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EFFECT OF PH ON GROWTH, PROTEIN PROFILS AND ANATOMY OF *PLECTRANTHUS AMBOINICUS* EXPLANTS

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ABSTRACT

In this study shoot tips of *P .amboinicus* family *Lamiaceae*, were used as explant for culture in which different levels of MS(Murasige and Skoog) medium salt strength and different pH media were used. Results clearly showed that, the best nutrient media for *P .amboinicus* propagation in establishment of shoot formation via tissue culture was 1/10 MS salt strength medium supplemented with 75 mg/L sodium dihydrogen phosphate (NaH_2PO_4), 30g/L sucrose, solidified with 5 g/L agar and in presence of plant growth regulator (BA 0.1 mg/L) compared to other used treatments. Treatment with pH 5 was the best pH used for *P. amboinicus* planted in greenhouse; on the contrary, pH 13 decreased all the tested parameters. It was appeared clearly that, pH 4 treatment enhanced the formation of two new polypeptides (30 and 60 kDa) proteins. In contrast, synthesis of these proteins was negatively affected by the other used treatments. It was clear that pH value stress induced variations in the appearance of new protein bands and disappearance of others high molecular weights proteins, whereas no changes in low molecular weights proteins were observed. On the other hand, pH 5 induced increase in the thickness of both midvein and lamina of leaf blades of *P. amboinicus*. It worth noting that, the thicker lamina induced by pH 5 was mainly due to increase in thickness of chloranchyma tissue, despite the presence of a decrease in the thickness of the upper epidermis. Also, the main vascular bundle of the midvein was increased in size the increment was mainly due to the increase in the length midvein bundel than control.

Key word: P.amboinicus, in vitro, MS salt strength medium, protein profiles, anatomy

INTRODUCTION

Plectranthus is one of the most important genera of the family *Lamiaceae*. It comprises about 300 species worldwide [1]. A large genus containing about 300 species found in Tropical Africa, Asia and Australia. Some species of *Plectranthus* are difficult to identify because the lack of clear-cut morphological criteria to discriminate not only among species within the genus but also among the closely related genera. The most frequently cited use of species of *Plectranthus* is for their medicinal properties, which accounts for over 85% of all uses. *Plectranthus sp*. are used to treat a wide range of diseases (13 categories) and accounts for about 68% of all traditional uses of the genus. Disorders of the digestive system are treated using 21 species of *Plectranthus*. Also some species are used to treat stomach pain, nausea, vomiting, diarrhea, mouth and throat infections and are used as purgatives, carminatives and as anthelmintic. For instance, *P.barbatus* is used for the treatment of stomachache and as a purgative [2, 3]. Plants of *Plectranthus* are well-known remedies in traditional medicine of many countries. They have been used for different digestive, skin and respiratory conditions, fever and infections, genital-urinary conditions, pain and muscular-skeletal conditions. For example, leaves of *P. igniarius* are used in Kenya to treat inflamed eyes. Numerous species including *P.*

amboinicus, P. barbatus, P. caninus, P. esculentus are reported to have cytotoxic and anti-tumour activity and can be used in the treatment of cancer [1]. Plants can be affected greatly by the water or substance being used to water it. The PH of the substance will affect the way the plant grows. Although the plants were the same type and height they each grew very differently because of the substance and its pH that it was using to water each plant. Neutral substances especially water are the best for plant growth. A substance very acidic would eat away the plant and a substance very alkaline would toxicate it. There is insufficient information about the effect of pH on explants grown *in vitro*. It seems that pH in the range of 5 - 6.5 supports growth, because lower pH (less than 4.5) and higher pH (more than 7) generally stop growth and development of plantlets [4].The objective of the present research aimed to study the effects of different pH levels on growth, some physiological parameters and the relationship between PH level with proteins and the anatomy of *P.amboinicus* obtained from tissue culture technique.

MATERIALS AND METHODS

Source of explants: experiments were carried out on *Plectranthus amboinicus*, of the family *Lamiaceae*, which were purchased from El Orman garden, Giza, Egypt. Mother stock plants, the strating material in this study, were kept at 27±2^{o}C in controlled green house. This work was carried out in Applied Research Center of Medicinal Plants (Tissue Culture and Biotechnology Labs.), National Organization for Drug Control and Research (NODCAR), Giza, Egypt and Agriculture Botany Department., Faculty of Agriculture., Zagazig University, Egypt, during the period of 2013 until 2014. The scientific work was divided into two parts, the first one was i*n vitro* propagation of *P. amboinicus* plants and the second part was acclimatization of *P.amboinicus* rooted shoots produced from *in vitro* (rooting stage) in green house. Prior i*n vitro* propagation, shoot tips of *P. amboinicus* explants were prepared to establish the *in vitro* culture. Shoot tips were washed several times by tap water and rinsed in water with a small amount of soap for 20 minutes for removal of external contamination. The shoot tips were then divided into parts (all parts contained individual bud) and soaked in fungicide solution (0.2% Benlet) + (% 0.25 NaOCl) for 10 minutes, then rinsed in sterile distilled water (three times) to remove all the traces of the disinfectant. Then surface sterilization was done under aseptic condition in laminar air flow cabinet, soaked in (0.625 % NaOCl) for 25 minutes, then rinsed in sterile distilled water (three times) to remove all traces of the disinfectant. The sterilized surface shoot tips becomes as explants. Shoot tips were planted in sterile jars containing 20 ml of basal medium supplemented with 30g/L sucrose and solidified by 2g/L phytagel [5]. The pH value was adjusted to 5.7 –5.8 by adding a suitable amount of 1 N HCL and 1 N KOH and autoclaved at 1.3 kg/cm^2 for 20 minutes. The cultures were incubated in a growth chamber under controlled temperature (27 ± 2˚C) and light (cool white fluorescent lamps of intensity 1000 Lux, 16 hr/day). The following experiments *in vitro* were conducted to study the effect of MS at different levels of salt strength, sodium di-hydrogen phosphate (NaH_2PO_4) and differenced pH values. The study also included adaptation stage.

