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Co-Inoculation Effect of Rhizobia and Endophytic Bacteria on *Vicia faba* Growth and Metabolism

Abbas, M. A. ; Samia A. Haroun ; A. M. Mowafy* and Mona S. Agha
Botany Department, Faculty of Science, Mansoura University, Egypt
***Corresponding Author: AmrMowfyE-mail: drammr79@yahoo.com**

ABSTRACT

Nodule-forming rhizobia and specific legume host are forming the mutualistic interactions between them which involve a series of signaling molecules leading to the establishment of a strong and functional symbiosis between the two partners. The success of symbiotic relationships and form nodule are depend on competitive ability and legume host specificity of rhizobia together with the ability of both rhizobia and legumes to release functionally divergent active molecules. The parameters showing the growth and metabolism of *Vicia faba* in response to cultivation with different Rhizobia and endophytic bacteria were determine. Combination of *Rhizobium* with different endophytic bacteria had a positive effect on growth parameters, photosynthetic pigments and carbohydrate content in shoot of *Vicia faba*, at flowering stage.

Keywords: Endophytic, Nodule, Rhizobia, *Vicia faba*.

INTRODUCTION

Nitrogen is a considerable essential macro-nutrient affecting growth, metabolism and yield of plants. It represents 2% of the plant dry weight (Miller and Cramer, 2005). It is a major component of amino acids which are the building blocks of both structural and functional proteins. It is also critical for nitrogen bases, DNA, RNA, alkaloids, vitamins, amides, coenzymes, hormones and many other vital components that constitute cell life and activity. The plant different physiological processes are significantly affected by nitrogen supply. The biosynthesis of chlorophyll and stem are all affected by the available nitrogen dose. Additionally, it's sufficient supply improve fruit quality as well as protein content of fodder plants. Synergistically it encourages the uptake and utilization of other nutrients including potassium and phosphorous (Bloom, 2015; Hemerly, 2016).

The process of biological nitrogen fixation (BNF) "or as it might be called as biological di-nitrogen fixation" is the reduction of atmospheric nitrogen to ammonia(De Bruijn, 2015), a process that could be done by different categories of prokaryotes that might be free living diazotrophs (*Rhodobacter* and *Azotobacter*) as well as those are living in close (*Azospirillum*) or in deep association with host plants which might be symbiotic nodule formers (*Rhizobium*) or endophytic (*Bacillus*)(Kaschuk and Hungria, 2017).

The endophytic bacteria which increases the efficiency of the Rhizobium species in one legume does not necessarily do the same in other legumes. The variable responses to co-inoculation underscores the need to identify appropriate combinations of rhizobia strain and endophytic bacteria for particular sites to enhance growth of common bean. The present study was therefore designed toevaluate the effect of co-inoculation of Rhizobia and *endophytic bacteria* on growth and metabolism of common bean.

MATERIALS AND METHODS

1. Seed material

The experiments were done by using a homogeneous lot of *Vicia faba* seeds which were obtained from the Agricultural Research Center, Ministry of Agriculture, Giza, Egypt and selected for apparent uniformity of size and shape. Some of the used chemicals were supplied from Sigma Chemical Company, while others were of analytical grade that supplied from different local companies. A semi-field experiment was carried out in the green house of Botany Department Faculty of Science, Mansoura University. This experiment was conducted to investigate the impact of rhizobia and endophytic bacteria inoculation on photosynthetic pigment and carbohydrates contents of *Vicia faba*.

2. Rhizobia and other endophytic bacteria

*Rhizobium*_MAP7 (P7) and other endophytic bacteria *Bacillus*_MAP3 (P3), *Brevibacillus*_MAP4 (P4), *Pseudomonas*_MAP5 (P5),*Pseudomonas*_MSAP8 (P8)were used in this experiment.

3. Seed treatment

The treatment of *Vicia faba* with rhizobia and endophytic bacteria was done as following (Mathivanan *et al.*, 2014). The seeds of common bean were surface sterilized with 80% ethanol and 0.1% mercuric chloride and washed the seeds with sterile distilled water three times. The seeds were mixed with rhizobium and other endophytic bacteria, either as individual organisms or combination of organisms as following:

1) Control (without bacteria)
2) P7.
3) P7 + P3.
4) P7 +P4.
5) P7 + P5
6) P7 + P8.
7) P7+ P4+ P5.
8) P7 + P3 +P4 + P5 +P8.

