Delta J. Sci. (12) (1) 1988, 261 - 275

# FORMATION OF AUXINS, GIBBERELLINS AND VITAMINS BY RHIZOSPHERE FUNGI AND THEIR EFFECT ON THE GROWTH OF BARLEY

Mostafa A. El-Sayed*, Abd El-Raheem R. El-Shanshoury*, and Hanaa S. Fahmy**

* Botany Department, Faculty of Science, Tanta University

**Botany Department, Faculty of Science, Zagazig University

Received: 26-1-1988

## ABSTRACT

Ninteen strains of locally isolated fungi were examined for their potentiality to produce vitamins and plant growth regulators in culture media. Vitamins c, folic acid and indole-3-acetic acid were produced by most of the isolates, whereas gibberellic acid was produced by a few organisms; Curvularia spicifera and Penicillium rugulosum proved to be the most active producers of the aforementioned biologically active substances and were selected for further studies. Pretreatment of barley grains with these fungi accelerated the plant growth probably due to the formation of vitamins and plant growth regulators. However, presoaking of barley grains or trickling the seedlings with P. rugulosum in the presence of superphosphate was more effective in enhancing the plant growth. Analysis of plant growth indicated that, the depth of the root system of barley was shorter, in spite of its high fresh weight and succulence in both presoaked grains and seedlings trickled by P. rugulosum, compared to the other experimental treatments. A marked increase in shoot length, fresh weight, dry

Delta J. Sci. (12) (1) 1988
Formation of auxins, gibberellins and vitamins by .....

weight and succulence were also reported in presoaked grains of the experimental plant in the spore suspension of P. rugluosum than that of the inoculated seedlings.

## INTRODUCTION

In spite of the fact that plants themselves synthesize the biologically active substances that they require, many of them respond positively to a supplementary application of these substances to the soil.

The production of vitamins by microorganisms has been previously demonstrated. Ascorbic acid was detected in the culture supernatant of fungi [1]. Cyanocobalamine ($B_{12}$) was produced by Streptomyces gresoaurantiacus [5].

Plant growth regulators of the auxin and gibberellin type are produced in culture supernatants of Azotobacter chroococcum[6,7,9], streptomycetes [8], soil fungi [20] and phytopathogenic fungi [23].

The influence of these biologically active substances produced by the rhizosphere organisms on cultivated plants were also studied. Ansell and Young [2] have elucidated the relationship between rhizosphere fungi and plants in the field. The effect of inoculation of soil cultivated with Zea mays with Azotobacter chroococcum has been descri-

bed [9]. The influence of fungal flora of rhizosphere on germination and growth of rice, alfalfa, barley, oats and wheat were recorded [16].

In the current work, the role of the experimental organisms in the growth of barley was undertaken.

## MATERIALS AND METHODS

The strains used in this study (Aspergillus flavus 1 and 2; A. niger 1,2,3 and 4; A. terreus 1 and 2; Penicillium rugulosum 1,2,3,4 and 5; P. chrysogenum 1 and 2, P. janthinellum 1 and 2; P. citrinum and Curvularia specifera) were isolated from the rhizosphere of different desert plants collected from El-Tall El-Kabeer and Ismaelia, Egypt. Cultures were maintained on Czapek agar slants. The common fungi were grown in 250 ml Erlenmeyer flasks containing 50 ml Czapek's broth. The fermentation medium was inoculated with standard inoculum. The cultures were incubated at 28°C for 7 days in surface cultures. The mycelial mat was removed and kept for further work. The liquid was filtered out under aseptic conditions using whatman No.1 filter paper and retained for analysis.

The nitrogent content of the fungal mats was determined by the micro-Kjeldahl method as described by Markham [17] and Jacobs [12].