I- *In vitro* cultivation of *P.amboinicus*

a- The effects of different levels of salt strength (1/20, 1/10, 1/8, 1/4 and full of MS salt strength) and different concentration of NaH_2PO_4 (0, 25, 50, 75 and 100 mg/L) supplemented with plant growth regulators (BA 0.1 mg/L) on growth and development of *P. amboinicus in vitro* during shoot formation in solid medium, were examined to determine the most suitable treatments. The experiment contained 25 treatments; each treatment had 3 replicates (Jars), each replicate (Jar) contained explants. After 8 weeks, percentage of explants survival, number of shoots, length of shoots (cm), number of leaves, fresh weight (g/shoot) and plant growth strength were recorded.

b- The effect of 1/10 salt strength of MS liquid medium supplemented with 75 mg/l NaH_2PO_4 were examined at different values of pH ranging from 1.0 to 14.0. This experiment was contained 14 treatments, each treatment had 15 replicates (Jars), and each replicate (Jar) contained two shoots of average length (5 - 75 cm). These shoots were obtained from those explants (shoot tips) that grown on the best medium (1/10 MS salt strength, 75 mg/L NaH_2PO_4 and plant growth regulator (BA 0.1 mg/L)). After 8 weeks the following parameters were recorded, survival %, root %, length of shoots (cm), number of roots, root length and changes in pH values.

II- Acclimatization of in vitro P. amboinicus rooted shoots produced from in green house (rooting stage).
In this experiment the well survived shoots produced on the best liquid medium for *in vitro* root formation (1/10 salt strength of MS liquid medium supplemented with 75 mg/L NaH_2PO_4 and eleven pH (ranging from3 to13) were used. Changing PH of media used modified pH values through growth and development of *P. amboinicus* results cultured *in vitro* for 8 weeks. were taken and washed from sucrose under tap water. Then, they were dipped in Benlet of 1% as fungicide solution shoots. Then, they were transferred to plastic pots of 12 cm diameter full of sterilized sand. The experiment was repeated twice at intervals of 8 weeks and then plants were transferred at sequenced steps to plastic pots of 20 cm full of sterilized sand for 12 weeks.

Essential oil extraction
Essential oils of the dry herbs were isolated by hydro-distillation and the percentage of extracted oil/g dry weight were estimated according to Egyptian Pharmacopoeia (1984) [6].

Incubation conditions
Cultures of all *in vitro* treatments were incubated in a growth chamber and maintained under controlled temperature (27 ± 2˚C) and light (cool white fluorescent lamps of intensity 1000 Lux, 16 hr/day) for 8weeks or 12 weeks according to the experiment.

Vegetative measurements
Three uniform plants were uprooted from each treatment at the full blooming stage 12 weeks from acclimatization and cultivation of plants in green house to measure morphological and physiological characteristics. The morphological parameters shoot length (cm), number of branches /plant, number of leaves /plant, fresh weight (g/plant), numbers of root, and oot length, were determined. Shoot growth was expressed in scores according to the method described by *Pattino (1981)* [7] as follows:
Negative growth results =1, below average growth =2, average growth =3, above average growth = 4 and excellent growth =5
Fresh and dry weights were estimated by drying each plant in electrical oven at 70°C to a constant weight.
After 12 weeks of the plant acclimatization in the green house, the following parameters were recorded as length of shoots (cm), number of branches /plant, number of leaves /plant, fresh weight (g/plant), number of roots, root length and estimation of the percentage of extracted oil/ g dry weight and total proteins.

Statistical analysis
The experiment was arranged in a completely randomized design and was analyzed using one-way analysis of variance (ANOVA). The values presented are all mean for three samples in each group. Differences among treatments were tested with least significant difference (LSD) at 5% and 1% level of significance according to [8] and *Steel and Torrie (1980)* [9].

