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Genetic Variability of Ecoanatomical Changes among Clay Soil and Soilless Money Plants

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ABSTRACT

Money plants *(Epipremnum aureum*) cultivated soilless and clay soil representing habitat types in soilless shown to significant anatomical characters to adept ecosystem. The leaf anatomy showing increase of blades thickness; palisade and spongy tissues of soilless plants than clay soil ones. In contrast the thickness of upper and lower epidermis layers were reduced. As well as thickness of midrib zone was slightly reduced. Moreover, vascular tissues lost their normal shape and arrangement as xylem arms. The most interesting finding, lengths of both, protoxylem and metaxylem were reduced while their widths were increased. Root length and diameter were reduced in soilless plants. Soilless root system showed several significant anatomical to adept ecosystem (piliferous layer, cortex, endodermis, pericycle, vascular tissues and pith). The number of vessel in bundles and its diameter of root were decreased incorporation to clay soil roots. Protein patterns and DNA fingerprint were revealed the molecular variability. SDS-PAGE appeared differences among soilless and soil plants in number and density of protein bands. As well as RAPD-PCR revealed polymorphism among soilless and clay soil plants, using three arbitrary primers. Seven polymorphic amplified fragments with 53.85% and three monomorphic amplified fragment with 23.08%. It was revealed 3 unique fragments (genetic marker) with 23.08% (700 and 500 for soilless and 600 bp for clay soil money plants). These results showed that, the somaclonal variation among soilless and clay soil money plants depending on genetic structure.

KEYWORDS: Money plants, Soilless, Hydroponic culture, Cross sections, SDS-PAGE, RAPD-PCR ; Genetic variability.

INTRODUCTION

Technical improvements in agrosystems in modern agriculture (soilless cultivation system) have technological advances in horticulture and vegetable production, in greenhouse; gardening and landscaping **(1).** Money plant (*Epipremnumaureum*) have unique place in houseplants grown indoors can be grown without soil in water filled bottles. The water remains clear once the roots have settled in well. It is propagate by stem cuttings of existing plant and put in bottles filled with water and transplanting in pot soil slightly moist and put a support like bamboo in soil, it helps money plant to climb. Money plant requires a temperature range of 15-30°C.Hance in order to get better growth and quality under water stress conditions(75 or 50 of F.C. of soil mixture) .It is preferable to inoculate the soil with mycorrhizae before planting **(2).**

Proteins are primary gene products of active structural genes; hence, any observed variation in protein systems induced by any mutagen is considered a mirror for genetic variations**(3)**. Variation in the DNA coding sequences frequently causes variation in the primary conformation of the proteins. Determination of protein molecular weight (MW) via SDS-PAGE **(4)** concluded that SDS-PAGE of proteins can be economically used to evaluate the genetic variation and relation in germplasm and also to differentiate mutants from their parent genotypes. Molecular biology have sensitive assays for detection of the variations at the DNA level, like Random Amplified Polymorphic DNA (RAPD), that is a very accurate method for screening the nucleotides sequence polymorphisms that are distributed in a random form throughout the genome, in both coding and noncoding regions and repeated or single copy (unique) sequences**(5**). RAPD-PCR technique has been successfully used to detect the mutations and damage of DNA and in some plant species induced by different types of genotoxic agents**(6)**.The aim of this study to assess the possible adaptation of money plant on soilless culture via anatomical characters, genetic variation by the levels of biochemical analyses of proteins using SDS-PAGE and DNA finger print using RAPD-PCR.

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MATERIALS AND METHODS

Sources of plants: Money plant (*Epipremnum aureum)*, stem cuttings of existing plant and put in filled bottles with water (soilless culture). The water was changed frequently (2-3 times per week) The other stem cuttings were transplanted in pots make a hole in clay soil. Money plants were grown in direct sun light in Botany Dept. Fac. Sci. Benha Univ. The leave and root samples were taken at 3 months from cultivation for analysis.

