

Studies on the Characteristics Related to Symbiotic Nitrogen Fixation of Legumes and the Rhizobium Resources in the Arid Area of the Northwestern China

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1. Abstract

The nodulation and nitrogen fixation of major cultivated and wild legumes dispersed over 36 genera, 98 species have been studied in some region of Gansu province and Ningxia Hui Autonomous Region. 360 strains of root nodules bacterial have been isolated from about 400 samples, among them of 44 strains were from 30 species that have not been reported yet in Allen's "The Leguminoae" and other literatures. The most of which were tested infecting in 22 model species that belong to 20 genera as host, 81.2% strains of tested rhizobia can nodulate with host plants. Above 85% of those nodule samples were effective for nitrogen fixation. The result showed that the activities of nitrogen fixation in nodules of various species varied greatly and all of them were low. 46.6% of them are under $1 \mu\text{molC}_2\text{H}_4/\text{g}_{\text{FW}}\text{h}$ and more than $10 \mu\text{molC}_2\text{H}_4/\text{g}_{\text{FW}}\text{h}$ is only 6.8%. The nitrogenase activity was closely related to the growth period of plants.

2. Introduction

The area of Gansu province and Ningxia autonomous region in the northwest China is approximately 450,000 Km^2 , there are small Mt. Longshan and west Qinling with semi-humid, semi-arid and deciduous broad-leaved forest warm temperate zone and wood-grass climate, Bailong River drainage area with semi-tropical continent humid climate, and Allah friendly plateau with middle Asia continent arid hungeriness climate. There are various nitrogen fixation plants; the ingredient of area is complicated, quite a few plants are legumes in the region. It is extremely important to exploit this part of symbiotic nitrogen fixation resources and study their physiological and ecological characteristics of symbiotic nitrogen fixation in order to fully display the potential of legumes to improve soil, maintain lasting productivity of soil and ecological environment.

3. Methods

3.1 Sampling

Digging the nodules at appropriate growth period of legumes, then labeling and recording the time, place, ecological vegetation, soil type, moisture conditions and so on.

3.2 Medium

YMA medium, Congo red YMA medium

3.3 Isolation and purification of rhizobium

Fresh nodules: washing the nodules clean and put them into 95% ethanol for 30 seconds then change them to 0.1% HgCl_2 for 5 minutes. After that wash them 5-6 times with sterile water. Use inoculation needle to take the organ or juice of the nodules and put them on the YMA medium then cultured at 28°C .

Dry nodules: put the dry nodules into sterile water for 5-6h or even 24h then disinfect according to the above.

Put the rhizobium on Congo red YMA medium to purify the single colony.

3.4 Returned vaccination of rhizobium

3.4.1 Medium

Non-nitrogen nutrient fluid

3.4.2 Seed expedite budding

Choose the health seeds and disinfect as we did on nodules. Then put them into the aseptic culture dish or 0.5% agar plates at 25°C - 28°C to germinate.

3.4.3 Vaccination and culture condition

Ferment the active rhizobium or put the suspension into culture dish. After germinates seeds being soaked for 30 minutes, they were put onto agar slants, vermiculite triangle flasks or large tubes. Culture conditions were as follows: illumination was 7000-8000lx; every day irradiation was 12-15h; temperature was 21°C - 24°C ; mat temperature was 13°C - 20°C ; relative humidity was 50%-70%.

3.4.4 Determination of acetylene reduction capability

This is according to the peak-height ratio method improved by Shanghai Institute of Plant Physiology Nitrogen Fixation Research.

4. Results

4.1 Nodulated legumes distribution in the Gansu province and Ningxia Hui Autonomous Region

The resources of legumes rhizobia in Gansu and Ningxia may be divided into approximate four regions according to the physical geography characteristic.

