, and the lowest was recorded in the pre flowering stage of Shaham (1.856 mg/g DW), which is early flowering type of date palm. In flowering phase minimum ascorbic acid content was noted in Khanezi (2.262 mg/g DW) and lowest was in Fardh (2.01 mg/g DW) variety (Figure 3).

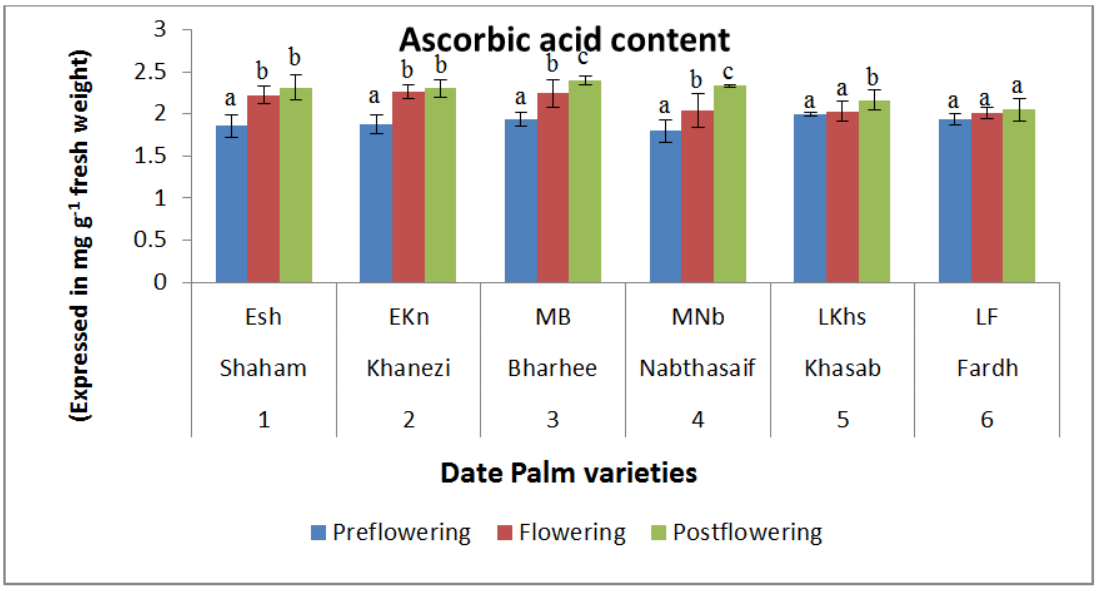

Figure 3: Variations in ascorbic acid content in date palm (*Phoenix dactylifera*) varieties during different flowering stages

Values are given as mean ±SD of six experiments in each group. Values, that are not sharing a common superscript (a,b,c) differ significantly at $P \leq 0.05$ (DMRT)

4.2.2 α-Tocopherol

The concentration of α-tocopherol was high during the flowering period in all the studied 6 varieties of date palms. However, the content diminished in post flowering stage, and in the preflowering stage the content was the minimum with an exception of Shaham variety, where it showed an opposite trend. A highest content of α-tocopherol was recorded in the flowering stage of Nabthasaif (1.196 mg/g DW) and lowest was in the preflowering stage of Nabthasaif (0.838 mg/g DW), which is a mid flowering variety (Figure 4).

4.2.3 Reduced glutathione

The content of reduced glutathione showed an increasing trend in flowering stages of all varieties but the content was far low in other two stages of flowering,

viz., preflowering and post flowering stages. The highest content was recorded in flowering stage of Khanezi (1.152 mg/g DW), which is early variety and the lowest was in postflowering stage of Shaham (0.592 mg/g DW) (Figure 5).

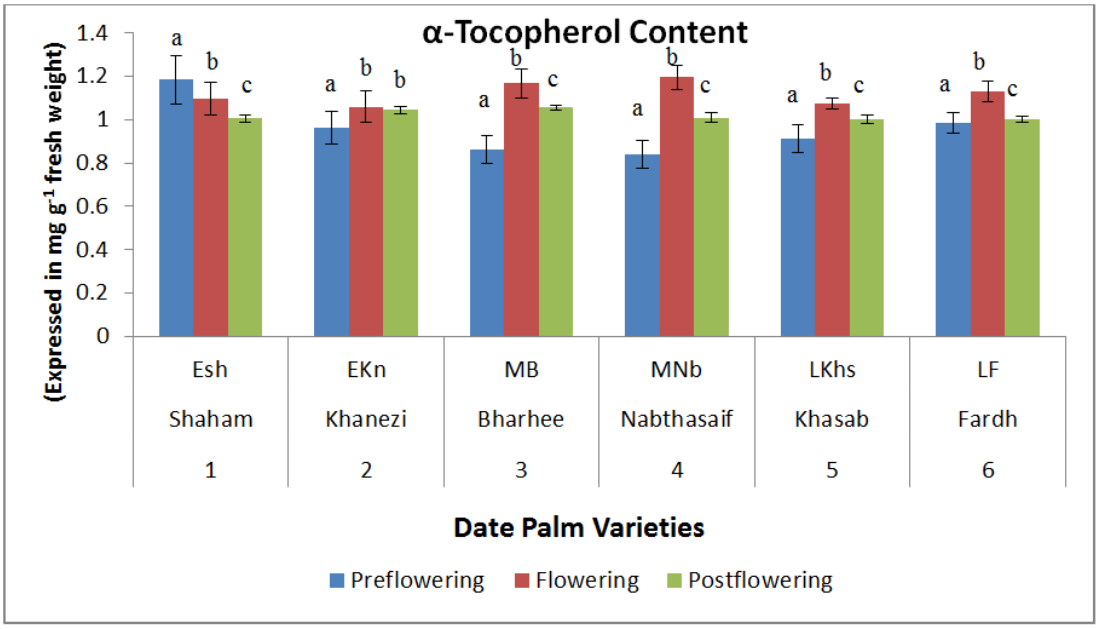

Figure 4: Variations in α-tocopherol content in date palm (*Phoenix dactylifera*) varieties during different flowering stages

Values are given as mean ±SD of six experiments in each group. Values, that are not sharing a common superscript (a,b,c) differ significantly at $P \leq 0.05$ (DMRT)

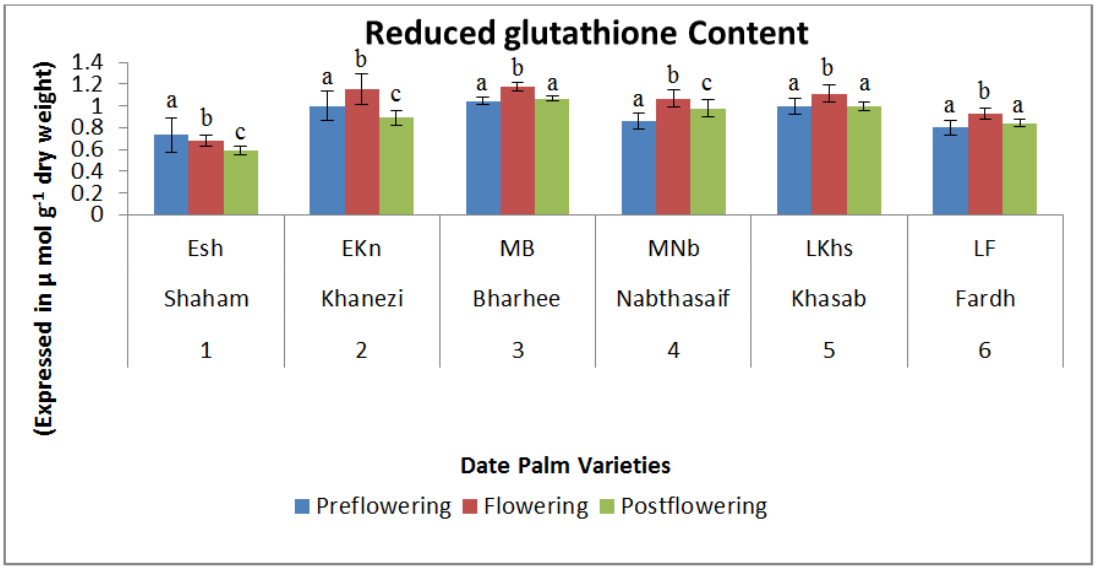

Figure 5: Variations in reduced glutathione (GSH) content in date palm (*Phoenix dactylifera*) varieties during different flowering stages

Values are given as mean ±SD of six experiments in each group. Values, that are not sharing a common superscript (a,b,c) differ significantly at $P \leq 0.05$ (DMRT)

4.3 Antioxidant enzymes

4.3.1 Ascorbate peroxidase activity (APX)

In ascorbate peroxidase activity (APX), the maximum observed in preflowering stage in all studied varieties. The highest activity was noted in Barhee variety (1.19 U mg/protein) and the lowest in Nabthasaif (0.659 U mg/protein) variety. The flowering and postflowering stages showed very little activities when compared to the preflowering stages (Table 1).