Anatomical studies: Samples (centimeter square) were taken from the fourth leaf developed on the stem and secondary root. In laboratory, the sections of samples were prepared by the method suggested by**(7)**. Plant materials were immediately preserved on F.A.A. solution. Samples were dehydrated in series of solutions of ascending concentrations from 50% to 100% ethyl alcohol. The samples were then embedded in paraffin wax using xylol as solvent. By using rotary microtome, sections were cut at the thickness of 15 microns and then mounted on slides with the aid of egg albumin as an adhesive. Wax dissolved in xylol and the slides were passed through descending series of ethyl alcohol solutions varying from 100% to 50% ethyl alcohol concentrations in descending order. The sections on the slides were stained with safranin and then the colored sections (light green) were kept as permanent preparations on the slides with Canada balsam as mounting medium. Sections in such cases were microscopically explored for the different microphotographs, which can be explored for the different tissues an components. All micrographs were prepared by Nikon Camera on a CarlZeiss Jena microscope. The micrographs were analyzed using programmer (SemAfore 521).The measurements confirmed morphological by using of measuring instrument(vernier caliper) and anatomically by micrometer .

Electrophoresis analysis of protein by SDS-PAGE:

Sodium dedocyl sulfite (SDS-PAGE) method was done according to **(8) as modified by(9)**. The gel was stained with Coomassie Brilliant blue (R-250) and shacked greatly for 24 h. The staining solution was replaced with destining solution and shacked gently for 24 h. The gels were viewed on a light-table and record the position of the bands, by photographing the gel. The gel analysis was applied by Gel documentation UV lamb.

Random amplified polymorphism DNA (RAPD) technique:

DNA extraction: The total DNA was extracted from fresh leaf samples of soilless and clay soil plants according to**(10)**. RAPD analysis was carried out using seven decamer random primers Sequence (5`→3`), OP-A9 (GGGTAACGCC); OP-B5 (TGCGCCCTTC); OP-B6(TGCTCTGCCC); OP-B7 (GGTGACGCAG); OP-B8 (GTCCACACGG); OP-B10 (CTGCTGGGAC); and OP-B14 (TCCGCTCTGG) which were purchased from Kits of Amersham pharmacia Biotech. The following ingredients were added to a sterile eppendorof tube placed on ice during pipetting as followed: 2.5 µl 25 mM MgCl2; 0.5 µl 40 mM dNTPs; 1 µl Taq DNA polymerase (1 unit/µl); 2 µl 0.4 uM 10-mer primer and 30 ng of each extracted DNA.The volume was completed to 25 µl dsH2O. The amplification protocol was doneby the using of PCR Program (Biometra) : Denaturation at 94ºC for 1 min., 35 cycles each consists of the following steps: Denaturation at 94ºC for 30 sec., Annealing at 45ºC for 1 min., Extension at 72ºC for 1 min., Final extension at 72ºC for 5 min.The amplified DNA (15 µl) for all samples was electrophoresed on 1% agarose containing ethidium bromide (0.5 µg/ml) in 1X TBE buffer (89 mMTris-HCl, 89 mM boric acid, 2.5 mMEDTA, pH 8.3) at 75 constant volt, and determine with UV transilluminator. The molecular weight of fragments were determined related to DNA ladder (manufactured by BioRoN) with molecular weights (100, to2000bp).The gel analysis was carried out by programme (UVI geltec version 12.4, 1999-2005, USA).

RESULTS

Histological leave: The presented data in Table (1) and Fig (1) illustrate the leave blade thickness in the leave cells of clay soil Money plant (Fig.1-Sl) was increased by +21.41% more than in the ones of soilless. This increment was corresponding to increase of palisade and spongy with +33.86 and +31.16% respectively table (1) fig. (1). As well as thickness of upper and lower epidermal layer increased by +15.87% and +20.78 %. On the contrary, the thickness of midrib zone was slightly reduced by +4.08% less than the clay soil Money plant. Such response in anatomical structure of leave was mainly correlated with the reduction of epidermis either upper or lower epidermal layers. Also, this effect was associated with evident changes on the main vascular bundle structures as comparing to clay soil money plant. Furthermore, the main vascular bundle of midrib zone tended to be incomplete

