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Photosynthesis Components in Vitro and Ex vitro Date Palm

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Abstract **– Date palm (Phoenix dactylifera L.) trees one of the important popular fruits were found in all Egypt cultivation areas, *in vitro* technique is a control condition as temperature, water, light and carbohydrates which can be affect plants photosynthesis processes and own production as, pigments, proteins, indoles and enzymes, ex vitro plantlets can be modify these effects, thus this work investigate effects of *in vitro* and *ex vitro* date palm on these components, the analysis of tissues in vitro shooting and rooting stages showed smallest contents of pigments, proteins and indoles, the antioxidant enzymes CAT and POD activities increased progressively from in vitro shooting and rooting stages to ex vitro plantlets in the greenhouse and open field in addition to offshoot correlated to the growth stages and changes in the environmental condition. In the C4 plants photosynthetic activities are take place between mesophyll and bundle sheath cells where the initial carboxylating enzyme is PEPC which greater in the offshoots and plantlets in the open field than in vitro tissues that have the lowest activity enzyme as well as Rubisco enzyme in the tested tissues assumed gradual increasing from in vitro to ex vitro in the greenhouse and open field.**

Keywords **– Photosynthesis Activity, Date Palm, Enzymes, Indoles, Pigments, Proteins.**

I. INTRODUCTION

Date palm (*Phoenix dactylifera* L.) is a monocotyledonous, angiosperm and dioecious trees have specific carbon – metabolism to produce different organic contents. Date palm tissue culture technique has provide large numbers of fruiting trees under specific condition as constant light, temperature, water also sufficient minerals and carbohydrates, this *in vitro* control condition continuous in all stages of tissue culture until the plantlets transferred to acclimatization stage in the greenhouse which facing different condition caused failure or decreasing success of *ex vitro* acclimatization, [1] Darwesh 2015, therefore, many scientists investigate the physiology characters *in vitro* and *ex vitro* as photosynthesis, enzymes, pigments and proteins. High air humidity, low gas exchange and thus a CO_2-shortage in the whole photoperiod, and relatively low photosynthetic photon flux density (PFD), induce disturbances in plant development and photosynthetic performance [2] Pospisilova *et al*. 1997 and [3] Kozai *et al*. 1997, Rubisco content was substantially decreased in vitro leaves under elevated CO_2 (1000 µmol.mol-1) was supplied [4] De La Vina et al. 1999, plantlets exhibit abnormal morphology, anatomy, and physiology as a response of in vitro condition as high air humidity, low irradiation, low CO_2 and high concentration of sugars as carbon source [5] Desjardins 1995 and [6] Kozai and Smith 1995, In vitro avocado, oak, and strawberry showed a decrease of Rubisco and significant decrease in total soluble proteins of avocado plantlets [7] Rival *et al*. 1999 on coconut and oil palm and [8] Premkumar *et al*. 2001, Moreover, these bad effects of in vitro can be changes in ex vitro greenhouse, that causing increasing of photosynthesis components as enzymes activity, proteins and chlorophyll contents, Increasing CO_2 level produced increasing photosynthesis rates also chlorophyll, carbohydrates contents and Rubisco activity [9] Cournac *et al*. 1992 and [10] Kadlecek *et al*. 2001, *Spathiphyllum floribundum* plantlets grown under in vitro conditions have differences in net photosynthetic rate, chl, and Fv/Fm which disappeared during the 15 days of ex vitro acclimatization and increase in net photosynthetic rate [11] Van Huylenbroeck, and Debergh 1996, in vitro of avocado (*Persea americana* Mill.) with highest concentration of sucrose showed significant decreasing of maximum photosynthetic rate, carbohydrates and starch [12] Ticha *et al*. 1998, significant rise in chlorophylls in ex vitro grapevine leaves after transplantation [13] Amancio et al. 1999, C3 plants found in cool and wet climates, with low light intensity, while C4 plants occur in hot and dry climatic conditions with high light intensity, C4 and CAM plants usually in arid environments. C4 plants have higher photosynthetic efficiency than C3 plants in arid, hot, and under high-light conditions, they possess an additional carbon fixation pathway and limit photorespiration [14] Taiz and Zeiger 2010, in C4 plant phosphoenolpyruvate carboxylase (PEPC) is the enzyme responsible for catalyzing the primary fixation of atmospheric CO_2, PEPC kinase (PEPCk), a protein kinase involved in this phosphorylation of PEPC [15] Agetsuma et al. 2005 which converted to oxaloacetate through phosphoenolpyruvate and carbon dioxide reaction [16]Méndez-Lucas et al. 2014, oxaloacetate converted to aspartate, which move to bundle sheath, in the bundle sheath cells, aspartate is converted back to oxaloacetate. PEPCK decarboxylates the bundle sheath oxaloacetate, releasing carbon dioxide, which fixed by the enzyme Rubisco [17] Christopher and Holtum 1996 and [18] Kanai and Edwards 1999, photosynthesis rate and growth were decreased of in vitro plantlets (*Samanea saman* Merr) and their enhancing after transfer to the greenhouse [19] Kriengkrai *et al*. 2004, the main bundle fibrous sheath thick of date palm leaves was different between different soft and dry cultivars [20] Abd El-Baky 2012, an incessant activity of Catalase, Ascorbate Peroxidase and Glutathione Reductase were also detected through one week of acclimatization of *Cardiospermum halicacabum* plantlets [21]Anushi and Anis 2014. The

