EVOLUTIONALLY FORMED GENETIC POLYMORPHISM OF COMMON BUCKWHEAT (FAGOPYRUM ESCULENTUM MOENCH)

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For the prospects of breeding, the important features are those that went beyond the morphological constitution of species. Their source is the kind of evolutionary mutation reserve of buckwheat, which is significantly different from intraspecific polymorphism.

The aim and tasks of the study. Some spontaneous mutations - the only source of the necessary genetic material expansion.

Material and methods. Genetic diversity of buckwheat was created and discovered by identifying genotypes from various ecogeographical groups; recombinogenesis using intra- or interspecific hybridization with further identification of genotypes by offspring; inbreeding for separation of buckwheat population into different genotypes.

Results and discussion. Determinate, green flowering are controlled by monogenic recessive alleles, short caulescent controlled by both recessive and dominant genes.

Feature "high-grained shortened central stalk" with specific regulation of photosystem is monogenic recessive controlled.

Heterostyled hybrids F_1 were obtained from from the wild type of self-fertile *F. homotropicum* (homostyly form) by introgression feature of self, which confirmi their hybridity and recessive control of homostyly.

We observed monogenic splitting in the hybrid combination F_2 (Form D / *Fagopyrum homotropicum*), where heterostyled dolichostylous phenotype appears

Conclusions. Identified valuable evolutionary mutations are determinant forms of three types of inflorescences, three types of green flowering, short caulescent, red flowering, leafless form, "truncated high-grained central stalk" and fasciations and qualify as genetic diversity of buckwheat features.

Key words*: buckwheat, mutation, determinant, green-flowering, red-flowering, `high-grained shortened central stalk`.*

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OPTIMIZATION OF CULTURE MEDIUM FOR A BRADYRHIZOBIUM JAPONICUM STRAIN INTRODUCED FROM RUSSIA

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Bradyrhizobium japonicum strain GF, which was introduced from Russia, was cultured in five media YMA, TY, PA, BSE and SM. It was found that the stain grew more rapidly in YMA medium than in the other four media. Then the carbon source in YMA medium was optimized, and the result proved that glucose was the optimal carbon source for the cultivation of *B. japonicum* strain GF in YMA medium. Finally, four components in YMA medium were optimized using a $L_9(3^4)$ orthogonal array. We chose the optimal mix for *B. japonicum* strain GF to be YMA

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medium at 10.0 g/l glucose, 0.8 g/l yeast powder, 0.2 g/l $MgSO_4 \cdot 7H_2O$, 0.8 g/l K_2HPO_4, 0.1 g/l NaCl and 0.01 g/l $CaCl_2$.

Key words: *Bradyrhizobium japonicum, introduced from Russia, medium, optimization rhizobia, nitrogen, symbiotic relationship, leguminous plants*

Introduction. The rhizobium-legume symbiosis is the most efficient in nitrogen fixation and thus provides the major proportion of available nitrogen to plants [1]. *Bradyrhizobium japonicum*, a species of legume-root nodulating, can convert atmospheric nitrogen into ammonia, which is directly available for leguminous plants. The nitrogen fixation of *B. japonicum* improves the nutritional status, yield and quality of legumes, but also provides more available nitrogen to soil and thus improves soil fertility [2].

Analysis of publications, pose the problem. Rhizobium inoculation in legumes is considered as a routine measure for improving crop yield and reducing fertilizer application all over the world, that is why rhizobium products are available in markets in many countries. With the development of pollution-free agriculture, inoculation of efficient soybean rhizobia will produce more significant social and environmental benefits [3].

Solid theoretical foundation about rhizobia and abundant practical experience have been accumulated for nearly 100 years of research in Russia [4, 5]. More than a dozen of *B. japonicum* strains, which were introduced from All-Russia Research and Development Institute of Soybean, Russian Academy of Agricultural Sciences, were studied, developed and finally applied by Heilong Academy of Agricultural Sciences according to the ecological characteristics and soybean cultivars in Heilongjiang Province. YMA medium is widely used for the isolation and cultivation of rhizobia in the laboratory, but not suitable for large-scale production, because rhizobia grow slowly on it. So, the components and their concentrations in medium should be optimized to improve the growth rate of rhizobia.