The Hexi area: the average elevation is 1900-2300m; their biological climate belongs to the temperate zone hungeriness grassland, their corresponding soil is gray calcium soil, gray-brown soil, brown desert soil and oasis irrigative-cultivated soil respectively. The region is far from sea, the climate is dry, the year rainfall reduces from east 250mm to west under 50mm, the evaporation increases from east under 2000mm increases to west more than 3500mm. 182 nodule samples were collected in the region, including 15 genera host legumes such as *Medicago*, *Melilotus*, *Vicia*, *Pisum*, *Thermopsis*, *Glycine*, *Trigonella*, *Swainsonia*, *Sophora*, *Glycyrrhiza*, *Kummerowia*, *Oxytropis* and so on.

Loess plateau area: the soil layer is deep, thickness is about 30-100m. Because of the loose soil, big hole, containing lots of grain-size silty sands the loess plateau is easily washed by rainstorm and eroded by running water. The area is the arid-semi-arid climatic zone, belonging to continental climate, the temperature varies greatly yearly and daily, year rainfall gradually reduces from east 600mm to west 300mm, even under 300mm. 129 nodule samples were collected in the region, including 21 genera host legumes such as *Medicago*, *Melilotus*, *Astragalus*, *Caragana*, *Onobrychis*, *Glycine*, *Vicia*, *Lespedeza*, *Robinia*, *Amorpha*, *Albizia*, *Indigofera*, *Gleditsia*, *Caesalpinia* and so on.

The Bailong River drainage area: the elevation is commonly about 1100~3100m, it is subtropics continental humid climate. The most of sunny slope is the mountainous chestnut-calcium soil, mountainous meadow soil, grassland meadow soil and alpine meadow soil. The shady side is mountainous brown soil, brown forest soil or the mountainous gray-brown forest soil. 59 nodule samples were collected in the region, including 14 genera host legumes such as *Medicago*, *Melilotus*, *Phaseolus*, *Hedysarum*, *Vicia*, *Lotus*, *Astragalus*, *Trifolium*, *Falcataria*, *Lathyrus*, *Lespedeza* and so on.

The Alashan wilderness area: the topographic features of the area alternates with denudation low mountain, incomplete hillock, Gobi and desert, with typical characteristic of the middle part of Asia temperate zone hungeriness climate, the year rainfall reduces from east 150mm to west 40mm, its soil is silt brown calcium soil, fixed sandy soil, semi-fixed sandy soil. 61 nodule samples were collected in the region, including 17 genus host legumes such as *Astragalus*, *Caragana*, *Lespedeza*, *Robinia*, *Glycyrrhiza*, *Coronilla*, *Swainsonia*, *Phaseolus*, *Thermopsis*, *Ammopiptanthus*, *Hedysarum* and so on.

4.2 Modulated legumes in the Gansu province and Ningxia Hui Autonomous Region

The wild and cultivated legumes 98 species belonging 39 genera have been studied, the result indicated that all legumes are nodulated except *Arachis hypogaea*, *Gleditsia triacanthus*, *Halimodendron halodendron*. Compared with the literatures, 32 new species have not been recorded. Most all of these plants belonged to *Papilionoideae*, accounting for 95%, 2 species in *Mimosoideae* accounting for 2%, 3 species in *Caesalpinioideae* accounting for 3%.

4.3 The species of rhizobia in Gansu and Ningxia region

360 strains of root nodules bacterial have been isolated from 36 genera, 99 species, 431 root nodule samples collected in Gansu and Ningxia region, among them of 44 strains were from 30 species legumes that have not been reported. The most of them were inoculated to 22 original host plants or thought close original host plants that belonged to 20 genera, 81.2% strains of tested rhizobia could nodule. The studies about species classification status of the rhizobium in Gansu and Ningxia region are needed further carrying out.