III- Determination of protein patterns in P. amboinicus plants
Total proteins were extracted from root, shoot and leaves of *P. amboinicus* plants grown in different pH treatments and were determined using SDS-polyacrylamide gel electrophoresis following *Laemmli (1970)* [10]. Briefly, equale weights of fresh samples were grinded under liquid nitrogen into fine powder and stored at - 80 °C until use [11]. The proteins in a fixed weights of fine grinded powder were boiled at 95°C for 5 min. after mixing with 5X sample buffer at ratio of 1:1 (V:V). The resolving and staking were prepared as described by *Laemmli, (1970)* [10]. Samples were loaded separately into slots in vertical gel electrophoresis (Multigel - Long cat. ≠ 010-400, Biometra®). The starting voltage was 80 Volt until the bromophenol blue dye reached the resolving gel then continued at 150 Volt for 5 hr. The protein bands were observed after staining the gel with silver nitrate as described by *Hussain et al., (1988)* [12]. Gels were photographed.

V - Anatomical studies
It was intended to carry out a comparative micrscopical examination on plant material, which showed the most prominent response of plant growth to the investigated treatments. Specimens of *P. amboinicus* were

taken from the fourth internode which resemble the median internode of the main stem as well as from the median leaf. The used plants for examination were taken at the age of 8 weeks after transferred to plastic pots of 20 cm. Specimens were killed and fixed for at least 48 hr in FAA (10 ml formalin, 5 ml glacial acetic acid and 85 ml of 70% ethyl alcohol). The selected materials were washed in 50% ethyl alcohol, dehydrated in normal butyle alcohol series, embedded in paraffin wax, sectioned to a thickness of 20 microns and double stained with crystal violet/erythrosin [13]. Then sections of the chosen treatments were examined to detected certain histological characters namely**,** stem diameter, epidermis thickness, cortex thickness, fiber strands thickness, phloem tissue thickness, xylem tissue thickness, vessel diameter and parenchymatous pith thickness. The observed histological manifestations (characters) were estimated and photomicrographed.

RESULTS AND DISCUTION

A- Effect of different MS salt strength medium and different concentrations of NaH_2PO_4 (0, 25, 50, 75 and 100 mg/L) in presence of plant growth regulator (BA 0.1 mg/L) on the in vitro growth and development of P. amboinicus.

Data in Table (1) showed that, the significant and highest values of survival percentage, number of shoot, shoot length, number of leaves, fresh weight and plants growth strength were recorded with 1/10 salt strength of MS and NaH_2PO_4 concentration of 75 mg/L medium. While all the above mentioned parameters recorded the lowest values with full MS salt strength and NaH_2PO_4 concentration of 75 mg/L medium. These results suggest that, the best nutrient medium for *P. amboinicus* propagation via tissue culture in establishment of shoot formation is 1/10 MS salt strength medium supplemented with NaH_2PO_4 (75 mg/L), 30g/L sucrose and solidified 5 g/L agar with plant growth regulator (BA 0.1 mg/L) compared with the other treatments as shown in Table (1).

Table (1)

Effects of different MS salt strength media and different concentrations of sodium dihydrogen phosphate (NaH_2PO_4) on in vitro growth and development of P. amboinicus for 8 weeks.

MS salt strength with ($NaH2PO_4$)		Survival %	Av. Number of shoot	Av. length of shoot	Av. number of leaves	Fresh weight g / plant	Av. Plants growth strength
1/20 salt	**0**	16	1.25	3.47	2.95	0.31	2.25
	25	47	2.29	3.43	3.18	0.83	3.28
	50	62	4.44	3.58	4.32	1.09	3.79
	75	88	5.98	4.65	6.01	1.45	4.28
	100	77	3.33	3.39	3.71	1.04	3.81
1/10 salt	**0**	18	1.55	3.77	1.95	0.42	2.52
	25	55	3.29	4.43	4.58	0.96	4.65
	50	76	4.44	5.28	5.72	1.34	4.70
	75	96	6.72	5.75	7.27	1.76	5.00
	100	85	3.65	4.33	4.26	1.21	4.42
1/8 salt	**0**	13	1.16	3.15	1.77	0.18	2.90
	25	45	1.40	3..55	2.44	0.41	3.15
	50	59	2.72	3.76	2.66	0.67	3.25
	75	71	2.98	3.88	2.27	1.02	3.45
	100	65	1.88	3..35	2.22	0.84	3.74
1/4 salt	**0**	9	1.03	2.33	1.31	0.12	2.50
	25	25	2.26	2.77	1.72	0.32	2.70
	50	38	2.67	2.93	1.95	0.56	3.15
	75	49	2.85	2.45	1.17	0.82	3.20
	100	45	2.33	2.87	1.83	0.71	3.13
Full salt	**0**	5	0.95	1.01	1.11	0.02	1.20
	25	21	1.14	1.08	1.21	0.07	1.25
	50	25	1.27	1.16	1.55	0.17	1.50
	75	33	1.09	1.24	1.17	0.29	1.21
	100	28	1.19	1.18	1.1 4	0.23	1.17
L.S.D. at 5%		-	**0.561**	**0.393**	**0.477**	0.153	**0.365**

B- Effects of 1/10 MS salt strength supplemented with 75mg/L NaH2PO4 liquid media with different pH values on in vitro growth and development of P. amboinicus for 8 weeks.