present work was done in order to investigate the effect of *in vitro* conditions on the photosynthesis components (pigments, proteins, indoles and enzymes) and changes after the plantlets transferring to *ex vitro* under different conditions in the greenhouse.

II. Materials and Methods

This work was done in 2015- 2016 in the Central Laboratory for Research and Development of date palm and Biochem. Dept., Fac of Agric., Ain Shams Univ. to measure photosynthesis components of date palm (*Phoenix dactylifera* L.) *in vitro* and *ex vitro*. *In vitro* leaves date palm samples were taken from shooting and rooting stages (cultured in MS media supplemented with 0.1 mg/l NAA + 0.05 BA + 30 g/l sucrose) placed in a growth chamber (27± C^0, 7000 lux), samples were taken as

1. *in vitro* shooting stage
2. *in vitro* rooting stage
3. *ex vitro* plantlets greenhouse (6 months from acclimatization stage)
4. *ex vitro* plantlets open field (one year from acclimatization stage)
5. offshoots

Samples *in vitro* and *ex vitro* were prepared to measure the following estimations

1. **Chlorophyll contents** a, b, total chlorophyll and carotenoids mg/g f.wt.: as described by [22] **Lichtenthaler** and **Wellburn** 1994
2. **Proteins** mg/g f.wt.: as described by [23] **Bradford** 1976
3. **Indoles** mg/g f.wt.: as [24] **Larsen** *et al.*1962
   Antioxidant Enzyme Activity CAT and POD
   Catalase CAT (EC 1.11.1.6) in leaves µmol /g.f.wt./min: as [25] **Aebi,** 1984
   Peroxidase POD (PRX, E.C.1.11.1.7) in leaves µmol /g.f.wt./min:: as [26] **Polle** *et al*., 1994
4. **Phosphoenolpyruvate carboxykinase** namely **PEPC (EC 4.1.1.31)** Unit /mg protein: as [27] **Gonzalez** 1998
5. **Ribulose1, 5-bisphosphate carboxylase/oxygenase (Rubisco)** (EC 4.1.1.39) Unit /mg protein: as [28] **Lilley** 1974

Experimental design: Complete randomized block design with three replicates and three plantlets for each one, two growth seasons (8 months for each). Data were analyzed by analysis of variances (ANOVA) and the means were compared following t- test using L.SD. values at 5 % level [29] **Snedecor** and **Chocran** 1990).