Water soluble vitamins were detected by thin layer chromatography. The solvent system was bidistilled water [10]. Glacial acetic acid : acetone : methanol : benzene (1:1:4:14) was used for thiamin HCL and cyanocoblamine [14]. The developed chromatogram was either sprayed with conc $H_2SO_4$ or subjected to UV light [11].

Plant growth regulators were detected by paper chromatography of the n-butanol extraction [19]. The solvent system was freshly mixed isopropanol: ammonia: water (10:1:1V/V/V) [3]. The developed chromatogram was either sprayed by 3% $H_2SO_4$ in methanol and 0.05% ferric chloride or subjected to UV light [19]. Indole-3- acetic acid aquired violet-red coloration whereas gibberellic acid showed blue green fluoresence under UV light.

The effect of the isolates on the growth of barley (Hordeum vulgare CV. Giza 117) was carried out in two different ways. One series of barley grains were soaked in spore suspension of C. specifera, P. rugulosum 4, C.specifera and P. rugulosum 4 for 30 minutes before planting. The grains were planted in pots containing 4kg steam sterilized clay-loamy alluvial soil. Control seeds were soaked for 30 minutes in sterile distilled water. Another series of grains were germinated in pots containing 4kg steam sterilized clay loamy alluvial soil. Seven days old seedlings were inoculated with

10 ml spore suspension of C. spicifera, P. rugulosum 4 or c. spicifera and P. rugulosum 4. The inoculum contained $10^4$ - $10^7$ spores/ml. Control was made by trickling 10 ml distilled water around the roots. Manures were added after 7 days of planting for both series. Plants were grown under green house conditions. Each pot was watered to field capacity every second day. Barley plants were grown for 30 days and growth parameters were then recorded

## RESULTS AND DISCUSSION

Ninteen isolates of 8 funga species were examined for a comparative study of the production of growth, total nitrogen, vitamins and plant growth regulators in culture media after seven days of incubation. From Table 1 it is obvious that, P. rugulosum 3 exhibited the highest dry weight whereas P. rugulosum 4 gained the least dry weight. The results also indicated that all the fungal mats gained nitrogen but in varying levels. Aspergillus flavus 1 showed the highest total nitrogen content.

Production of vitamins by microorganisms has been noted previously [1,5]. In Czapek's liquid medium (Table 1), all the tested strains produced vitamin c in various levels, the largest amounts were produced by A. flavus 2, P. rugulosum 4 and C spicifera. Folic acid was also produced by most of the isolates under investigation. Among the fungi studied,

the highest vitamin content was recorded for C. spicifera. Minute amounts of vitamins $B_1$ , $B_{12}$ nicotinamide and riboflavins were detected only in the culture filtrate of few isolates of the fungi studied, this may be due to the unsuitability of the culture medium.

The data for auxins and gibberellic acid production (Table 1) suggested that indole-3-acetic acid (IAA) was produced by most of the fungi studied, whereas, gibberellic acid ($GA_3$) was detected on the culture filtrate of only few isolates. These results are in accordance with those obtained from previous studies on rhizospere actinomycetes, bacteria and fungi (4,13,22). The versatility of auxins and $GA_3$ production may be attributed to strain variation. Such versatility in the different isolates was recorded by many investigators (8,18,19,21). The results indicated that C. spicifera and P. rugulosum 4 were the most active producers for the biologically active substances studied, and subsequently were selected for the foregoing work.

Presoaking of barley grains in spore suspension of C. spicifera or and P. rugulosum 4 and trickling the spores of these fungi around the seedlings exerted a variable but mild action on the plant growth. Camparing to the control plant, Tables 2 and 3 illustrates the effect of each fungal spore suspension in the presence of different manures on

the seedlings of barley. From the tables, it is obvious that the growth parameters of the plant seedlings were moderatly affected. However, this effect was not going in one direction, as it was stimulatory in some treatments and inhibitory in the others. This could be attributed to the nature of the manure, which might be inhibitory or promoting for the fungal spores. In other words, the manure could act as a suitable or unsuitable medium for the induction of growth of some fungal spores and hence affecting the production of the corresponding vitamins, auxius and gibbrellic acid. From Tables 2 and 3, it is clear that the most inhancing medium for the shoot parameters was the mixture of the spere suspension of P. rugulosum 4 and superphosphate, this was rather convenient wheather the spore suspension had been used for presoaking the grains or for inoculating the seedlings.