circular on shape and lost it's normal shape and it's arrangement as xylem arms as comparing to the untreated plants. The same was happened with the vascular tissue. Length of protoxylem vessel was reduced by -32.40%, while, it's width increased by -21.96% more than clay soil Money plant. Also, length of metaxylem vessel was reduced by +18.4% but its width enhanced by – 6.03% more than clay soil Money plant. On the other hand There are many crystals with two shape Druses (about 8) and Raphids (about 8) in soil Money plant. While There no druses crystals but Raphids crystals are present (about 5) soilless Money plant. Numerous of stomata on the lower epidermis (about 19) with stomatal cavity diameter (20µm) in soil Money plant .no of stomata in soilless plant (about 24) with stomatal cavity diameter (25µm).clear modification was observed in soilless plant by the presence of stomata on the upper epidermis.

Table (1): Histological characters of leave cross section of soilless Money plant compared to arid soil Money plant.

Characteristics (µm.)	Soilless Plants	clay soil Plants	% of change
Thickness of leaflet blade	150.95*	192.35*	+21.51**
Thickness of upper epidermal layer	15.75	18.25	+13.69
Thickness of lower epidermal layer	9.25	13.76	+32.77
Thickness of palisade tissue	45.61	68.96	+33.86
Thickness of spongy tissue	78.30	113.75	+31.16
Thickness of midrib zone	1225.65	1275.75	+3.92
No. of differentiated vascular bundles	3	5	+40.0
Length of protoxylem vessel	19.25	25.50	+24.50
Width of protoxylem vessel	25.65	21.03	-21.96
Length of metaxylem vessel	40.75	48.25	+15.54
Width of metaxylem vessel	37.65	35.38	-6.41
Druses crystals	-	8	+100
Raphids crystals	5	8	+37.5
No. stomata	24	19	-26.31
stomatal cavity	25	20	-25

*Average of absolute valuesµm ** % of soil money plants

Histological root: Soilless root system showed several eco anatomical changes characteristic to adopted ecosystem .Tallest length and fresh weight were reduced .The number of vessel of in bundles and its diameter of root were decreased and also root hair. Piliferous layer is less lignified. Xylem vessels were less and small in area and less lignified . Cells of cortex were not complete and unarranged . Layer of root hair was not destroyed so hairs were clear. Root in Arid soil money plant; Piliferous layer is more lignified .layer of root hair was destroyed so hairs were not clear. Cells of cortex were complete and arranged. Xylem vessels were numerous, large in diameter and more lignified. More modification observed by the absence of endodermis and pericycle layer in soilless, the presence of starch grains, lignified parenchyma and more lignified fibers in clay soil plant.

Table (2): Histological characters of root cross section of soilless Money plant compared to arid soil Money plant .

Root characteristics	Soilless money plant	Clay soil money plant	change (%)
Diameter of root (um)	22.75	24.25*	+6.19 **
Piliferous layer is less lignified	Less	more	
Layer of root hair (mm^2)	0.05	0,25	+80
Cells of cortex	Not complete &unarranged.	complete& arranged.	
Calcium oxalate crystal in cortex (%)	25	79	68.35
Area of vascular bundle (mm^2)	0.75	0,95	+21.05
Area of xylem vessel elements (mm^2)	0.6	0.75	+20
Xylem vessels (No)	12 Lignified	14 lignified	+14.28

