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Effect of Mineral Acids (H_3PO_4) on Rooting Response of Fresh Mung Bean (*Phaseolus aureus* Roxb.) Cuttings Via Indole Acetic Acid Level

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Abstract

The influence of H_3PO_4 as a mineral acid on rooting response of fresh mung bean cuttings has been studied on the level of IAA . The date revealed highly gignificant increase in rooting response of fresh cuttings treated with 0.0001 , 0.001 , 0.01 % concentration of H_3PO_4 solution , while highly concentrations 0.1 , 0.5% revealed highly significant decrease in rooting response compared to control (d / H_2O) .Quantitative estimation of IAA by spectrophotometric method verified a highly significant increase of IAA content in hypocotyls of fresh cuttings in optimal concentration of H_3PO_4
Key words :Mineral acids , macronutrients , IAA biosynthesis , rooting response , stem cuttings , mung bean

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1- Introduction

Plant root systems are highly plastic in their development and can adapt their architecture in response to the prevailing environmental conditions[1].This plasticity is possible because root systems develop by the continual propagation of new meristems [2].Plants root systems perform many essential adaptive functions including water and nutrient uptake, anchorage to the soil and the establishment of biotic interactions at the rhizosphere[3].Three major processes affect the overall architecture of the root system (RSA) , First, cell division at the primary root meristem (i.e. of initial cells) . Second lateral root formation and third, root – hair formation [3].

Soil nutrients are critical elements for plant growth and productivity. The bioavailability of nutrients in the soil solution may determine root growth,root proliferation and specific functional responses that depend on the prevailing nutrient status of the plant . Nitrogen (N), phosphorus (P), and sulfur (S) are among the nutrients that have been reported to alter post- embryonic root developmental process [3]. phosphate is often the limiting nutrient for plant growth because of its low mobility in soil , Therefore , it is not surprising that phosphate can have a profound effect on root system architecture (RSA). In barley (*Hordeum Vulgare*) Phosphate – rich patches have been shown to promote lateral root development in phosphate-starved plants [4]. Bean (

Phaseolus Vulgaris) plants grow on low phosphate change the angle of growth of basal roots in favor of outward rather than downword growth [5].

Adventitious root formation is a developmental process involving sequences of histological events with each stage having different requirements for growth substances (auxins , cytokinins , gibberellic acid , etc) . Indole -3- acetic acid (IAA) was identified as a naturally occurring compound having considerable auxin activity. Synthetic indol acetic acid (IAA) was subsequently tested for its activity in promoting roots on stem segments, and in 1935 investigators demonstrated the particl use of this material in stimulating root formation on cuttings [6].

Adventitious root formation occur on two basic phases , the first-Initiation phase and the second- Growth and Development phase [7]. In many bean cuttings Jarvis[8] divided the initiation phase into three Secondary phases :

1- Induction Phase which characterize with accumulation of auxin in the region of initiation and companied with decrease of IAA- oxidase activity.

2-Early Initiation Phase – Accumulation of auxin causes cell division, consequently this leads to formation of root premordia.

3- Late Initiation Phase – diminishing auxin level because of IAA- oxidase action and hence , lead to promoting of root premordia and finally development. In second phase(Growth and Development Phase), root premordia converted to visible roots as result to boron action in increase of IAA oxidase causing diminish in IAA level as result to formation complexes between boron and phenolic compounds.

The main objective of this study was to evaluate the influence of H_3PO_4 as a weak mineral acid on rooting response of fresh mung bean cutting on the level of IAA.

2. materials and methods

2.1 Cultivation of Stock Plants

Seeds of mung bean (*Phaseolus aureus* L.) were soaked overnight , sown in moistened (with distilled H_2O or tested solutions) sterilized sawdust in plastic trays. Seedlings were raised in growth chamber provided with a continuous light (light intensity 3,00- 3,500 Lux),temperature $25C^0 \pm 1^0C$ and relative humidity 60% - 70 % for ten day

2.2 Peraration of Cuttings

Cuttings were prepared according to Hess [9] from 10 days old light grown seedlings .These cuttings described by having small terminal bud,pair of fully expanded primary leaves, a whole epicotyls and hypocotyls (3 cm length) under cotyledonary nodes , after removal of root system .

