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Amelioration Of Flooding Stress Of Wheat Plants By Pseudomonas sp. Inoculation And (Or) Exogenous Ascorbic Acid Application

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Abstract

The experiments included in this study were carried out during the convenient seasons of the years 2012-2013. The study was done to investigate the effect of deleterious effects of water logging (150% and 200% field capacity (F.C)) on growth and some physiological changes of wheat (Triticum aestivum L.) local cultivar grown in pot experiments.

The study also investigated the effect of Pseudomonas sp. inoculation and (or) Ascerbic acid (AsA) foliar application on growth and some metabolic activities of wheat grown under such water-stress conditions. Pot experiments were conducted in the greenhouse of the botanical garden of the Faculty of Natural Resources, Omar El-Mukhtar University .

In pot-experiments wheat plant (Triticun aestivum L.) tolerated water flooding stress up to 2.0 field capacity (F.C.) level. Plant growth parameters (length, leaf-area, pigments, fresh and dry weight) were decreased by increasing water stress level. Pseudomonas inoculation and (or) ascorbic acid (1 mM) foliar application significantly enhanced growth parameters of wheat grown at different levels of water flooding stress. Pseudomonas inoculation increased the height of wheat seedlings by 16.1%, 16.0% and 7.1% as compared with the absolute control at 1, 1 and 2 field-capacity, respectively. Pseudomonas inoculation or ascorbic acid application enhanced the accumulation of shoot and root soluble carbohydrates up to 3 and five time at 2 field capacity. Pseudomonas inoculation and (or) ascorbic acid application increased wheat shoot and root crude-protein as compared with the corresponding control treatments. Water flooding decreased the accumulation of Na+, K+ and P+3 in wheat tissues, however, bacterization and (or) ascorbic acid significantly stimulated K+, P+3 but not Na+ accumulation in wheat shoot system as compared with control plants. Hydrogen peroxide (H2O2) accumulation in wheat root-system was at high levels by elevating water-stress. However, the lowest values of H2O2 was recorded by bacterization and (or) ascorbic acid application at 2 F.C. level of water-stress. Individual amino-acids increased in plants exposed to 2 field capacity compared with control plants. The amino-acid Proline was recorded in higher concentrations with ascorbic acid or when it was applied with Pseudomonas in mixed treatments at 2 field-capacity level.

الملخص

أجريت تجارب في أصص لدراسة الدور الذي يلعبه التلقيح ببكتيريا السيدوموناس أو رش الأوراق بحمض الأسكوربيك عند تركيز 1 مللي مول على نمو وبعض التغيرات الفسيولوجية لنبات القمح النامي تحت الإجهاد المائي (الغمر)، ويمكن تلخيص نتائج الدراسة كالآتي:

1- قاوم نبات القمح الإجهاد المائي (الغمر) حتى مستوى 2 سعه حقلية.

2- أدى الإجهاد المائي إلى تناقص قياسات النمو المختلفة (طول النبات . مساحة سطح الورقة . الوزن الرطب والجاف)، وخاصة عند تركيز 2 سعة حقلية.

3- أدى التلقيح ببكتيريا السيدوموناس إلى زيادة في طول النبات بمقدار 16.1% - 16% - 7.1 عند 1 - 1.5 - 2 سعة حقلية على التوالي وذلك بالمقارنة بالنبات الكنترول المطلق (بدون أي معاملة).

4- أدى رش الأوراق بحامض الأسكوربيك عند 1 مللي مول إلى الزيادة في طول المجموع الخضري للنبات بمقدار 5.4% - 10.7%، 16.7% وذلك على الترتيب عند 1- 1.5 - 2 سعة حقلية بالمقارنة بالنبات الكنترول.

5- ازدادت مساحة سطح الورقة بواسطة التلقيح البكتيري أو الرش بحمض الأسكوربيك بمقدار 53.0% - 34.0% - 15.0% - 23.5% - 20.9% - 37% و17.6% - 34.4% عند 1، 1.5، 2 سعة حقلية بالمقارنة بالنبات الكنترول على الترتيب.

6- أدى التلقيح ببكتيريا السيدوموناس مع (أو) الرش بحمض الأسكوربيك إلى الزيادة بشكل معنوي في محتوى أصباغ كلوروفيل B والكاروتينات عند جميع مستويات الرطوبة المختبرة بالمقارنة بالنبات الكنترول.

7- ازداد المحتوى الكلي للسكريات الذائبة في كل من المجموع الخضري والجذري بزيادة نسبة مستوى رطوبة التربة وذلك بالمقارنة بالنبات النامي عند 1 سعة حقلية، كما أدى التلقيح البكتيري أو المعاملة بحمض الأسكوربيك إلى زيادة إضافية في محتوى تراكم السكريات الكلية.

8- ازداد محتوى البروتين الخام للمجموع الجذري والخضري لنبات القمح وذلك بالتلقيح البكتيري أو الرش بحمض الأسكوربيك مقارنةً بالنبات الكنترول عند جميع مستويات الرطوبة المختبرة.

9- أدى التلقيح البكتيري مع (أو) الرش بحمض الأسكوربيك إلى زيادة تراكم عناصر البوتاسيوم والفسفور دون الصوديوم في المجموع الخضري لنبات القمح مقارنةً بالنبات الكنترول وذلك في المجموع الجذري والخضري.

10- ازداد تراكم تركيزات فوق أكسيد الهيدروجين في أنسجة المجموع الجذري لنبات القمح وذلك بزيادة الإجهاد المائي (الغمر). بينما سجلت المعاملة بالتلقيح البكتيري أو الرش بحمض الأسكوربيك تركيزات منخفضة من فوق أكسيد الهيدروجين داخل أنسجة نبات القمح.

11- ازدادت تركيزات الأحماض الأمينية المنفصلة بزيادة الإجهاد المائي وذلك في كل من المجموع الجذري والخضري لنبات القمح، كما تراكمت تركيزات مرتفعة من الحامض الأميني (البرولين) وذلك عند المعاملة بالرش بحمض الأسكوربيك بشكل منفصل أو عند خلطه مع اللقاح البكتيري وذلك عند التركيز المرتفع للسعة الحقلية (2 سعة حقلية).