The root depth of pretreated plant with P. rugulosum 4 and superphosphate was shorter than in plants cultivated under the other experimental treatments. On the other hand, the fresh weight of the root system and succulence were higher than the other experimental treatments (Tables 2 and 3). This may be attributed to the secretion of a high quantity of IAA which stimulated water uptake by root and thus increasing the fresh weight and succulence of shoot. This finding is supported by the results obtained by El-Shourbagy et al. [9].

Heftmann, E. Reinhold publishing corporation, New York (1967)P. 708.

15- Kirilenko, T.S. 1973. The effect of fungi isolated from barley and oat rhizosphere on seedling growth, Mikal fitopathol. 7 (1): 3-7.

16- Kirilenoko, T.S. 1978. Fungal flora of soils in the Ukrainian SSR VSSR. Microbiol. 2H (Kiev). 40 (2): 214 - 223

17- Markham, R. 1942. Biochem. J. 36: 790.

18- Nita, A.R. 1964. The action of microorganism metabolites on higher plants. An, Inst. Ceret. Pentrusi-plante Tek. Fundulea ser. B, 32: 443 - 455.

19- Podojil, M. and Sevcik, V. 1960. Quantitative estimation of gibberellic acid by paper chromatography. folia Microbiol. 5, 192.

20- Selim, M.S.N., Abdulla, M. El-S and Radi, S.H. 1985. Production of gibberellins and indoles by some soil fungi. Proc. Egypt. Bot. Soc. 4: 316 - 330.

21- Smalii, V.T. and Bershova, O.T. 1957. Heteroauxin formation in Azotobacter culture. Microbiologiya 26: 526 - 532.

22- Strzelczyk, E. and Pokojska-Burdziej, A. 1984. Production of auxins and gibberellin like substances by mycorrhizal fungi, bacteria and actinomycetes isolated from soil and the mycorrhizo-

sphere of pine (Pinus silvestris L.). Plant and Soil. 81 (2); 185 - 194.

23- Tamura, S. 1979. Plant growth regulators produced by microorganisms. In: Advances in Pesticide Science PP., 356 - 365. Edited by: H. Geissbuhler, C.T. Brooks and P.C. Kearner. Pergamon Press. Oxford, New York, Toronto, Sydney, Paris, Frankfurt.

Table 1 : Growth, total nitrogen, vitamins, auxins and gibberellic acid produced by fungi isolated from rhizosphere of some desert plants.