The aim and tasks of the study. In the present study, the components and their concentrations in YMA medium, which was the most suitable for the cultivation of *B. japonicum* strains, were optimized with an attempt to provide a scientific basis for high-density and large-scale cultivation of rhizobia.

Materials and methods.

1.1 Materials

1.1.1 Strain. *B. japonicum* strain GF is a fast-growing soybean rhizobium provided by the All-Russia Research and Development Institute of Soybean, Russian Academy of Agricultural Sciences.

1.1.2 Media. Five media YMA, SM, TY, PA and BSE were selected in this study.

1.2 Methods

1.2.1 Activationand inoculation of B. japonicum. Stock solution of *B. japonicum* was inoculated to a slant and cultured at 28 ^oC for 2 days and then inoculated into YMA liquid medium, cultured at 28 ^oC, at 150 r/min for 4 days.

1.2.2 Expanding culture of B. japonicum. The above-obtained *B. japonicum* liquid medium was inoculated at the ratio of 1% (*V*/*V*) into four media in 250 flasks, each containing 100 ml of medium, cultured at 28 ^oC and at 150 r/min.

1.2.3 Comparison of culture media. The *OD* value of each bacterial culture medium during different growth periods was read at 600 nm using a spectrophotometer to plot the growth curves of *B. japonicum* in YMA, PA, BSE, TY and SM media.

1.2.4 Screening of carbon sources. After the optimal medium was determined, carbon source in the medium was optimized. In detail, mannitol in YMA medium was replaced by an equal amount of glycerol, lactose, dextrose, sucrose or maltose, while other components remained unchanged. Then, bacterial liquid was inoculated into these liquid media at the inoculation ratio of 1%, cultured at 28 ^oC and at 150 r/min. The *OD* values of the media were determined 12, 24, 36 and 48 h later.

1.2.5 Optimization of medium components. Four components in YMA medium were optimized using a $L_9(3^4)$ orthogonal array after the optimal carbon source was determined (table 1).

Table 1

Orthogonal array

No.	Orthogonal design					Medium compo-nents (g)			
	A	B	C	D		Glucose	Yeast extract	K_2HPO_4	$MgSO_4 \cdot 7H_2O$
1	1	1	1	1		10	0.4	0.5	0.1
2	1	2	2	2		10	0.6	0.8	0.15
3	1	3	3	3		10	0.8	1.0	0.2
4	2	1	2	3		12	0.4	0.8	0.2
5	2	2	3	1		12	0.6	1.0	0.1
6	2	3	1	2		12	0.8	0.5	0.15
7	3	1	3	2		15	0.4	1.0	0.15
8	3	2	1	3		15	0.6	0.5	0.2
9	3	3	2	1		15	0.8	0.8	0.1

Results and discussion.

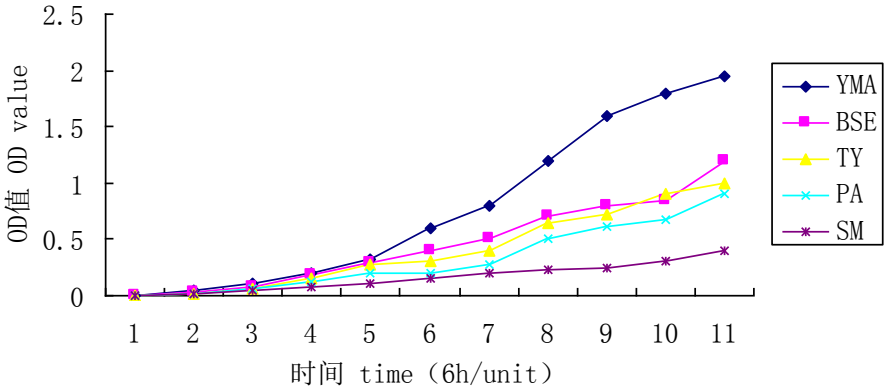

Fig. 1. Growth rate of *B. japonicum* strain GF in different media

2. 1 Growth of B. japonicum strain GF in different media. The *OD* values of *B. japonicum* cells grown in YMA, TY, SM, PA and BSE media were measured and plotted versus the incubation time. As shown in Fig. 1, the strain GF grew more rapidly in YMA medium than in other media. The bacterial growth rates in BSE, TY and PA media were similar, all higher than that in SM medium. So, YMA medium, which is the most commonly used for the culturing *B. japonicum*, was selected, and its components were optimized in this study.