Introduction

Water logging occurs over a vast regions throughout the world, and adversely affecting approximately 10% of the global land area (FAO, 2002). It usually occurs when rainfall or irrigation water deposits on the soil surface or subsoil for prolonged period of time. It can also occur when the amount of water added through rainfall or irrigation is more than what can percolate into the soil within one or two days (Kozlowski, 1984). Water logging occurs in many wheat growing regions throughout the world, especially irrigated and high rainfall environments. About 10 – 15 million ha of the world's wheat growing areas are affected by water logging each year (Sayer et al., 1994), representing 15-20% of the 70 million ha annually cultivated for wheat production (Settler et al., 2009). Water logging is most widespread in rice-wheat rotation, since soils are generally puddled to restrict water percolation for rice cultivation which leads to develop a soil pan. The soil pan is often left undisturbed at cultivation for wheat that follows rice and may cereate a barrier for water movement causing water logging in case of excessive irrigation or rainfall (Samad et al., 2001; Hossain and Uddin, 2011). Water logged plants are affected by the reduction in soil and plant oxygen concentration (Hypoxia), which leads to decrease cell-respiration and ATP generation (Lee et al., 2011). Thus many of the physiological processes are inhibited e.g. sucrose, amino-acids, protein and lipid synthesis, moreover, mineral nutrient deficiencies, microelement toxicities, and altered plant hormone levels (Mengel and Kirkby, 2001; Settler and Waters, 2003.(

Waterlogging induces progressive reductions in soil O2 concentration (Anoxia) and in redox potential, which contribute to the de-energizing of photosystems, such as photorespiration and Mehler reaction and increase the production of Reactive Oxygen Species (ROS) such as hydrogen peroxide (H2O2), superoxide and hydroxyl radical (Yiu et al., 2009). These ROS Cause lipid peroxidation and consequently membrane injury, protein degradation, enzyme inactivation and disruption of DNA strands, thus, they need to be scavenged for the maintenance of normal plant growth. In order to overcome and control ROS effects, exogenous application of some antioxidants such as proline, ascorbic acid, carotenoids or some enzymes such as SOD, CAT and GR, and the application of diamine precursors e.g. spermine (Spm), and spermidine (Spd) serve as ROS scavengers, and convert them to molecular oxygen and H2O (Tseng et al., 2007; Yiu et al., 2009 and Ejaz et al., 2012).

Ethylene which is produced by most plants mediates a range of different plant responses and developmental steps beginning with seed germination, tissue differentiation, root elongation, flowering initiation, pollination and fruit ripening (Abeles et al., 1992; Grichko and Glick, 2001 and Rzewuski and Sauter, 2008). Under various stresses in plant, the increase in ethylene synthesis serves as a common step in the chain of events leading to a variety of responses. For example, under flooding stress (Hypoxia) overproduction of ethylene affect booth the transport and perception of indole -3- acetic acid (IAA), and lead to its accumulation at the stem base (Suttle, 1988; Lui and Reid, 1991 and Geigenberger, 2003). Thus, the negative effect of ethylene overproduction under Anoxia condition can be controlled by the exogenous application of IAA transport inhibitors (Grichko and Glick, 2001). The tryptophan from root exudates may be utilized by soil bacteria for IAA synthesis (Patten and Glick, 1996), and bacterial IAA may act synergistically with plant-synthesized IAA. Moreover, in higher plants the intermediate precursor of ethylene is 1-aminocyclopropane -1- Carboxylic acid (ACC) which is produced as a result of Yang cycle (Abeles et al., 1992). Expression of the ACC deaminase, which cleave ACC to ammonia and α-ketobutyric acid, provides an alternative means of lowering plant ethylene dangerous effect under flooding conditions (Klee et al., 1991; Sheehy et al., 1991 and Klee and Kishore, 1992).

In flooding, roots release a high amount of ACC into the surrounding soil (Else et al., 1995). Most soil bacteria are able to degrade and utilize ACC (Glick and Karaturovic, 1995). For example, when 103 soil microorganisms isolated from various regions in South Africa were screened, 81 isolates were able to utilize ACC (Campbell and Thompson, 1996). The establishment of microbial population able to break down ACC may be an effective non-invasive alternative approach to amelioration the damage to plants caused by flooding. This approach utilizes the beneficial relationships of a plant and ACC deaminase-containing plant growth-promoting bacteria (PGB) (e.g. Pseudomonas, Rhizobium and Azospirillums that can significantly decrease ACC levels in plants (Glick et al., 1994; 1998; Grichko and Glick, 2001 and Bajiran et al., 2008).

Plant growth-promoting bacteria can affect plant growth in a variety of ways. They increase seed germination and viability, root respiration and formation, stimulate root and whole plant growth, and increase plant membrane permeability for more efficient nutrient uptake (Hamdia and El-Komy, 1998; El-Komy et al., 2003). Inoculants of beneficial bacteria may solubilize phosphorus and other minerals thereby reducing dependence on chemical fertilizers (El-Komy, 2005).

Thus, the present study was carried out to evaluate the possible mode of interaction between flooding stress and pseudomonas sp., a plant-growth promoting bacterium, inoculation and (or) exogenous ascorbic acid foliar-application on growth and some physiological changes of wheat (Triticum aestivum L.) grown in pot-experiments.

MATERIALS AND METHODS

Experiments included in this study were carried out during the convenient seasons of the years 2012-2013. The study was done to investigate the effect of deleterious effects of waterlogging (150% and 200% field capacity (F.C)) on growth and some physiological changes of wheat (Triticum aestivum L.) local cultivar grown in pot experiments.

The study was also investigated the effect of Pseudomonas sp. inoculation and (or) Ascerbic acid (AsA) foliar application on growth and some metabolic activities of wheat grown under such water-stress conditions. Pot experiments were conducted in the green house of the botanical garden of the Faculty of Natural Resources, Omar El-Mukhtar University.

Microorganism and growth conditions:

Pseudomonas sp. kindly supplied by the Botany department, Faculty of science, El-Minia University, Egypt was used as inoculants in the pot experiments. Bacterial strain was grown in nutrient broth (NB) medium (El-Komy, 1992) overnight at 30ºC on a shaker at 200 rpm. Bacterial cells were harvested by centrifugation (6000 rpm), washed twice in sterile distilled water, and the number of bacterial cells used for inoculation was adjusted to 10^7 CFU/seed by the dilution of the cell suspension of a known cell densities at 450 nm absorbance using a spectrophotometer (JENWAY 6300, U.K.).