Data in Table (2) show the effect of 1/10 salt strength of MS liquid medium supplemented with 75 mg/L NaH_2PO_4 at different values of pH on shoot and root production from shoots explants of *P. amboinicus* after 8 weeks. It was obvious that, the selected salt strength of MS (1/10) and 75 mg/L NaH_2PO_4 medium with PH of values (1, 2 and 14) had bad effect on survival percentage, shoot length, number of roots, root production and the ability of modifying the pH of media as well as the juice of the plant cells of *P. amboinicus*, while the pH of values (3 to 13 pH) achieved the highest values of the aforementioned parameters (Table 2). In addition, data obtained in table (2) showed that, pH 3, 4 and 5 were the best pH used for in vitro growth and development of P. amboinicus. Although the used culture pH media in this study were between 1to14, the measured pH of cell juice was between the ranges 5.1 to 5.2 (Table 2), these results were under level of *in-vitro* treatment. On the other hand, the decreased values of all parameters at pH 14, conforming that pH affects plant growth. It is well known that pH can affect the nutrients availability and the absorption of nutrients by plant roots. In this regard, pH values above 7.5 cause iron, manganese, copper, zinc and boron ions to be less available to plants, while pH values below 6 cause drop in the solubility of phosphoric acid, calcium and magnesium [14].On the other hand, changing the pH towards the basic or acidic regions has remarkable decreasing effects on growth and development. These results are in line with Camloh and Gegola (1992) [15]. Many in vitro plant cells and tissues will tolerate pH in the range of about 1.0-7.2, those inoculated into media adjusted to pH 2.5-3.0 or 8.0 will probably die, best results are usually obtained in slightly acid condition [16].

Table (2)

Effects of pH values on the growth of P. amboinicus cultured for 8 weeks in 1/10 MS strength liquid medium and supplemented with 75 mg/L sodium di-hydrogen phosphate.

pH values	Survival %	Root %	av. Shoot length (cm)	av. root number	av. root length (cm)	Changes in pH values of medium From	To	Changes in pH juice cell of plants
1	0	0	0	0	0	1	0	0
2	0	0	0	0	0	2	0	0
3	100	100	8.27	3.91	4.52	3	4.45	5.13 – 5.14
4	100	100	8.32	4.24	3.88	4	5.28	5.13 – 5.14
5	100	100	8.83	4.55	4.88	5	5.69	5.13 – 5.15
6	100	100	7.91	3.62	4.52	6	5.96	5.14 – 5.15
7*	100	100	7.64	3.29	4.11	7*	6.68	5.15 – 5.16
8	100	100	7.42	2.91	3.18	8	6.72	5.15 – 5.16
9	100	100	7,13	2.72	2.49	9	6.94	5.16 – 5.17
10	100	100	7.01	2.31	1..91	10	7.11	5.16 – 5.17
11	100	100	6.82	2.14	1.24	11	7.49	5.17– 5.18
12	100	95	6.61	1.98	1.07	12	7.86	5.18 – 5.18
13	100	85	6.44	1.35	1.01	13	8.12	5.19 – 5.20
14	0	0	0	0	0	14	0	0
LSD at 0.05	-	-	0.421	0.352	0.384	-	0.537	-

** PH 7 = MEDIA OF PLANT CONTROL*

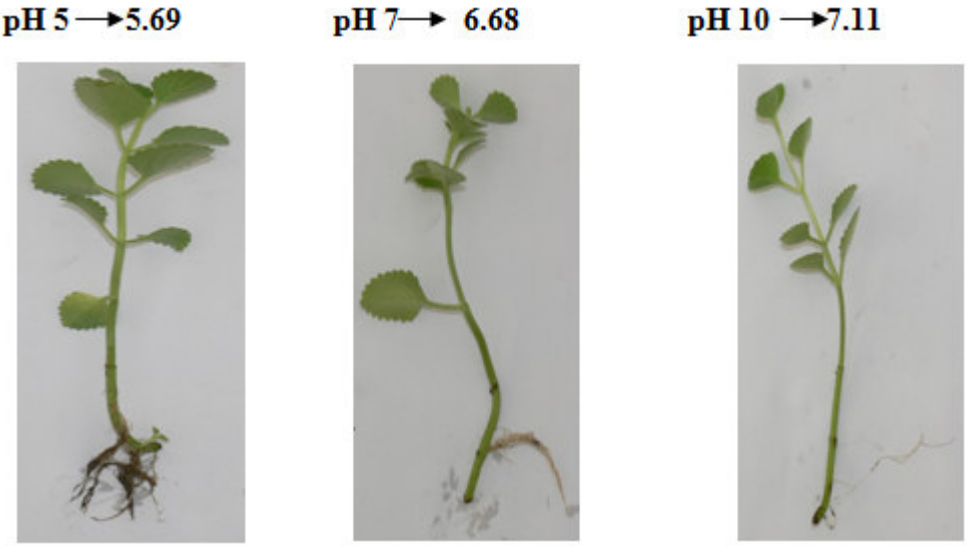

Figure (1)

Effect of pH value on *in vitro P. amboinicus* growth cultured for 8 weeks in 1/10 MS strength liquid medium supplemented with 75 mg/L sodium di-hydrogen phosphate.