4.4 The modality of legumes nodules in Gansu and Ningxia region

The nodule is formed via symbiosis between the rhizobium and its host plant. The nodules and nitrogen fixation in legumes focus on the nodules of root system. The result of investigation about legumes nodule in Gansu and Ningxia region indicated that the life habit of legumes was various and the nodules have many characters. Most of the legumes which always grew in hilly meadow, native grassland and forest marsh were annuals. The nodules were annuals and always round or long circle and pink, while the nodules of perennials legume arbor and frutex which distributed in sunny zone were generally long cylindrical-shape, furcellate, white, brown or yellow. The nodules were generally inserted on branch roots, fibre, and few on taproots. The modalities of the leguminous plant's nodules were related with the plants. The nodules of different species in same genus

were often similar. The nodules were often round or long-circle if host plants infected by rhizobia had no or few infection threads, or were short of permanent meristematic tissues. The host plants were infected via infection threads, the nodules were often limitlessly vegetal, long cylindric-shape, clubbed or digitiform if the host plant had permanent meristematic tissue. So the modality of the leguminous plant's nodules was related with the host plants' genes, and the species of legume didn't matter. The nodules that had the nitrogen fixation activity were pink because of leghemoglobin. The leghemoglobin played an important role in nodule, and provided low concentration and high flow oxygen. It was absolutely necessary condition for nitrogen fixation. We also confirmed the point according to diagnoses in the Gansu Ningxia region.

4.5 The symbiosis nitrogen fixation of rhizobium-legume

In order to know the characteristic of rhizobium and legume nodule, acetylene reduction activity of more than 400 nodule samples collected in Gansu and Ningxia region was tested by the method of peak height (Table 1). The result showed that more than 85% nodules were efficient, different nodule's nitrogen fixation activity varied greatly. Most of them was low, under $1\mu\text{molC}_2\text{H}_4/\text{gFWH}$ was 46.6%, more than $10\mu\text{molC}_2\text{H}_4/\text{gFWH}$ was only 6.8%, the maximum was up to $37.73\mu\text{molC}_2\text{H}_4/\text{gFWH}$. The history of legumes cultivated in Gansu Ningxia region was long. The larger cultivated area of *Medicago sativa*, *Vicia villosa* and *P.sativum* possessed high activities.

The legume-rhizobium symbiosis nitrogen fixation was related with the genes and the physiological characteristics of legumes and rhizobia. Because rhizobia in different region differed, the nitrogen fixation activity of different plant and same plant in different region varied greatly (Table 1).

Table 1 Some Nitrogen Fixation Activity of the Nodules in the Northwestern China

Botanical name	Sampling place	Growth period	Root nodule form	($\mu\text{molC}_2\text{H}_4/\text{gFWH}$)
<i>Albizia kalkora</i>	Maiji shan	nutritive stage	round	0.43
<i>Astragalus adsurgens</i>	Sha Potou	nutritive stage	digitiform	0.66
<i>A.coronilloides</i>	Gaoliushan	flowering and seeding stage	long circle	0.27
<i>Caragana acanthophylla</i>	Sha Potou	nutritive stage	clubbed with bifurcate	0.18
<i>C.jubata</i>	Lanzhou	seeding stage	long-stick	0.29
<i>C.microphylla</i>	Sha Potou	seeding stage	round	1.69
<i>C.pygmaea</i>	Linxia	flowering stage	round	5.46
<i>C.rosea</i>	Sha Potou	nutritive stage	clubbed	0.18
<i>C.sinica</i>	Weiwu	nutritive stage	clubbed	0.35
<i>C.tibetica</i>	Sha Potou	flowering stage	clubbed,digiti form	1.27
<i>Cercis hinensis</i>	Maiji shan	nutritive stage	round, clubbed	0.33
<i>Coronilla emerus</i>	Sha Potou	nutritive stage	clubbed	0.09
<i>Eremosparton songoricum</i>	Minqin county	flowering stage	clubbed	0.42
<i>Falcata japonica</i>	Gaoliushan	nutritive stage	round	0.81
<i>G.glabra</i>	Minqin county	seeding stage	round	0.70
<i>G.infrata</i>	Sha Potou	seeding stage	round	0.24
<i>G.korsninskii</i>	Sha Potou	flowering stage	round	0.11
<i>G.uralensis</i>	Sha Potou	flowering stage	round	0.08
<i>H.multijugum</i>	Wudu	flowering and seeding	round	0.74
<i>Kummerowia stipulacea</i>	Fankan enxian	nutritive stage	round	5.97
<i>Lathyrus ilosus</i>	Baijiamen Lintan	nutritive stage	clubbed	1.96
<i>L.pratensis</i>	Huangxianggou	nutritive stage	clubbed	0.52
<i>Lespedeza bicolor</i>	Sha Potou	flowering stage	round	0.47
<i>Lotus corniculatus</i>	Liujiaping Wenxian	seeding stage	clubbed	0.13
<i>Onobrychis viciaefolia</i>	Ganchuan wudu	flowering stage	clubbed digitiform	0.31
<i>Oxytropis aciphylla</i>	Sha Potou	nutritive stage	clubbed	0.24
<i>O.glabra</i>	Minle county	flowering stage	oval	9.42
<i>O.gueldestacdtioides</i>	Linxia	nutritive stage	oval	37.73