Pot experiments:

Seeds of wheat (*T. aestivum* L. local cultivar) were obtained from Department of Agronomy, Faculty of Agriculture, Omar El-Mukhtar University, Libya. Seeds were selected and surface sterilized with a mixture of ethanol (90%) and H_2O_2 (25%) in a ratio of 1:1 (V:V) for 3 minutes, followed by several washings with sterile distilled water.

Sterilized seeds were germinated in sterile Petri dishes containing damp sterile filter paper. Sterile water was added at intervals to keep the paper and germinated seeds wet. Dishes were incubated at 30ºC for 2-3 days or until the radicals were 2-3 cm long.

Five germinated seeds were planted into each pot, immediately after planting, the seeds were inoculated with the bacterial inoculants. Pots were kept in the wire proof greenhouse. Five kilograms of dried clay soil was put into each pot. When the growing plants were about 12cm length, they were thinned down to three per pot, and pots were divided into four groups:

- Seedlings of the first pots group were inoculated with *pseudomonas* sp. and their soil moisture content was adjusted to 100%, 150% and 200% field capacity (control and flooding – stress treatments).
- The second group of pots, seedling (2 weeks old) were sprayed 3 times with 1 mM ascorbic acid (AsA) (10 ml per pot) and their moisture content was adjusted to the corresponding water field – capacity (100%, 150% and 200% F.C.).
- Pots of the third group were inoculated with *Pseudomonas* sp. and sprayed with ascorbic acid (1 mM) and were adjusted to the corresponding water field capacity (100%, 150% and 200% F.C.).
- The fourth group of pots were adjusted to the corresponding water field capacity but left without bacterial inoculation or AsA application (as control treatments). Pots were then irrigated with tap water to maintain the required field capacities. Pots were arranged in a complete randomized design with three replicates for each treatment. After 40 days of sowing, plants were harvested, shoot and root system were separated for further analysis.

Growth criteria:

At the end of the experiment, shoot and root length and their fresh and dry matter yields were determined.

Leaf area was determined according to Norman and Campbell (1994) by measuring leaf length and maximum leaf width according to the formula:

Leaf area = K (leaf length × leaf maximum width)

Where the coefficient K = 0.7 for monocot plants

To determine the dry matter yield, fresh shoots were dried in an oven at 70ºC. Successive weighing was carried out until the constant dry weight was reached.

Estimation of photosynthetic pigments:

The photosynthetic pigments were extracted from a known fresh weight of leaves in 85% aqueous acetone to a certain concentration for spectrophotometric measurements. The photosynthetic pigments (chlorophyll a, b and carotenoids) were determined spectrophotometric method as described by Metzner *et al.*, (1965). The pigments extract was measured against a black of pure 85% aqueous acetone at three wavelengths of 452.5 644 and 663 nm. Taking into consideration the dilution factor, it was possible to determine the concentration of pigment fractions (chl. a, b and carotenoides) as mg/ml using following equations:

- Chlorophyll a = $10.3\ E_{663} - 0.918\ E_{644}$ = mg/ml
- Chlorophyll b = $19.7\ E_{644} - 3.87\ E_{663}$ = mg/ml
- Charotenoids = $4.2\ E_{452.5} \begin{cases} 0.0264\ \text{chl.a} \\ + \\ 0.4260\ \text{chl.b} \end{cases} = \text{mg/ml}$

Finally these pigment fractions were calculated as mg/g fresh matter.

Total carbohydrate:

To estimate total carbohydrates, a known weight of the dried tissue material was hydrolysed by 2N HCl in a water bath for one hour. After cooling the hydrolysate was filtered and then completed to a defined volume. The total carbohydrates were determined by the method of anthrone sulphoric acid which was carried out by (Fales, 1951). The anthrone sulphuric acid reagent consists of 0.2 gm anthrone, 8 ml absolute ethyl alcohol, 30 ml distilled water and 100 ml concentrated H_2SO_4 (D = 1.84). These were successively mixed in a conical flask under continuous cooling. This reagent must be always freshly prepared.

The procedure following was to use one ml of carbohydrate solution, which give a colour equivalent to the range of 0.2 g glucose. This solution was put in a clean Pyrex test tube of about 16 x 160 mm and mixed with 4.5 anthrone reagent. Then this sample was heated at 100°c in water bath for 7 minutes, and directly cooled under tap water. The extinction of the developed blue green colour was measured at wavelength of 620 nm against a blank, which contained only distilled water and anthrone reagent using spectrophotometer. Then 4.5 ml of anthrone reagent, was added to 0.1 ml of the prepared unknown solution in a clean dried test tube, the total carbohydrate content was calculated as mg/g dry weight of the plant organ.

Estimation of total nitrogen in shoot and root tissues:

0.2 g fine ground dried tissue was digested in 2 ml concentrated sulfuric acid and 1 ml of H_2O_2 (50%) as a catalyst were added in a digestion flask, and kept on a hot-plate until the contents become colourless. The solution was completed with H_2O to a fixed volume, and nitrogen content spectrophotometrically at 420 nm using the Nessler's method as described by (Hesse, 1971). The percentage of crude protein plant tissues was found by multiplying the total nitrogen content (%N) by the factor 6.25 according to the Association of official Agricultural Chemistry (A. O. A. C.) (1975).

Determination of free individual amino acids:

The individual free amino acids in wheat tissues were extracted as described by (Speckman *et al.*, 1958). One gram of dry plant material was ground with 10 ml of 3% sulfosalicylic acid, then centrifuged at 7000 xg for 20 minutes. The supernatant was transferred to 50 ml measuring flask and the precipitated residual material in the centrifuge tube was transferred back to the mortar and the same extraction procedure repeated once more. The combined extract was made to a known volume with 3% sulfosalicylic acid then 5 ml were dried then dissolved in 1 ml of 0.2 M citrate buffer and kept of analyses.

Estimation:

The amino acids were analyzed using a Backman 100 Cl amino acid analyzer at the Agricultural Research Center, Giza, Egypt. The following conditions:

Column	6×460
Resin type	W3
Buffers	pH 3.25 (0.2N) Na^+
	pH 3.95 (0.4 N) Na^+
	pH 6.4 (1.0 N) Na^+
Buffer flow rate	44 ml/h

Ninhydrin flow rate	22 ml/h
Column temperature	50ºC - 65ºC
Record chart speed	6 inches/h

Wave length 440 nm for proline and 570 nm for all other amino acids injection volume 0.1 ml (for both standard and sample).