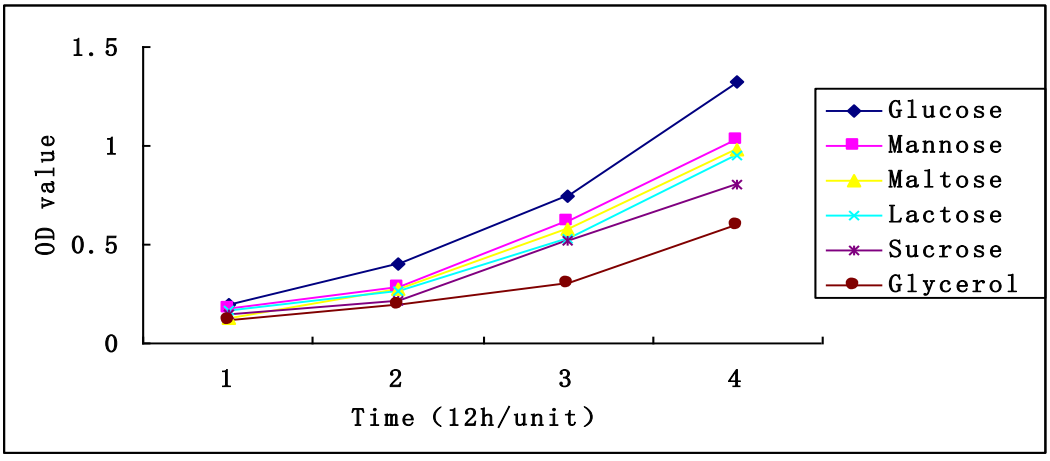

Fig. 2. Effects of different carbon sources on growth of *B. japonicum* strain GF

2.2 Optimization of YMA medium components

2.2.1 Carbon source. The growth rate of B. japonicum strain GF in YMA medium supplemented with different carbon sources was measured. The results showed that the strain grew the most rapidly, when glucose was used as a carbon source in YMA medium. The strain grew better in YMA media supplemented with mannitol, sucrose and glycerol than in YMA medium supplemented with lactose. Therefore, glucose was selected as the optimal carbon source and its concentration in YMA medium was optimized in later tests.

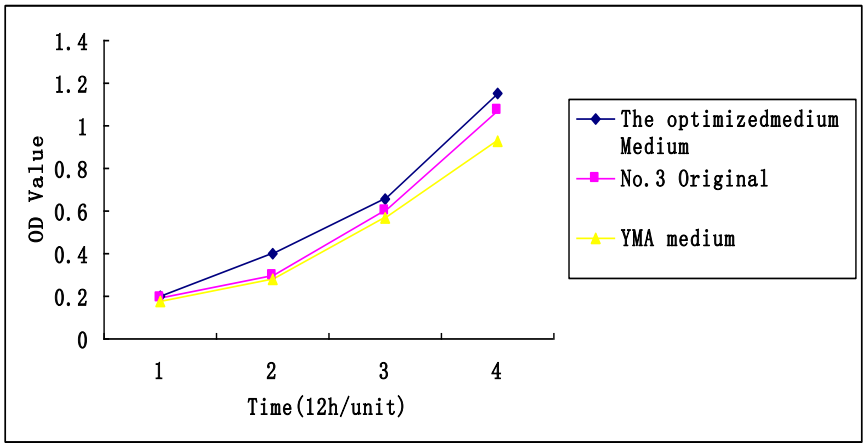

Fig. 3. Growth of *B. japonicum* strain GF in the optimized medium, medium No. 3 and original YMA medium

Table 2

Results of orthogonal test

Medium No.	1	2	3	4	5	6	7	8	9
OD	0.982	1.073	1.132	0.912	0.871	0.882	0.635	0.786	0.792