Table 1: Ascorbate peroxidase (APX) activity in date palm (*Phoenix dactylifera*) varieties during different flowering stages

S. No	Variety Name	Category	Flowering stages		
			Preflowering	Flowering	Post flowering
1	Shaham	Early Flowering	1.146±0.070^{a}	0.768±0.033^{b}	0.794±0.042^{b}
2	Khanezi		1.028±0.097^{a}	0.842±0.026^{b}	0.985±0.038^{b}
3	Bharhee	Mid Flowering	1.190±0.031^{a}	0.990±0.040^{b}	1.000±0.023^{b}
4	Nabthasaif		0.832±0.039^{a}	0.797±0.028^{a}	0.659±0.063^{c}
5	Khasab	Late Flowering	1.040±0.074^{a}	0.796±0.024^{b}	1.033±0.050^{a}
6	Fardh		0.935±0.040^{a}	0.842±0.064^{b}	0.802±0.022^{b}

Values are given as mean ±SD of six experiments in each group. Values, that are not sharing a common superscript (a,b,c) differ significantly at P≤0.05 (DMRT)

4.3.2 Superoxide dismutase (SOD)

The super oxide dismutase (SOD) activity was maximum in postflowering stage of Barhee (0.597 U/mg protein) and lowest in Fardh (0.295 U mg/protein). Anyhow, the changes were not significant in varieties during different flowering seasons (Table 2).

Table 2: Superoxide dismutase (SOD) activity in date palm (*Phoenix dactylifera*) varieties during different flowering stages

S. No	Variety Name	Category	Flowering stages		
			Preflowering	Flowering	Post flowering
1	Shaham	Early Flowering	0.429±0.037^{a}	0.371±0.019^{b}	0.300±0.026^{c}
2	Khanezi		0.391±0.024^{a}	0.247±0.017^{b}	0.302±0.018^{c}
3	Bharhee	Mid Flowering	0.502±0.006^{a}	0.540±0.026^{b}	0.597±0.015^{c}
4	Nabthasaif		0.333±0.018^{a}	0.388±0.032^{b}	0.407±0.026^{c}
5	Khasab	Late Flowering	0.338±0.027^{a}	0.311±0.020^{a}	0.305±0.044^{a}
6	Fardh		0.313±0.022^{a}	0.303±0.044^{b}	0.295±0.020^{b}

Values are given as mean ±SD of six experiments in each group. Values, that are not sharing a common superscript (a,b,c) differ significantly at P≤0.05 (DMRT)

4.3.3 Catalase (CAT)

In all the studied varieties, the catalase (CAT) activities showed higher values during the flowering season. However, the values decreased slightly during post flowering and still reduced in preflowering stages. The maximum value was 1.249 U mg/protein in postflowering stage of Barhee variety, and lowest value in preflowering stage of Fardh variety (0.813 U mg/protein) (Figure 6). In all the studied varieties, the preflowering stage had the lowest values.

4.3.4 Polyphenol oxidase (PPO)

The polyphenol oxidase (PPO) activity was not significantly altered in different stages of flowering except Nabthasaif variety, which is a mid flowering type of palm. A slight increase during flowering stage was noted, but a decrease during post and preflowering stage was clear. The highest value of PPO in Barhee flowering stage (0.584 U mg/protein) (Figure 7).

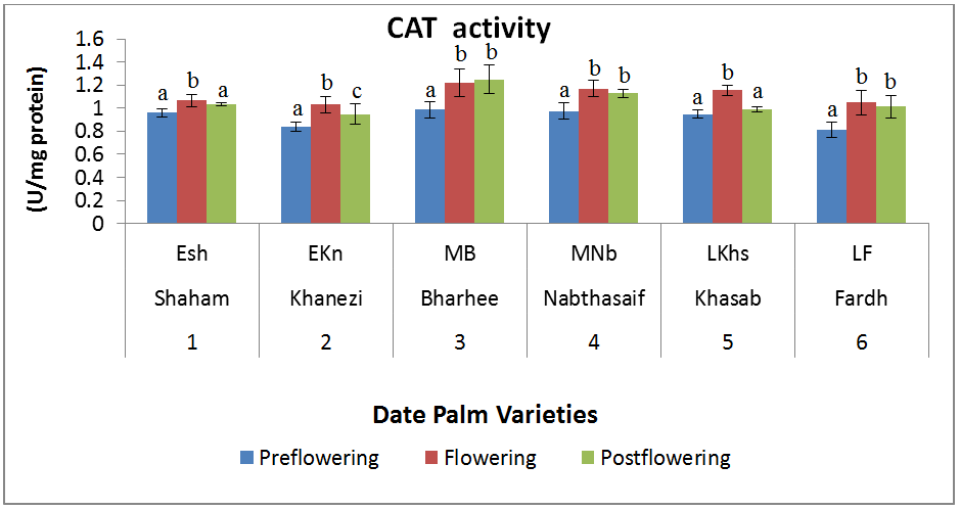

Figure 6: Catalase (CAT) activity in date palm (*Phoenix dactylifera*) varieties during different flowering stages

Values are given as mean ±SD of six experiments in each group. Values, that are not sharing a common superscript (a,b,c) differ significantly at P≤0.05 (DMRT)

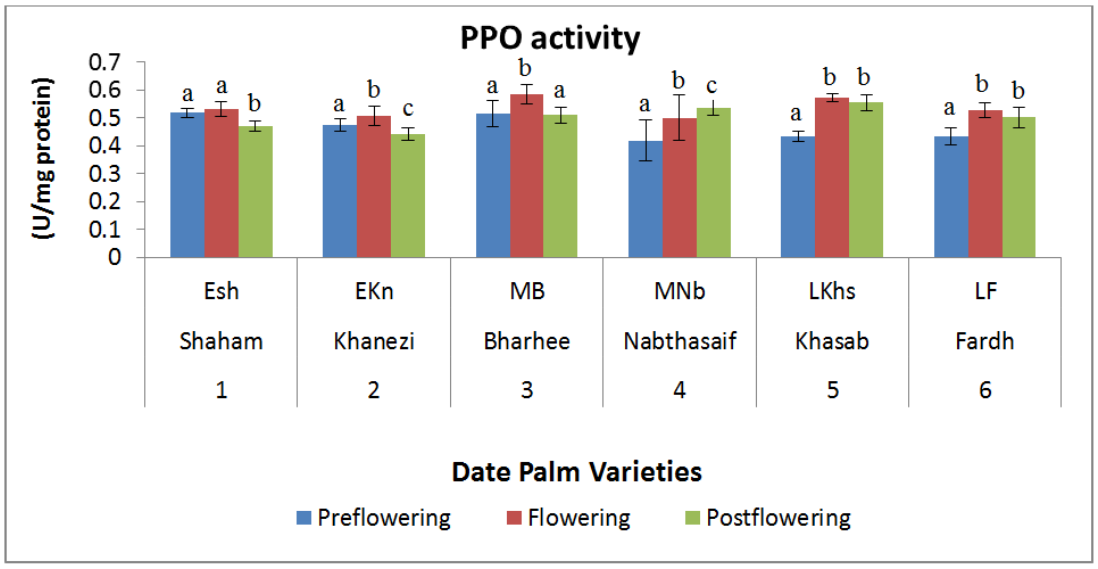

Figure 7: Polyphenol oxidase (PPO) activity in date palm (*Phoenix dactylifera*) varieties during different flowering stages

Values are given as mean ±SD of six experiments in each group. Values, that are not sharing a common superscript (a,b,c) differ significantly at $P \leq 0.05$ (DMRT)

4.3.5 Peroxidase activity (POX)

The peroxidase (POX) activities showed only slight increase during the flowering of date palm. There was a trend in slight increase during flowering stage but a decrease during post and preflowering stage. The POX highest values were in Fardh variety flowering stage (0.527 U mg/protein) (Figure 8).

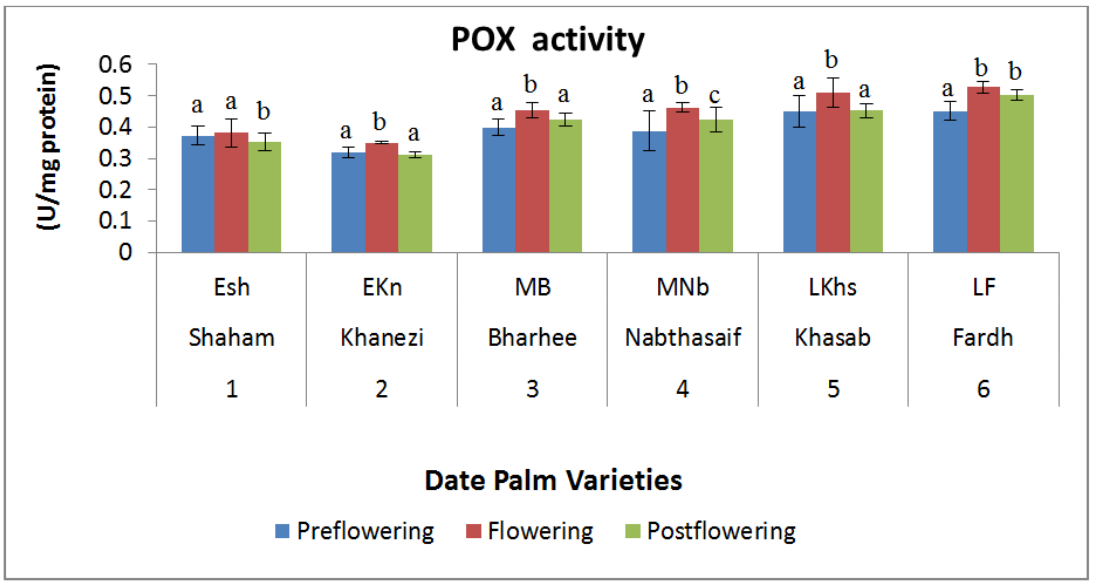

Figure 8: Peroxidase (POX) activity in date palm (*Phoenix dactylifera*) varieties during different flowering stages

Values are given as mean ±SD of six experiments in each group. Values, that are not sharing a common superscript (a,b,c) differ significantly at P≤0.05 (DMRT)

4.4 Isoenzyme electrophoresis analysis

4.4.1 Peroxidase (POX)

In peroxidase (POX) (Figure 9), the band patterns of enzymatic activity were not consistent for the most of the sample in relation to pre-flowering, flowering and post-flowering periods and in early mid and late varieties of date palm. Maximum five bands and variation observed in the POX, among this most of the varieties of date palm had POX enzymatic activity banding patterns were rather consistent but some minor qualitative variances in the staining strength. Some peroxidase bands occupying the same position. Particularly highest enzyme banding intensity was in lane EKn, MB, MNb and it showed their activity varied in the time of flower

induction. The activity staining of the enzyme, with guaiacol as substrate, showed a single band corresponding to the position of the POX activity.