*Average of absolute valuesµm ** % of soil money plants

Soil plant soilless plant

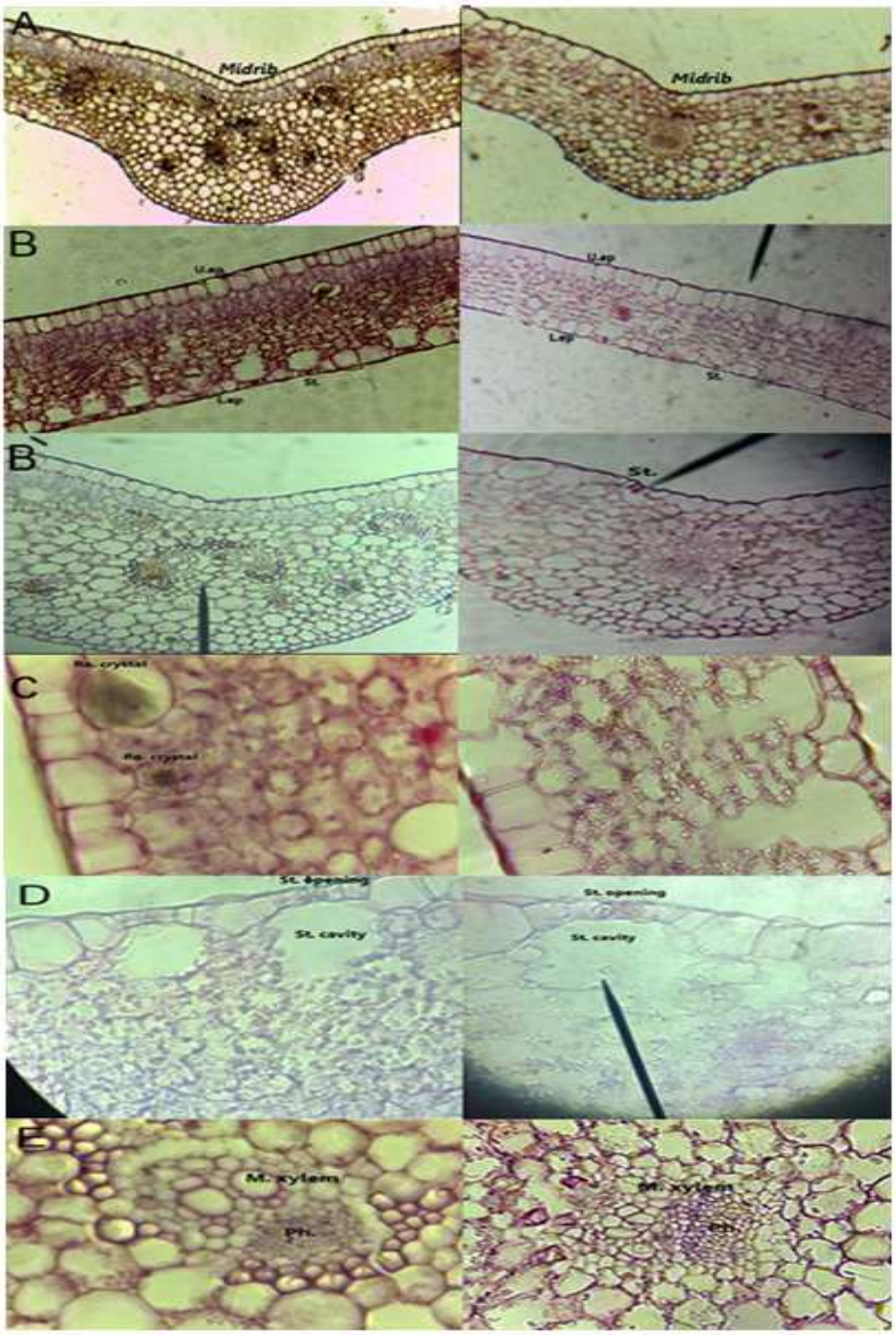
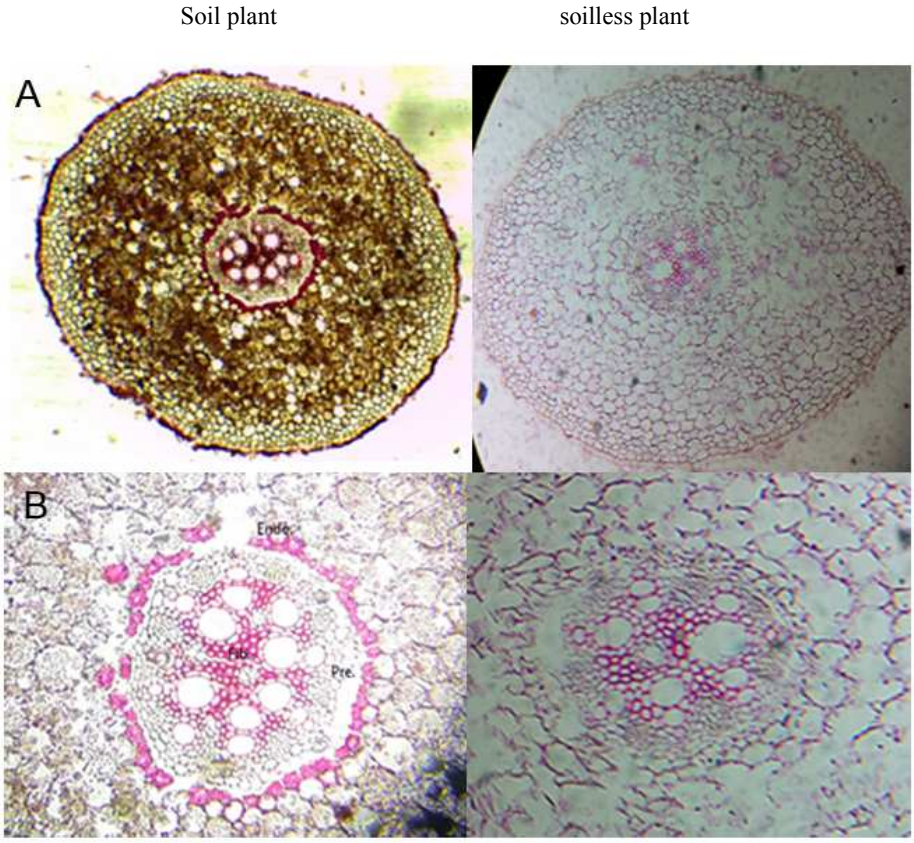