II- The effect of different pH levels on some morphological parameters of P. amboinicus grown for 12 weeks on soil medium (sand) in greenhouse (rooting stage).

Data in Table (3) cleared that, pH 5 was the best pH used for *P. amboinicus* growth and oil percentage cultured in greenhouse but pH 13 decreased all parameters. This indicated that pH values ranging from 4 to 7 are extensively distributed throughout the tropical and subtropical regions of the world [17]. The limited plant growth in acid soils may be due to a variety of factors, including the direct effect of pH (excess H ion concentration) as well as pH-induced toxicities (e.g., Al, Mn) and/or (e.g., Ca, Mg, P, Mo) [20]. Increase in the hydrogen ion concentration of the medium generally causes a decrease in the rate of absorption of cations, probably as a result of the competition between the similar charged ions for binding and carrier sites [18]. Similarly, the role of high pH has often been considered to be detrimental in causing deficient nutrient availability and ionic imbalance [18]. On the other hand, sand and peat moss are an excellent growing material. Peat addition tended to decrease microbial biomass and some of soil enzyme activities, whereas some other activities were elevated [19]. The increased soil moisture in the peat treated plots were expected to have positive influences on microbe. On the other hand, peat treatment decreased pH, electric conductivity, soluble P, Ca and nitrate [19].

Table (3)

Effects of different pH levels on some morphological parameters of P. amboinicus grown for 12 weeks on soil medium (sand) in greenhouse (rooting stage).

pH values	av. Branches number	av. Shoot length(Cm)	av. Leaf number	av. root number	av. root length(cm)	av. Fresh weight g/plant	Oil % 100 g dry weight
3	**1.21**	**38.49**	**49.72**	**7.52**	**4.52**	**107.34**	**0.19**
4	**1.55**	**41.68**	**58.37**	**8.88**	**4.88**	**112.25**	**0.20**
5	**1.72**	**51.22**	**97.04**	**9.22**	**6.61**	**124.99**	**0.22**
6	**1.63**	**44.92**	**73.43**	**8.98**	**5.92**	**118.59**	**0.16**
7	**1.31**	**48.34**	**88.37**	**8.41**	**6.28**	**104.72**	**0.15**
8	**1.13**	**47.54**	**91.96**	**7.68**	**5.28**	**101.51**	**0.16**
9	**1.08**	**44.87**	**82.31**	**6.99**	**4.99**	**95.12**	**0.16**
10	**1.05**	**40.55**	**76.44**	**6..59**	**3..91**	**86.97**	**0.16**
11	**1.03**	**33.24**	**57.69**	**5.74**	**2.74**	**82.75**	**0.18**
12	**1.01**	**26.98**	**47.06**	**4.47**	**2.47**	**77.44**	**0.19**
13	**1.00**	**21.35**	**39.72**	**3.21**	**1.21**	**73.55**	**0.17**
LSD at 0.05	**0.044**	**0.141**	**1.497**	**1.121**	**1.121**	**1.971**	**-**

I1I-Protein patterns

Protein patterns had been analyzed in roots, stems and leaves of *P. amboinicus* plants (Figure.2). It was clear that pH value stress induced variations in the appearance of new protein bands and disappearance of others high molecular weights proteins, whereas no changes in low molecular weights proteins were observed. It was appeared clearly, that pH 4 treatment enhanced the formation of a 30 and 60 kDa proteins. In contrast, syntheses of these proteins bands were negatively affected by other treatments. The new bands of high molecular weights of protein in pH stressed plants might be due to de novo synthesis of these proteins [20]. These new proteins may have a specific function to protect *P. amboinicus* plants from further dehydration damage and considered as a defense mechanism to drought stress. Changing of pH values induced polypeptides had been observed in many studies and are assumed to play a role in pH stress [21]. The effect of pH 8 on the approximately 220, 100 and 60 MW protein in the leaves and 60 MW protein in the stem of *P. amboinicus* plants, as well as the effect of PH 12 and pH 13 on the approximately 30 MW proteins in the stems of *P. amboinicus* plants were shown in figure (3). Disappearance of certain polypeptides in pH stressed plants may be related to increase in the hydrolyzing enzyme RNAase activity [22]. The effect of pH was cleared, suggesting an interaction between the protein synthesis and pH values. However, it was cleared that, the severe pH stress treatment had no effect on the accumulation of the protein subunits. These subunits particularly that found at 30 KDa were so condensed when PH stressed plants were treated. These data suggest that accumulation of the low MW subunits proteins (between 30- 20 KDa) was insensitive to PH stress. Nevertheless, pH value stress might have accounted for the delayed onset of high MW protein subunits, relative to the onset of the low MW subunits, under pH stressed. These results were consistent with [23], who reported that, soyabean seeds produced under pH stress had a variation in β-subunit of the β-conglycinin, probably because of degradation of proteins in the shriveled seeds produced under pH stress.