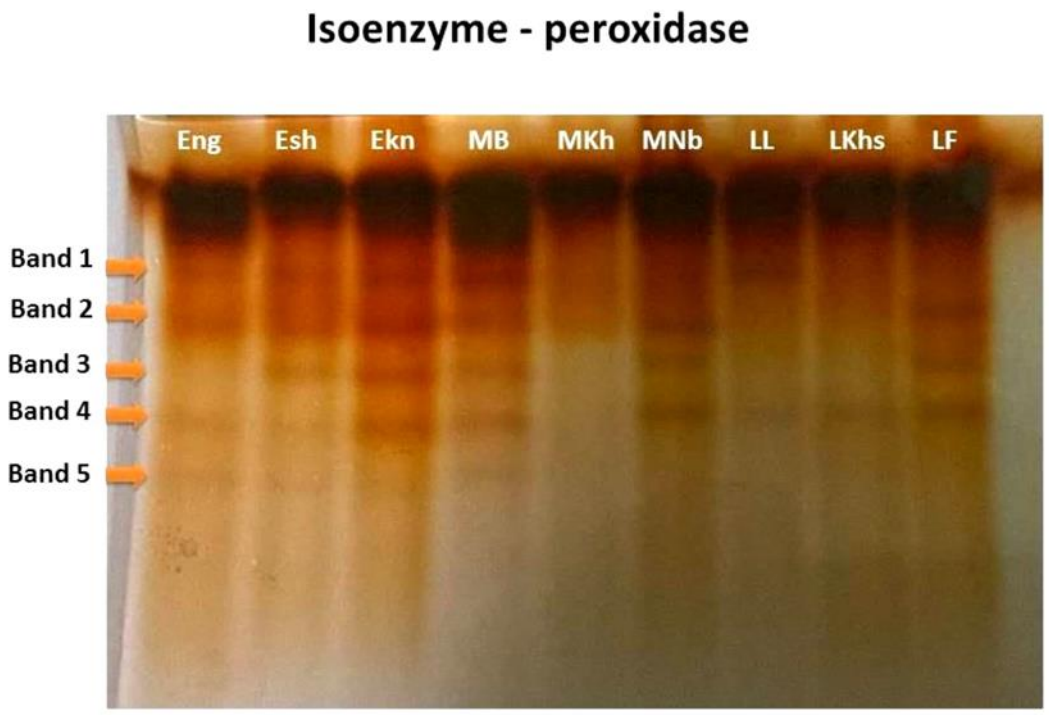

Figure 9: Peroxidase (POX) isozymes activity bands in in date palm (*Phoenix dactylifera*) varieties during different flowering stages

4.4.2 Polyphenol oxidase (PPO)

In polyphenol oxidase (PPO) there are two banding patterns were observed in the midst of nine different date palm varieties (Figure 10). Among these banding MKh and MB had highest intensity and noted strong staining as compared to other verities. Other varieties showed similar intensity of PPO staining and banding patterns.

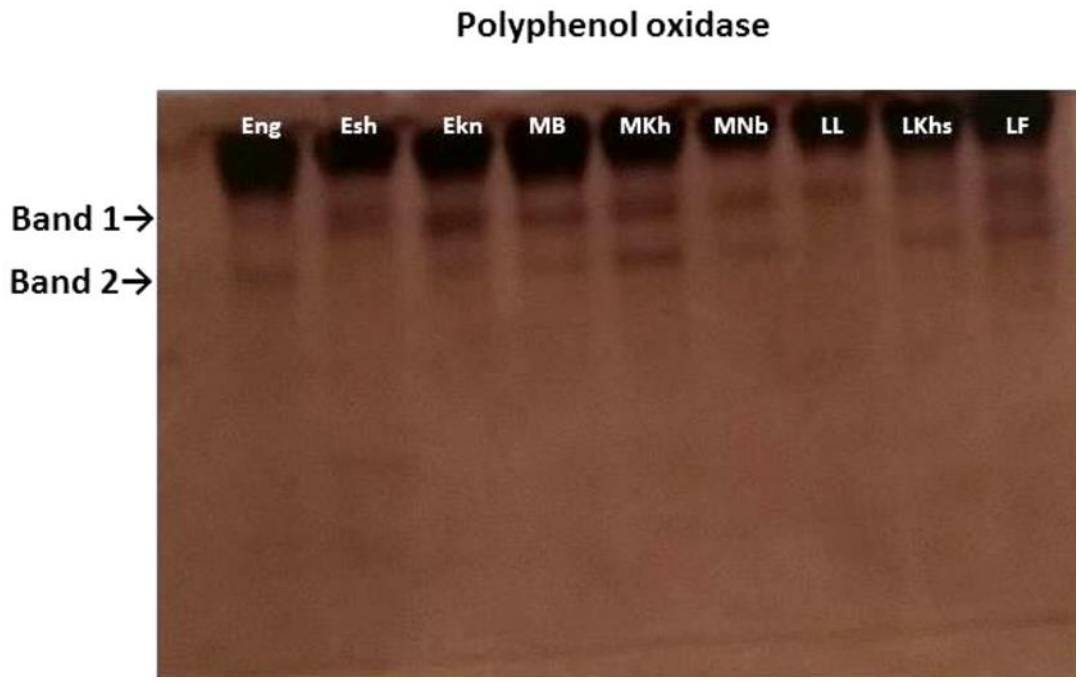

Figure 10: Polyphenol oxidase (PPO) isozymes activity bands in date palm (*Phoenix dactylifera*) varieties during different flowering stages

Table 3: Similarity index between early, mid and late varieties of date palm (*Phoenix dactylifera*) from UAE

	ENg	Esh	EKn	MB	MKh	MNb	LL	LKhs	LF
ENg	1.0000000								
Esh	0.7500000	1.0000000							
EKn	0.6250000	0.8750000	1.0000000						
MB	0.7500000	0.7500000	0.6250000	1.0000000					
MKh	1.0000000	0.7500000	0.6250000	0.7500000	1.0000000				
MNb	0.6250000	0.6250000	0.5000000	0.6250000	0.6250000	1.0000000			
LL	0.8750000	0.8750000	0.7500000	0.8750000	0.8750000	0.5000000	1.0000000		
LKhs	0.7500000	0.7500000	0.6250000	0.7500000	0.7500000	0.3750000	0.8750000	1.0000000	
LF	0.7500000	0.7500000	0.6250000	0.7500000	0.7500000	0.3750000	0.8750000	1.0000000	1.0000000

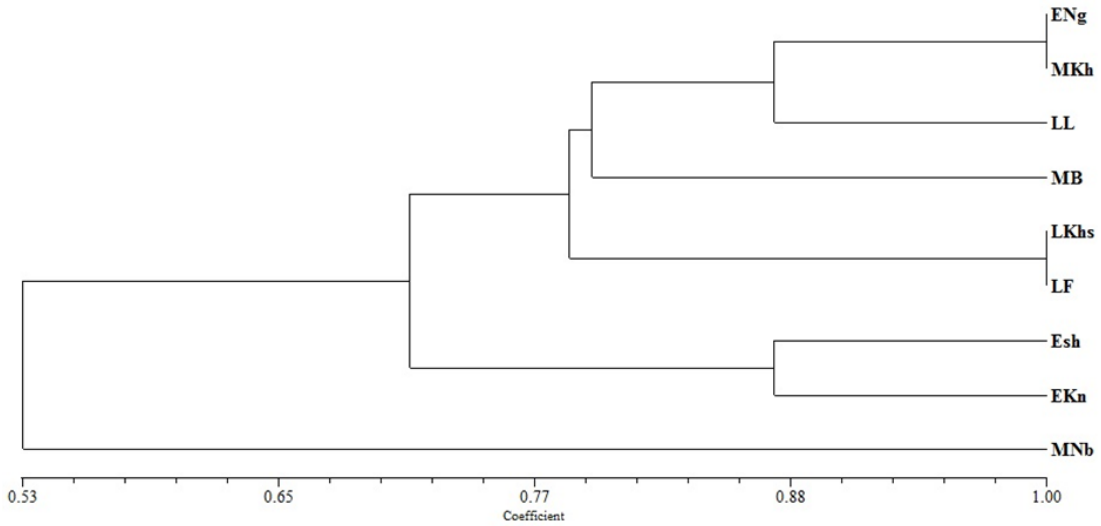

Figure 11: Dendrogram based on Jaccard's similarity level showing the genetic relationships between early, mid and late varieties of date palm (*Phoenix dactylifera*) from UAE

The similarity matrix was calculated based on Jaccard coefficient. The dendrogram was constructed based on the similarity coefficient, which resulted in two major clusters. The cluster 1 was subdivided into two sub clusters. In sub cluster 1, all the three late varieties grouped together with two mid varieties (MKh and MB) and one early variety (ENg). This shows that all the late varieties have more genetic similarities compared to other two flowering types. In sub cluster 2, two early varieties Esh and EKn grouped together, indicating the high genetic similarity of the tested early varieties. In cluster 2, the Mid variety MNb was grouped alone which showed a similarity value of 0.375 with cluster 1. The similarity value for isozyme analysis was found to be a minimum of 0.375 observed between MNb with LKhs and MNb with LF. The similarity value was maximum between ENg and MKh and also between late varieties LF and LKhs. In the case of early varieties, the similarity value between the varieties ranged from 0.625 to 0.875. In mid flowering varieties, the genetic similarity ranged from 0.625 to 0.75. Compared to early and mid-flowering

varieties, Late flowering varieties showed maximum similarities between the tested varieties, which ranges from 0.875 to 1.

