Morphology, physiology and anatomy in vitro affected acclimatization ex vitro date palm plantlets: A Review

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***Abstract*—**Date palm tissue culture technique aimed to production of huge numbers of plants to farmers in order to increasing dates which have high nutritional value. Date palm plantlets from pre-acclimatization stage in vitro were transferred to acclimatize in the greenhouse cultured in peat moss + perlite 2:1 and kept in the plastic covered inside 100% humidity and 28 C0 till new leave or root was grown. The plantlets of date palm in vitro technique facing problem in the acclimatization stage as high mortality concerning failure in this stage, in vitro condition saturated atmosphere, relatively low light intensity, constant temperature 20-28 C0, high humidity, water continuously found, this in vitro condition lead to deficiency of plants morphology, physiology and anatomy as tiny shoots and leaves, small leaves and roots, low rates of photosynthesis, gas exchange, lowest contents of pigments, absent of cuticular wax and epicuticular, Limited palisade layer, impaired stomata, roots epidermal and subepidermal layer could not regular, cortical cells smaller in size and impaired root function. Date palm plantlets when transferred to ex vitro become stressful by different condition compared in vitro, these plantlets must be acclimatize in the greenhouse for modified these morphology, physiology and anatomy effects in order to increasing survive percent of plantlets for allowing enormous numbers of plants to farmers.

***Keywords*—** acclimatization, anatomy, date palm, ex vitro, morphology and physiology.

I. Introduction

MORPHOLOGICAL characters of plantlets *in vitro* and ex vitro

Plantlets from *in vitro* have shortest shoots and roots and lowest numbers of leaves and roots than *ex vitro*, increasing of these parameters was found after *ex vitro* acclimatization in the different periods weeks or months, following scientists were proved these, Total dry mass leaves of *Nicotiana tabacum* were higher in *ex vitro* than *in vitro* (**Kadlecek** *et al*. 1998), also Plant height, number of leaves, total dry mass and leaf mass area ratio of *Nicotiana tabacum* (**Pospisilova** *et al.* 1999). Leaves and height of *Fraxinus excelsior* L. were smaller *in vitro* than *ex vitro* (**Lebedev** and **Schestibratov** 2013).a relationship between plantlet morphology and survival under conditions inducing water stress (**Mohammed** and **Vidaver** 1990). Curled dark leaves of date palm derived *in vitro* condition (**El- Bahr** *et al*. 2003). Leaf length and shoot number and shoot and root dry weights were increased after ex vitro than in vitro of (*Uniola paniculata* L.) sea oats (**Aracama** *et al.* 2008). *In vitro* conditions compromise plantlets' leaf morphology, leaf blade expansion and dry matter accumulation these characteristics negatively impact the capacity for ex vitro acclimatization, but the degree to which plants are affected by the *in vitro* environment depends on the plant species (**Ziv** 1991, **Kozai** *et al*., 1992 and **Llorente** and **Apóstolo** 1998). stem diameter, shoots numbers of *Alpinia purpurata* were increased in ex vitro compared to in vitro (**Medina** *et al*. 2007). The survived-plantlets in *ex vitro* continually grew acclimatized had varied survivability from 73-93% with 82% in average, increased in leaf and root number and length and height of shoots of *Ruscus hypophyllum* L. (**Winarto** and **Setyawati** 2014). Number of roots, mean root length and shoot height of Teak (*Tectona grandis*) were increment after 45 days from acclimatization stage in the greenhouse (**Yasodha** *et al*. 2005). *Coffea Arabica* plants that were transferred to the greenhouse these plants reached about 30 cm length after 5 months after acclimatization (**Zok** 1986). New leaves of Red Raspberry *Rubus idaeus* L**.** were increased after 4 weeks of acclimatization, the number of transitional leaves formed on transplants seems to depend on the degree of hardening of the cultured plantlets and the stress of the new ex vitro environment (**Dong** and **Donnelly** 1993). The 3 week-old *Vitis vinifera* plantlets were more advantageous with enhanced vigor than the 4 and 5 week-old plantlets (**Thomas** 1998). the survival rate of *Prunus domestica* plantlets was affected by the shoot height rather than the number and length of roots (**Padilla** *et al.* 2003). Leaf numbers, plant height root number of *Gladiolus grandiflorus* L cv**.** Vinks Glory were increased in *ex vitro* after one month (**Sheena** and **Sheela** 2010). Micropropagation technique enable to mass production of high quality, disease-free and uniform planting material (**Kumar** and **Rao** 2012). Survival rate 85%of Vietnamese ginseng (*Panax vietnamensis* Ha et Grushv.). numbers of leaves and axillary shoots, fresh and dry weights of leaves and shoots of *Malus hupehensis* were increased in the glasshouse(**Ur-Rahman***et al.* 2007). (**Bariya** and **Pandya** 2014). After 3