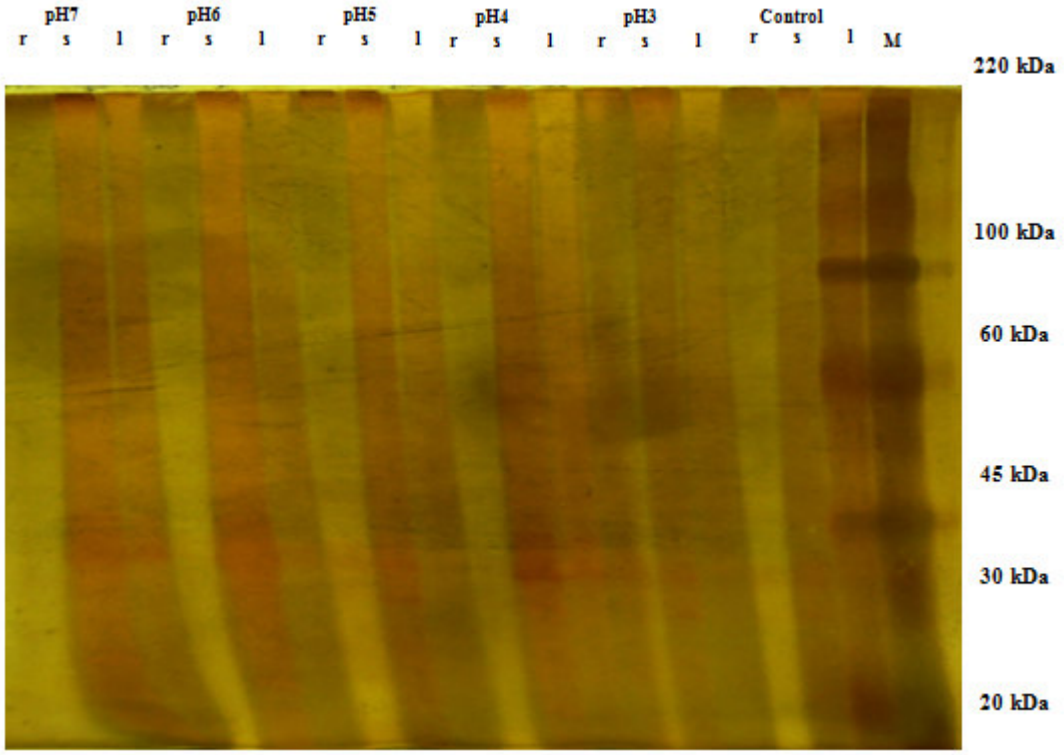

Figure (2)

One dimensional SDS-PAGE analysis of proteins extracted from roots, stems and leaves of P. amboinicus plants which grown at different pH levels in greenhouse for 20 weeks from cultured. r = root, s = stem, l = leaf & M = marker

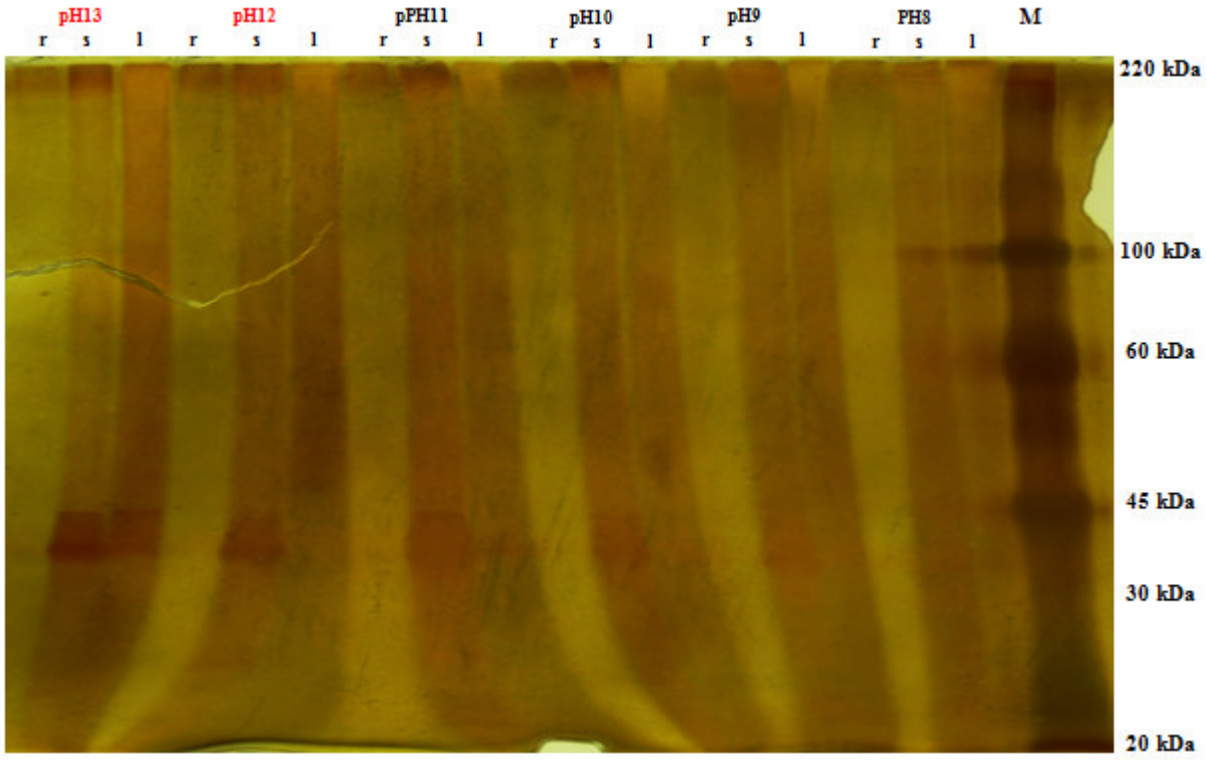

Figure (3)