2.3 Basal Treatment of Cuttings

Dipping of the whole hypocotyls (3 cm depth in glass vials required 15 ml of tested solutions . Fresh cuttings were treated for 24 h with d/ H_2O or tested solutions (12 cuttings / treatment), then transferred to boric acid (10 µg /ml) for 6 days, before counting the root numbers.The area of 1st true tri-foliated leaf in cuttings measured according to stickler *et al* [10]. completely randomized design(CRD)was conducted in all experiments for statistical analysis according to Spiegel [11].

2.4 Preparation of solution

Boric acid solution : prepared at (10µg/ml) and employed as rooting medium[12].
Synthetic auxin solution : Indol-3- Acetic Acid (IAA) was initially dissolved in small amount of absolute alcohol to prepare 5x 10^{-4} M (0.00876%) [13].
Phosphoric acid solution:H_3PO_4 (85%)was prepared as (%) percent solution (v/v), by dissolving 1ml of H_3PO_4 in 99 ml of d/H_2O to achieve 1% as stock solution ,then diluted to the required concentration.

2.5 Quantitative determination of I AA

Naturally accurring auxin(IAA)was measured spectrophotometrically in hypocotyts of fresh cuttings , according to stoessl and venis [14] and Plieninger et al [15]. The above procedure (were modified) included the reaction of IAA with acetic anhydride to form 2- methyl-indole-α pyrone. Synthetic IAA was used for standard curve.

3.Results

3.1 Physiological Part

3.1.1 Effect of H_3PO_4 in rooting response of fresh cutting Table 1 shows the effects of H_3PO_4 in rooting response of fresh cutting , when supplied to cuttings immediately. the results revealed that the means of roots number , roots length (mm), leaf area (cm^2) as the mean of one cutting developed in fresh , untreated cuttings (general control d/H_2O)are(9.5 root, 3.941 mm, 0 cm^2) respectively .These means in cuttings treated with auxin (special control IAA) are(36.374 root, 1.587 mm and 0.054 cm^2) respectively , while these in cuttings treated with H_3PO_4 are (30.25 ,18.375 , 20.875, 0 and 0) root,(5.429 , 3.055 , 2.241, 0 and 0) mm and (0 , 0 , 0 , 0 and 0) cm^2 at pH (5.5 , 4.02 , 3.06 , 2.38 , 1.9) respectively. Statistically , cuttings treated with (0.0001, 0.01 %) of H_3PO_4 at pH (5.5 and 3.06) respectively were positively highly significant ($P \geq 0.05$). At the same time , cuttings treated with (IAA) PH (4.38)was positively significant at the same probability level compared to control treatment (d/ H_2O) , while cuttings treated with 0.001 % of H_3PO_4 at pH (4.02) was positively significant ($P \leq 0.05$). Generally, high concentration of H_3PO_4 (0.1 and 0.5%) at pH(2.38 , 1.9) respectively have no significant effect on rooting response in all treatments , on the other hand , low concentrations of H_3PO_4 0.0001 , 0.001 and 0.01 at pH 5.5 , 4.02 , 3.06 respectively have no significant effect in mean root length , while high concentrations (0.1 and 0.5 %) have negative significant difference on ($P \geq 0.05$) compared to control (d/H_2O) . At the same time, cuttings treated with (IAA) have negative significant difference on ($P \leq 0.05$) level as compared to control (d/H_2O). Statistically , cutting treated with H_3PO_4 have no significant effect in mean leaf area in all treatments except cuttings treated with (IAA) which have positive significant difference on ($P \leq 0.05$) level as compared to control treatment (d/ H_2O).