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months, rooted and shoots *Gerbera jamesonii* had grown to a height of more than 20 cm. Plants grown of *Anthurium andraeanum* (Linden ex andre) towards an effective increase in height up to 29 weeks of growth ex vitro, greatest number of leaves being evident 182 days after transplant (**Viegas** *et al.* 2007). Plant height, number of leaves/plant, fresh and dry weight of leaves and shoot and root/plant of *Pterocarpus santalinus* L. were increased in ex vitro (**Rajeswari** and **Paliwal** 2008). The increase in height of *Calendula officinalis* in soil was an average of 19.9 cm., The highest growth was verified between the third and fourth weeks. In approximately two and a half months, the plants began to develop adventitious shoots (**Victório** et al. 2012). After two months of acclimatization of *Panax Vietnamensis* A total of 170 (85%) surviving plants were obtained out of which had two shoots per plant instead of one, increasing the average root length and shoot height was observed (**Nhut** *et al.* 2011). Good root growth of the plantlets would support shoot growth during acclimatization period (**Borkowska** 2001). The success rate of this transplantation of *Gerbera jamesonii* was 95%. After 3 months, rooted shoots had grown to a height of more than 20 cm (**Aswath** and **Choudhary** 2002). Some of the substrates medium and different treatments were produced positive effects on determinations of leaves and roots as, The *Stemona curtisii* Hook.f. plantlets which were cultivated in coconut fiber or hydroponic culture showed 100% survival rate with the highest average number of leaves was found with coconut fiber, the mean root length of the plantlets grown in sand soil (**Palee** *et al.* 2012). plantlets of Pineapple(*Ananas Comosus*.L.Meer.cv.Del Mont grown in culture medium containing only peat moss was significantly increased in plant length , number of leaves /plant, fresh weights of vegetative growth and roots (**AL-Taha** 2013).). 12 weeks of acclimatization The best treatment for increasing roots length which penetrated out from the pots through the holes at the bottom of the pots or grew in circle on the bottom of the pots, and also highest shoot height, leaf number an shoot diameter of oil palm plantlets *Elaeis guineensis* Jacq in the *ex vitro* condition was by dipping the basal end of the plantlets in 2 -8 mM IAA,IBA and NAA solution (**Sumaryono** and **Riyadi** 2011).