One dimensional SDS-PAGE analysis of proteins extracted from roots, stems and leaves of *P. amboinicus* plants which grown at different pH levels in greenhouse for 20 weeks from cultured. r = root, s = stem, l = leaf & M = marker

V- Anatomical studies

It was aimed in this investigation to follow the internal structure of vegetative growth which exhibited the most noticeable response to the tested treatments. The aforementioned findings concerning the morphological characters of vegetative growth of *P. amboinicus* treated with pH 5 achieved the most remarkable effects among the various tested levels of pH. This may justify (need) a further study on the internal structure of *P. amboinicus* that grown at pH 5 where the microscopical examination of the main stem fourth internode as well as the leaf of *P. amboinicus* were examined.

1. Anatomy of the main stem

Microscopical measurements of certain histological characters in transverse sections through the fourth internode of the main stem of *P. amboinicus* treated with pH 5 and those of control are given in Table (4). Likewise, microphotographs illustrating these histological characters are shown in Figure (4). It is obvious from Table (4) and Figure (4) that pH 5 treatment increased stem diameter by 23.3 % over the control. This increment in stem diameter was mainly due to the prominent increase in thickness of cortex, fiber strands, phloem tissue thickness, xylem tissue thickness and paranchymatous area of the pith were 62.5, 14.2, 16.5, 36 and 13.4% respectively, as compared to the control.moreover, vessel diameter was increased by 13.2 % over the control.On the other hand, pH 5 had not effect on epidermal thickness

Table (4)

Measurements in microns of certain histological characters in transverse sections of the fourth internode of the main stem of** P. amboinicus**, at the age of 8 weeks as affected by pH 5 treatment (Means in microns of three sections from three specimens).

Characters	Treatments		
	Control	pH 5	% to control±
Stem diameter	2241.8	2764.1	+23.3
Epidermis thickness	18.1	18.1	-
Cortex thickness	144.8	235.3	+62.5
Fiber strands thickness	42.2	48.2	+14.2
Phloem tissue thickness	36.2	42.2	+16.5
Xylem tissue thickness	150.8	205.1	+36
Vessel diameter	45.2	51.2	+13.2
Parenchymatous pith thickness	1435.9	1629	+13.4

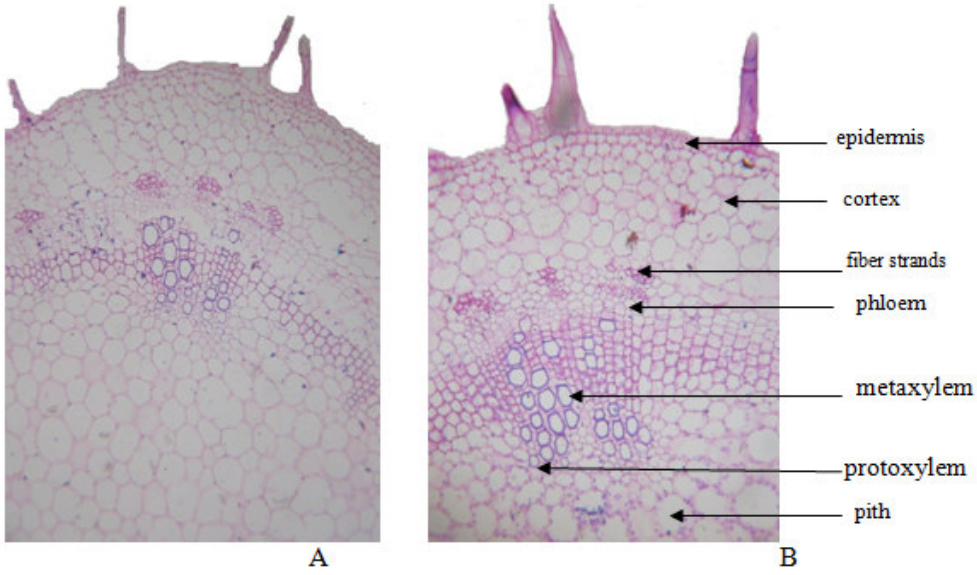

Figure (4)

Transverse sections of the fourth internode of the main stem of P. amboinicus, at the age of 8 weeks, as affected by pH 5 treatment. (x40)

A) From untreated Plant (control). B) From Plant treated with pH 5.