3.2 Biochemical part

3.2.1 Quantization Determination of I AA

3.2.1.1 Effect of H_3PO_4 on IAA level in fresh cuttings

Fig.2 shows IAA level in hypocotyls of fresh mung bean cuttings treated in optimal concentration of H_3PO_4 and IAA . IAA level in 1 g hypocotyls of fresh cuttings (general control d/ H_2O) is 11.316 m molar, whereas , IAA level in 1 g hypocotyls of cuttings treated with synthetic IAA (special control treatment) is 15.095 m molar. IAA level 1 g hypocotyls of cuttings treated with H_3PO_4 solution (0.0001 % pH = 5.5) is 14.212 m molar .Treatment with IAA and H_3PO_4 revealed positive highly significant($P \geq 0.05$)as compared to control treatment(d/H2O).On the other hand, treatments revealed significant decrease ($P \leq 0.05$) in content of IAA in cuttings treated with H_3PO_4 compared to control treatment IAA.

4.Discussion

The ability of plants to respond appropriately to nutrient availability is of fundamental importance for their adaptation to the environment . Nutrients such as nitrate , phosphate , sulfate and iron act as signals that can be perceived. These signals trigger molecular mechanisms that modify cell division and cell differentiation processes within the root and have a profound impact on root system architecture [16].However the significant rooting response of fresh mung bean cuttings Table 1. may be attributed to the following factors.

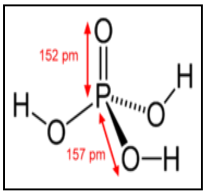
1) The effect of phosphoric acid

H_3PO_4 is capable for trapping free radicals , because of presence of high electronic conjugation in this compound. Although H_3PO_4 is anon-toxic , inorganic and weak acid, it is a very polar molecule; therefore it is highly soluble in water .the oxidation state of phosphorus (p) in phosphoric acid is +5; oxygen atoms (O) is -2 and all the hydrogen atoms(H)is+1.Triprotic acid means that an phosphoric acid molecule can dissociate up to three times , giving up H^+ each time , which typically combines with a water molecule forming positively charged cation , Hydronium (H_3O^+) and their salts , as shown in these reaction :

$$H_3PO_{4\,(s)} + H_2O_{(1)} \rightleftharpoons H_3O^+_{(aq)} + H_2PO_{4\,(aq)} \quad Ka_1 = 7.25X10^{-3}$$

$$H_2PO_4^-{}_{(aq)} + H_2O_{(1)} \rightleftharpoons H_3O^+_{(aq)} + HPO_4^{-2}{}_{(aq)} \quad Ka_2 = 6.31x10^{-8}$$

$$HPO_4^{-2}{}_{(aq)} + H_2O_{(1)} \rightleftharpoons H_3O^+_{(aq)} + PO_4^{-3}{}_{(aq)} \quad Ka_3 = 4.80x10^{-13}$$



Because of the triprotic dissociation of phosphoric acid , the fact that its conjugate bases cover a wide pH range, and,because phosphoric acid/phosphate solutions are, in general , non toxic, mixtures of these types of phosphates are often used as buffering agents or to make buffer solutions , where the desired pH depends on the proportions of phosphates in the mixtures .The activity of H_3PO_4 attributed to the triprotic dissociation of acid when capable for absorption from the plant , absorbent phosphate ions by the plant continuously constituted by phosphoric acid dissociation according to the base of Lechatleh. Oxidation-reduction reactions gave a strong charactur for H_3PO_4 as a strong oxidizer agent which pull H^+ through bonds that formed with oxygen. Phosphates are found pervasively in biology, especially in the compounds derived from phosphorylated sugars such as DAN , RNA and ATP .This confirmed by in organic chemistry studies [17,18,19].

2) The effect of phosphorus element

The significant rooting response may be attributed to the capability of phosphorus element as one of the fifth group and third cycle elements in periodic table.Phosphorus atoms are characterized by presence of external envelope which contain individual electrons act as internal suppressors of free radicals through formation covalent bonds and lowering the effect of oxidative products[19].