4.5 Hormone profiling

In pre-flowering season, the highest amount of Gibberellic Acid (µg/g) was noted in Shaham (61.5) and Khanezi (65.73). The lowest contents were in late flowering plants (33.02 in Khasab and 23.65 in Fardh). IAA and ABA were lowest in Barhee (0.13 IAA and 0.27 ABA) and highest in Fardh (2.77 IAA and 2.11 ABA) plants. But in Nabthasaif plants, there was no significant variation in IAA content during preflowering and preflowering stages. Nabthasaif plants had highest amount of zeatin (µg/g) and which was 35.83.

In flowering season, early varieties (Shaham and Khanezi) showed the highest amount of GA3 (µg/g) (78.89 and 76.91) respectively. Barhee and Nabthasaif showed lower values for this hormone. ABA content was low in late flowering varieties in flowering stage (2.35 in Khasab and 2.32 in Fardh). In Fardh variety there was no significant variation in ABA content during preflowering and flowering seasons (Table 4).

Table 4: Phytohormone concentrations in different date palm (*Phoenix dactylifera*) varieties during different flowering stages

Variety	Type	Growth Stage	GA (µg/g)	IAA (µg/g)	ZAn (µg/g)	ABA (µg/g)
ESh	Early season	Preflowering	61.05±2.84^{a}	0.16±0.01^{a}	10.77±0.59^{a}	0.41±0.01^{a}
		Flowering	78.89±2.65^{b}	1.22±0.09^{b}	19.54±0.90^{b}	4.89±0.40^{b}
		Postflowering	16.5±0.99^{c}	0.04±0.01^{a}	12.4±0.53^{a}	0.08±0.00^{a}
EKn		Preflowering	65.73±3.49^{a}	0.64±0.09^{a}	13.01±0.75^{a}	0.41±0.01^{a}
		Flowering	76.91±2.53^{b}	1.60±0.11^{b}	4.14±0.42^{b}	4.22±0.38^{b}
		Postflowering	41.87±1.38^{c}	1.22±0.08^{b}	0.15±0.01^{c}	0.29±0.01^{a}
MBr	Mid-season	Preflowering	29.39±1.80^{a}	0.13±0.04^{a}	10.60±0.48^{a}	0.27±0.01^{a}
		Flowering	16.36±0.84^{b}	1.12±0.08^{b}	4.41±0.39^{b}	2.56±0.07^{b}
		Postflowering	18.19±0.75^{b}	0.66±0.01^{a}	10.56±0.88^{a}	2.19±0.07^{b}
MNb		Preflowering	38.86±1.75^{a}	1.15±0.18^{a}	35.83±1.50^{a}	1.02±0.05^{a}
		Flowering	12.41±0.72^{b}	1.29±0.08^{a}	12.56±0.78^{b}	2.58±0.19^{b}
		Postflowering	14.10±0.68^{b}	1.46±0.12^{b}	5.71±0.33^{c}	0.25±0.02^{c}
LKh	Late season	Preflowering	33.02±1.58^{a}	0.83±0.02^{a}	16.90±0.80^{a}	1.19±0.09^{a}
		Flowering	17.72±0.99b	0.59±0.01^{a}	13.20±0.69^{b}	2.35±0.09^{b}
		Postflowering	22.38±0.98^{c}	1.56±0.17^{b}	24.62±1.35^{c}	0.23±0.01^{c}
LFr		Preflowering	23.65±1.30^{a}	2.77±1.20^{a}	18.15±1.63^{a}	2.11±0.09^{a}
		Flowering	19.50±0.90^{b}	0.50±0.01^{b}	14.90±0.75^{b}	2.32±0.09^{a}
		Postflowering	16.49±0.78^{c}	0.86±0.01^{b}	25.08±1.30^{c}	0.23±0.01^{b}

GA: Gibberellic Acid, IAA: Indole Acetic Acid, ZAn: Zeatin, ABA: Abscisic Acid, ESh: Shaham, EKn: Khanezi, MBr: Barhee, MNb: Nabthasaif, LKh: Khasab, LFr: Fardh.

Values are given as mean ±SD of six experiments in each group. Values, that are not sharing a common superscript (a,b,c) differ significantly at $P \leq 0.05$ (DMRT)

Chapter 5: Discussion

The present investigation was conducted to determine enzyme activity and hormonal profiling in six date varieties of United Arab Emirates. The results on biochemical constituents, non-enzymatic and enzymatic antioxidants, isoenzymes and phytohormones profile are discussed hereunder.

5.1 Biochemical contents

5.1.1 Protein and phenol contents

From the results it is clear that the protein content was high in early flowering varieties during the preflowering period. This might be the reason in early flowering in these plants. There are reports that the protein involvement in flowering time initiation in different plants. Notaguchi et al. (2008) explained that the proteins like FT protein in *Arabidopsis* are an important part of the mobile signal that promotes flowering in all the varieties irrespective of the seasons or flowering development. Anyhow, the highest contents were noted on flowering and post flowering stages in mid and late varieties. Cruz et al. (1970) reported the various biochemical factors including protein which influence the grain production in rice. Mainly the transition from vegetative to flowering is one of the important phase in plant life, which triggered by the changes in many of the biochemicals (Paul et al., 2008).

The phenol contents were less during preflowering and flowering induction stages, but were high during the post flowering stages in all the varieties of date palm. Engelsma (1979) revealed that the presence of high amount of phenols may delay flowering, and subsequently, its reduction will induce flowering as shown in *Salvia occidentalis*. It might be due to the presence of phenols in the cytoplasm of

leaf cells inhibits the synthesis or the transport of a flowering hormone Engelsma (1979). Keller and Hrazdina (1998) reported increase in phenol during post flowering stage of grapes and throughout ripening. del Baño et al. (2003) reported the role of phenolic diterpenes during the development of plant organs especially flowers of *Rosmarinus officinalis*.

5.2 Antioxidant compounds

5.2.1 Ascorbic acid content

Mohamed and Akladious (2014) observed the increased ascorbic acid content in soybean plants under oxidative pressure. The ascorbic acid content showed a variation under abiotic stress in maize and wheat, suggesting its significant role in oxidative stress response (Nayyar and Gupta, 2006). The increased ascorbic acid content observed in the leaves of chickpea plant (Pradeep et al., 2011; Patel and Hemantaranjan, 2012), sunflower and bhendi plants under drought stress (Rabert et al., 2014). Wu et al. (2017) recently reported a potential relation of glutathione-ascorbate cycle in flowering Chinese cabbage. In this study, there was a reduction in ascorbic acid in preflowering stage. Lower levels of ascorbic acid are a prerequisite for the production of flower induction hormones such as IAA and gibberellic acid (GA). There is a transient rise in the ascorbic acid content which could be seen in flowering and postflowering stages in all the varieties. Ascorbic acid protects the date palm in the time of winter to overcome the abiotic stress resulted from the winter season during flowering season.

5.2.2 α-Tocopherol

An important rise in the content of α-tocopherol was observed at the flowering stage in all varieties of date palm. α-Tocopherol is a strong antioxidant that it induces

tolerance to stress (Tlili et al., 2009). Normally many alterations take place during flower bud formation and opening and secondary metabolites due to well defined sequence like cell division, cellular differentiation membrane permeability, cell elongation (Sood and Nagar, 2003). The elevation in the α-tocopherol content can be been correlated with the response of photosynthetic tissues to a variety of abiotic stresses (Keles and Oncel, 2002). In the present study, the postflowering stage of the crop overlaps with the summer season leading to high fluctuations in temperature to protect the plants from the oxidative stress. The variation in α-tocopherol could be endorsed to the low temperature stress observed at the flowering stage in the date palm, as the α-tocopherol will be expressed in the plants under stress.

5.2.3 Reduced glutathione (GSH)

In mammalian cells, non-protein thiol GSH participates in a variety of detoxification reactions (Lu, 2009). GSH is a major protectant for cell, which can directly catch ROS, other oxygen-centered free radicals and radical centers on DNA or other molecules (Lobo et al., 2010). In line with the above findings our results showed, depleted GSH levels in preflowering while in the flowering season the levels were found to be raised when compared to other studied varieties. The GSH biosynthesis rate increased due to stress inducible activation of glutamyl cysteine synthetase at the posttranscriptional levels (Ogawa et al., 2001), which may have enhanced the stress-associated promotion of flowering. Hence, this finding may benefit the date palm cultivation industry since flowering is induced only during low-temperature months. Chilling stress is known to cause an oxidative stress and to induce changes in the GSH content of plants (Caverzan et al., 2012). This was observed in this study where noticeable changes in the content of GSH occurred in all the three varieties.

5.3 Antioxidant enzymes

Stresses commonly produce the overproduction of reactive oxygen species (ROS), such as superoxide radical (O^{2-}), H_2O_2 and hydroxyl radical (HO) in plant cells (Demidchik, 2017). Different mechanisms participate in ROS detoxification and drought resulted in a significant increase in superoxide dismutase activity (Sharma et al., 2017). During the flowering stages and post flowering stages the palms are under stress with winter and summer respectively, which might enhance the antioxidant metabolism in palms.