Physiology effects

Shoots in vitro conditions were continuously founded in a high sugars concentration which is a source of carbohydrates, constant light, constant temperature thus the metabolism process and carbon dioxide fixation is depressed, photosynthetic pigments was low, pigments synthesis may be impaired under constant and low light intensity also concentrations of starch is very low, when un change in light, temperature and water the evapotranspiration is very low or lacking, CO_2 uptake is relatively low. Therefore, The growth of plantlets *in vitro* is often greater under photoautotrophic conditions than under heterotrophic conditions, provided that the *in vitro* environment is properly controlled for promoting photosynthesis (**Kozai** 1990). The ability of *Pseudotsuga menziesii* (Mirb.) plantlets to utilize high irradiance and to photosynthesize under conditions of water stress was enhanced by the presence of many roots. Carbon dioxide uptake rate increased with increasing root number and decreased as the needle surface area/root surface area ratio decreased. High root number, low needle surface area/root surface area ratio, and elongated roots were associated with low apparent rates of shoot and root respiration (**Mohammed** *et al.* 1992). in grape (Vitis L. hybrid) Improved transplant growth using ex vitro CO_2 enrichment has been found (**Lakso** *et al.* 1986). CO_2 is one of the most important factors directly affecting the growth and photosynthesis of plantlets because they should produce complex organic compounds from CO_2 as a carbon source using energy from light. Therefore, it is necessary for enhancing photoautotrophic growth to provide a sufficient optimal concentration of CO_2 (**Tanaka** 1991). Increasing the photosynthetic photon flux density (PPFD) and CO_2 in the growth chamber remarkably increased the number of leaves and roots, and shoot and root fresh and dry weights compared with plantlets under the same level of CO_2 under low PPFD, however, there was a remarkable decrease in photosynthetic capacity (**Norikane** *et al*. 2013 on *Oncidesa* (formerly Oncidesa Gower Ramsey 'U-1'). The ability to induce greater biomass by increasing the carbon dioxide (CO2) concentration through photoautotrophic micropropagation which has proven benefits in terms of productivity (**Kozai** *et al.* 2005) same results were found with (**Teixeira** da Silva et al. 2007) who stated The photoautotrophic growth of several orchid plantlets was stimulated using 3000 mmol mol21 CO_2 enrichment: C3 Cymbidium, decrease in photosynthetic capacity, chlorosis and browning, which were observed in plantlets grown at 10 000 mmol mol21CO_2 under high photon flux density PPFD (photon flux density). implying that higher CO_2 enrichment (.3000 mmol mol21) could have a positive effect on the photoautotrophic growth of orchid plantlets plantlets grown at 10 000 mmol mol21 CO_2 under high PPFD showed decreased photosynthetic capacity and total Rubisco activity tended to decline (**Sharma** *et al.* 1999, **Norikane** and **Tanaka** 2010 and **Norikane** *et al*. 2010). CO_2 enrichment in vitro produced the plantlets had the heaviest shoot weight as a result of the accumulation of carbohydrates in the Pseudobulb as a direct consequence of photosynthesis (**Hew** and **Ng** 1996). Increasing light levels and improving the availability of carbon dioxide, either through venting or artificial enrichment, allows plant tissue to be grown photoautotrophically in a vessel. Photosynthetic rates and growth of strawberry plantlets were greater under photoautotrophic conditions with carbon dioxide enrichment (**Fujiwara** *et al.,* 1988). Leaves formed during the acclimatization period may still have a lower photosynthetic capacity than leaves of greenhouse-grown plants (**Carvalho** *et al.* 2001). Leaf yellowing is attributed to photoinhibition, nutrient deficiency, premature senescence and other causes (**Sicher** 2008). Plantlets under acclimatization stage ex vitro were exposed to high relative humidity (RH) in the plastic covers and low light intensity for many weeks or months then RH was gradually decreased and adjustment light level. During ex vitro acclimatization of red raspberry *(Rubus idaeus* L.) greenhouse- or field-grown (control) type anatomy and physiology developed gradually (**Donnelly** and **Vidaver** 1984a, **Donnelly** *et al.* 1984 and 1985). Ex vitro