Calculations:

The peak area of each individual amino acid (for standard or sample) was calculated according to the following equation:

$A = h \times W\frac{1}{2}h$

Where:

A= Peak area
h = Height of peak
$W\frac{1}{2}h$ = Width at $\frac{1}{2}$ height

The concentrations of the individual amino acid in the sample was determined according to the following equation:

$Cs = \frac{As\ Ct}{At}$

Where:

Cs = concentration of amino acid.
AS = Peak area of amino acid in sample.
At = Peak area of standard amino acid.
Ct = Concentration of each amino acid (250 n mol/0.1 ml).

Finally the concentration of each amino acid was expressed in mg g^{-1} d.wt and the percent of relative amount of each amino acid was determined.

Determination of some minerals:

Extraction:

A known weight (0.2g) of the dried tissue material was digested in 2ml concentrated sulfuric acid and 1 ml of H_2O_2 in the digestion flask on a hot-plate until the contents become colourless. After cooling the solution was filtered and then diluted to a definite volume. Sample of this solution were taken for Na, K and P determinations and the data were expressed as mg/g dry matter.

Sodium and Potassium determinations:

Sodium (Na^+) and Potassium (K^+) were determined by flame-Photometer following the method of (Williams and Twin 1960).

Phosphorus determination:

Phosphorus was determined according to the method which is based on the formation of molybdate complex, where phosphates acts as catalyser. The intensity of molybdate blue colour is a function of the phosphate concentration of the reaction (Olsen and Sommers, 1982).

Determination of H_2O_2 content:

Hydrogen peroxide levels were determined according to the method of (Sergiev *et al.*, 1997). 0.5 g of fresh root was homogenized with 5 ml 0.1% (W : V), trichloro acetic acid (TCA). The homogenate was then centrifuged at 12,000 xg for 15 min and 0.5 ml of the supernatant was added to 0.5 ml potassium phosphate buffer (pH 7.0) and 1 ml 1M KI. The absorbancy of the supernatant was read at 390 nm. The content of the H_2O_2 was estimated as absorbancy /1g fresh root. Determinations were performed in 3 replicates.

Statistical analysis:

Data were statistically analyzed after using the Completely Randomized Design (CRD). Comparisons among means were done by the Least Significant Difference (LSD) method using the Costat program.

RESULTS AND DISCUSSION

Results presented in Tables (1 and 2) show that in control non-treated plants increasing soil water content than the field capacity (flooding stress) resulted in decreased plant growth parameters (shoots length, leaf-area, fresh and dry weight). On the other hand *Pseudomonas* inoculation and (or) Ascorbic acid (AA) application resulted in significant increases in these growth parameters at all levels of soil moisture content as compared with both the corresponding and absolute (control of 1-field – capacity) control treatments. For example, the growth parameters recorded 16.1%, 16.0% and 7.1% increase in shoot length by *Pseudomonas* inoculation at 1, $1\frac{1}{2}$ and 2 field – capacity levels as compared with the absolute control treatment, respectively. Ascorbic acid (AA) folior application resulted in 5.4%, 10.7% and 16.1% increase in shoot length at 1, $1\frac{1}{2}$ and 2 field capacity levels compared with the absolute control treatment, respectively. Ascorbic acid foliar application resulted in 5.4%, 10.7% and 16.1 increase in shoot length at 1, $1\frac{1}{2}$ and 2 field-capacity levels compared with the absolute control, respectively. Moreover, *Pseudomonas* and Ascorbic acid mixed treatment recorded 14.3% and 19.6% increase in shoot length at 1

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and $1\frac{1}{2}$ field capacity levels as compared with the absolute control treatment, respectively. Similarly, wheat leaf-area was increased by *Pseudomonas* and (or) Ascorbic acid application and recorded 53.0%, 34.0%, 15.0% and 23.5%, 20.9%, 37% and 17.6%, 34.4% increases as compared with absolute control treatment, respectively (Table 1). Shoot and root water content was increased by *Pseudomonas* and (or) Ascorbic acid (AA) application at all the field capacity levels as compared with the corresponding control treatments (Table 1).

Table (1): Effect of *Pseudomonas* sp. inoculation and (or) Ascorbic acid application on shoot, root length (cm/pot) and leaf area (cm^2/plant) of wheat (*T. aestivum* L.) grown at different field capacity levels.

Field capacity	Treatments	Shoot-length		Root-length		Leaf-area	
		cm/pot	%	cm/pot	%	cm^2/plant	%
1	C	28.0	100	15.5	100	59.1	100
	I	32.5	116.1	26.5	170.5	91.0	153.9
	A	29.2	105.4	28.5	183.9	73.0	123.5
	M	32.0	114.3	29.0	187.1	69.5	117.6
$1\frac{1}{2}$	C	28.5	101.8	20.5	132.2	58.2	98.4
	I	32.5	116.1	26.0	167.7	79.6	134.7
	A	31.5	110.7	20.5	132.3	71.5	120.9
	M	33.5	119.6	29.0	187.1	79.3	134.4
2	C	25.0	89.3	22.5	145.2	53.2	40.0
	I	30.0	107.1	23.5	156.6	68.2	115.4
	A	32.5	116.1	24.0	154.8	81.2	137.4
	M	26.0	92.9	23.5	151.6	57.3	97.0
L.S.D. 5%	-	2.5	-	2.3	-	3.4	-

C: Control. I: Inoculated plants
A: Ascorbic –acid M:Mixed-treatment

Table (2): Effect of *Pseudomonas* sp. inoculation and (or) Ascorbic acid application on fresh, dry weight (g/pot) and water content (%) of wheat grown at different field capacity levels.