Table 3

Range analysis on the data of $L_9(3^4)$ orthogonal array

	A	B	C	D
*k*1	1.062	0.843	0.883	0.882
*K*2	0.888	0.91	0.926	0.863
*K*3	0.738	0.935	0.879	0.943
R	0.324	0.092	0.047	0.08
Optimal level	A1	B3	C2	D3
Sequence		A＞B＞D＞C		

2.2.2 Optimal concentrations of four major components in YMA medium. After the optimal carbon source was determined, the concentrations of four major components in YMA medium were optimized using a $L_9(3^4)$ orthogonal array. As shown in Table 2, the strain GF grew more rapidly in medium No. 3 than in all other media. Range analysis on the data revealed that the effects of the four components in YMA medium on the growth rate of the strain GF can be ranked in the following order: glucose > yeast extract > $MgSO_4 \cdot 7H_2O$ > K_2HPO_4. As shown in Table 3, A1B3C2D3 was the best combination. So, optimal mixture for *B. japonicum*strain strain GF was chosen to be YMA medium at 10.0 g/l glucose, 0.8 g/l yeast powder, 0.2 g/l $MgSO_4 \cdot 7H_2O$, 0.8 g/l K_2HPO_4, 0.1 g/l NaCl, 0.01 g/l $CaCl_2$and 4 ml/l Rh solution.

2.2.3 Verification of the optimized medium. The optimal medium (A1B3C2D3) we screened through the orthogonal design was compared with medium No. 3 and original YMA medium in the orthogonal test. The *OD* values of *B. japonicum* cells cultured in the three media

were measured 12, 24, 36 and 48 h after inoculation. The results proved that the OD value of the optimized medium was higher than those of medium No. 3 and original YMA medium (Fig. 3), indicating that the optimized medium was better than original YMA medium for culturing *B. japonicum.*

Conclusions. In summary, among the five media we tested, YMA was the best for the culturing *B. japonicum* strain GF, which was introduced from Russia, and glucose was the best carbon source in YMA medium. According to the orthogonal tests, we chose the optimal mixture for *B. japonicum* strain GF to be YMA medium at 10.0 g/l glucose, 0.8 g/l yeast powder, 0.2 g/l $MgSO_4 \cdot 7H_2O$, 0.8 g/l K_2HPO_4, 0.1 g/l NaCl, 0.01 g/l $CaCl_2$and 4 ml/l Rh solution. The optimized YMA medium makes it possible to produce rhizobia rapidly at a large scale, which is necessary for the industrial production of rhizobia [6].

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ОПТИМІЗАЦІЯ ЖИВИЛЬНОГО СЕРЕДОВИЩА ДЛЯ ІНТРОДУКОВАНОГО З РОСІЇ ШТАМУ БУЛЬБОЧКОВИХ БАКТЕРІЙ BRADYRHIZOBIUM JAPONICUM

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Мета і задачі дослідження. Дослідження спрямоване на визначення компонентів та їх концентрації для живильного середовища YMA, які були б найбільш придатні для культивування бульбочкових бактерій сої *Bradyrhizobium japonicum.* Це забезпечить наукову основу для виробництва бульбочкових бактерій у промислових масштабах.

Матеріали і методи. Вихідним матеріалом були бульбочкові бактерії сої *Bradyrhizobium japonicum*, штам GF, який було інтродуковано з Всеросійського НДІ сої РАСГН. Живильні середовища – YMA, SM, TY, PA, BSE.

Концентрований розчин бактерій культивували протягом двох діб при 28 ^oC, потім розподіляють у рідкому живильному середовищі YMA і культивують чотири доби. Після цього *B. japonicum* розподіляли у чотирьох середовищах в 250 колбах по 100 мл. Оптимальне середовище визначали в різні періоди росту бактерій за допомогою спектрофотометра за кривими зростання. Після цього було оптимізовано склад живильного середовища та концентрація його компонентів. Зокрема, маніт у середовищі YMA було замінено на таку ж кількість гліцерину, лактози, декстрози, сахарози, мальтози, в той час як інші компоненти залишалися незмінними.

Обговорення результатів. Результати дослідження показали, що клітини *B. japonicum* найшвидше виростали у середовищі YMA у порівнянні з іншими – SM, TY, PA, BSE. Таким чином, живильне середовище YMA було вибране для оптимізації складу.