III. Results

3.1. ***Leaves chlorophyll a, b and total chlorophyll mg/g f.w.:*** determined of chlorophyll a, b as well as total chlorophyll (Fig 1 and 7) on the different types tissues in vitro and ex vitro produce the evidence that the chlorophyll a, b and total chlorophyll were increased significantly from shooting tissues in the shooting stage as 0.13 mg/g f.wt. for chl. a, 0.15 mg/g f.wt. for rooting tissues, 0.2 mg/g f.wt. for ex vitro plantlets in the greenhouse and have the insignificant differs 0.4 mg/g f.wt. between ex vitro plantlets in the open field and offshoots. Assayed chlorophyll b showed significant gradually raising from shooting tissues 0.02 mg/g f.wt., 0.13 mg/g f.wt. for rooting tissues, 0.12 mg/g f.w. for ex vitro plantlets greenhouse, 0.2 mg/g f.wt. for ex vitro plantlets in the open field and offshoots with insignificant variance in between. Consequent results found on the total chlorophyll produced from in vitro shooting tissues 0.15mg/g f.wt., 0.25 mg/g f.wt. for in vitro rooting stage, 0.32 mg/g f.wt. for ex vitro greenhouse, insignificant distinction 0.6 mg/g f.wt. obtained between ex vitro plantlets open field and offshoots

3.2. ***Proteins contents mg/g f.wt***: different types of tissues in vitro and ex vitro (Fig 2 and 7) presented significantly higher different values, smaller values of proteins associated to in vitro shooting tissues 1.1 mg/g f.wt. which ascending by rooting tissues 2.2 mg/g f.wt., moreover ex vitro tissues exhibited significant increasing of proteins 2.3 mg/g f.wt. for plantlets greenhouse, the plantlets in the open field and offshoots produced insignificant distinguish in between 2.8 and 2.9 mg/g f.wt. respectively

3.3. ***Indoles contents mg/g f.wt.*:** in vitro shooting tissues (Fig 3 and 7) recorded smallest indole contents 3.6 mg/g f.wt. followed by in vitro tissue of rooting 10.2 mg/g f.wt. meanwhile ex vitro plantlet in the open field and offshoots recorded highest indole contents 11.5 and 12.1 mg/g f.wt. respectively without significant differs between them

3.4. ***Antioxidant enzymes CAT and POD µmol/ g. f.wt./min***: increasing activity of antioxidant enzymes CAT and POD (Fig 4 and 7) significantly pronounced enhancing at the ex vitro tissues in the greenhouse and open field meanwhile the assaying enzymes activity in the in vitro tissues shooting and rooting stages gave minimum activity. non- significant differences were found for enzymes activity between plantlets in the open field and offshoot for two enzymes.

3.5. ***PEPC and Rubisco enzymes* :** spectrophotometer data on the determination of photosynthesis enzymes (Figs 5, 6 and 7) Phosphoenolpyruvate carboxykinase namely PEPC (EC 4.1.1.31) and ribulose 1, 5-biphosphate carboxylase-oxygenase (EC 4.1.1.39; Rubisco) showed significant variance from in vitro and ex vitro tissues, the significant differences were observed between the different types in vitro and ex vitro tissues related to PEPC and Rubisco activity which recorded highest activity for offshoots meanwhile in vitro tissues bringing out lowest activity followed by rooting stage, greenhouse and open field.

IV. Discussion

Date palm (*Phoenix dactylifera* L.) is the important genus in Arecaceae distributed in the arid and semi arid area [30] Jain 2012 and the main source of food in these regions of North Africa, middle east and South-Asian countries [31]