Field capacity	Treatments	Fresh weight				Dry - weight				Water content	
		Shoot	%	Root	%	Shoot	%	Root	%	Shoot	Root
1	C	2.2	100	1.7	100	0.6	100	0.3	100	72.7	82.4
	I	3.5	159.1	2.4	141.2	0.7	116.7	0.6	200	80.0	75.0
	A	2.6	118.2	2.5	147.1	0.7	116.7	0.5	166.7	73.1	80.0
	M	2.5	113.2	2.3	135.3	0.6	100	0.5	166.7	76.0	78.3
$1\frac{1}{2}$	C	1.7	77.3	1.3	76.5	0.5	83.3	0.4	133.3	70.6	69.2
	I	2.4	109.1	1.5	88.2	0.6	100	0.5	166.7	75.0	66.6
	A	2.6	118.2	2.2	129.4	0.8	133.3	0.8	266.7	69.2	63.6
	M	2.3	104.5	1.8	105.9	0.6	100	0.6	200	73.9	66.6
2	C	1.5	68.2	1.3	76.5	0.4	66.7	0.2	66.6	73.3	84.6
	I	2.4	109.1	2.2	129.4	0.5	83.3	0.3	100	87.5	86.4
	A	5.9	268.2	2.7	158.8	1.2	200	0.5	166.7	79.7	81.5
	M	2.2	100	2.1	123.5	0.5	83.3	0.4	133.3	77.3	80.1
L.S.D. 5%	-	0.7	-	0.8	-	0.2	-	0.1	-	-	-

Previous studies on the reduction in plant growth due to water stress has been widely reported (El-Komy *et al.*, 2003; Malik and Ashraf, 2012). In the present study, *Pseudomonas* inoculation and (or) Ascorbic acid (AA) application improved wheat growth compared to non-treated plants which indicated that these treatments helped wheat plants to mitigate adverse effects of water stress. Bacteria that are beneficial to plants are of two general types: those form a symbiotic relationship, which involves formation of specialized structures or nodules on host plant roots, and those that are free-living in the soil (El-Komy, 1992). Numerous free-living soil bacteria are considered to be plant growth-promoting bacteria (PGPB).

Moreover fluorescent *Pseudomonas* are well known for their ability to colonize the root tissues of wide crop plants and promote the plant growth (Grichko and Glick, 2001). There are several ways in which plant growth promoting bacteria can directly facilitate plant proliferation. They may fix atmospheric nitrogen, synthesize siderophores which solubilize minerals such as phosphorus, and synthesize some less well characterized low molecular mass compounds or enzymes that can modulate plant growth and development (El-Komy, 2005; Jaleel *et al.*, 2007).

Moreover, results of the present study on the role of Ascorbic (AA) in amiloration the adverse effects of flooding stress on plant fresh biomass are in accordance with other earlier reports. For example, Ejaz *et al.*, (2012) reported the ameliorative effect of Ascorbic acid (0.5 mM on fresh biomass of saccharum spp. when exposed to water-stress (Singh *et al.*, 2001; Azzedine *et al.*, 2011 and Malik and Ashraf, 2012).

The chloroplast pigments, chlorophyll a, b and carotenoid play an important role in phytochemical reactions. The present study showed that flooding stress (at 2 F.C.) significantly reduced the leaf pigment content (Table 3). This is in line with what has been earlier reported in many researches (Hamdia and El-Komy, 1998; Jaleel *et al.*, 2009 and Yiu *et al.*, 2009). The decrease of chlorophyll content under water-stress conditions is reported to take place because of its photo-oxidation and degradation by the activity of chlorophyllase enzyme (Abdel-Samed, 2005), as well as due to the production of reactive oxygen species (ROS) in the thylakoids (Sairam *et al.*, 2009). Results of this study also showed that *Pseudomonas* inoculation and (or) Ascorbic acid application significantly elevated the photosynthetic pigments especially chl. b and carotenoid at all levels of soil moisture-content compared with both the corresponding and absolute control treatments (Table 3). Exogenous application of Ascorbic acid (AA) helped plants maintaining the chlorophyll pigments and hence mitigated the adverse effects of flooding stress. These finding are in line with some earlier reports on Cassia, Okra, wheat and maize.

Reactive oxygen species (ROS) produced under stress conditions have been reported to cause pigment degradation (Anjum *et al.*, 2011). However, ascorbic acid being an antioxidant activity scavenges these ROS, thereby reducing the chlorophyll degradation under stress (Ashraf, 2009). Similarly, several reports show that the exogenous application of brassinolide, spermidine (diamine precursor) and methyl jasmonate improved flooding water stress with increased activities of SOD, CAT and APX enzymes, and total improved carotenoid contents in maize (Li *et al.*, 1998) and welsh onion (Yiu *et al.*, 2009).

Results of Table (4) indicate that total soluble carbohydrates in the shoot and root system of wheat was increased by increasing flooding stress level compared with the absolute control plant (1 F.C.). Maximum increasing in carbohydrate accumulation in control plants was reported at 2 field-capacity which recorded three and five time increasing in the shoot and root system, respectively. *Pseudomonas* inoculation and (or) Ascorbic acid application further enhanced the accumulation of shoot and root total soluble carbohydrate.

Table (3): Effect of *Pseudomonas* sp. inoculation and (or) Ascorbic acid application on chlorophyll (a), chlorophyll (b) and carotenoid (mg/g fresh wt.) of wheat (*T. aestivum* L.) grown at different field capacity levels.

Field capacity	Treatments	Chlorophyll (a)		Chlorophyll (b)		Carotenoid	
		mg/g	%	mg/g	%	mg/g	%
1	C	4.5	100	1.2	100	1.4	100
	I	4.6	102.2	1.7	141.6	2.1	150.0
	A	4.8	106.6	2.3	191.7	2.2	157.1
	M	4.5	100.0	2.8	233.3	3.2	228.6
$1\frac{1}{2}$	C	5.4	120.0	2.2	183.3	3.2	228.6
	I	4.8	106.6	3.3	275.0	1.7	121.4
	A	4.9	108.9	2.3	191.7	2.8	200.0
	M	4.5	100.0	2.8	233.3	3.2	228.6
2	C	2.1	46.7	0.7	58.3	1.1	78.6
	I	5.4	120.0	2.5	208.3	3.7	264.8
	A	3.5	77.8	1.6	133.3	3.1	221.4
	M	4.5	100.0	2.9	241.7	3.0	214.3
L.S.D. 5%	-	0.3	-	0.2	-	0.3	-

Table (4): Effect of *Pseudomonas* sp. inoculation and (or) Ascorbic acid application on total soluble carbohydrates (mg/g) in the shoot and root system of wheat grown at different field capacity levels.