All of these bacteria separately having a cell load of $1\chi10^{9}$ CFU/ml^{-1}. *Vicia faba* seeds were soaked in liquid media contain bacterial treatments for one hour (Remans *et al.*, 2007).

The seeds were sown in (clay: sandy) (2:1) soil 3 kg soil per pot and use two types for the supplemented fertilizer, first fertilizer is urea was added to supplement N that applied following the local practice and represent the control treatment (1 g/ pot). The second fertilizer P_2O_5 as diammonium Pi and K_2O as KCL were applied for all treatments (1 g/ pot).

The samples were taken after 50 day at flowering stage.for assessment of growth parameters (shoot length, number of nods, number of leaves, shoot fresh weight, shoot dry weight, root length, root fresh weight and root dry weight) and photosynthetic pigments (chlorophyll a, chlorophyll b, carotenoids, total chlorophylls (a+b), chlorophyll a/b and consequently total pigment). as well as some metabolic aspects such as carbohydrates fractions in shoot (glucose, sucrose, total soluble sugars, polysaccharides and total carbohydrates).The full data of the differently treated groups were statistically analyzed and comparison among means was carried out by computer programming method. (satgraphic- vers-4-2 display ANOVA), as described by (Snedecor and Cochran)

4. Pigments analysis

The spectrophotometric method was used to determine Photosynthetic pigments (chlorophyll a, chlorophyll b and carotenoids) as recommended by (LICHTENTHALER and Wellburn, 1983) for chlorophylls and (Horvath *et al.*, 1972) for carotenoids as adopted by (Haroun *et al.*, 2010)Then, theμg/g fresh weightused to calculate the amount of photosynthetic pigments as of the differently treated plant leaves.

5. Estimation of carbohydrates

The different carbohydrate fractions such as glucose and sucrose were estimated usingthe methods which adopted by (Yemm and Willis, 1954) and (Singh and Luthra, 1988). Total soluble sugars (TSS) content was determined using the modified procedures of(Yemm and Willis, 1954). The method used for estimation of polysaccharides study was that of (Thayermanavan, 1984).

Total carbohydrates were calculated as the summation of the amount of glucose, sucrose, total soluble sugars and polysaccharides of the same sample, as they estimated in the previously explained procedures.

RESULTS

1. Changes in growth parameters

Considering shoot length of *Vicia faba* plant at flowering stage table 1 showed that, treatment with P7+P3, P7+P4, P7+P5, P7+P4+P5 and P7+P3+P4+P5+P8 caused a significant increase but other treatments (P7 and P7+P8) had non-significant increase in this parameter compared to control.

Table 1. Effect of bacterial treatments on growth parameters of *Vicia faba* shoot during flowering stage

Treatment	Shoot length (cm/plant)	Number of leaves/ plant	Shoot fresh wt. (g/plant)	Shoot dry wt. (g/plant)
Control	53.5	11.75	15.40	1.95
P7	59	10.75	17.06	2.37
P7+P3	67.5*	13	19.30	2.35
P7+P4	68.75*	12.25	20.23	2.28
P7+P5	63*	12	27.67*	3.01*
P7+P8	60.25	12	21*	2.61
P7+P4+P5	71*	14	30.88*	3.23*
P7+P3+P4+ P5+P8	65.25*	12	21.7*	2.56

Results of 1-way ANOVA analysis at p ≤ 0.05

Treatments means are with ±SD of 3 replica. Treatments with identical letters are not significant at P≤0.05. . P3=*Bacillus MAP3*, P4=*Brevibacillus MAP4*, P5=*Pseudomonas MAP5*, P7=*Rhizobium MAP7*, P8=*Pseudomonas MAP8.*

Treatment with P7+P3, P7+P4, P7+P5, P7+P8, P7+P4+P5 and P7+P3+P4+P5+P8 caused non-significant increase the number of leaves/plant value while P4 treatment caused non- significant decrease compared to control value.

Considering shoot fresh weight *Vicia faba* plant at flowering stage, P7+P5, P7+P8, P7+P4+P5 and P7+P3+P4+P5+P8 treatments, increased this parameter significantly while treatment with P7, P7+P3, P7+P4 showed non-significant increment in this parameter compare to control plant.