Fig (1): Anatomic gram showing, cross sections of leave of clay and soilless money plants. Where:- A: Midrib B: Leaf epidermis& stomata on upper and lower epidermis B' :Appearance of stomata on the upper surface of soilless plant(200µM) C:Raphids and Rose crystals D: Stomata E: Vascular bundle(500 µM).



Fig(2) Anatomic gram, of cross sections of root in clay and soilless money plants.
Where:- A:detailed sector of root (200µM) B:vascular cylinder(500 µM)

Genetic variability

Adaptive-related protein: Protein profile of total proteins extracted from the soilless and clay soil plant leaves are presented in (Fig. 3).SDS-PAGE analysis exposed 14 protein bands with different molecular weights ranged from 75 to 20kDa as shown in (Table 3).These bands were varied in number and molecular weight among soilless and clay soil money plants (table 3 and fig.2). On the other hand, 7 expressed protein markers with (75,63,55,50,38,30 and 20kDa) were newly induced in the soilless money plants which they disappeared in clay soil ones . two bands were induced in clay soil plants (60 and 22kDa) . Such newly induced bands are considered as protein markers for adaptive in water culture and could be used as marker in money plant in soilless culture.

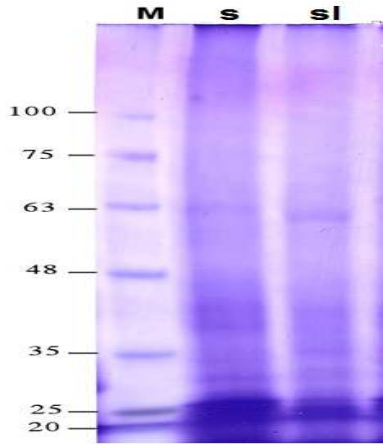


Fig. (3) .Acrylamide gel (12%) showing Protein pattern of Money plant cultivated in soilless (sl); soil (s) by SDS-PAGE determined molecular weight with protein marker (M).

Table (3).Protein pattern of **Money plant** cultivated in clay soiland soilless by SDS-PAGE .

Molecular weight (kD)	Soilless plant	Soil plant	Polymorphism
75	+	-	Unique
63	+++	-	Unique
60	-	+++	Unique
55	+	-	Unique
50	+	-	Unique
48	++	+	Monomorphic
40	++	+	Monomorphic
38	++	-	Unique
35	+++	+++	Monomorphic
30	++	-	Unique
28	++	++	Monomorphic
25	+++	++	Monomorphic
22	-	++	Unique
20	++	-	Unique

RAPD analyses of money plant under soil stress:

Total DNA isolation is found definitive for RAPD-PCR. The DNA yield was determined spectrophotometrically as 5.5 µg/1.0 g leave tissues. The DNA purity as indicated by 260/280 was 1.5. It was found that DNA quality was a good template per PCR sharp and clear amplification products.