Field capacity	Treatments	Shoot		Root	
		mg/g	%	mg/g	%
1	C	63.6	100	57.2	100
	I	73.4	115.4	74.2	129.7
	A	73.1	114.9	75.1	131.3
	M	75.2	118.2	74.1	129.5
$1\frac{1}{2}$	C	74.4	116.9	69.8	122.0
	I	75.3	118.4	74.2	129.0
	A	74.5	117.1	69.0	120.6
	M	78.2	122.9	77.1	134.8
2	C	186.4	293.1	285.0	498.3
	I	137.4	216.1	232.0	405.6
	A	287.5	452.1	204.0	356.6
	M	142.5	224.1	240.1	419.6
L.S.D. 5%	-	2.3	-	3.5	-

Previous studies on the carbohydrate contents in plants grown under water-stress indicated that stress induced profound changes in both total and relative components of carbohydrate pool. Some authors have reported carbohydrate accumulation in various plants grown under water stress conditions (Arafat, 2003; El-Komy *et al.*, 2003 and Sairam *et al.*, 2009). Others observed that at low and moderate water-stress level, sugars and total carbohydrates were decreased (Tattini *et al.*, 2002 and Abdel-Samad, 2005).

The accumulation of sugar was attributed to the raised synthesis of carbohydrates more than to their utilization in new cells and tissues formation. In addition, the monosaccharides glucose and fructose might be of general important in osmotic adjustment at high levels of water stress (Hamdia and El-Komy, 1998; Geigenberger, 2003 and Sairam *et al.*, 2009). Sugar accumulation may be responsible for the relative maintenance of turgidity during plant growth under drought stress. Moreover, some authors concluded that sugar accumulation depends on the plant species and the level and duration of stress (Abdel-Samad, 2005 and Zahran, 1999).

The observed increases in sugar accumulation by rhizobacterial inoculation and (or) Ascorbic acid application was recorded previously in our laboratory (El-Komy *et al.*, 2003 and Abdel-Samad *et al.*, 2003). These authors attributed the increase in growth parameters following rhizabactial inoculation to general improvement of physiological status of the inoculated plants including saccharides content. Recently, El-Refaey *et al.*, (2011) indicated that the increase levels of soluble carbohydrates under *Azospirillum* inoculation was perhaps due to the necessity of its protective role on chloroplast integrity leading to enhanced photosynthesis under water-stress condition. Our results (Table 1) with regards to leaf-area increasing by *Pseudomonas* inoculation and (or) AA application, revealed improved conductance by these treatments which may allow better gas exchange and enhancement of photosynthesis. Grichko and Glick (2001) reported that tomato plants grown under flooding condition and inoculated with bacteria with ACC-deaminase activity (*Enterobacter cloaceae, Pseudomonas putida*) stimulated plant growth, and leaf chlorophyll A and B content compared to flooded non-bacterized plants. The last authors indicated that bacterization of tomato seeds lead to lower the amount of ACC(1-aminocyclopropane-1-carboxylate) available for oxidation to ethylene in the shoots of flooded plants by the bacterization of roots by plant growth promoting bacteria expressing ACC-deaminase gene, which is transcriptionally controlled by the anaerobically regulated promoter that normally regulates the expression of ACC deaminase in these bacteria.

Thus, under flooding-condition ACC-synthase genes are induced in the root of tomato plants, while ACC oxidation (to ethylene by ACC oxidase) is arrested because of root hypoxia (Abeles *et al.*, 1992). ACC is therefore transported by the xylem to shoots where it is oxidized to ethylene (Jackson, 1985). However, plants treated with ACC-deaminase containing bacteria exhibited a significant higer tolerance to flooding than did non-bacterized control plants. Thus, protection against flooding was achieved as a result of the presence of roots treated with ACC-deaminase bacteria which significantly increased overall plant growth, leaf chlorophyll content and subsequently decreased ethylene production in leaf tissues (Grichko and Glick, 2001).

Data presented in Table (4) show that the observed increases of saccharides in the shoot and root of control non treated wheat plants was accombined by increasing in the percent of crude protein Table (5). *Pseudomonas* inoculation and (or) Ascorbic acid application further increased plant shoot and root protein content as compared with the corresponding control treatments. These results are in accordance with previously reported findings of (Saravanakumar and Samiyappan, 2007 and Dolatabadian and Saleh Jouneghani, 2009). Thus rhizobacteria might play an important role in the protein biosynthesis either directly (through fixation of nitrogen) or indirectly by enhancing the uptake of soil nitrogen due to their hormonal effects or nitrate-reductase activities (El-Komy *et al.*, 2003 and Baniaghil *et al.*, 2013). Ascorbic acid (AA) had variable effect on plant protein content at water stress conditions and it depends on Ascorbic acid (AA) concentration (Dolatabadian *et al.*, 2010). The application of 50 mg/L AA had no effect on number of leaves, height and protein percentage at the vegetative stage of corn plant compared with non-treated plants. However, protein percentage increased due to 150 mg/L Ascorbic acid

(AA) foliar application at the same mentioned stage of growth (Dolatabadian *et al.*, 2010).

It is reported previously that Ascorbic acid scavenges reactive oxygen species and prevent protein oxidation and degradation (Noctor and Foyer, 1998). Moreover, it was reported that by AA application under flooding stress condition, about 20 anaerobic proteins were synthesized in maize roots, while synthesis of the normal proteins were drastically repressed (Sachs *et al.*, 1980). Many of these induced proteins were identified as enzymes of the glycolytic and fermentation pathways (Dolferus *et al.*, 2003). The identified anaerobiosis induced proteins (ANP) such as sucrose-synthase, phosphohexose isomerase, and pyruvate decarboxylase were reported (Chung and Ferl, 1999).

Table (5): Effect of *Pseudomonas* sp. inoculation and (or) Ascorbic acid application on N% and crude-protein content (%) in the shoot and root system of wheat grown at different field capacity levels.

Field capacity	Treatments	Shoot		Root	
		%N	Crude-protein	%N	Crude-protein
1	C	1.5	9.3	0.9	5.6
	I	1.7	10.6	1.6	11.9
	A	1.4	8.2	1.3	8.1
	M	1.8	10.6	1.7	10.6
$1\frac{1}{2}$	C	0.9	5.6	1.1	6.9
	I	1.1	6.8	1.7	10.6
	A	1.3	8.9	1.9	11.8
	M	1.4	8.8	1.8	11.3
2	C	1.2	7.5	1.3	8.1
	I	1.4	9.3	1.5	9.4
	A	1.3	8.1	1.8	11.3
	M	-	-	2.0	12.5
L.S.D. 5%	-	-	-	-	-

Results presented in Tables (6 and 7) indicated that in control non-treated plants Na^{+}, K^{+} and P^{+3} accumulation was decreased by increasing soil moisture content both in the shoot and root systems. However, *Pseudomonas* inoculation and (or) Ascorbic acid (AA) foliar application significantly enhanced K^{+} and P^{+3} but Na^{+} accumulation especially in the shoot-system compared with the absolute (1 F.C.) or the corresponding control treatments.