Studies mentioned that P in the plant is found largely as phosphate esters – including the sugar – phosphates , which play such an important role in photosynthesisand termediary metabolism. Other important phosphate esters are the nucleotides that make up DNA and RNA as well as the phospholipids present in membranes. Phosphorus in the form of ATP, ADP and Pi , phosphorylated sugars, and phosphorylated organic acids also plays an integral role in the energy metabolism of cells [20].

An excess of phosphorus stimulates growth of roots over shoots thus reducing the shoot/root ratio ; Fertilizers with high phosphorus content , such as bone meal , are often applied when transplanting perennial plants in order to encourage establishment of a strong root system [20] thereafter , explain the decline in means of leaf area in cuttings treated with H_3PO_4 comporred to IAA treatment which revealed significant increase ($P \leq 0.05$) compared to control treatment (d/ H_2O).

3) The effect of pH

The significant rooting response of mung bean cuttings maybe attributed to the acidic pH.The data revealed that adventitious root formation in mung bean cuttings positively affected by acidic pH.This was confirmed by prior studies by using the same kind of cuttings [21,22,23].Plant can grow in soils in a pH range of 3 to 9. Some plant grow in more acidic soil while some grow in more alkaline soil [24].pH has two major effects-competition and injury. A low pH is believed to reduce cation uptake by competition between hydrogen ions and the substrate cations for sites on acarrier. At high pH values hydroxyl or bicarbonate ions might compete with substrate anions , thus reducing anion uptake Acidity or alkalinity therefore has a profound influence on the relative absorption of anions and cations [25].

An alternative interpretation of pH effects is that the external level of hydrogen ions (protons) influence the magnitude of proton extrusion across the plasma membrane .The Movement of protons out provides agradient for the movement of cations in. Similarly the uptake of anions will be driven by an efflux of hydroxyl ions. The presence of metabolically-important anions, such as phosphate often stimulates the uptake of other ions ,presumably through an effect on metabolism. Ultimately absorption is dependent on growth and this depends on the availability of all essential elements [25].

4)The effect of hormonal balance

Plant growth regulators control most of the characteristics of root systems, including primary root growth and the formation of lateral roots and root hairs. Many plant species respond to the exogenous application of auxins by producing large numbers of lateral roots , and to auxins and ethylene by increasing the density and length of root hairs [26,27].These findings suggest that root responses to nutrients may originate from hormonal signals that are triggered by specific nutrient pathways.

In white lupin and *Arabidopsis*,an important role for auxins transport in the formation of lateral roots has been recently demonstrated .These results show that auxins are required for the formation of lateral roots under P-limiting conditions. Suggesting that changes in auxin concentration, transport and sensitivity may play an important role in the effect of P on root system architecture[28].

Cytokinins suppress lateral root initiation in low–p- grown plants[28]. Martin etal [29]showed that the increase in the ratio of shoot/root growth that occurs in response to low p is paralleled by a decrease in cytokinin concentration. Exogenous cytokinins repressed the expression of low- P- regulated gens suggesting that these hormones not only control root architecture but also control other aspects of the low-prescue response. thereafter, explain the significant increase of IAA content in hypocotyls of fresh mung bean cuttings fig.2 which reflected the significant increase of rooting response. This was confirmed by prior studies by using the same kind of cuttings[21,22,23].