Темп росту *B. japonicum* посилюється за доповнення живильного середовища вуглецем з різних джерел. Серед декількох джерел (глюкоза, маніт, сахароза, гліцеролтан) було визначено, що кращим є глюкоза. За темпами росту штаму GF було встановлено, що оптимальним співвідношенням компонентів і концентрацій є 10,0 г/л глюкози, 0,8 г/л порош-

кових дріжджів, 0,2 г/л $MgSO_4 \cdot 7H_2O$, 0,8 г/л K_2HPO_4, 0,1 г/л NaCl, 0,01 г/л $CaCl_2$ і 4 мл/л розчину Rh.

Оптимізоване середовище порівнювали з оригінальним YMA та із середовищем № 3. Оцінювали результати культивування бактерій через 12, 24, 36 і 48 годин після інокуляції. Доведено, що оптимізоване середовище за цим показником було краще, ніж оригінальне і № 3.

Висновки. У результаті дослідження встановлено, що серед п'яти живильних середовищ кращим для інокуляції бульбочкових бактерій сої *B. japonicum* штам GF є YMA. Його склад оптимізовано і встановлено, що кращими компонентами і концентрацією є 10,0 г/л глюкози, 0,8 г/л порошкових дріжджів, 0,2 г/л $MgSO_4 \cdot 7H_2O$, 0,8 г/л K_2HPO_4, 0,1 г/л NaCl, 0,01 г/л $CaCl_2$ і 4 мл/л розчину Rh. Оптимізоване середовище YMA надасть можливість виробництва бульбочкових бактерій в промислових масштабах у стислий час.

Ключові слова: *Bradyrhizobium japonicum, інтродукція з Росії, середовище, оптимізація, бульбочкові бактерії, азот, симбіоз, бобові рослини*

ОПТИМИЗАЦИЯ ПИТАТЕЛЬНОЙ СРЕДЫ ДЛЯ ИНТРОДУЦИРОВАННОГО ИЗ РОССИИ ШТАММА КЛУБЕНЬКОВЫХ БАКТЕРИЙ BRADYRHIZOBIUM JAPONICUM

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Цель и задачи исследования. Исследование направлено на определение компонентов и их концентрации для питательной среды YMA, которые были бы наиболее пригодными для культивирования клубеньковых бактерий сои *Bradyrhizobium japonicum.* Это обеспечит научное основание для производства клубеньковых бактерий в промышленных масштабах.

Материалы и методы. Исходным материалом были клубеньковые бактерии сои *B. japonicum*, штамм GF, интродуцированный из Всероссийского НИИ сои РАСХН. Питательные среды – YMA, SM, TY, PA, BSE.

Концентрированный раствор бактерий культивировали на протяжении двух суток при 28 ^oC, потом распределяли в жидкой питательной среде YMA и культивировали четверо суток. После этого *B. japonicum* распределяли в четырех средах в 250 колбах по 100 мл. Оптимальную среду определяли в разные периоды роста бактерий с помощью спектрофотометра по кривым возрастания. После этого был оптимизирован состав питательной среды и концентрация ее компонентов. В частности, манит в питательной среде YMA был заменен на такое же количество глицерина, лактозы, декстрозы, сахарозы, мальтозы, в то время как другие компоненты оставались неизменными.

Обсуждение результатов. Результаты исследования показали, что клетки *B. japonicum* быстрее всего вырастали в питательной среде YMA в сравнении с другими – SM, TY, PA, BSE. Таким образом, питательная среда YMA была выбрана для оптимизации состава.

Темп роста *B. japonicum* усиливается при добавлении в питательную среду углерода из разных источников. Среди нескольких источников (глюкоза, манит, сахароза, глицеролтан) было определено, что лучшим является глюкоза. По темпам роста штамма GF было установлено, что оптимальным соотношением компонентов и концентраций является 10,0 г/л глюкозы, 0,8 г/л порошковых дрожжей, 0,2 г/л $MgSO_4 \cdot 7H_2O$, 0,8 г/л K_2HPO_4, 0,1 г/л NaCl, 0,01 г/л $CaCl_2$ и 4 мл/л раствора Rh.