RAPD-PCR analyses were performed using random primers; three primers (OP-A9;OP-B6 and OP-B8) from seven primers appeared polymorphism between the soil and soilless money plants (Table 4,Fig4). The total number of fragments developed through the PCR reaction was 48were differed from soil to soilless money plants. As well as Polymorphism levels differed from primer to the other in the soil and soilless money plants (Table 4).

Primer(OP-A9) exhibited 10 amplified fragments , one fragment at molecular marker of 1650 bp appeared in the soil money plants, while three fragments with molecular size,1700,1500,500 bp in the soilless money plants and 5 fragments in both two plants (Table 4, Fig4). Primer(OP-B6) appeared 5 molecular markers with(1900,1550,1500,600 bp)In the soil money plants Thus, soilless money plants appeared 5 amplified fragments with(1700,1650,1000,700,500bp)and 4fragments in both two plants (Fig.4 and Table4).Primer(OP-B8) exhibited two fragments at molecular markers, (1900,1550 bp) appeared in the soil money plants, while three fragments with molecular size,1700,1500,500 bp in the soilless money plants and 5 fragments in both two plants (Table 4, Fig4).

The amplified DNA fragments of the soil and soilless money plants were assorted in number, density and molecular weight. The variability analysis exhibited some DNA amplified fragments absent or/and present among the soil and soilless money plants (Table4).RAPD analyses showed the polymorphism among 2 plants revealed 13 amplified fragments (7 polymorphic amplified fragments (specific fragments) with 53.85% ; 3 monomorphic amplified fragments (common fragments) with 23.08 % and 3 unique amplified fragments (genetic marker) with 23.8%.

Table (4): RAPD amplified polymorphism fragments (polymorphic ,monomorphic and markers)for soil and soilless money plants by three random primers .

Fragment	OP-A9		OP-B6		OP-B8		Polymorphism
Size	S	SL	S	SL	S	SL	
1900	+	++	++	-	+	-	Polymorphic
1750	+	+	+	+	-	+	Polymorphic
1700	-	+	-	+	-	+	Polymorphic
1650	++	-	-	+	+++	+	Polymorphic
1600	++	+++	+++	+	++	+	Monomorphic
1550	+	++	++	-	+	-	Polymorphic
1500	-	++	++	-	+	++	Polymorphic
1400	+	+++	++	++	+	+++	Monomorphic
1000	-	+	-	++	-	+	Polymorphic
800	++	+++	+++	+	++	+++	Monomorphic
700	-	-	-	+	-	-	Unique
600	-	-	+++	-	-	-	Unique
500	-	-	-	+++	-	-	Unique
Total Fragment	7	9	8	9	7	8	

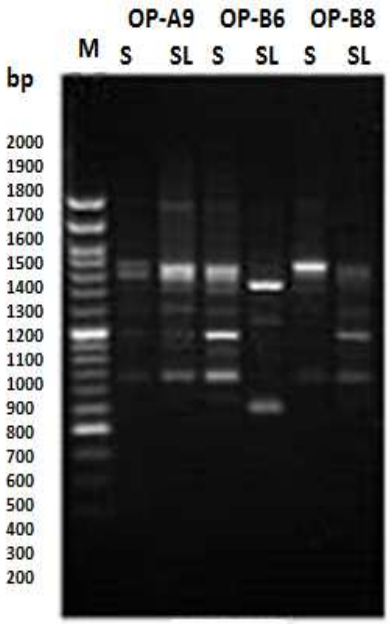


Fig.(4) : Agarose gel 1% showing RAPD-PCR products amplified from DNA extracts of two plants St1, St2, St3, and St4 – M: DNA molecular weight marker (100bp ladder)

DISCUSSION

Plants are exhibited to different harsh environmental stresses that adversely affect their growth, metabolism and yield. Among the environmental stresses water and drought stress is one of the most counter factors for plant growth and productivity**(11);(12) and (13)**.