FAO 2013, date palm is dioecious species and very heterozygous separate male and female palms, propagation by seedlings produced over 50% male (one male tree for pollen 25 female trees) in addition male and female seedlings can be distinction flowering stage, offshoots as a traditional propagation of date palm produced limited numbers of trees which insufficient for new reclamation areas, the survival rate of offshoots is low when separated from mother tree by incorrectly practice or infection by palm diseases and pets, thus in vitro micropropagation become the most suitable for providing great numbers of homogenous and healthy female date palm in this respect many researchers as [32] Eke et al. 2005, [33] Al-Mazroui et al., 2007, [34] Al- Khateeb 2008b, [35] Mustafa et al. 2013 and [36] Mazri and Meziani 2015, on the other hand in vitro culture control conditions resulted in abnormal morphology, anatomy and physiology of plantlets and might be impaired many photosynthesis functions thus needed to acclimatization stage to correct these abnormalities, after transfer from the in vitro to the ex vitro greenhouse [37] Fila et al. 1998, [38] Bolar et al. 1998. ***In concerning to leaves contents of chlorophyll, proteins and indoles***, chlorophyll can play a vital role for building growth components as proteins, auxins etc., results showed the progressively increasing of chlorophyll a and b also total chlorophyll as well as increasing of proteins and indoles contents, increase in chlorophyll a and b from in vitro to ex vitro of date palm plantlets demonstrated the induction of chlorophyll synthesis enzymes indispensable for chlorophyll biosynthesis, increase in Chlorophyll a and b synchronous with the new leaves during the acclimatization, Chlorophyll *a* fluorescence has been used as an indicator of photochemical efficiency [39] Krause and Weis, 1999, Leaves formed during the acclimatization period may still have a lower photosynthetic capacity than leaves of greenhouse-grown plants [40] Carvalho *et al.* 2001, decreasing chlorophyll of Rhododendron 'Alfred' value and increasing vitality index value (Rfd) during first 4 weeks of acclimatization plantlets proved changing from mixotrophic metabolism of plantlets *in vitro* to autotrophic in ex vitro [41] BoŜena Matysiak 2004, growing plants maintain the optimum nutrient levels that led to high chlorophyll activity which may be correlate with high amount of protein contents [42] Arigita et al. 2005, higher photosynthetic rates P(N) of sun leaves (ginkgo and beech) and stomatal conductance were also reflected by higher values for the Chl fluorescence decrease ratios. [43]Lichtenthaler et al. 2007 and [44]2013, chlorophyll a, b and total chlorophyll increased in hardening and acclimatization stages of *Boswellia serrate* Roxb. Plantlets [45] Suthar and Purohit 2011, ex vitro plants have greater chlorophyll a and b contents of *Nepeta nuda* ssp. *nuda* than in vitro plants [46] Dragolova et al. 2015, chlorophyll and total soluble proteins were increased in the glasshouse in the acclimatization stage of *Tectona grandis* L. [47] Akram and Aftab 2015, increasing protein and chlorophyll contents at the end of acclimatization period [48] Hofman et al. 2002, protein content increased at the end of shoot formation stage [49] Sharifi 2012, indoles contents increased at the end of pre-acclimatization stage [50] Darwesh *et al.* 2011, and increasing of chlrorphyll a and b and indoles contents in acclimatization stage was found in date palm plantlets in the greenhouse [51] Ibrahim et al. 2012.

Activity of antioxidant enzymes: assaying CAT and POD activity showed increasing activity sequenced from in vitro shooting stage to ex vitro plantlets in the greenhouse and in the open field and offshoots which come out from the differs of environmental conditions from in vitro and ex vitro also different growth stages with different photosynthesis products, antioxidants helped in preventing the damage occurred in cells by free radicals that are released during normal metabolic process of oxidation [52] Young and Woodside 2001, antioxidant activity of polyphenols due to redox properties which are important in decomposing peroxides [53] Karou et al., 2005, superoxide dismutase and guaiacol peroxidase activities increased with increasing light intensity, Catalase and glutathione reductase increased during the first three weeks of acclimatization, while an increase in guaiacol peroxidase and ascorbate peroxidase observed later in the acclimatization, [54] Van Huylenbroeck et al. 2000, rise activity of SOD after one week of transplantation plantlets showed its role in preventing oxidative stress of *Rauvolfia tetraphylla*, increasing carotenoids content reflects the functional response of photosynthetic apparatus to the different light environment of *Tylophora indica* [55] Faisal and Anis 2009 and [56] 2015, catalase and glutathione peroxidase plays an important role in preventing cell injuries [57] Starlin and Gopalakrishnan, 2013, CAT decomposition of H_2O_2 into oxygen and water associated with guard systems for AOS in cells [58] Agnieszka and Edyta 2014, the activity of catalase and peroxidase changes with fluctuations of endogenous H_2O_2 content [59] Konieczny et al. 2014.