Оптимизированную питательную среду сравнивали с оригинальной YMA и со средой № 3. Оценивали результаты культивирования бактерий через 12, 24, 36 и 48 часа после инокуляции. Доказано, что оптимизированная питательная среда по этим показателям была лучше, чем оригинальная и № 3.

Выводы. В результате исследования установлено, что из пяти питательных сред лучшей для инокуляции клубеньковых бактерий сои *B. japonicum* штамм GF является YMA. Ее состав оптимизирован и установлено, что лучшими компонентами и их концентрацией

являются 10,0 г/л глюкозы, 0,8 г/л порошковых дрожжей, 0,2 г/л $MgSO_4 \cdot 7H_2O$, 0,8 г/л K_2HPO_4, 0,1 г/л NaCl, 0,01 г/л $CaCl_2$ и 4 мл/л раствора Rh. Оптимизированная питательная среда YMA предоставит возможность производства клубеньковых бактерий в промышленных масштабах в сжатые сроки.

Ключевые слова: *Bradyrhizobium japonicum, интродукция из России,* питательная среда*, оптимизация, клубеньковые бактерии, азот, симбиоз, бобовые растения*

OPTIMIZATION OF CULTURE MEDIUM FOR A BRADYRHIZOBIUM JAPONICUM STRAIN INTRODUCED FROM RUSSIA

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The aim and tasks of the study. The study was aimed at determination of ingredients and their concentrations to culture medium YMA, which would be the most suitable for cultivation of soybean nodule bacteria *Bradyrhizobium japonicum*. This will provide a scientific rationale for the production of nodule bacteria on an industrial scale.

Materials and methods. The starting material was soybean nodule bacteria *B. japonicum*, strain GF, introduced from the All-Russian Research Institute of Soybean of the Russian Agricultural Academy. Culture media were YMA, SM, TY, PA, BSE.

Concentrated solution of bacteria was cultured for two days at 28 ° C, then was spread in liquid nutrient medium YMA and cultured for four days. Thereafter, *B. japonicum* was devided among four media in 250 flasks of 100 ml. Optimal medium was spectrophotometrically determined for different periods of bacterium growth by growth curves. Thereafter, nutrient medium composition and concentration of its ingredients were optimized. In particular, mannitol in nutrient medium YMA was replaced with the same amount of glycerol, lactose, dextrose, sucrose, and maltose, while the other ingredients remained unchanged.

Results and discussion. The results showed that *B. japonicum* cells grew the fastest in nutrient medium YMA as compared with the others - SM, TY, PA, BSE. Thus, culture medium YMA was chosen to optimize the composition.

The growth rate of *B. japonicum* enhanced, when carbon from different sources was added to nutrient medium. Among several sources (glucose, mannitol, sucrose, glycerytan), glucose was the best. In terms of strain GF growth it was found that the optimal ratio of ingredients and concentrations was 10.0 g / L of glucose, 0.8 g / L of yeast powder, 0.2 g / L of $MgSO_4 \cdot 7H_2O$, 0.8 g / L of K_2HPO_4, 0.1 g / L of NaCl, 0.01 g / L of $CaCl_2$, and 4 ml / L of Rh solution.

Optimized culture medium was compared with original medium YMA and medium 3. Results of culturing bacteria 12, 24, 36 and 48 hours after inoculation were assessed. It was proved that optimized nutrient medium by these parameters was better than original one and number 3.

Conclusions. The study revealed that of the five culture media YMA was the best to inoculate soybean nodule bacteria *B. japonicum,* strain GF. Its composition was optimized, and it was demonstrated that the best ingredients and their concentrations were 10.0 g / L of glucose, 0.8 g / L of yeast powder, 0.2 g / L of $MgSO_4 \cdot 7H_2O$, 0.8 g / L of K_2HPO_4, 0.1 g / L of NaCl, 0.01 g / L of $CaCl_2$, and 4 ml / L of Rh solution. Optimized culture medium YMA will enable the production of nodule bacteria on an industrial scale in a short time.

Key words: *Bradyrhizobium japonicum, introduction from Russia, nutrient medium, optimization, nodule bacteria, nitrogen, symbiosis, legumes*