acclimatization can be expensive in terms of labor, controlled environment facilities, and plant losses. Manipulating the culture environment to alter the CIP toward photoautotrophy and hardening could abbreviate or eliminate the ex vitro acclimatization micropropagation [**Donnelly** and **Tisdall** 1993). Plantlets of *Swertia Chirata* derived in vitro are heterotrophic in their mode of nutrition must to be acclimatized to can be culture in the open field (**Pant** *et al.* 2010). A reduction in relative humidity leads to increases in plant transpiration with associated development of functional stomata for controlling plant water loss. (**Diaz-Perez** et al., 1995b). Roots developed in vitro are believed to compensate for water loss caused by malfunctioning stomata. Improved performance and increases in dry weight of these in vitro-rooted plants may be due to extra nutrient uptake through the roots. After acclimatization the water retention capacity of in vitro-formed apple leaves was lower than in vivo-formed leaves (**De Klerk,** 2002). Growth of carnation plantlets under high light levels was greater on a sugar-free medium using nutrient components widely used in hydroponic culture than either on a sugar-free medium with 1/2 Murashige and Skoog (MS) (**Murashige** and **Skoog** 1962) or sugar containing hydroponic or 1/2 MS media (**Kozai** *et al.* 1988). plantlets can grow in vitro in a sugar-free culture medium provided the environment is conducive to photosynthesis. In a closed vessel, plantlets are unable to achieve their photosynthetic capacity as the carbon dioxide concentrations are too low during most of the light period (**Kozai** *et al*., 1991). Saccharose and agar in medium, and CO_2 concentration in vessel atmosphere can affect subsequent acclimation to ex vitro conditions, leaves formed in vitro may be photosynthetically competent, these leaves are frequently replaced soon after transfer to the greenhouse by leaves with higher photosynthetic activity (**Van Huylenbroeck** and **Debergh** 1996). Transfer of microshoots from in vitro to ex vitro conditions under direct sunlight can be cause photoinhibition and chlorophyll (Chl) photobleaching. Thus, the exposure of *Calathea louisae* and *Spathiphyllum floribundum* plantlets to high irradiance immediately after transplantation caused photoinhibition and even Chl photobleaching (**Van Huylenbroeck** *et al.,* 1995).

Anatomy effects

Plantlets in vitro shoots and roots showed different characteristics compared to plants in the open field, *In vitro* shoots and plantlets are tiny, Saturated water makes reducing dry matter accumulation per area Thin cell wall, inhibit cell wall deposition and sclerenchyma, collenchyma formation Absent of cuticular wax and epicuticular, Thinner epidermal layer or irregular epidermal cells, limited palisade layer and sometimes strangely shaped palisade cells, palisade development is affected by different light levels and reduced as a consequence of relatively low light intensity, limited palisade layer and sometimes strangely shaped palisade cells, palisade development is affected by different light levels and reduced as a consequence of relatively low light intensity, stomatal had slow response times or impaired function, Loosely organized spongy mesophyll, Roots *in vitro* were irregular, epidermal and subepidermal layer could not regular, xylem vessels were not completed, pericycle and endoderms were darkly, in this respect, A large portion of the plantlets were loss during hardening, there are various problems in the acclimatization stages of *Stevia rebaudiana*, these problems could be summarized as follows: The shoots were very thin, very long internodes and a few shoots were vitrified. Low efficient rooting and low rate of survival upon transfer to soil (**Hassanen** and **Khalil** 2013). In addition, plantlets were very thin, higher and have non – functional roots and they could not survive and eventually died in the greenhouse. Thus, it was needed to acclimatize the plants *in vitro*, where they receive a special treatment before they can be transferred to greenhouse (**Hassanen** and **Khalil** 2013). in vitro grown plants lack wax on the cuticle necessary to reduce transpirational losses. (**Grout** and **Millam** 1985). Plantlets through tissue culture are heterotrophic, lack cuticle on their epidermis (**Laemmli** 1970.) as well as having non- functional stomata (**Murashige,** T. and F. **Skoog,** 1962). Leaves from in vitro grown *Prunus cerasus*, *Vaccinium corymbosum* or *N. tabacum* plantlets had ring shaped stomata, but in leaves of ex vitro transferred plants, stomata were elliptical also, Guard cells of in vitro grown Rosa hybrid plantlets contained numerous ribosomes and mitochondria, starch rich plastids and relatively large vacuoles indicating that they may exhibit metabolic activity similar to normal guard cells (**Drew** *et al*, 1992). deposition of protective epicuticular wax on the surface of the leaves is the most important factor responsible for excessive loss of water, leading to poor transplantation success (**Hazarika** *et al.* 2000). Root cortical cells of date palm plantlets, appear variable in shape and size. The inner region of the cortical zone was degenerated and disrupted whereas the outer cortical zone (3-4 layers) was normal and compact in shape. The central zone of the vascular tissues (stele) was devoid from differentiation and development of vascular elements except a mass of small, parenchymatous cells, epidermal and subepidermal layers could not be clearly distinguished for their compactness and dark staining, less number and size of stomata was observe each stoma was surrounded by four epidermal cells differing in shape and size from the other epidermal cells (**El- Bahr** *et al.* 2004) on the other hand roots of date palm offshoots showed epidermal and sclerenchyma cell thickness also cortical region showed thickness and greatest maximum vascular region thickness (**Fatima** *et al.* 2010). In vitro leaves of Teak lacked cuticles but had unicellular and uniseriate trichomes scattered all over the leaf surface, stomatal structure in vitro grown leaves differed markedly from normal seedlings were larger in size and circular in shape with a larger stomatal aperture, didn't close in the dark, meanwhile in ex vitro the lamina expanded, a thin layer of cuticle was formed and stomata became functional. (**Yasodha** *et al.* 2005). Lack of vascular connections between roots and shoots of Malus cultivars was implicated in low survival of in vitro rooted apple plantlets after transfer to the soil (**Yepes** and **Adwinckle** 1994). Date palm Greenhouse-grown plants had the greatest wax deposits followed by the acclimatized plantlets.*In vitro* plantlets had an average of 15% of the wax of greenhouse plants, The increase in wax deposition as a result of polyethylene glycol treatment, explains the decreased water loss observed in acclimatized