PEPC and Rubisco enzymes: findings on PEPC and Rubisco activity showed graduated value activity from date palm in vitro tissues which scored lowest activity values to maximum activity values belonging to ex vitro tissues. C3 and C4 plants use photosynthesis process to convert light to energy and CO_2 into (carbohydrates). increase of both biomass and CO_2 fixation with light intensity in C4 faster than C3, led to use of solar energy in C4 plants, C4 plants following angiosperms and monocotyledon with high daytime temperatures, intense sunlight, C4 plants can grow well at 35-40 $^{\circ}$C, grow in 20-25 $^{\circ}$C soil temperature [60] Oberhuber et al. 1993 and [61] Wang et al. 2012, Date palm can assimilate CO_2 through two independent carboxylation pathways, one occurs in the chloroplasts through, another pathway occurs in the cytosol through the Phosphoenolpyruvate carboxylase pathway [62] Kwa et al., 1997, C4 plants, CO_2 is fixed by phosphoenolpyruvate carboxylase (PEPC) in the mesophyll cells into four-carbon acids and flow into an inner ring of bundle sheath cells, which decarboxylated and the CO_2 is fixed again by Rubisco, [63] Doubnerová and Ryšlavá 2011, [64] Freschi and Mercier 2012 and [65] Jasper et al 2012, C4 plants produced carbon dioxide fixation is a 4-carbon compound,

C4 plants have a uniform mesophyll layer with developed bundle sheath around each vein, water use efficiency of the plants [66] Stephen 2014. *In vitro* plants are photomixotrophic, low rates of photosynthesis with low light intensities, low CO_2 and high amount of sucrose in the medium which inhibits photosynthesis [67] Reuther 1991), Rubisco and PEPC were greater in the plantlets have 3-4 leaves than in vitro date palm [68] Masmoudi et al. 1999, increasing of FBPase and Rubisco when transition from photoheterotropic to photo-autotrophic conditions of Banana plants [69] Regev et al. 1997, photosynthesis rates improve when cultures are grown under elevated CO_2 levels [70] Mitra et al. 1998, *in vitro* conditions led to limit leaves photosynthetic incorporation of CO_2, after transplanting tobacco plants to natural conditions reached to normal values [71] Katya et al. 1996, lack of photosynthetic capacity is the main factors responsible for decreasing survival and slow growth of microcuttings [72] Carmen et al. 2007, [73] Debergh et al. 2000, in vitro condition the lowest intensities of light, little concentrations of CO_2 and inhibition of photosynthesis led to lower net photosynthetic and dark respiration rate than *ex vitro* plants [74] Slavtcheva and Dimitrova 2001 and [75] Zhang et al. 2009, in vitro plantlets detect degradation of Rubisco. In ex vitro plantlets, these degradation were no longer detected, decrease in soluble sugars and starch gave an indication of a faster acquisition of autotrophic characteristics [76] Carvalho et al. 2005, at the beginning of acclimatization low vitality *in vitro* plants were noted and indicate poor functioning of dark phase of photosynthesis, vitality of new leaves reflected to higher photosynthetic potential [77] Fuentes et al., 2005, [78] LLisandra et al. 2006 on *Malus domestica* – cv M9 and [79] Pedroso et al. 2010.

V. Conclusion

Date palm in vitro tissues showed decreasing leaves contents of chlorophyll which as strong indicator of photosynthesis process and its components as proteins and indoles in addition to many enzymes as antioxidant enzymes and the main photosynthesis enzymes for date palm as PEPC and Rubisco that reflected on the growing plants, these effects can be modified after acclimatization in the greenhouse, as well as the plantlets derived tissue culture technique exhibited normal process in the open field.