5.3.1 Ascorbate peroxidase activity (APX)

The APX activity was increased in soybean (Zhao et al., 2017) and in chickpea (Pradeep et al., 2011) under water stress. Pandey and Shukla (2015) reported that the APX greatly increased in rice under oxidative stress. Hence, it was possible that the upturns in catalase activity at these stages could have resulted from the accumulation of H_2O_2 due to a higher rate of respiration which in turn might have resulted from the low temperature dominant during the flowering season (Kasraoui et al., 2014).

5.3.2 Superoxide dismutase (SOD)

Superoxide dismutase is the most predominant enzymatic antioxidant found in sperm cells (Makkeret al., 2009). It is a natural scavenger of reactive oxygen species and superoxide radicals and does this by combining with active oxygen free radicals specifically superoxide ions in order to prevent lipid peroxidation of the cell membrane and damaging metabolites' formation (Bailly et al., 1996).

The consolidated impact of both antioxidant enzymes (CAT and SOD) is converting the poisonous superoxide radical (O_2) and H_2O_2 to water and oxygen (O_2), and in this manner keep up the cells under dry seasons (Noctor et al., 2000). Likewise, the elevation in SOD activity in shoots of date palm may be because of the enactment of radical scavenging ability. Changes in the action of antioxidant compounds, which are signs of plant adaption to stress conditions.

5.3.3 Catalase (CAT)

CAT enzyme showed a significant high activity in genotype at all intervals and reached its maximum activity at moderate drought stress (Hassan et al., 2015). Abassi et al. (2014) reported a manganese deficiency is associated with burst of catalase activity during bud development which peaked during fruit set in apple, contrary to the results obtained from the mid flowering variety of the date palm in this study.

The elevation in CAT action may be valuable in disproportioning H_2O_2 that is the key item in diminishing senescence under extreme environmental stress. In peroxisome the CAT does a basic part in the evacuation of poisonous H_2O_2, which is continuously formed during photorespiration by the dismutation of the superoxide radicals generated in the NADH subordinate electron transport system of the peroxisomal layer (Corpas et al., 2001). The results on early and late flowering are in conformity with Abassi et al. (2014), as there was a significant increase of CAT activity during early flowering at all the three stages, except in late flowering stage.

5.3.4 Polyphenol oxidase (PPO)

The different PPO isozymes patterns could be due to differentiation, aggregation in composition of amino acid, various percentage of covalently linked carbohydrates, partial proteolysis and other post translational modifications (Kumar et al., 2014). During the blooming season, there was no notable increase in PPO action among varieties. This might be clarified by the way that PPO are compartmentalized in plant cells yet thus of pathogenic attack, membrane interruption may happen starting the presentation of vacuole phenolics to luminal PPO (Pourcel et al., 2007). During the flowering season, there was no significant difference in PPO activity among all the varieties. This may be explained by the fact that PPO are compartmentalized in plant cells but as a result of pathogenic attack, membrane disruption may occur initiating the exposure of vacuole phenolics to lumenall PPO (Kant et al., 2015).

5.3.5 Peroxidase activity (POX)

Activity of POX is considered necessary in the oxidation of auxin (IAA) (Hiraga et al., 2001) and have proven IAA oxidase activity (Schwambach et al., 2008). This example has been seen in many plant species. If the POX activity is higher during the preflowering stage would exhaust the high IAA necessary for flower induction, so it is sensible to have diminished POX action at first (Cheniany et al., 2010). POX have been shown to function in cell growth and expansion (Schmidt, 2016) and the synthesis of lignin and suberin (Syros et al., 2004). Enzyme POX are oxidoreductases that catalyze the oxidation of a different types of organic compounds utilizing hydrogen peroxide as a definitive electron acceptor (Karigar and Rao, 2011).

5.4 Isoenzyme electrophoresis analysis

As per Sharma et al. (2012), the generation of new isozymes can be because of their biosynthesis de novo, or by change of dynamic or idle antecedents into new molecular structures. However, Plants have a large number of POX isoenzymes that may differ by more than 50% in amino acid sequence (Krainer, 2014). The expansion in dissolvable POX action could be because of a general increment in all isoenzymes to an increment specifically isoenzymes effectively present or to the presence of new isoenzymes (Nag et al., 2013). According to Dubrovskaya (2017), the formation of new isozymes can be due to their biosynthesis de novo, or by transformation of active or inactive precursors into new molecular forms in POX. The band patterns of peroxidase enzymatic activity were not consistent for most of the sample in relation to pre-flowering, flowering and post-flowering periods and in early mid and late varieties of date palm.

PPO has been reported to have properties associated with adventitious root formation (Verstraeten et al., 2014), and may be useful in defining the beginning and duration of the walnut rooting phases in micro shoots as observed in embryo-cotyledonary linking areas and cotyledon pieces by Cheniany et al. (2010). POX and PPO contribute to the formation of defense barriers for reinforcing the cell structure (Thakker and Dhandhukia, 2012). The different polyphenol oxidase isozyme patterns could be due to differentiation, aggregation in composition of amino acid, various percentage of covalently linked carbohydrates, partial proteolysis and other post translational modifications (George and Christoffersen, 2016).

The results of Jaccard similarity index suggest that there is high level of genetic similarity in isozyme production in all the tested late flowering varieties

compared to early and mid-varieties. This is considered as a preliminary study on genetic variation of isozyme in different flowering varieties. Further studies with larger population will provide more information about the genetic diversity of flowering varieties of date palm.

5.5 Hormone profiling

Cheruth et al. (2015) reported the possible roles of innate hormones in the regulation of flower development in date palm varieties. High amount of ABA may contribute to the delayed flowering in the mid and late varieties. The number of Panicle in the Litchi under the treatment of ABA gradually increased as exogenous ABA was added prior to the emergence of the panicle primordial (Cui et al., 2013). A low GA level is significant for transporting nutrients from the vegetative organs into reproductive organs (Aldaej et al., 2014). There are various processes take place during stages like flower induction, initiation, flower opening until senescence, with the notable changes in concentration of innate hormones and secondary metabolites (Klessig, 2017). Flowering is sensitive to physiological parameters and to current environmental conditions (Guilbaud et al., 2015).

Chapter 6: Conclusion

The study examined the possible role of antioxidant compounds and enzymes on flower development of date palm with special emphasis on hormonal status during various flowering stages. The study was conducted in 3 different types of dates, early, mid and late flowering palms. The antioxidant activity of *P. dactylifera* varies according to its varieties and flowering stages. Recently there are many reports on the antioxidant activity and their possible role in health related effects of date fruits, but our study is different from those aspects. So, this type of study provides insight on palm's physiological basis of flowering.

It should be highlighted that no data have so far been published on the role of antioxidant enzymes in date flowering. Most studies relate the pharmacological potentials of *P. dactylifera* to its antioxidant activity. Phenolic compounds are found to be potent modifiers of catalase, peroxidase, and polyphenol oxidase activity, as both inhibitors and stimulators in date palm varieties. Diminutive evidences are obtainable on hormonal changes during different stages of flowering in date palm. Therefore, the hormonal profiling and enzymatic activities of three different seasons of date palm varieties assumes greater significance. These data clearly indicated the important role of hormones which can play a significant effect in influencing the rate and quality of date palm plants. This study showed the possible role of endogenous hormones, antioxidant contents, and activities of antioxidant enzymes in regulating flower development in date palm varieties. Future work will evaluate the importance of these metabolites in all cultivars of the early, mid, and late flowering date palm varieties at various stages of flower development.

References

Abassi, R., Namsi, A., Bennasri, M., Abdalah, H. B., Mâachia, S. B., Ouerghi, Z., & Duran-Vila, N. (2014). Manganese deficiency is associated with histological changes in date palm fronds showing brittle leaf disease symptoms. Journal of Plant Pathology, 96(1), 29-34.

Abohatem, M., Zouine, J., & El Hadrami, I. (2011). Low concentrations of BAP and high rate of subcultures improve the establishment and multiplication of somatic embryos in date palm suspension cultures by limiting oxidative browning associated with high levels of total phenols and peroxidase activities. Scientia Horticulturae, 130(1), 344-348.

Aggarwal, B. B., Sundaram, C., Prasad, S., & Kannappan, R. (2010). Tocotrienols, the vitamin E of the 21st century: its potential against cancer and other chronic diseases. Biochemical Pharmacology, 80(11), 1613-1631.

Aldaej, M. I., Alturki, S. M., Shehata, W. F., & Ghazzawy, H. S. (2014). Effect of potassium nitrate on antioxidants production of date palm (*Phoenix dactylifera* L.) in vitro. Pakistan Journal of Biological Sciences, 17(12), 1209-1218.

Allaith, A. A. A. (2008). Antioxidant activity of Bahraini date palm (*Phoenix dactylifera* L.) fruit of various cultivars. International Journal of Food Science & Technology, 43(6), 1033-1040.

Al-Maasllem, I. S. (1996). Date Palm (Phoenix dactylifera L.) Vol. 7. Encyclopedia Works Publishing & Distribution

Al-Shahib, W., & Marshall, R. J. (2003). The fruit of the date palm: its possible use as the best food for the future?. International Journal of Food Sciences and Nutrition, 54(4), 247-259.

Angelov, G. B., & Ivanova, T. A. (2012). Isoenzyme variation and genetic affinities among four species of the genus *Festuca* L.(Poaceae). Biodiversity: Research and Conservation, 28, 3-8.