plantlets when transferred*ex vitro*. (**Zaid** and **Hughes** 1995). The plantlets may quickly wilt as water loss of their leaves is not restricted. In addition, water supply can be limiting because of low hydraulic conductivity of roots and root-stem connections (**Fila** et al. 1998). Transplants of Red Raspberry may be considered fully acclimatized ex vitro if they have functional stomata and photosynthetic capacity and acclimatization is completed (**Deng** and **Donnelly** 1993). stomatal conductance was found to be higher in the first fortnight and it gradually decreased by the end of second fortnight. This might be due to the leaves of *in vitro* grown plants showed open stomata and collapsed guard cells, while acclimatized leaves presented closed stomata as well as decreased stomatal density and aperture (**Romano** and **Martins-Loucao** 2003). the decrease in stomatal conductance was due to the reduced water loss (**Tari** 2003). Low stomatal conductance indicates reduced water loss and it is important in the maintenance of plant water status (**Bishnoi** *et al.* 1994). malfunction of stomata, less development of cuticle or epicuticular wax on the surface of *in vitro* leaves are contributing to excessive water loss resulting in the high mortality of plantlets acclimatization (**Hazarika** 2006). The decrease in relative humidity induced the epicuticular wax formation of plantlets (**Lamhamedi** *et al.* 2003). Roots of plantlets produced *in vitro* are usually very weak and without root hairs (**Hazarika** 2006). The reduced leaf area index might be due to the reduction in plant height and leaf size (**Rajendiranm** and **Ramanujan** 2003). Early acclimatization period, the roots do not function normally to support the plants as anchors or physiological role to uptake water and nutrients (**Almeida** *et al.* 2005). In the plantlets of *Carlina onopordifolia* Basser the connection between vascular bundles of shoots and roots was poorly formed, This restricted water uptake from the root into the shoot. Leaves that developed in culture deteriorated rapidly after transplanting to ex vitro conditions, and new leaves were formed in the second week of acclimatization (**Trejgell** and **Tretyn** 2011). the abnormalities of plantlets need to be repaired. The plantlets usually need some weeks of acclimation under shade with the gradually humidity. The transpiration rate gradually decreases according to the development of effective stomatal regulation of gas exchange (accompanied with changes in leaf anatomy, especially distribution and density of stomata); the epicuticular waxes develop after transplanting slowly than the effective stomatal regulation (**Noe** and **Bonini** 1996 and **Abd- El Baky** 2012 on date palm cvs) Any abnormalities of *Drosera intermedia* observed *in vitro* gradually disappeared during further 4 weeks (**Rejthar** *et al.* 2014).