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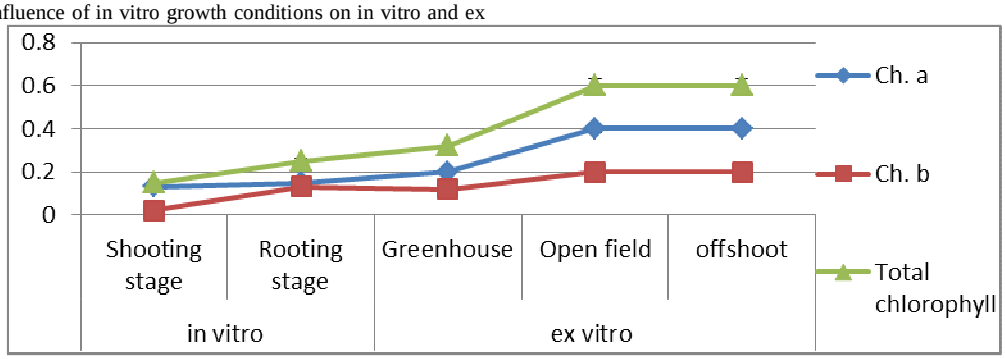

Fig. 1. Different type's tissues affected chlorophyll contents

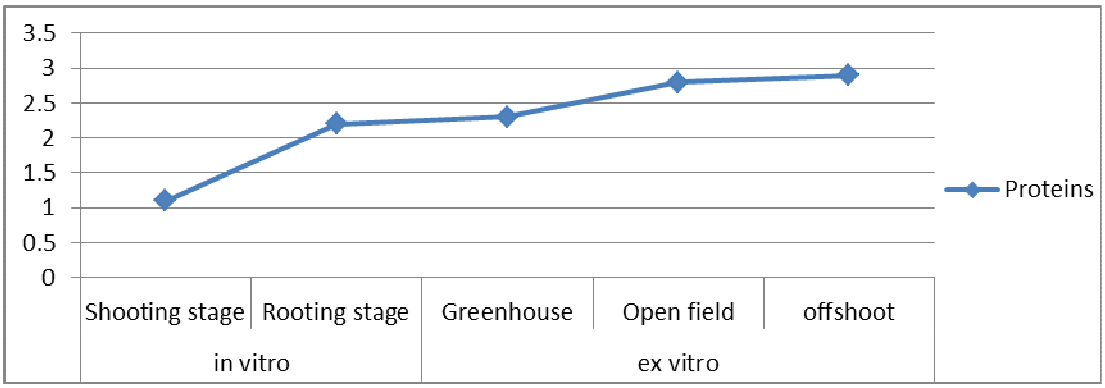

Fig. 2. Different type's tissues affected proteins contents

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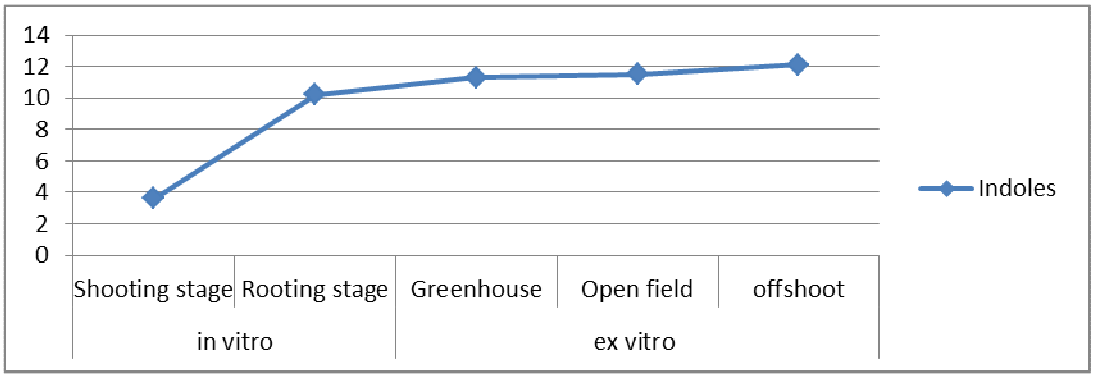