As shown in table 1, one way ANOVA test demonstrated that, during flowering stage shoot dry weight *Vicia faba* by P7 + P5 and P7+P4+P5 treatment increased it significantly. P7, P7 + P3, P7 + P4, P7 + P8 and P3 + P4 + P5 + P7 + P8 treatments non-significantly increased this parameter comparing to control plant. The highest value of the previous parameters was recorded in case of treatment with P7+P4+P5.

2. Changes in pigments contents

One way ANOVA test demonstrated that during flowering stage P7+P4, P7+P8 and P7+P4+P5 treatments led to significant decrease in chl.a while P7, P7+P3, P7+P5 and P7+P3+P4+P5+P8treatments significantly increased this parameter compared to control. The highest value was recorded in case of treatment with P7+P5.

At flowering stage P7+P3, P7+P5, P7+P8, P7+P4+P5 and P7+P3+P4+P5+P8 treatments caused significant increase in chl.b. Regarding P7+P4 treatment a non-significant increase was obtained in this pigment. In the other hand, only P7 treatment led to significant decrease of chl.b compare to control treatment The highest value was recorded in case of treatment with P7+P8.

In relation to control ratio, chlorophyll a/ chlorophyll b. non-significantly increase treatment by P7 caused in this relation, but the other treatments decreased this ratio either significant, by P7+P3, P7+P4, P7+P5, P7+P8, P7+P4+P5 and P7+P3+P4+P5+P8 treatments or non-significant, by P7+P4 treatment. The highest value was recorded in case of treatment with P7.

Chlorophyll a+b content of *Vicia faba* leaves, at flowering stage, increased significantly in response to treatment with P7+P3, P7+P5, P7+P8, P7+P4+P5 and P7+P3+P4+P5+P8. and increased non-significantly by P7 and P7+P4 treatment compered to control value. The highest value was recorded by treatment with P7+P8 as in case of chl.b.

Concerning carotenoids content of *Vicia faba* leaves, the data given in table 3 showed that, at flowering stage, this pigment increased significantly by P7+P3, P7+P5, P7+P8, P7+P4+P5 and P7+P3+P4+P5 +P8 treatments, but under P7 and P7+P4 treatment, this pigment decreased and increase non- significantly respectively.The highest value was recorded in case of treatment with P7+P8.

The response of total pigments content of *Vicia faba* leaves, at flowering stages to the used treatments are more or less comparable to that of carotenoids compared to control.

3. Carbohydrates content of *Vicia faba* shoot:

As observed in table 3 glucose content in shoot of *Vicia faba* was significantly increased in response to P7+P5 and P7+P8 treatments, While P7, P7+P3, P7+P4, P7+P4+P5 and P3+P4+P5+P7+P8 treatments caused non-significant increase value. The highest value was recorded in case of treatment with P7+P8.

Sucrose contents in shoot of *Vicia faba* showed significant decrease in response to P7, P7+P4 and P7+P5 treatments and non-significant increased by P7+P3 and P7+P8 treatments as represented in table 3. Sucrose content was non-significant decreased in response to P7+P4+P5 and increased significantly with P7+P3+P4+P5+P8 treatment as compared to the control values The highest value was recorded in case of treatment with P7+P3+P4+P5+P8.

Total soluble sugars in shoot of *Viciafaba* showed significantly increased by P7, P7+P3, P7+P4, P7+P8 and P7+P4+P5 compared to control value but other treatments P7+P5 and P7+P3+P4+P5+P8 showed non-significant decrease in this metabolite. The highest value was recorded in case of treatment with P7+P8.

As represented in table 3 all treatments decresed polysaccharides *Viciafaba* shoot either significant with P7, P7+P4, P7+P5, P7+P8, P7+P4+P5 and P7+P3+P4+P5+P8 or non-significant by P7+P3 treatment. The highest value was recorded in case of treatment with control.

As apparent from the recorded data of carbohydrates which cleared that, total carbohydrates in shoot were decreased non-significantly under the used treatments except for P7+P3 that increased this parameter non-significant compared to control value.