Asada, K. and Takahashi, M. (1987) Production and scavenging of active oxygen in photosynthesis. In Photoinhibition (Kyle, D.J. et al., eds), pp. 227–287, Elsevier.

Asgher, M., Khan, N. A., Khan, M. I. R., Fatma, M., & Masood, A. (2014). Ethylene production is associated with alleviation of cadmium-induced oxidative stress by sulfur in mustard types differing in ethylene sensitivity. Ecotoxicology and Environmental Safety, 106, 54-61.

Ashraf, Z., & Hamidi-Esfahani, Z. (2011). Date and date processing: a review. Food Reviews International, 27(2), 101-133.

Awad, M. A., Al-Qurashi, A. D., & Mohamed, S. A. (2011). Antioxidant capacity, antioxidant compounds and antioxidant enzyme activities in five date cultivars during development and ripening. Scientia Horticulturae, 129(4), 688-693.

Azeqour, M., Majourhat, K., & Baaziz, M. (2002). Morphological variations and isoenzyme polymorphism of date palm clones from in vitro culture acclimatized and established on soil in South Morocco. Euphytica, 123(1), 57-66.

Bailly, C., Benamar, A., Corbineau, F., & Come, D. (1996). Changes in malondialdehyde content and in superoxide dismutase, catalase and glutathione reductase activities in sunflower seeds as related to deterioration during accelerated aging. Physiologia Plantarum, 97(1), 104-110.

Baker, H., Frank, O., DeAngelis, B., & Feingold, S. (1980). Plasma tocopherol in man at various times after ingesting free or acetylated tocopherol. Nutrition Reports International, 21(4), 531-536.

del Baño, M. J., Lorente, J., Castillo, J., Benavente-García, O., del Río, J. A., Ortuño, A., & Gerard, D. (2003). Phenolic diterpenes, flavones, and rosmarinic acid distribution during the development of leaves, flowers, stems, and roots of *Rosmarinus officinalis* antioxidant activity. Journal of Agricultural and Food Chemistry, 51(15), 4247-4253.

Barrow, S. C. (1998). A monograph of *Phoenix* L. (palmae: Coryphoideae). Kew Bulletin, 513-575.

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry, 72(1-2), 248-254.

Caughey, W. (Ed.). (2012). Biochemical and clinical aspects of oxygen. Elsevier.

Caverzan, A., Passaia, G., Rosa, S. B., Ribeiro, C. W., Lazzarotto, F., & Margis-Pinheiro, M. (2012). Plant responses to stresses: role of ascorbate peroxidase in the antioxidant protection. Genetics and Molecular Biology, 35(4), 1011-1019.

Chandlee, J. M., & Scandalios, J. G. (1984). Analysis of variants affecting the catalase developmental program in maize scutellum. Theoretical and Applied Genetics, 69(1), 71-77.

Chao, C. T., & Krueger, R. R. (2007). The date palm (Phoenix dactylifera L.): overview of biology, uses, and cultivation. HortScience, 42(5), 1077-1082.

Chaudhari, H. V., Dakhale, G. N., Chaudhari, S., Kolhe, S., Hiware, S., & Mahatme, M. (2012). The beneficial effect of vitamin C supplementation on serum lipids in type 2 diabetic patients: a randomized double blind study. International Journal of Diabetes and Metabolism, 20, 53-58.

Cheniany, M., Ebrahimzadeh, H., Masoudi-nejad, A., Vahdati, K., & Leslie, C. (2010). Effect of endogenous phenols and some antioxidant enzyme activities on rooting of Persian walnut (*Juglans regia* L.). African Journal of Plant Science, 4(12), 479-487.

Cherry, J. H. (1963). Nucleic acid, mitochondria, & enzyme changes in cotyledons of peanut seeds during germination. Plant Physiology, 38, 440–446.

Cheruth, A. J., Kurup, S. S., & Subramaniam, S. (2015). Variations in hormones and antioxidant status in relation to flowering in early, mid, and late varieties of date palm (*Phoenix dactylifera*) of United Arab Emirates. The Scientific World Journal, Article ID 846104, 8 p., doi:10.1155/2015/846104.

Corpas, F. J., Barroso, J. B., & del Río, L. A. (2001). Peroxisomes as a source of reactive oxygen species and nitric oxide signal molecules in plant cells. Trends in Plant Science, 6(4), 145-150.

Cosio, C., & Dunand, C. (2009). Specific functions of individual class III peroxidase genes. Journal of Experimental Botany, 60(2), 391-408.

Cruz, L. J., Cagampang, G. B., & Juliano, B. O. (1970). Biochemical factors affecting protein accumulation in the rice grain. Plant Physiology, 46(5), 743-747.

Csiszár, J., Fehér-Juhász, E., Kotai, E., Ivankovits-Kiss, O., Horváth, G. V., Mai, A., & Erdei, L. (2005). Effect of osmotic stress on antioxidant enzyme activities in transgenic wheat calli bearing MsALR gene. Acta Biologica Szegediensis, 49(1-2), 49-50.

Cui, Z., Zhou, B., Zhang, Z., & Hu, Z. (2013). Abscisic acid promotes flowering and enhances LcAP1 expression in Litchi chinensis Sonn. South African Journal of Botany, 88, 76-79.

Demidchik, V. (2017). Reactive oxygen species and their role in plant oxidative stress. Plant Stress Physiology, CAB International, Oxford, 24-58.

Doaigey, A. R., Al-Whaibi, M. H., Siddiqui, M. H., Al Sahli, A. A., & El-Zaidy, M. E. (2013). Effect of GA 3 and 2, 4-D foliar application on the anatomy of date palm (*Phoenix dactylifera* L.) seedling leaf. Saudi Journal of Biological Sciences, 20(2), 141-147.

Dubrovskaya, E. V., Pozdnyakova, N. N., Golubev, S. N., Grinev, V. S., & Turkovskaya, O. V. (2017). Peroxidases from alfalfa roots: Catalytic properties and participation in degradation of polycyclic aromatic hydrocarbons. Russian Journal of Plant Physiology, 64(2), 174-183.

Eberhardt, M. V., Lee, C. Y., & Liu, R. H. (2000). Nutrition: Antioxidant activity of fresh apples. Nature, 405(6789), 903-904.

Eitenmiller, R. R., & Lee, J. (2004). Vitamin E: food chemistry, composition, and analysis. CRC Press.

El Hadrami, A., Daayf, F., Elshibli, S., Jain, S. M., & El Hadrami, I. (2011). Somaclonal variation in date palm. In Date Palm Biotechnology (pp. 183-203). Springer Netherlands.

Engelsma, G. (1979). Effect of day length on phenol metabolism in the leaves of Salvia occidentalis. Plant Physiology, 63(4), 765-768.

Eris A. (1995). "The Physiology of Horticulture" Uluda˘g University Press.

Erskine, W., Moustafa, A. T., Osman, A. E., Lashine, Z., Nejatian, A., Badawi, T., & Ragy, S. M. (2004, May). Date palm in the GCC countries of the Arabian Peninsula. In Proc. Regional Workshop on Date Palm Development in the Arabian Peninsula, Abu Dhabi, UAE.

Fki, L., Bouaziz, N., Kriaa, W., Benjemaa-Masmoudi, R., Gargouri-Bouzid, R., Rival, A., & Drira, N. (2011). Multiple bud cultures of 'Barhee'date palm (*Phoenix dactylifera*) and physiological status of regenerated plants. Journal of Plant Physiology, 168(14), 1694-1700.

Fridovich, I. (1975). Superoxide dismutases. Annual Review of Biochemistry, 44(1), 147-159.

Gallé, Á., Csiszár, J., Secenji, M., Guóth, A., Cseuz, L., Tari, I., & Erdei, L. (2009). Glutathione transferase activity and expression patterns during grain filling in flag leaves of wheat genotypes differing in drought tolerance: response to water deficit. Journal of Plant Physiology, 166(17), 1878-1891.

Garay- Arroyo, A., De La Paz Sánchez, M., García- Ponce, B., Azpeitia, E., & Álvarez- Buylla, E. R. (2012). Hormone symphony during root growth and development. Developmental Dynamics, 241(12), 1867-1885.

George, H. L., & Christoffersen, R. E. (2016). Differential latency toward (–)-epicatechin and catechol mediated by avocado mesocarp polyphenol oxidase (PPO). Postharvest Biology and Technology, 112, 31-38.

Gopal Singh, B., & Verma, D. P. S. (2001). Glutathione: An antioxidant to withstand oxidative stress in transgenic lines of tobacco. Indian Journal of Plant Physiology, 6(3), 229-232.

Gordon, S. A., & Paleg, L. G. (1961). Formation of auxin from tryptophan through action of polyphenols. Plant Physiology, 36(6), 838-845.

Griffith, O. W. (1980). Determination of glutathione and glutathione disulfide using glutathione reductase and 2-vinylpyridine. Analytical Biochemistry, 106(1), 207-212.

Guilbaud, C. S., Dalchau, N., Purves, D. W., & Turnbull, L. A. (2015). Is 'peak N'key to understanding the timing of flowering in annual plants?. New Phytologist, 205(2), 918-927.

Hameed, A., Bibi, N., Akhter, J., & Iqbal, N. (2011). Differential changes in antioxidants, proteases, and lipid peroxidation in flag leaves of wheat genotypes under different levels of water deficit conditions. Plant Physiology and Biochemistry, 49(2), 178-185.

Hartmann, H. T., Kester, D. E., Davies, F. T., & Geneve, R. L. (1997). Plant propagation: principles and practices (No. Ed. 6). Prentice-Hall Inc..