5) Ionic balance

The concentration of free ions in the soil solution is generally low,with the major portion of the cations absorbed on to the negatively – charged site of clay micelles and organic materials in the soil. Anions (phosphate,nitrate,and sulphate) are absorbed by plant almost entirely from the soil solution, where as cations may, under some conditions, be exchanged directly between roots and soil particles for hydrogen ions by carbonic acid exchange. However, as with anions, the movement of cations by both diffusion and mass flow in the soil solution can be the limiting step for uptake from the soil . Removal of ions from the soil by plant roots will liberate more ions from exchange complexes , maintaining a dynamic equilibrium between the roots,the soil solution and the exchange complex , water uptake by plant roots results in amass flow of soil solution towards the root surface , which will enhance the supply of nutrient ions to the root .The microflora in the rhizosphere surrounding the root surface , also influences the uptake of ions by the plantwhich increase the capability of the plant to accumulate certain nutrients (particularly phosphate) from the soil [25]. In general active uptake of ions by plant cells is sensitive to a wide variety of factors . Of a greatest significance are those which affect the energy supply , those which affect growth , and those which concern the composition and concentration of the external medium.The energy supply may be affected by factors such as O_2 , CO_2,temperature , light and metabolic inhibitors.As active transport is dependent on metabolism it is not surprising that ion uptake is sensitive to all metabolic inhibitors particularly respiratory inhibitors , such as CO which inhibit electron transport . In addition to its influence on the availability of ions the surface area of cell which ions are transported , the internal ionic, concentration of a cell , pH values , and external concentration[25].The presence of metabolically – important anions such as phosphate and nitrate , often stimulates the uptake of the ions , presumably through an effect on metabolism ultimately absorption is dependent on growth and this depends on the availability of all essential elements[25].

RSA is greatly influenced by the soil environment and especially the availability and distribution of nutrients [30].The ability of root system to respond to phosphate availability was found to be independent of sucrose supply and auxin signaling . In contrast, shoot phosphate status was found to influence the root system architecture response to phosphate availability[31] .Furthermore, the overal architecture of the root system can be affected by the nutrient status of the plant , such that in nutrient- limiting conditions the RSA may be very different from the RSA in nutrient-rich environments [32].

Substantial genetic variation for p efficiency (i.e. ability to grow and yield on low P soil) exists within bean germplasm [33].And appears to be associated with contrasting root architecture [34], rather than contrasting rhizosphere modification or microbial symbiosis [35]. Plant hormones, mainly auxins , cytokinins and ethylene , appear to be key factors in these nutrient mediated pathways .The nutrient pathways may affect hormone biosyntheses transport and/ or sensitivity [28]. A few genes that control essential steps in the nitrate -and phosphate-signaling pathways have been identified.These include ANR1 and PHR1 from an *Arabidopsis* and the HAR1 gene of Lotus *japonicus* [36].

5. Conclusion

Generally and as a conclusion , significant rooting response of fresh mung bean cuttings treated with different concentrations of H_3PO_4 may be attributed to many factors .H_3PO_4 is capable for trapping free radicals ,because of presence of high electronic conjugation in this compound.AlthoughH_3PO_4 is non- toxic, inorganic and weak acid it is a very polar molecule and triprotic acid which means that an phosphoric acid molecule can dissociate up to three times , giving up an H^+ each time , which combines with a water molecule forming (H_3O^+) and their salts (phosphate anions). So, phosphorus element as one of the fifth group and the second cycle elements in periodic table, it's atoms are characterized by presence of external envelope which contain individual electrons act as internal suppressors of free radicals through covalent bonds formation and lowering the effects of oxidative products that occurs during treatment. However, as well as the above explantion (H_3PO_4) confirm the rooting response of fresh mung bean cutting through the following factors : a) Hormonal factors and IAA content (hormonal balance). b) Ph . c) ionic balance.

d) Osmosic balance . e) Enternal and external of environmental effects. f) shoot phosphate status. g) Nutrients availability. h) Stock plants nutrition . i) Genetic effects.Not with stanting , the forgoing suitable factors may lead to decline the oxidative processes , causing increase of rooting response in fresh mung bean cuttings.