Acclimatization of plantlets

In vitro condition which described high humidity, sufficient water and carbohydrates sources constant temperature, these condition attributed to abnormality of morphology, physiology and anatomy of plantlets, therefore in vitro plantlets need to acclimatized in the greenhouse to modified these abnormal characters. Healthy date palm plantlets 3-4 leaves, 10-15 cm in length and 2-3 roots were transferred into ex vitro for acclimatized in the greenhouse, these plantlets washed carefully with tap water and treated with fungicide, plantlets cultured in peat moss + perlite 2:1 and kept in the plastic covered inside 100% humidity and 28 C^0 till new leave or root was grown, the plastic covers were opening daily at 5 – 10 minutes to renewal air in the plastic covers. Acclimatization stage was necessary to produce huge numbers of offshoots to open field. Plantlets under acclimatization in *ex vitro* were facing difficult conditions and stress than *in vitro*. Acclimatizing, hardening-off, or conditioning plantlets from the *in vitro* to the ambient environment can be a challenge that may result in death or damage to a large percentage of micropropagated plants (**Preec**e 2010). Therefore, after *ex vitro* transplantation plants usually need some period of acclimatization with gradual lowering in air humidity (**Bolar** *et al.*, 1998). Water loss from leaves is not restricted, the plantlets may wilt quickly, and water absorption limited for low hydraulic conductivity of root and root stem connections (**Fila** *et al.* 1998). Plantlets when transplanted in vitro to greenhouse, they may desiccate or wilt rapidly and can die as a result of the change in environment, unless substantial precautions are taken to accommodate them (**Poole** and **Conover** 1983). In the greenhouse; in particular, differences in light, relative humidity, nutrients and other growth promoters, the gaseous composition and the medium substrate (**Seelye** et al., 2003 and **Chandra** *et al.*, 2010). Moreover it must be reduced humidity gradually in the greenhouse to recovery plantlets, 90% of *Acacia mearnsii* plantlets survived when acclimatized in transparent plastic containers under greenhouse conditions (**Sascha** *et al*., 1998) on the other hand, gradual lowering in air humidity it's the big problem facing acclimatization of plantlets; however it must be overcome this problem to increasing survival (**Lavanya** *et al.* 2009). 90% survival of *Holostemma annulare* of four weeks hardening by adjusting humidity with removing the polytheline covers for 1 h during first week then increasing gradual exposure time (**Sudha** *et al*. 1998). *Bambusa tulda* acclimatization stage taking 30-45 days included gradual hardening from *in vitro* to natural environment *ex vitro*, new leaves were emerged after 8 weeks (**Mishra** *et al*. 2011). **To increasing acclimatization survival percent many treatments were used as pre-acclimatization in vitro,** date palm cv. Karama plantlets treated with PEG in media at 4.0g/L or 8.0g/L which was effective to enhance root hardening, 72.72 percent of plantlets and increasing root thickness and length (**Mahdia** and **Abd-Alla** 2010). PEG at 30% led to increase the percentage of acclimatization (100%) of date palm; while treatment with PEG at 25% led to increase the percentage of acclimatization 80% (**Al-Meer** *et al*. 2008) . *Centella asiatica* (L.) plantlets were transferred to ¼ MS strength medium having 3% sucrose devoid of PGR for seven days and transferred to polybags containing a mixture of soil: sand: FYM manure (1:1:1) and kept for two weeks in mist-chamber under controlled condition (tamp-25oC ± 2oC), humidity 65% ± 5% (**Bhandari** *et al*.2013). Paclobutrazol Pbz in the rooting of citrus plantlets makes them more wilt tolerant and increases the survival of plantlets in the greenhouse (**Hazarika** *et al*., 2002). Moreover, ABA treatment after transition to acclimatization stage had slight positive effect on Ch content and other photosynthetic