Table 2. Effect of bacterial treatments on carbohydrates content of *Viciafaba* shoot during flowering stage

Treatment	Chl.a (mg/g dwt.)	Chl.b (mg/g dwt.)	Chl.a \ Chl.b (mg/g dwt.)	Chl.a + Chl.b (mg/g dwt.)	Carotenoids (mg/g dwt.)	Total pigments. (mg/g dwt.)
Control	4.75	3.52	1.35	8.28	2.97	11.25
P7	5.74*	2.60*	2.21	8.35	2.55	10.90
P7+P3	5.79*	6.71*	0.86*	12.51*	4.51*	17.02*
P7+P4	4.42*	3.94	1.13	8.37	3.00	11.37
P7+P5	6.29*	6.43*	0.99*	12.72*	4.66*	17.38*
P7+P8	6.20*	3.39*	1.84*	9.59*	4.69*	14.29*
P7+P4+P5	3.46*	7.11*	0.48*	10.58*	4.66*	15.24*
P3+P4+P5+P7+P8	4.95*	8.58*	0.57*	13.54*	4.69*	18.23*

Results of 1-way ANOVA analysis at $p \leq 0.05$

Treatments means are with ±SD of 3 replica. Treatments with identical letters are not significant at $P \leq 0.05$. . P3=*Bacillus MAP3*, P4=*Brevibacillus MAP4*, P5=*Pseudomonas MAP5*, P7=*Rhizobium MAP7*, P8=*Pseudomonas MAP8.*

Table 3. Effect of bacterial treatments on carbohydrates content of *Viciafaba* shoot during flowering stage

Treatment	Glucose (mg / g dwt.)	Sucrose (mg / g dwt.)	Total soluble sugars (mg / g dwt.)	Polysaccharides (mg / g dwt.)	Total carbohydrates (mg / g dwt.)
Control	5.11	48.63	24.41	126.48	204.30
P7	5.38	11.83*	29.56*	93.52*	140.29
P7+P3	5.78	51.22	38.86*	123.26	219.12
P7+P4	6.23	18.84*	40.54*	77.50*	143.11
P7+P5	7.43*	35.17*	23.13	94.95*	160.68
P7+P8	8.49*	51.02	46.01*	98.45*	203.97
P7+P4+P5	7.01	44.33	39.61*	92.80*	183.75
P7+P3+P4+ P5+P8	7.32	55.85*	23.48	106.67*	193.32

Results of 1-way ANOVA analysis at $p \leq 0.05$

Treatments means are with ±SD of 3 replica. Treatments with identical letters are not significant at $P \leq 0.05$. . P3=*Bacillus MAP3*, P4=*Brevibacillus MAP4*, P5=*Pseudomonas MAP5*, P7=*Rhizobium MAP7*, P8=*Pseudomonas MAP8*

DISCUSSION

Co-inoculation of *Rhizobium* MAP7 along with *Brevibacillus* MAP4 and *Pseudomonas* MAP5 significantly increase the shoot dry weight if compared to the treatment with *Rhizobium* MAP7 alone. This effect might be attributed to the ability of the accompanying isolates to produce IAA, ammonia and HCN in addition to the significant hydrolytic activity against lipids and cellulose which all may directly or indirectly promote shoot growth(Verma *et al.*, 2012). The present study showed the presence of endophytic non-rhizobial bacteria in common bean nodules that are belonging to the most common known plant growth promoting rhizobacteria (PGPR) such as *Bacillus*, *Brevibacillus* and *Pseudomonas*. The addition of rhizobia along with *Brevibacillus* MAP4 and *Pseudomonas* MAP5 strains can be used together to improve growth of common bean. These results indicate that this combination of isolates can be used as potential new bio-fertilizer to improved common bean growth and metabolism.

Compared with the control "un-inoculated treatment ", treating plants with rhizobia led to a significant increase in growth parameters (length, fresh weight, dry weight and number of leaves).

The improved growth of plants subjected to *Rhizobium* inoculation is effectively attributed to its positive effect due to the symbiotic relationship evolved between both partners through the exchange of nitrogen from the side of rhizobia with the organic carbon from the side of legume (Newton and Burgess, 1983; Ndakidemi and Semoka, 2006; Bambara and Ndakidemi, 2010).

The same results were previously reported by several studies in which different rhizobial strains were tested with their legumes (Ndakidemi and Semoka, 2006; Bambara and Ndakidemi, 2010; Ravikumar, 2012; Tairo and Ndakidemi, 2013; Nyoki and Ndakidemi, 2014). Also, Leaf area along with the chlorophyll contents have been improved in presence of the rhizobial inoculant.