Table 1: Influence of H_3PO_4 on rooting response of fresh mung bean cuttings

Solution	Concentration%	Mean root No./cutting	Mean root length/ cutting (mm)	Mean of 1st true trifoliated leaf area (cm^2)	pH
d/H_2O	0	9.5	3.941	0	6.65
Indole acetic acid (IAA)	0.00876 ($5x10^{-4}$ M)	36.375**	*1.587	0.054*	4.38
H_3PO_4	0.0001	30.25**	5.429	0	5.5
	0.001	18.375*	3.055	0	4.02
	0.01	20.875**	2.241	0	3.06
	0.1	**0	**0	0	2.38
	0.5	**0	**0	0	1.9

Stem cuttings were taken from seedlings grown in d /H_2O for 10 days .Then treated for 24h µg/ml) for 6 days. . in above concentration of (H_3PO_4) .Thereafter, transferred to boric acid (10
Mean of root number LSD (0.01) = 8.999 LSD(0.05) = 6.255 , mean of root length LSD (0.01)= 3.192 LSD (0.05) = 2.218 , mean of leaf area LSD (0.01) = 0.060 LSD (0.05) = 0.041 .

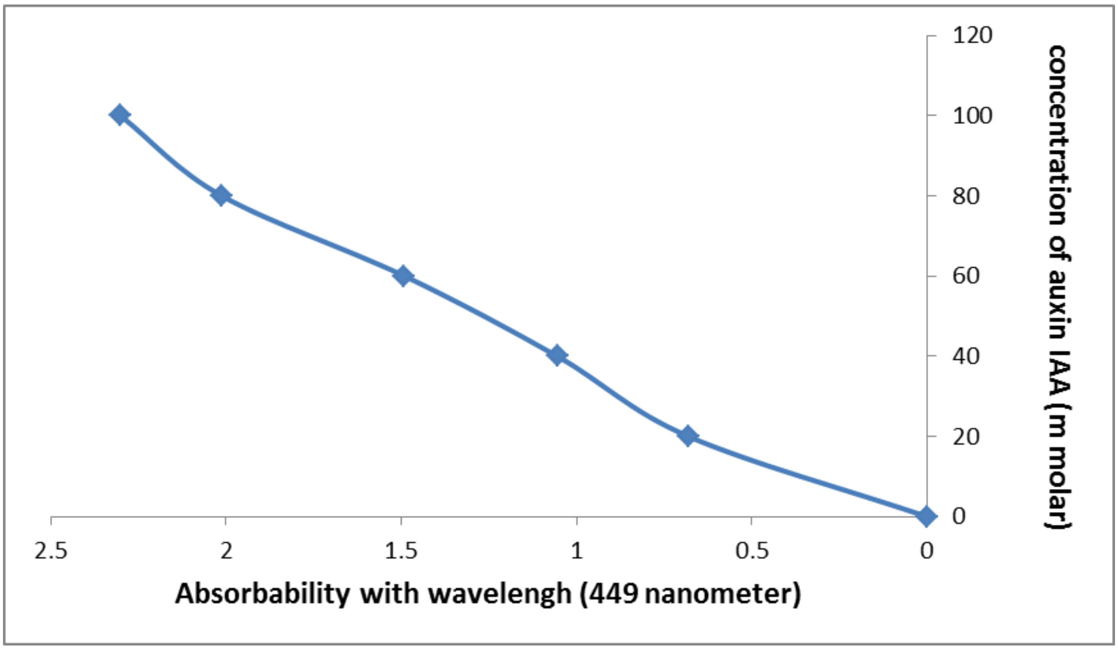


Fig.1 : Standard carve of different concentrations of auxin (IAA) and Absorbability with wave length (449nanometer)