Fig. 3. Different type's tissues affected proteins contents

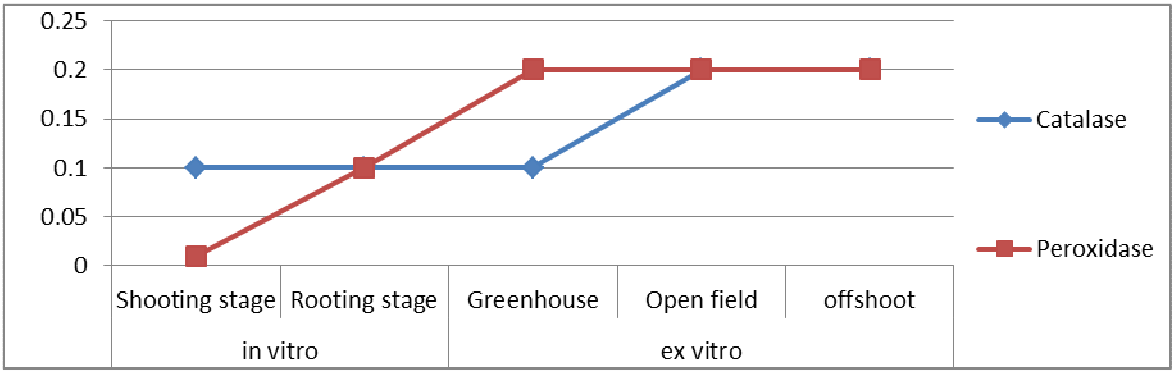

Fig. 4. Different type's tissues affected CAT and POD activity

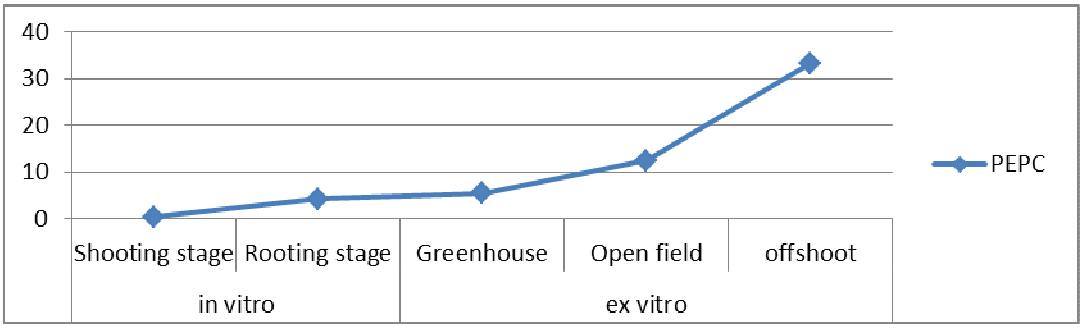

Fig. 5. Different type's tissues affected PEPC activity

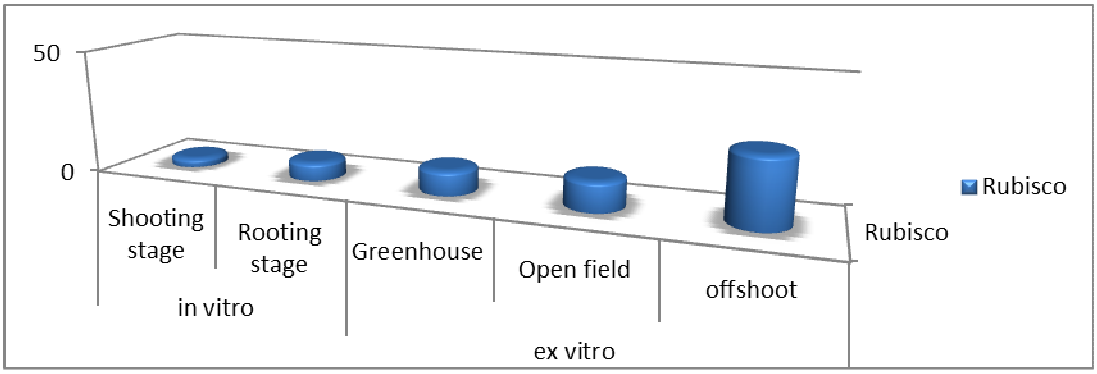