This study cleared anatomical variations between money plants grown in soilless and clay soil. The results showed that thickness of either upper or lower layers and midrib zone were reduced in soilless than those of clay soil ones. The thickness of leaflet blades, palisade and spongy tissues were reduced in soilless than those of clay soil. In the contrary the width of whether potoxylem or metaxylem vessels were higher in soilless than those of clay soil ones. However, It was also, noticed that the number of differentiated vascular bundles were decreased in stressed cells comparing with healthy ones. We conclude from the previous data that the plant make some modification to tolerant the stress (excess of water) .the epidermis layer usually cover the entire leaf surface to protect the tissues within from drying out and from mechanical injury and for more protection it covered by cuticle layer but in the case of soilless plant there is no drying out so the thickness of either upper or lower layers were reduced. Midrib zone also reduced due to more availability of water and this cause distortion of cells and loss their normal shape. The veins or vascular bundles that consist of xylem and phloem conduct water, mineral salts and food and also mechanically support the mesophyll tissues. In soilless plant the number of conducting elements reduced because the excess of water and the plant in this case do not need many of conducting elements**(14).**

Anatomical changes caused by water shortage in higher plants are better observed indicators to protect and adept to this stress; they can be directly applied to agriculture and handled**(15).**Plant tissues responses to water stress depend on the anatomic features that adjust the transmission of the water stress effect to the cells **(16) and(17).**Tissues prone environments with a shortage of water have generally shown decrease in cell size and increase in vascular tissues and cell wall diameter**(18)**.several features of vascular structure have been inspected , like modifications to the wall architecture and variation of xylem/ phloem ratio which are thought to be inserted in the resistance of the plant to environmental stresses**(19)**.**(20)** reported that the number of xylem elements (protoxylem and metaxylem)with lignified walls was higher in plants tolerant to excess Mn than in the control plants. In this regard **(21)**found that, the most important changes due to sweet potato chlorotic stunt virus (SPCSV) infection were confined to the vein region. Similar results were obtained by**(22),** who

reported that, infected phloem tissues showed less active sieve elements, and thickness of phloem radial and secondary phloem fibers were reduced also, the thickness of xylem tissue and vessels diameter were also reduced. **(23)** showed that, papaya ring spot virus brings about histological and histochemical changes in papaya upon infection. In diseased leaves, palisade cells were markedly distorted .The spongy cells lost their normal round shape with complete disintegration**. (24)** reported that salinity induced structure changes in xylem in root. In salt stress plants vascular cell thickness was much larger than control and it was reduced with cinnamic acid .Salt stress plants showed higher thickness in vascular tissues and cortex thickness was reduced in increasing concentration of Nacl in comparison to cinamic acid treated plants.

In this paper , two varied marker techniques, protein (gene expression) and DNA patterns were used to illustrate the adaptation plant under soil and soilless to show the adaptation markers that could be linked to the adaptation genes and protein expression in plants. The polymerase chain reaction (PCR) is a process used in molecular biology to amplify a single copy or a few copies of a piece of DNA across several orders of magnitude, generating thousands to milloins of copies of a particular DNA sequence. This technique used in medical and biological research labs for a variety of applications **(25)** However, no many informative reports could be obtained that establishing any type of PCR markers neither in plants under soilless. Consequently, such topics are not well documented yet and this study is the first record in plants. Molecular biology have sensitive assays for detection of the variations at the DNA level, like Random Amplified Polymorphic DNA (RAPD), that is a very accurate method for screening the nucleotides sequence polymorphisms that are distributed in a random form throughout the genome, in both coding and noncoding regions and repeated or single copy (unique) sequences **(5).**RAPD-PCR technique has been successfully used to detect the mutations and damage of DNA and in some plant species induced by different types of genotoxic agents **(6).**

SDS-PAGE used for detection of gene expression variability among types of money plants via determination quantitative and qualitative of the total proteins. The results obtained from SDS-PAGE analysis of total soluble proteins could emphasized the foremost effects of soil and soilless. The plants cultivated in soil and soilless revealed variable protein expression based on the presence or absence of protein bands. In addition to, soil related proteins compared in soilless protein synthesis showed that seven proteins were enhanced in soilless plants. The five protein were the most prominent. These proteins were distributed in soluble subcellular fractions.

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