parameters and enhanced plant growth (**Pospisilova** *et al.*, 1988). 0.5 gm.L-1 NPK and half strength of Hoagland solution increase the acclimatized plantlets 80%, and increasing leave chlorophyll content of the plantlets (**Muhsen** *et al*. 2013). Furthermore, ex vitro plantlets derived from Compact 3U system developed better than those from Neon system on Cymbidium 'Tim Hot', Lilium longiflorum and Fragaria vesca cv. 'My Da' (**Nhut** *et al.* 2006). date palm *Phoenix dactylifera* L.were transferred at the liquid medium (1/4 MS) as the pre acclimatization stage for three subcultures (3 weeks for each one). Then these plantlets were transferred to the greenhouse for acclimatization under tunnels (90% humidity) for three month, with 82 % survival (**Darwesh** and **Mohamed** 2009 on cv Bartoumoda, **Othmani** *et al.* 2009 on date palm c. Deglet Nour**,** **Darwesh** *et al*,2011 on cv. Bartoumoda, **Ibrahim** *et al.* 2012 on cv. Barhee). The maximal ex vitro rooting Fraxinus pennsylvanica occurred when shoot explants of the three clones were dipped in 1 mM IBA (**Kim** et al. 1998). Growth retardants increased the survival percentage in acclimatization of *Stevia rebaudiana* under greenhouse conditions (**Hassanen** and **Khalil** 2013). **Different culture soil media was used as**, **Tisserat** (1984 and **Montes** *et al.* 1999 on *Anthurium cubense*) who they elucidated that high survival rate was obtained when date palm plantlets were transplanted in pots containing a mixture of peat moss and vermiculite. (**Puchooa** and **Sookun** 2003) stated that, *Anthurium andraeanum* well-developed roots after transplantation to vermiculite and grow under low light intensity and high humidity in the greenhouse. The date palm cv Kheneizi plantlets were hardened and acclimatized and planted in pots, containing 1:1:1 peat, sand and dehydrated cow manure, which resulted in over 60% ex vitro plant survival (**Kurup** et al. 2014). 95% survival percentage when plantlets of *Pterocarpus santalinus* L. in the mixture of coarse sand, clay and farmyard manure 1:1:1, with high humidity, high successful was found in the dipping of auxine (**Rajeswari** and **Paliwal** 2008 and **Bhandari** *et al.* 2013 on *Centella asiatica*) these mixture media of acclimatization was reported by (**Quaraishi** et al. 2004 on *Azadirachta indica).* Rooted plantlets blueberry cultivar 'Northland are transferred to plug trays containing 1:1:1 peat: sand: vermiculite adjusted to pH 4.5 with ferrous sulphate, and readily acclimatized under greenhouse conditions (**Zhao** et al. 2011). (**Abou Dahab** 2006) found that, the tallest *Hydrangea macrophylla* plants and the greatest number of leaves were observed when using peat moss and sand (1:1 v/v) and 100%. Plantlets *Vitis vinifera* L. were potted in 1 soil: 1 perlite: 1 peat and grown under greenhouse conditions, at 24 0C, healthy, good green color and rapid growth (**Faisal** *et al*. 2005 on *Tylophora indica* (Burm. F.) Merrill and **Shatnawi** *et al.* 2011). Plantlets of Swertia Chirata were enhanced growth after 30 days from acclimatization in the peat: sand: manure 1:1:1 with 85 % survival (**Pant** et al. 2010). plantlets grown in plastic pots filled with sterile sand, soil and vermiculate (2:1:1) kept in the controlled atmosphere chamber room (26 ºc ± 2ºc, 55 ± 5% Humidity and 2000 Lux) for hardening (**Pandey** et al. 2013 on *Psoralea corylifolia* and **Bariya** and **Pandya** 2014 on *Mentha arvensis* L). Plantlets of *Tylophora indica* (Burm. f.) Merr transferred to polycups containing, vermicompost and autoclaved garden soil (1:3). Plants were covered with inverted glass beakers to maintain high humidity for harden showed 75% survival (**Verma** et al. 2010). 