Fig. 6. Different type's tissues affected Rubisco activity

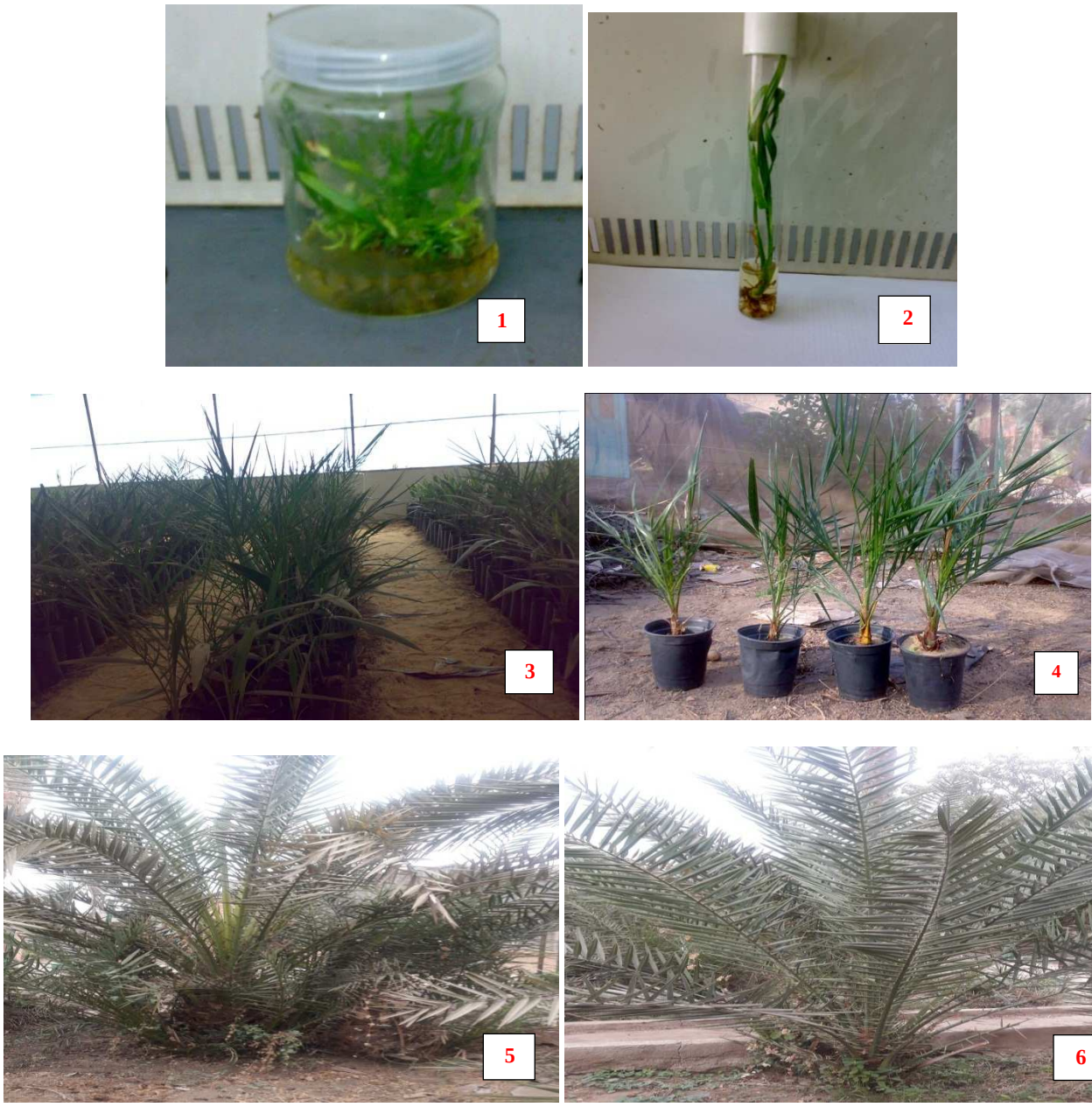

Fig. 7. In vitro and ex vitro date palm stages. In vitro stages as 1= shooting stage 2 = rooting stage).
Ex vitro stages as 3 and 4 = plantlets in the greenhouse 5 = plantlets in the open field 6 = offshoot.