| Fungi | Dr.wt. mg/100ml | T.N. mg/g dry weight | vitamins | | | | | | plant growth regulators | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | C | Folic acid | $B_1$ | $B_{12}$ | Nicotinamide | Riboflavin | IAA | $GA_3$ |
| Aspergillus flavus 1 | 1020 | 44.52 | + | + | ND | ND | ND | ND | ++ | ND |
| A. flavus 2 | 420 | 29.90 | ++ | + | ND | ND | ND | ND | ND | ++ |
| A. niger 1 | 1200 | 23.90 | + | ND | ND | ND | + | ND | ND | + |
| A. niger 2 | 260 | 19.3 | + | + | ND | ND | + | ND | + | ND |
| A. niger 3 | 1220 | 21.4 | + | + | ND | ND | ND | ND | ++ | ND |
| A. niger 4 | 1080 | 26.5 | + | ND | ND | ND | ND | ND | + | ND |
| A. terreus 1 | 1060 | 36.8 | + | + | ND | ND | + | ND | ND | ND |
| A. terreus 2 | 1320 | 39.7 | + | + | ND | ND | + | ND | ND | + |
| Penicillium rugulosum 1 | 1300 | 25.8 | + | + | ND | ND | ND | ND | ND | ND |
| P. rugulosum 2 | 1140 | 20.4 | + | + | ND | ND | ND | ND | + | ND |
| P. rugulosum 3 | 1520 | 33.0 | + | + | ND | ND | ND | ND | ND | + |
| P. rugulosum 4 | 140 | 20.6 | ++ | ND | ND | ND | ND | ND | ++ | ++ |
| P. rugulosum 5 | 1160 | 24.4 | + | + | ND | ND | ND | ND | ND | ND |
| P. chrysogenum 1 | 1200 | 31.8 | + | + | ND | ND | ND | ND | ND | ++ |
| P. chrysogenum 2 | 340 | 39.8 | + | ND | ND | ND | ND | ND | + | ND |
| P. Janthinellum 1 | 1280 | 29.1 | + | + | ND | ND | + | ND | + | ND |
| P. Janthinellum 2 | 1320 | 31.9 | + | + | ND | ND | + | ND | + | ND |
| P. citrinum | 540 | 42.4 | - | ND | + | ND | ND | ND | ++ | ND |
| Curvularia spicifera | 1240 | 34.2 | ++ | ++ | ND | ND | ++ | ++ | ++ | ND |

ND = not detected under the experimental conditions.

+ = moderately positive; ++ = strongly positive.

T.N = total nitrogen

Table 2 : Effect of presoaking barley grains in the fungal spore suspensions in the presence of manures on growth of barley

| Treatment / Manure | Growth parameters ( after 30 days ) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Root system | | | | shoot system | | | |
| | Depth | F.wt. | D.wt. | succulence | length | F.wt. | D.wt. | succulence |
| Urea / Control | 3.4 | 0.05 | 0.03 | 0.02 | 13.5 | 1.5 | 0.40 | 1.0 |
| C. spicifera | 3.7 | 0.11 | 0.04 | 0.07 | 23.0 | 0.97 | 0.31 | 0.66 |
| P. rugulosum | 5.5 | 0.05 | 0.02 | 0.03 | 18.8 | 0.83 | 0.28 | 0.55 |
| C. spicifera + P. rugulosum | 3.5 | 0.09 | 0.06 | 0.03 | 15.0 | 0.51 | 0.17 | 0.34 |
| Supernitrate / Control | 3.6 | 0.11 | 0.03 | 0.08 | 13.5 | 0.50 | 0.11 | 0.30 |
| C. spicifera | 3.6 | 0.10 | 0.05 | 0.05 | 17.8 | 1.5 | 0.36 | 1.04 |
| P. rugulosum | 6.2 | 0.10 | 0.05 | 0.05 | 19.4 | 0.84 | 0.29 | 0.55 |
| C. spicifera + P. rugulosum | 3.3 | 0.10 | 0.04 | 0.06 | 18.3 | 0.70 | 0.26 | 0.44 |
| Superphosphate / Control | 3.1 | 0.03 | 0.03 | 0.02 | 19.0 | 0.3 | 0.10 | 0.20 |
| C. spicifera | 3.3 | 0.10 | 0.03 | 0.07 | 18.0 | 1.0 | 0.31 | 0.69 |
| P.rugulosum | 2.5 | 0.13 | 0.05 | 0.08 | 24.4 | 1.45 | 0.44 | 1.07 |
| C. spicifera + P. rugulosum | 2.5 | 0.06 | 0.02 | 0.04 | 14.2 | 0.13 | 0.12 | 0.16 |

Table 3 : Effect of trickling different spore suspensions around barley seedlings in presence of manures.