Water-stress play an important role in the uptake and internal accumulation of minerals in different plant species (Zahran, 1999 and Abdel-Samad, 2005). The change in Na^{+}, K^{+}, Ca^{+2}, Mg^{+2} and P^{+3} accumulation may play a role in the difference of water stress tolerance among plant species. Several species tend to take up more Na^{+} and exclude K^{+} with increasing water stress (Werner and Finkelstein, 1995). Drought tolerant species of *Triticum* had lower Na^{+} accumulation than the senstive species (Sultana *et al.*, 2002). However, active sequestration of Na^{+} in plant tissues grown in extreme water drought conditions may be one of the responses determinately to the tissue (Fortmeir and Schuber, 1995). Potassium accumulation could be replaced by Na^{+} at the sites of uptake of alkali cations at the plasmalemmae of root cortical cells with increasing NaCl in the medium (Abbas *et al.*, 1991). The inhibition of Ca^{+2} transport and accumulation was reported previously under water-stress conditions (El-Komy *et al.*, 2004).

Results of this study are also in accordance with findings of several investigators (Drew, 1988; Gutlerez Boem *et al.*, 1996; Steeffens *et al.*, 2005 and Grichko and Glick, 2001) in respect to deficiency of mineral uptake under flooding-stress conditions. On waterlogged soil, plants show chlorosis and necrotic spots on older leaves. Both Mn^{+2} toxicity and N deficiency may be induced by the low redox potential in waterlogged soils that produces plant-available Mn^{+2} and promote denitrification of NO_3^-. Under these anaerobic conditions, N^{+}, P^{+3}, K^{+} and Ca^{+2} uptake was decreased by *Brassica napus* (Guterez Boem *et al.*, 1996). On the other hand, waterlogging changes the available ion concentration of the soil solution. Due to electron excess, Fe^{III} and Mn^{IV} are reduced to Fe^{II} and Mn^{II}, respectively. Rice roots can avoid uptake of the accumulated Fe^{II} and Mn^{II} ions by release of oxygen into the rhizosphere for Fe and Mn oxidation (Mengel and Kiekby, 2001). Plants such as wheat and barely are not able to oxidize Fe and Mn so that a toxicity of these minerals may occur under waterlogged conditions (Drew, 1988). Steeffens *et al.*, (2005) reported that under water logged conditions oxygen deficiency did not induce nutrient toxicity of Mn and Fe, but caused sub-optimum nutrient supply of N, P, K, Mn and Zn of wheat and barely plants.

Table (6): Effect of *Pseudomonas* sp. inoculation and (or) Ascorbic acid application on the accumulation of Na, K and P (mg/g) in the shoot system of wheat (*T. aestivum* L.) grown at different field capacity levels.

Field capacity	Treatments	Na		K		P	
		mg/g	%	mg/g	%	mg/g	%
1	C	3.4	100	2.4	100	3.9	100
	I	2.1	61.7	5.3	220.8	5.2	133.3
	A	3.1	91.2	6.3	262.5	3.3	84.6
	M	1.1	32.3	3.8	158.3	5.8	148.7
$1\frac{1}{2}$	C	3.2	94.1	1.4	58.3	3.4	87.2
	I	2.9	85.3	4.7	195.8	4.6	117.9
	A	2.0	58.8	2.8	116.6	2.8	71.8
	M	0.9	26.5	2.9	120.8	5.1	130.8
2	C	3.0	88.3	1.1	45.8	4.3	110.3
	I	2.7	79.4	3.2	133.3	6.1	156.4
	A	1.1	32.4	5.8	241.7	4.0	102.6
	M	2.1	61.8	2.7	112.5	5.1	130.8
L.S.D. 5%	-	0.2	-	0.4	-	0.3	-

Table (7): Effect of *Pseudomonas* sp. inoculation and (or) Ascorbic acid application on the accumulation of Na, K and P (mg/g) in the rootsystem of wheat (*T. aestivum* L.) grown at different field capacity levels.

Field capacity	Treatments	Na		K		P	
		mg/g	%	mg/g	%	mg/g	%
1	C	6.3	100	2.4	100	3.9	100
	I	4.2	66.7	1.6	66.7	5.2	133.3
	A	5.1	80.9	1.8	75.0	3.3	84.6
	M	5.3	84.1	1.4	58.3	5.7	146.2
$1\frac{1}{2}$	C	5.0	79.4	1.1	45.8	3.3	84.6
	I	6.1	96.9	1.3	54.2	4.1	105.1
	A	5.3	84.1	1.4	58.3	2.9	74.4
	M	2.7	42.9	1.1	45.8	5.1	130.8
2	C	4.9	77.8	1.2	50.0	4.3	110.3
	I	4.2	66.7	1.1	45.8	6.6	169.3
	A	5.5	87.3	1.7	70.8	3.9	100
	M	4.4	69.8	1.2	50.0	4.1	105.1
L.S.D. 5%	-	0.4	-	0.3	-	0.6	-

Results of this study indicate that the physiological status of *Pseudomonas* inoculated plants was changed including mineral ions accumulation. The increases of K^+ and P^{+3} accumulation was accompanied by reduction in Na^+ concentrations. The explanation of the increased nutrients uptake after *Pseudomonas* inoculation under water-stress conditions based mainly on the stimulation of root development, and root hairs proliferation (Bashan and Holguin, 1997; El-Komy *et al.*, 2004 and Rejli *et al.*, 2008). Some benefit rhizobacteria for example *Pseudomonas*, *Bacillus* and *Azespirillum* species can solubilize insoluble inorganic phosphate in vitro, and enhance phosphorus mobilization into plant tissue (El-Komy, 2005).

Recently, Baniaghil *et al.*, (2013) reported that maximum amount of Mn accumulation was related to plant growth promoting rhizobacteria (PGPR) inoculation under water-stress conditions. The authors showed that PGRR inoculation facilitate microelements uptake. Fe, Mn and Zn uptake may be related to ability to produce plants siderophore or microbial siderophores. Siderophores are organic compounds with low molecular weight and high affinity to complex with some cations such as Fe siderophore production in PGPR such as *pseudomonas*, *Azospirillum* and *Azotobacter* has been demonstrated (Arzanesh *et al.*, 2009).