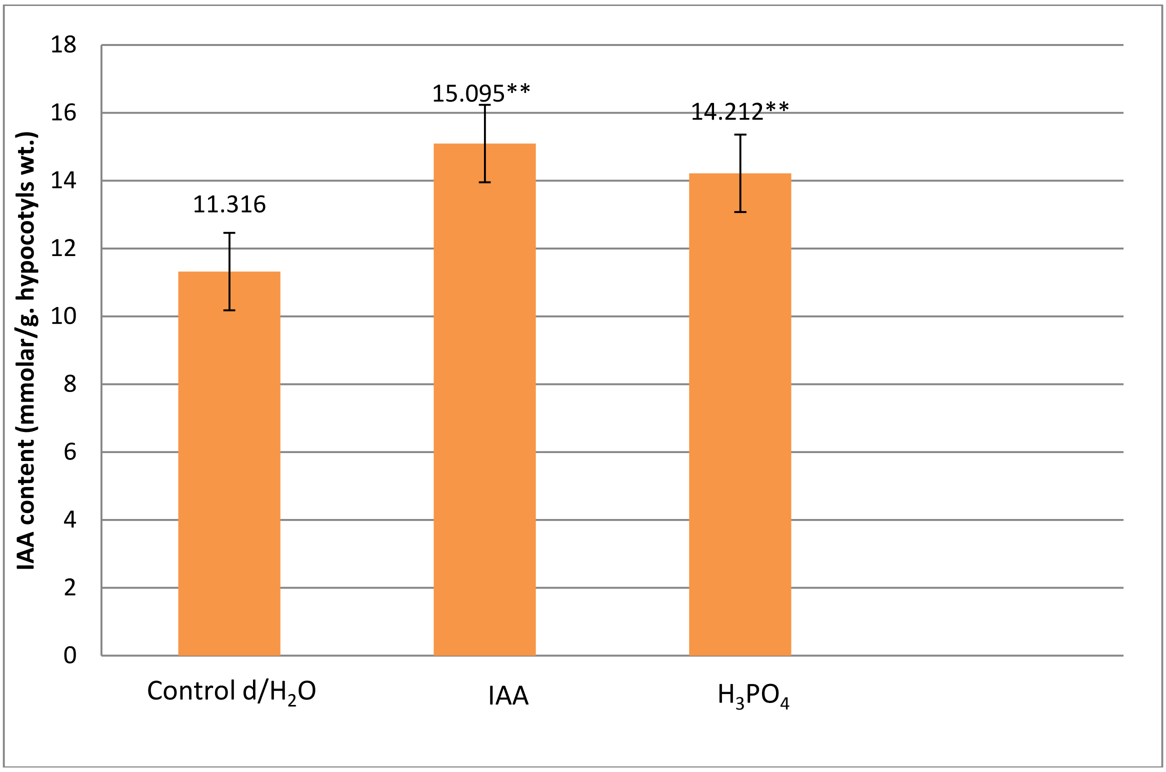


Fig. 2 IAA content (mmolar/g hypocotyls wt.) of fresh mung bean cuttings treated with 0.0001% concentration (pH 5.5) of H_3PO_4 solution and $5x10^{-4}$ M concentration (pH 4.38) of IAA solution. LSD (0.05) = 0.844, LSD (0.01) = 1.630

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تأثير الحامض المعدني H_3PO_4 في استجابة تجذير عقل الماش الطرية في مستوى اندول حامض الخليك (*Phaseolus aureus* Roxb.)

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الخلاصة :

تمت دراسة تأثير حامض الفسفوريك (H_3PO_4) بصفته حامض معدني في استجابة تجذير عقل الماش الطرية ، في مستوى اندول حامض الخليك (IAA). اظهرت النتائج زيادة معنوية في استجابة تجذير عقل الماش الطرية المعاملة بتركيز 0.0001, 0.001, 0.01% من محلول H_3PO_4 . بينما كشفت التراكيز العالية 0.1 ، 0.5 % انخفاض معنوي في استجابة التجذير مقارنة بعينة السيطرة (d /H_2O) . ان التقدير الكمي للاوكسين (IAA) بالطريقة الطيفية اكدت زيادة معنوية لمحتوى(IAA)في السويقات الجنينية السفلى (hypocotyls) للعقل الطرية بالتراكيز المثلى من H_3PO_4 .

الكلمات المفتاحية : الحوامض المعدنية ، المغذيات الكبرى ، تخليق الاوكسين الحيوي ، استجابة التجذير ، العقل الساقية ، الماش

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