<i>O.ochrantha</i>	Liupan shan	nutritive stage	clubbed	0.11
<i>Piptanthus concolor</i>	Maiji shan	seeding stage	digitiform	0.14
<i>Robinia pseudoacacia</i>	Tianshui	flowering and seeding stage	oval	0.95
<i>R.hispida</i>	Maiji shan	flowering stage	oval	3.15
<i>R.sp</i>	Maiji shan	seeding stage	peanut-shaped	1.28
<i>Sophora alopecuroides</i>	Sishui Gulang	flowering stage	long circle	0.28
<i>S.japonica</i>	Sha Potou	nutritive stage	digitiform	12.08
<i>Swainsonia sasulla</i>	Jinta county	flowering stage	clubbed	5.74
<i>Thermopsis lanceolata</i>	Shandan	flowering stage	clubbed	4.87
<i>Trigonella foenum graecum</i>	Xiaochuan Cheng county	nutritive stage	digitiform	19.54

The factors that affected nitrogen fixation activity of legume nodule were very complicated. The activity was related with the growth period and life habit of host plant except for the environment (Table 1). The annual cultivated *Dolichos lablab* possessed higher nitrogen fixation activity at nutritive stage before flowering stage. The activity of *Dolichos lablab* in different region was 6.388 $\mu\text{molC}_2\text{H}_4/\text{g}_{\text{FWH}}$, 4.908 $\mu\text{molC}_2\text{H}_4/\text{g}_{\text{FWH}}$ and 2.37 $\mu\text{molC}_2\text{H}_4/\text{g}_{\text{FWH}}$ respectively. But along with plant growing the activities dropped greatly, just 1.99 $\mu\text{molC}_2\text{H}_4/\text{g}_{\text{FWH}}$, 1.25 $\mu\text{molC}_2\text{H}_4/\text{g}_{\text{FWH}}$, 0.492 $\mu\text{molC}_2\text{H}_4/\text{g}_{\text{FWH}}$ at flowering and seeding stage.

But as to annual cultivated plant *Trigonella foenum-graecum*, the activity was low to 1.384 $\mu\text{molC}_2\text{H}_4/\text{g}_{\text{FWH}}$, 1.023 $\mu\text{molC}_2\text{H}_4/\text{g}_{\text{FWH}}$ at before flowering stage and nutritive stage, and high up to 16.53 $\mu\text{molC}_2\text{H}_4/\text{g}_{\text{FWH}}$, 19.54 $\mu\text{molC}_2\text{H}_4/\text{g}_{\text{FWH}}$ at the flowering stage, 16-19 times more than before flowering stage and nutritive stage. The wilding *Medicago falcate* also had the same trend 4 in 5 studied plants had the highest activity.

5. References

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