Data shown in Table (8) indicate the results of hydrogen peroxide (H_2O_2) accumulation in wheat root-tissues at harvesting. In control untreated plants, H_2O_2 increased by increasing the level of water flooding-stress, and the highest H_2O_2 concentration was recorded at 2 field-capacity

At 1 and $1\frac{1}{2}$ field-capacity, H_2O_2 accumulation was not affected by *Pseudomonas* inoculation and (or) Ascorbic acid folior-application.

However, at 2 field-capacity the accumulation of H_2O_2 was decreased significantly by these treatments, and the lowest values of H_2O_2 was decreased recorded by AA and *pseudomonas* mixed treatment. These results are in accordance with several reports that under flooding-conditions (Hypoxia) H_2O_2 is recorded. Hydrogen peroxide accumulation under flooding-condition (Hypoxia) has been shown in the roots and leaves of *Hordeum vulgare* (Kalashinkov *et al.*, 1994) and in wheat roots (Biemelt *et al.*, 2000). The presence of H_2O_2 in the apoplast and in association with the plasma membrane has been visualized by transmission election microscopy under hypoxic conditions in four plant species (Blokhima *et al.*, 2001).

Table (8): Effect of *Pseudomonas* sp. inoculation and (or) Ascorbic acid application on H_2O_2 root system of wheat grown at different field capacity levels.

Field capacity	Treatments	Absorbance	% from absol. control	% from Corresponding control
1	C	0.32	100.0	100.0
	I	0.42	131.0	131.2
	A	0.39	121.9	121.9
	M	0.34	106.3	106.3
$1\frac{1}{2}$	C	0.59	184.4	100.0
	I	0.63	196.8	106.8
	A	0.57	178.1	96.6
	M	0.55	171.8	93.2
2	C	0.90	281.1	100.0
	I	0.43	134.4	47.8
	A	0.33	103.1	36.7
	M	0.30	93.7	33.3

H_2O_2, OH^- and other reactive oxygen species (ROS) are responsible for a state of oxidative stress in the whole plant tissues (Grichko and Glick, 2001). H_2O_2 is a strong oxidant and its higher concentration is injurious to cell, resulting an oxidative damage, lipid peroxidation and disruption of metabolic function and losses of cellular integrity at sites where it accumulates (Glick, 2005). In the present study and other studies, the decreased levels of H_2O_2 by Ascorbic acid (AA) and (or) *pseudomonas* application by the action of activation of antioxidant enzymes (Athar *et al.*, 2009). These higher levels of antioxidant enzymes e.g. POD and SOD might be attributed to their property to help develop the plant's resistance against oxidative damage. Ather *et al.*, (2009) reported an increase in antioxidant enzymes in wheat plants after ascorbic acid (AA) application. Thus, earlier work suggested that an increase in the activity of antioxidant enzymes (as indicated by the reduction in H_2O_2 level) helps the plants to maintain their growth under stress conditions and can be used as an indicator of stress conditions and can be used as an indicator of stress tolerance (Ejaz *et al.*, 2012).

Results presented in Table (9) show the accumulation of individual amino acids in wheat shoot and root system as estimated as percentage of relative amount. Individual amino acids increased in plants exposed to flooding stress (2 field-capacity). Data indicated that the amino-acid proline was reported in higher concentrations in ascorbic acid and mixed treatments both in the root and shoot system at 2 field-capacity level. The accumulation of amino-acids under water and salt-stress contribute mainly to osmotic adjustment (Abdel-Samed, 2005). Results of this study indicated that the numbers and concentrations of individual amino acids in wheat shoot and root system were higher in water-stressed plant (2 field capacity) than those grown at 1 field-capacity.

The results also indicated that proline was accumulated sharply by ascorbic acid or *Pseudomonas* inoculation. Proline accumulation was used as an index of plant resistance to stress-conditions in several studies (Hamdia and El-Komy, 1998). Shifting was also recorded in argnine and glutamic acid accumulation to proline by rhizobacteria inoculation or ascorbic acid-application was previously reported in several studies (Zahran,1999;Abdel-Samad,2005).

Table (9): Effect of *Pseudomonas* sp. inoculation and (or) Ascorbic acid application at different field capacity levels on the individual amino acids (% relative amount) in the shoot and root system of wheat grown at 1 and 2 field-capacity.

Field capacity	Treatments	Alanine	Lysine	Glycine	Glutamic acid	Threonine	Aspartic acid	Proline	Methionire	Histidine	Phenykine alanine	Agrinine	Isoleueine	Leucine	Cystine	Valine	Serine	Tyrosine
1 F.C	C	-	9.5	20.5	-	-	-	-	-	-	-	-	-	-	-	-	-	-
(Shoot)	I	-	2.4	6.7	-	6.2	2.1	-	8.3	-	82.5	-	-	-		-	-	-
	A	-	7.1	-	-	60.0	-	-	6.5	-	30.2	20.0	24.3	-	3.7	-	-	-
	M	-	20.6	-	19.4	-	-	-	-	-	-	-	-	-		-	-	-
2 F.C.	C	-	2.4	6.7	-	2.0	-	-	8.3	-	82.5	-	-	-		-	-	-
(Shoot)	I	-	0.5	3.0	75.9	2.7	-	-	-	-	-	2.5	1.4	-	12.3	-	-	-
	A	-	3.0	-	30.6	2.3	-	20.0	3.8	-	19.2	19.4	-	-	1.2	-	-	-
	M	-	5.5	-	40.5	9.5	-	30.1	-	-	-	24.0	-	-		-	-	-
1 F.C.	C	-	8.3	-	-	-	-	-	-	-	-	10.4	53.6	-	18.2	-	-	-
(Root)	I	7.2		-	10.6	6.7	-	12.1	-	-	14.6	-	30.1	-	16.1	1.8	-	-
	A	-	1.8	-	-	-	-	20.7	-	-	13.8	18.2	-	-	0.8		-	-
	M	-		-	64.4	27.9	-	3.7	-	-	-	-	6.8	-	0.6	11.4	-	-
2 F.C.	C	-	23.4	-	-	-	-	-	-	-	-	-		-	25.0	23.5	-	-
(Root)	I	-	0.6	-	-	-	0.8	-	-	-	-	-	35.4	-	3.4		59.8	-
	A	-		-	2.3	-	-	42.6	-	-	5.0	-	5.9	-	0.8	7.5	2.3	28.0
	M	5.8	1.6	-	-	-	-	90.1	-	-	-	-		-	-	2.4	-	-

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