83% ***in vitro*** and *ex vitro* rooted plantlets of wild citrus trees (*Citrus halimii* Stone) when transferred to a soil mixture soil, sand and organic material (1:1:1) (**Normah** *et al*., 1997). date palm cv. Maktoom (plantlets) were transplanted in peat moss and perlite (2:1) and placed in plastic tunnels in a greenhouse. with 85% survival percentage (**Khierallah** and **Bader** 2007). The substrate consisted of mixed coconut fiber and soft soil. The survival rate was over 90% after 3 weeks, by which time plants had well-developed leaves (**Minh** 2005) stated that, Melina (*Gmelina arborea* Roxb.) showed 70% on peat and 40% on sand after six weeks of culture on ex vitro conditions. A mixture of peat moss and allows a certain degree of water retention, and permits good drainage and aeration of roots (**Jimenez** et al., 2011 on *Drosera capensis* L.) . Best response (65%) *Gentiana*. *lutea* was obtained in peat: perlite: sand (2:1:1 v/v/v), after eight weeks of transplantation in *ex vitro* conditions (**Petrova** *et al*. 2011). Successful adaptation of vitro plantlets of date palm cv. Zaghlool when planted in pots contained equal volumes of peat moss and vermiculite under high humidity conditions (**Bekheet** 2013). Plantlets of an endangered orchid, *Orchis catasetum* were transplanted to pots filled with perlite, wood pieces, ionolite and mineral cartridge shell (1:1:1:1), also perlite individually produced 100% of plants survived (**Ghaziani** *et al***.** 2014). *Coffea Arabica* (Oudayni, Hammady and Dawaeiry) resulted in 100% of acclimatized plants and reached about 30 cm length after 5 months after acclimatization (**Zok** 1986 and **Ebrahim** *et al*. 2007). Date palm plantlets showed highest survival efficiency 83 % after 8 weeks of ex vitro transplantation and great thickness (**Khan** and **Bi Bi** 2012). Also 86, 82, 77.9, 70 and 80% and survival of *Prunus serotina*, *Ruscus hypophyllum* L., microshoots of Teak and wild strawberry in the greenhouse conditions (**Espinosa** *et al.* 2006, **Winarto** and **Setyawati** 2014, **Tiwari** et al. 2002 **Bhatt** and **Dhar** 2000 and **Mazri** 2013 on date palm). plantlets were successfully acclimatized under the mist in greenhouse of cherry (Gisela 5 and Gisela 6), plum (Fereley Jaspi) and pear (Pyrodwarf) at 61.8,100, 93.2 and 90.9 respectively for different cultivars (**Vujovic** *et al.* 2012). To increase relative humidity around the newly transferred plantlets of date palm , a micro-tunnel covered by transparent plastic was used. Every 2 to 3 days, plantlets were sprayed with a fungicide to prevent crown and leaf rot. Under such conditions, plantlets having 2 to 3 leaves, a well formed and closed crown and 3 to 4 roots showed a high survival rate of about 80-90% **(Abahmane** 2013). Plantlets acclimatization can be considered as a major phase for successful establishment of micropropagated plantlets (**Kumar** and **Rao** 2012).

II. Conclusion

Plantlets in vitro facing particular condition, constant temperature and light, contentious source of water and carbohydrates, this condition cause abnormal morphology, physiology and anatomy characteristics, therefore in vitro

plantlets must be subject under acclimatization stage in the greenhouse to modified these abnormal in high recommended humidity 100% under plastic covers, $28C^0$ for 1-3 month, plastic covers were open daily for 5 minutes, plantlets remain under plastic covers to new root or leave were grown, pre-aclimatization stage also recommended to increasing success plantlet acclimatized.

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