Hasanuzzaman, M., Nahar, K., Gill, S. S., Alharby, H. F., Razafindrabe, B. H. N., & Fujita, M. (2017). Hydrogen peroxide pretreatment mitigates cadmium-induced oxidative stress in *Brassica napus* L.: An intrinsic study on antioxidant defense and glyoxalase systems. Frontiers in Plant Science, 8, 115. http://doi.org/10.3389/fpls.2017.00115

Hassan, N. M., El-Bastawisy, Z. M., El-Sayed, A. K., Ebeed, H. T., & Alla, M. M. N. (2015). Roles of dehydrin genes in wheat tolerance to drought stress. Journal of Advanced Research, 6(2), 179-188.

Hiraga, S., Sasaki, K., Ito, H., Ohashi, Y., & Matsui, H. (2001). A large family of class III plant peroxidases. Plant and Cell Physiology, 42(5), 462-468.

Hong, Y. J., Tomas-Barberan, F. A., Kader, A. A., & Mitchell, A. E. (2006). The flavonoid glycosides and procyanidin composition of Deglet Noor dates (*Phoenix dactylifera*). Journal of Agricultural and Food Chemistry, 54(6), 2405-2411.

Hornero-Méndez, D., Gallardo-Guerrero, L., Jarén-Galán, M., & Mínguez-Mosquera, M. I. (2002). Differences in the activity of superoxide dismutase, polyphenol oxidase and Cu-Zn content in the fruits of Gordal and Manzanilla olive varieties. Zeitschrift für Naturforschung C, 57(1-2), 113-120.

Hwang, S. Y., Lin, H. W., Chern, R. H., Lo, H. F., & Li, L. (1999). Reduced susceptibility to waterlogging together with high-light stress is related to increases in superoxide dismutase and catalase activities in sweet potato. Plant Growth Regulation, 27(3), 167-172.

Jaradat, A. A., & Zaid, A. (2004). Quality traits of date palm fruits in a center of origin and center of diversity. Journal of Food Agriculture and Environment, 2, 208-217.

Jassim, S. A., & Limoges, R. G. (2014). Date palm tree's defense mechanisms from viral infection and solar ultraviolet radiation. Advances in Microbiology, 4, 1-5.

Johnson, M., Nanthini, A. U. R., & Malar, T. R. J. J. (2010). Isozyme variation and genetic relationships among three plumbago species. Journal of Ecobiotechnology, 2(5), 54-59.

Kant, M. R., Jonckheere, W., Knegt, B., Lemos, F., Liu, J., Schimmel, B. C. J., & Egas, M. (2015). Mechanisms and ecological consequences of plant defence induction and suppression in herbivore communities. Annals of Botany, 115(7), 1015-1051.

Karigar, C. S., & Rao, S. S. (2011). Role of microbial enzymes in the bioremediation of pollutants: a review. Enzyme Research, Article ID 805187, 1-11.

Kasraoui, M. F., Duquesnoy, I., Winterton, P., & Lamaze, T. (2014). Soluble and cell wall bound peroxidase activities are markers of flower bud development stages in lemon (*Citrus limon* L.). Journal of Applied Botany and Food Quality, 87, 1-8.

Kelen, M., Demiralay, E. C., ŞEN, S., & ALSANCAK, G. Ö. (2004). Separation of abscisic acid, indole-3-acetic acid, gibberellic acid in 99 R (*Vitis berlandieri*

x *Vitis rupestris*) and rose oil (Rosa damascena Mill.) by reversed phase liquid chromatography. Turkish Journal of Chemistry, 28(5), 603-610.

Keleş, Y., & Öncel, I. (2002). Response of antioxidative defence system to temperature and water stress combinations in wheat seedlings. Plant Science, 163(4), 783-790.

Keller, M., & Hrazdina, G. (1998). Interaction of nitrogen availability during bloom and light intensity during veraison. II. Effects on anthocyanin and phenolic development during grape ripening. American Journal of Enology and Viticulture, 49(3), 341-349.

Khan, M. J. U., Billah, M., & Latif, M. A. (2016). A study on different arabian date palm (*Phoenix dactylifera* L.) accessions in Bangladesh. The Agriculturists, 13(2), 36-43.

Kiffin, R., Bandyopadhyay, U., & Cuervo, A. M. (2006). Oxidative stress and autophagy. Antioxidants & Redox Signaling, 8(1-2), 152-162.

Klessig, D. F. (2017). How does the multifaceted plant hormone salicylic acid combat disease in plants and are similar mechanisms utilized in humans?. BMC Biology, 15(1), 1-11.

Krainer, F. W., Pletzenauer, R., Rossetti, L., Herwig, C., Glieder, A., & Spadiut, O. (2014). Purification and basic biochemical characterization of 19 recombinant plant peroxidase isoenzymes produced in Pichia pastoris. Protein Expression and Purification, 95, 104-112.

Kumar, K. B., & Khan, P. A. (1982). Peroxidase and polyphenol oxidase in excised ragi (*Eleusine corocana* cv PR 202) leaves during senescence. Indian Journal of Experimental Biology, 20(5), 412-416.

Kumar, O. A., Rupavathi, T., & Tata, S. S. (2013). Genetic variation of isozyme Polyphenol oxidase (PPO) profiles in different varieties of *Capsicum annuum* L. Notulae Scientia Biologicae, 5(4), 454-457.

Kumar, O. A., Tata, S. S., & Rupavathi, T. (2014). Evaluation of genetic diversity in 21 cultivars of chili pepper (*Capsicum annuum* L.) using isozyme markers. European Journal of Experimental Biology, 4(6), 44-49.

Kurup, S. S., Hedar, Y. S., Al Dhaheri, M. A., El-Heawiety, A. Y., Aly, M. A., & Alhadrami, G. (2009). Morpho-physiological evaluation and RAPD markers-assisted characterization of date palm (*Phoenix dactylifera* L.) varieties for salinity tolerance. Journal of Food Agriculture and Environment, 7(3), 503-507.

Laemmli, V. K. (1970). Determination of protein molecular weight in polyacrylamide gels. Nature, 227, 680-685.

Lobo, V., Patil, A., Phatak, A., & Chandra, N. (2010). Free radicals, antioxidants and functional foods: Impact on human health. Pharmacognosy Reviews, 4(8), 118-126.

Lu, S. C. (2009). Regulation of glutathione synthesis. Molecular aspects of Medicine, 30(1), 42-59.

Mahmoudi, H., Hosseininia, G., Azadi, H., & Fatemi, M. (2008). Enhancing date palm processing, marketing and pest control through organic culture. Journal of Organic Systems, 3(2), 29-39.

Makker, K., Agarwal, A., & Sharma, R. (2009). Oxidative Stress & Male Infertility. Indian Journal of Medical Research, 129, 357-367.

Malick, C. P., & Singh, M. B. (1980). Analysis of total phenols. Plant Enzymology and Histolnzymology, Kalyani Publishers, New Delhi, India. p. 286.

Mansouri, A., Embarek, G., Kokkalou, E., & Kefalas, P. (2005). Phenolic profile and antioxidant activity of the Algerian ripe date palm fruit (*Phoenix dactylifera*). Food Chemistry, 89(3), 411-420.

Mayer AM, Harel E (1973). Polyphenol oxidases in plants. Phytochemistry 18, 193-215.

Miransari, M., & Smith, D. L. (2014). Plant hormones and seed germination. Environmental and Experimental Botany, 99, 110-121.

Mohamed, H. I., & Akladious, S. A. (2014). Influence of garlic extract on enzymatic and non enzymatic antioxidants in soybean plants (*Glycine max*) grown under drought stress. Life Science Journal, 11(3s), 46-58.

Mullineaux, P. M., & Rausch, T. (2005). Glutathione, photosynthesis and the redox regulation of stress-responsive gene expression. Photosynthesis Research, 86(3), 459-474.

Munné-Bosch, S., & Alegre, L. (2002). The function of tocopherols and tocotrienols in plants. Critical Reviews in Plant Sciences, 21(1), 31-57.

Nag, S., Paul, A., & Choudhuri, M. (2013). Changes in peroxidase activity during adventitious root formation at the base of mung bean cuttings. International Journal of Scientific & Technology Research, 2, 171-177.

Nakurte, I., Keisa, A., & Rostoks, N. (2012). Development and validation of a reversed-phase liquid chromatography method for the simultaneous determination of indole-3-acetic acid, indole-3-pyruvic acid, and abscisic acid in barley (*Hordeum vulgare* L.). Journal of Analytical Methods in Chemistry, Article ID 103575, 1-6.

Nayyar, H., & Gupta, D. (2006). Differential sensitivity of C3 and C4 plants to water deficit stress: association with oxidative stress and antioxidants. Environmental and Experimental Botany, 58(1), 106-113.

Nei, M., & Li, W. H. (1979). Mathematical model for studying genetic variation in terms of restriction endonucleases. Proceedings of the National Academy of Sciences, 76(10), 5269-5273.

Noctor, G., Veljvoic-Jovanovic, S. and Foyer, C.H. (2000) Peroxide processing in photosynthesis: Antioxidant coupling and redox signalling. Philosophical Transactions of the Royal Society of London, 355, 1465-1475.

Notaguchi, M., Abe, M., Kimura, T., Daimon, Y., Kobayashi, T., Yamaguchi, A., & Araki, T. (2008). Long-distance, graft-transmissible action of *Arabidopsis*

flowering locus T protein to promote flowering. Plant and Cell Physiology, 49(11), 1645-1658.

Ogawa, K. I., Tasaka, Y., Mino, M., Tanaka, Y., & Iwabuchi, M. (2001). Association of glutathione with flowering in *Arabidopsis thaliana*. Plant and Cell Physiology, 42(5), 524-530.