| Treatment / Manure | Growth parameters ( after 30 days ) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Root | | | | Shoot | | | |
| | Depth | F.wt. | D.wt. | Succulence | Length | F.wt. | D.wt. | Succulence |
| Urea / Control | 6.4 | 0.07 | 0.06 | 0.01 | 15.5 | 0.80 | 0.31 | 0.39 |
| C. spicifera | 9.0 | 0.07 | 0.06 | 0.01 | 16.4 | 0.65 | 0.23 | 0.07 |
| P. rugulosum | 8.6 | 0.07 | 0.06 | 0.01 | 18.2 | 0.43 | 0.20 | 0.06 |
| C. spicifera + P. rugulosum | 6.5 | 0.08 | 0.07 | 0.01 | 14.8 | 0.53 | 0.26 | 0.01 |
| Supernitrate / Control | 5.1 | 0.06 | 0.05 | 0.01 | 20.0 | 0.53 | 0.17 | 0.36 |
| C. spicifera | 4.2 | 0.05 | 0.03 | 0.02 | 13.6 | 0.36 | 0.24 | 0.12 |
| P. rugulosum | 4.1 | 0.06 | 0.04 | 0.02 | 15.4 | 0.37 | 0.13 | 0.24 |
| C. spicifera + P. rugulosum | 4.5 | 0.05 | 0.03 | 0.02 | 16.1 | 0.50 | 0.21 | 0.29 |
| Superphosphate / Control | 4.2 | 0.06 | 0.04 | 0.02 | 20.1 | 0.90 | 0.48 | 0.42 |
| C. spicifera | 6.2 | 0.07 | 0.03 | 0.04 | 23.0 | 0.63 | 0.27 | 0.36 |
| P. rugulosum | 4.1 | 0.08 | 0.03 | 0.04 | 24.1 | 0.91 | 0.48 | 0.61 |
| C. spicifera + P. rugulosum | 6.3 | 0.07 | 0.03 | 0.04 | 19.0 | 0.50 | 0.20 | 0.30 |

# انتاج الاكسينات،الجبريللينات والفيتامينات بوساطة الفطريات الجذرمحيطية وتأثيرها على نمو نبات الشعير

مصطفي احمد السيد ، عبد الرحيم رمضان الشنشورى وهناء فهمى
قسم النبات- كلية العلوم- جامعة طنطا
قسم النبات- كلية العلوم- جامعة الزقازيق

يهدف هذا البحث الى اختبار مقدرة تسعة عشر سلالة من الفطريات الجذرمحيطية والمعزولة محليا على انتاج الاكسينات ، الجبريللينات والفيتامينات فى المزارع الصناعية ودراسة تأثيرها على انماء نبات الشعير. اثبتت النتائج ان لمعظم السلالات المقدرة على انتاج فيتامينات ج وحمض الفوليك ، وكذلك اندول- ٣- حمض الخليك. بينما اظهر عدد محدود منها المقدرة على انتاج حمض الجبريللين. اختيرت فطرتى كيرفيولاريا اسبيسفرا وبنسليوم ريجيلوسم لمقدرتهما الكبيرة على انتاج المركبات النشطة بيولوجيا سابقة الذكر وعوملت بهما حبوب وبادرات نبات الشعير. وقد لوحظ ان المعاملة السابقة لزراعة الحبوب او بادرات النبات بجراثيم فطرة بنسليوم ريجيولوسم فى وجود السوبر فوسفات افضل المعاملات فى زيادة نمو النبات ، حيث لوحظ قصر المجموع الجذرى بالرغم من زيادة وزنه الرطب والجاف ومحتواه المائى وقد يكون ذلك نتيجة المواد الانمائية التى يفرزها الفطر ، اما المجموع الخضرى فقد لوحظت زيادة ملحوظة فى طول النبات ووزنه الرطب والجاف وكذلك محتواه المائى وذلك فى حالة النقع المسبق لحبوب النبات فى معلق جراثيم فطرة بنسليوم ريجيولوسم عنه فى النباتات المضاف الى بادراتها معلق جراثيم الفطر.