The amount of leaf chlorophyll of common bean showed a significant increase not only in presence of rhizobia but also when co-inoculated with other plant growth promoting bacteria (Bambara and Ndakidemi, 2009). This improvement might be attributed to the increase in nitrogen supply via the biological nitrogen fixer *Rhizobium* inoculant and subsequently increase the chlorophyll contents. The results in our study showed that *Rhizobium* inoculation increased leaf chlorophyll, results that have been reported previously (Bambara and Ndakidemi, 2009; Tairo and Ndakidemi, 2013; Nyoki and Ndakidemi, 2014). The promising results obtained from our study concluded that rhizobia inoculation may substitute or at minimum reduce the use of inorganic N fertilizers.

Few reports are available on the effect of combination between *Rhizobium* and different endophytic bacteria. The results obtained in this study revealed that the combination between *Rhizobium* and endophytic bacteria is better than the other single inoculations in leguminous plants. This combination showed significant increase in growth and nutrient level. The biochemical constituents of vicia faba shoot this investigation like glucose, sucrose, polysaccharides and total soluble sugars were increased significantly in combination between *Rhizobium* , *Bacillus* and *Pseudomonas* in leguminous plants.

Despite of, a few reports are available on the effect of combination between *Rhizobium* and different endophytic bacteria from the current study it could be concluded that the inoculation of *Rhizobium, Bacillus* and *Pseudomonas* in *Vicia faba* showed a positive effect of these microbes on host plant's growth (shoot growth parameters, photosynthetic pigments and carbohydrates) when compared to control. This is supported by that (Simons *et al.*, 2005).

REFERENCES

Bambara, S. and P. A. Ndakidemi (2009). Effects of Rhizobium inoculation, lime and molybdenum on photosynthesis and chlorophyll content of Phaseolus vulgaris L. African Journal of Microbiology Research 3(11): 791-798.

Bambara, S. and P. A. Ndakidemi (2010). Effects of Rhizobium innoculation, lime and molybdenum on nitrogen fixation of nodulated Phaseolus vulgaris L. African Journal of Microbiology Research 4(9): 682-696.

Bambara, S. and P. A. Ndakidemi (2010). Phaseolus vulgaris response to Rhizobium inoculation, lime and molybdenum in selected low pH soil in Western Cape, South Africa. African Journal of Agricultural Research 5(14): 1804-1811.

Bloom, A. J. (2015). The increasing importance of distinguishing among plant nitrogen sources. Current opinion in plant biology 25: 10-16.

De Bruijn, F. J. (2015). Biological nitrogen fixation. Principles of Plant-Microbe Interactions, Springer: 215-224.

Haroun, S., W. Shukry and O. El-Sawy (2010). Effect of asparagine or glutamine on growth and metabolic changes in Phaseolus vulgaris under in vitro conditions. Bioscience Research 7(1): 01-21.

Hemerly, A. (2016). Genetic controls of biomass increase in sugarcane by association with beneficial nitrogen-fixing bacteria''. Plant and Animal Genome XXIV Conference. Plant and Animal Genome, during month of January.

Horvath, G., J. Kissimon and Á. Faludi-Dániel (1972). Effect of light intensity on the formation of carotenoids in normal and mutant maize leaves. Phytochemistry 11(1): 183-187.

Kaschuk, G. and M. Hungria (2017). Diversity and Importance of Diazotrophic Bacteria to Agricultural Sustainability in the Tropics. Diversity and Benefits of Microorganisms from the Tropics, Springer: 269-292.

Korir, H., N. W. Mungai, M. Thuita, Y. Hamba and C. Masso (2017). Co-inoculation effect of rhizobia and plant growth promoting rhizobacteria on common bean growth in a low phosphorus soil. Frontiers in plant science 8: 141.

LICHTENTHALER, H. K. and A. R. Wellburn (1983). Determinations of total carotenoids and chlorophylls a and b of leaf extracts in different solvents, Portland Press Limited.

Mathivanan, S., A. Chidambaram, P. Sundaramoorthy, L. Baskaran and R. Kalaikandhan (2014). The Effect of plant growth promoting rhizobacteria on groundnut (Arachis hypogaea L.) seed germination and biochemical constituents. Int J Curr Res Aca Rev 2: 187-194.