Omaye, S. T., Turnbull, J. D., & Sauberlich, H. E. (1979). Selected methods for the determination of ascorbic acid in animal cells, tissues, and fluids. Methods in Enzymology, 62, 3-11.

Pandey, V., & Shukla, A. (2015). Acclimation and tolerance strategies of rice under drought stress. Rice Science, 22(4), 147-161.

Patel, P. K., & Hemantaranjan, A. (2012). Salicylic acid induced alteration in dry matter partitioning, antioxidant defence system and yield in chickpea (*Cicer arietinum* L.) under drought stress. Asian Journal of Crop Science, 4(3), 86-102.

Paul, A. L., Folta, K. M., & Ferl, R. J. (2008). 14-3-3 Proteins, red light, and photoperiodic flowering: A point of connection?. Plant Signaling & Behavior, 3(8), 511-515.

Pourcel, L., Routaboul, J. M., Cheynier, V., Lepiniec, L., & Debeaujon, I. (2007). Flavonoid oxidation in plants: from biochemical properties to physiological functions. Trends in Plant Science, 12(1), 29-36.

Pradeep, K. P., Hemantaranjan, A., Sarma, B. K., & Singh, R. (2011). Growth and antioxidant system under drought stress in Chickpea (*Cicer arietinum* L.) as sustained by salicylic acid. Journal of Stress Physiology & Biochemistry, 7(4), 130-144.

Rabert, G. A., Manivannan, P., Somasundaram, R., & Panneerselvam, R. (2014). Triazole compounds alters the antioxidant and osmoprotectant status in drought stressed *Helianthus annuus* L. plants. Emirates Journal of Food and Agriculture, 26(3), 265-276.

Rai, M. K., Shekhawat, N. S., Gupta, A. K., Phulwaria, M., Ram, K., & Jaiswal, U. (2011). The role of abscisic acid in plant tissue culture: a review of recent progress. Plant Cell, Tissue and Organ Culture, 106(2), 179-190.

Sadiq, I. S., Izuagie, T., Shuaibu, M., Dogoyaro, A. I., Garba, A., & Abubakar, S. (2013). The nutritional evaluation and medicinal value of date palm (*Phoenix dactylifera*). International Journal of Modern Chemistry, 4(3), 147-54.

Sairam, R. K., Rao, K. V., & Srivastava, G. C. (2002). Differential response of wheat genotypes to long term salinity stress in relation to oxidative stress, antioxidant activity and osmolyte concentration. Plant Science, 163(5), 1037-1046.

Saleh, E. A., Tawfik, M. S., & Abu-Tarboush, H. M. (2011). Phenolic contents and antioxidant activity of various date palm (*Phoenix dactylifera* L.) fruits from Saudi Arabia. Food and Nutrition Sciences, 2, 1134-1141.

Salem, N., Msaada, K., Hamdaoui, G., Limam, F., & Marzouk, B. (2011). Variation in phenolic composition and antioxidant activity during flower development of safflower (*Carthamus tinctorius* L.). Journal of Agricultural and Food Chemistry, 59(9), 4455-4463.

Samuolienė, G., Urbonavičiūtė, A., Šabajevienė, G., & Duchovskis, P. (2008). Flowering initiation in carrot and caraway. Sodininkystė ir Daržininkystė, 27(2), 17-25.

Schmidt, R., Kunkowska, A. B., & Schippers, J. H. (2016). Role of reactive oxygen species during cell expansion in leaves. Plant Physiology, 172(4), 2098-2106.

Schwambach, J., Ruedell, C. M., de Almeida, M. R., Penchel, R. M., de Araújo, E. F., & Fett-Neto, A. G. (2008). Adventitious rooting of Eucalyptus globulus× maidennii mini-cuttings derived from mini-stumps grown in sand bed and intermittent flooding trays: a comparative study. New Forests, 36(3), 261-271.

Sergeeva, E., Liaimer, A., & Bergman, B. (2002). Evidence for production of the phytohormone indole-3-acetic acid by cyanobacteria. Planta, 215(2), 229-238.

Shabani, F., Kumar, L., Nojoumian, A. H., Esmaeili, A., & Toghyani, M. (2016). Projected future distribution of date palm and its potential use in alleviating micronutrient deficiency. Journal of the Science of Food and Agriculture, 96(4), 1132-1140.

Sharma, P., Jha, A. B., Dubey, R. S., & Pessarakli, M. (2012). Reactive oxygen species, oxidative damage, and antioxidative defense mechanism in plants under stressful conditions. Journal of Botany, Article ID 217037, 1-26.

Sharma, S., Kiran, U., & Sopory, S. K. (2017). Developing Stress-Tolerant Plants by Manipulating Components Involved in Oxidative Stress. In Plant Biotechnology: Principles and Applications (pp. 233-248). Springer Singapore.

Silva, S., Gomes, L., Leitao, F., Coelho, A. V., & Boas, L. V. (2006). Phenolic compounds and antioxidant activity of *Olea europaea* L. fruits and leaves. Food Science and Technology International, 12(5), 385-395.

Sofo, A., Scopa, A., Nuzzaci, M., & Vitti, A. (2015). Ascorbate peroxidase and catalase activities and their genetic regulation in plants subjected to drought and salinity stresses. International Journal of Molecular Sciences, 16(6), 13561-13578.

Sood, S., & Nagar, P. K. (2003). Changes in abscisic acid and phenols during flower development in two diverse species of rose. Acta Physiologiae Plantarum, 25(4), 411-416.

Stefanini, M. B., Rodrigues, S. D., & Ming, L. C. (2002). Ação de fitorreguladores no crescimento da erva-cidreira-brasileira. Horticultura Brasileira, 20(1), 18-23.

Syros, T., Yupsanis, T., Zafiriadis, H., & Economou, A. (2004). Activity and isoforms of peroxidases, lignin and anatomy, during adventitious rooting in cuttings of *Ebenus cretica* L. Journal of Plant Physiology, 161(1), 69-77.

Tan, Y., Liang, Z., Shao, H., & Du, F. (2006). Effect of water deficits on the activity of anti-oxidative enzymes and osmoregulation among three different genotypes of *Radix astragali* at seeding stage. Colloids and surfaces B: Biointerfaces, 49(1), 60-65.

Thakker, J. N., Patel, S., & Dhandhukia, P. C. (2012). Induction of defense-related enzymes in banana plants: effect of live and dead pathogenic strain of Fusarium oxysporum f. sp. cubense. ISRN Biotechnology, Article ID 601303, 1-6.

Tlili, N., Nasri, N., Saadaoui, E., Khaldi, A., & Triki, S. (2009). Carotenoid and tocopherol composition of leaves, buds, and flowers of *Capparis spinosa* grown wild in Tunisia. Journal of Agricultural and Food Chemistry, 57(12), 5381-5385.

Tran, L. T., & Constabel, C. P. (2011). The polyphenol oxidase gene family in poplar: phylogeny, differential expression and identification of a novel, vacuolar isoform. Planta, 234(4), 799- 813.

Vallejos, C. E. (1983). Enzyme activity staining. Isozymes in Plant Genetics and Breeding, 1, 496-516.

Vayalil, P. K. (2002). Antioxidant and antimutagenic properties of aqueous extract of date fruit (*Phoenix dactylifera* L. Arecaceae). Journal of Agricultural and Food Chemistry, 50(3), 610-617.

Verstraeten, I., Schotte, S., & Geelen, D. (2014). Hypocotyl adventitious root organogenesis differs from lateral root development. Frontiers in Plant Science, 5, 1-13.

Wetter, L. R., & Constabel, F. (Eds.). (1982). Plant tissue culture methods. Saskatoon: National Research Council of Canada.

Wu, Z., Liu, S., Zhao, J., Wang, F., Du, Y., Zou, S., & Huang, Y. (2017). Comparative responses to silicon and selenium in relation to antioxidant enzyme system and the glutathione-ascorbate cycle in flowering Chinese cabbage (*Brassica campestris* L. ssp. chinensis var. utilis) under cadmium stress. Environmental and Experimental Botany, 133, 1-11.

Young, I. S., & Woodside, J. V. (2001). Antioxidants in health and disease. Journal of Clinical Pathology, 54(3), 176-186.

Zaid, A., de Wet, P.F. (2002) in Date palm cultivation, Climatic requirements of date palm, ed Zaid A. (Food and Agriculture Organization Plant Production and Protection Paper no. 156. Food and Agriculture Organization of the United Nations, Rome, Italy), pp 57–72.

Zhao, X., Wei, P., Liu, Z., Yu, B., & Shi, H. (2017). Soybean Na+/H+ antiporter GmsSOS1 enhances antioxidant enzyme activity and reduces Na+ accumulation in *Arabidopsis* and yeast cells under salt stress. Acta Physiologiae Plantarum, 39(1), 19. doi.org/10.1007/s11738-016-2323-3

Zheng, Y., Yu, B., De La Cruz, L. K., Roy Choudhury, M., Anifowose, A. and Wang, B. (2017). Toward hydrogen sulfide based therapeutics: Critical drug delivery and developability issues. Medicinal Research Reviews. *In Press*. doi:10.1002/med.21433.

Zhu, F., Cai, Y. Z., Sun, M., Ke, J., Lu, D., & Corke, H. (2009). Comparison of major phenolic constituents and in vitro antioxidant activity of diverse Kudingcha genotypes from Ilex kudingcha, Ilex cornuta, and *Ligustrum robustum*. Journal of Agricultural and Food Chemistry, 57(14), 6082-6089.