2- Anatomy of the leaf

Microscopical measurements of certain histological characters in transverse sections through the blade of the fourth leaf developed on the main stem of control plants of *P. amboinicus* and of those treated with pH 5 are presented in Table (5). Likewise, microphotographs illustrating these treatments are shown in Figure (5).

It was realized from Table (5) and Figure (5) that, treated with pH 5 induced increase in the thickness of both midvein and lamina of leaf blades of *P. amboinicus* by 39.6% and 15.4% respectively, as compared the control. It was noted that, despite the observed decrease in thickness of upper epiderms by 20 % than control, the thicker lamina induced by pH 5 was mainly due to the increase in thickness of chloranchyma tissue by 18.4 % as compared to the control. Data also indicated that the main vascular bundle of the midvein was increased in size. The increment was mainly due to the increase in length by 11% over the control. Moreover, xylem vessels had wider cavities, being 83.3% more than the control.

Table (5)

Measurements in microns of certain histological characters in transverse sections of the blade of the fourth leaf on the main stem of P. amboinicus, at the age of 8 weeks, as affected by pH 5 treatment (Means in microns of three sections from three specimens).

Characters	Treatments		
	Control	pH5	± % to control
Thickness of midvein	609.3	850.7	+ 39.6
Thickness of lamina	506..8	585.2	+ 15.4
Thickness of upper epiderms	30.16	24.13	- 20
Thickness of lower epiderms	18.1	18.1	-
Length of midvein bundel	108.6	102.6	+ 11
Thickness of chloranchyma tissue	458.5	543	+ 18.4
Vessels diameter	18.1	33.1	+83.3

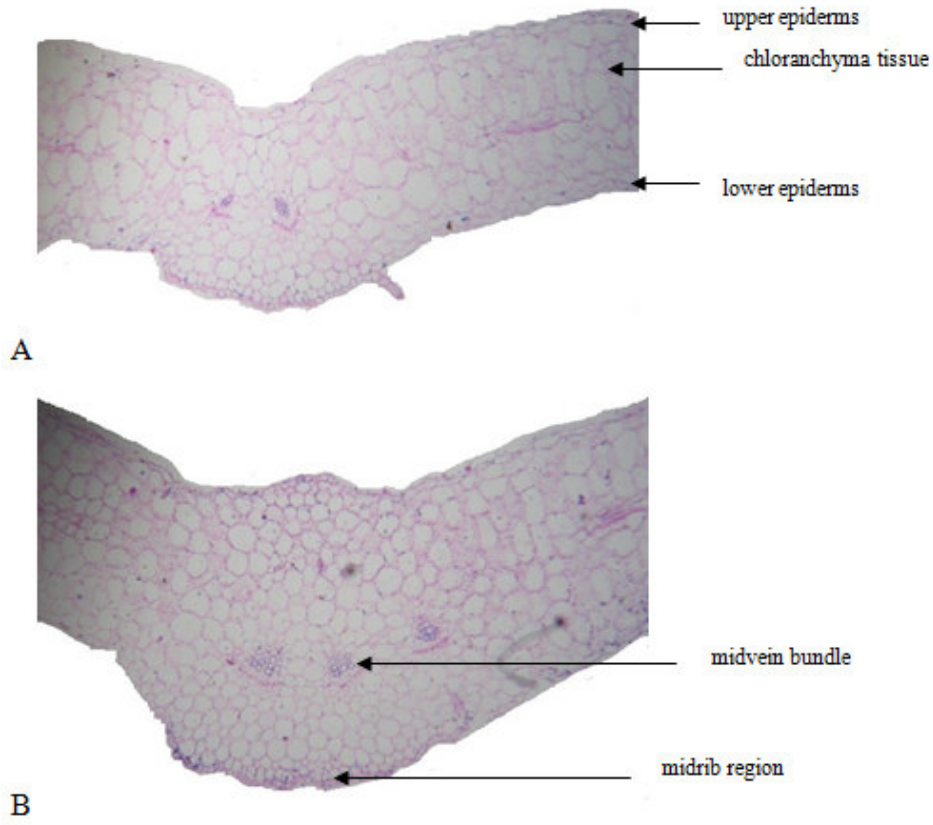

Figure (5)

Transverse sections through the blade of the fourth leaf on the main stem of P. amboinicus, at the age of 8 weeks, as affected by pH 5 treatment. (X 40)

A- From untreated plant (control). B- From plant treated with pH 5.

CONCLUSION

Plant proteins have become a standard tool in plant-stress biology. These technologies have mainly been applied to model systems and have greatly enlarged the knowledge of mechanisms of tolerance. The various abiotic stresses cause changes in plant processes at all levels of organization (morphological, physiological, biochemical and molecular). The list of proteins were unregulated in response to stress is rapidly increasing. Functions for some of these polypeptides are close to being identified and their likely role in stress physiology is being determined. The understanding of mechanisms that regulate proteins will expand the ways in which plants can be utilized. The molecular analysis of stress responses has arrived at a stage where

research can build upon a large collection of characterized proteins.The use of novel approaches combining genetic, physiological and molecular techniques should provide excellent results in the near future [24].

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