Miller, A. and M. Cramer (2005). Root nitrogen acquisition and assimilation. Root Physiology: from Gene to Function, Springer: 1-36.

Ndakidemi, P. and J. Semoka (2006). Soil Fertility Survey in Western Usambara Mountains, Northern Tanzania1. Pedosphere 16(2): 237-244.

Newton, W. E. and B. K. Burgess (1983). Nitrogen fixation: its scope and importance. Nitrogen Fixation, Springer: 1-19.

Nyoki, D. and P. A. Ndakidemi (2014). Effects of phosphorus and Bradyrhizobium japonicum on growth and chlorophyll content of cowpea (Vigna unguiculata (L) Walp). American Journal of Experimental Agriculture 4(10): 1120.

Ravikumar, R. (2012). Growth effects of Rhizobium inoculation in some legume plants. International Journal of Current Science 1: 1-6.

Remans, R., A. Croonenborghs, R. T. Gutierrez, J. Michiels and J. Vanderleyden (2007). Effects of plant growth-promoting rhizobacteria on nodulation of Phaseolus vulgaris L. are dependent on plant P nutrition. European Journal of Plant Pathology 119(3): 341-351.

Simons, M., J. Gloy, A. Ganner, A. Bullerkotte, M. Bashkurov, C. Krönig, B. Schermer, T. Benzing, O. A. Cabello and A. Jenny (2005). Inversin, the gene product mutated in nephronophthisis type II, functions as a molecular switch between Wnt signaling pathways. Nature genetics 37(5): 537.

Singh, N. and R. Luthra (1988). Sucrose metabolism and essential oil accumulation during lemongrass (Cymbopogon flexuosus Stapf.) leaf development. Plant Science 57(2): 127-133.

Snedecor, G. and W. Cochran GStatistical Methods. 1994. New Delhi: Oxford and IBM, Publications 265.

Tairo, E. V. and P. A. Ndakidemi (2013). Bradyrhizobium japonicum inoculation and phosphorus supplementation on growth and chlorophyll accumulation in soybean (Glycine max L.). American Journal of Plant Sciences 4(12): 2281.

Thayermanavan, V. (1984). Qual Plant Foods Hum Nutr., 34, 253-257. Quoted from Biochemical Methods. Sadasivam, S. and A. Manickam, eds) 11-12. New ag. inter. Limit. Publ. New Delhi, India.

Verma, J. P., J. Yadav and K. N. Tiwari (2012). Enhancement of nodulation and yield of chickpea by co-inoculation of indigenous mesorhizobium spp. and Plant Growth–Promoting Rhizobacteria in Eastern Uttar Pradesh. Communications in soil science and plant analysis 43(3): 605-621.

Yemm, E. and A. Willis (1954). The estimation of carbohydrates in plant extracts by anthrone. Biochemical journal 57(3): 508.

تأثير تلقيح بكتريا الريزوبيم والبكتريا المتطفله داخليا على نمو وايض نبات الفول

محمد على عباس ، سامية على هارون ، عمرو محمد موافى و منى صلاح أغا
قسم النبات - كليه العلوم - جامعه المنصوره

التفاعلات المتبادلة بين بكتريا الريزوبيم المكونة للعقد الجذرية من ناحية والعائل البقولى المتخصص من ناحية اخر تتضمن سلسلة من الاشارات مؤدية الى انشاء علاقه تكافليه قويه ووظيفيه بين الطرفين. القدرة التنافسية وتخصص العائل البقولى لبكتريا الريزوبيم مع قدرة الريزوبيم والبقوليات على اطلاق جزئيات نشطه متعدده الوظائف والتى تحدد نجاح العلاقة التكافلية وتكوين العقد الجذرية. وقد تم تحديد المعايير التى توضح نمو وايض نبات الفول استجابة لزراعتة مع اليزوبيم و انواع مختلفة من البكتريا المتطفله داخليا. ولقد اتضح ان دمج الريزوبيم مع البكتريا المتطفله داخليا لها تأثير ايجابى على معايير النمو والاصباغ المستخدمة فى البناء الضوئى والمحتوى السكرى فى المجموع الحضرى لنبات الفول فى مرحلة الازهار.