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抗病性不同大豆品种根面及根际微生物区系的变化

Ⅱ. 连作大豆(重茬)根面及根际微生物区系的变化

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摘要：采用平板计数法测定了两个抗根腐病连作大豆品种生育期间根面和根际微生物区系动态变化。结果表明，两个品种大豆根面细菌随生育期增加呈递减趋势,品种间无差异。合丰 25(H25)的根际细菌数量随生育期呈递减趋势,绥农 10 号(S10)根际细菌数量从三叶期到鼓粒初期不断增加,到成熟期又急剧减少。感病品种 H25 根瘤重明显低于抗病品种 S10。H25 根面真菌和镰孢霉总数在三叶期和成熟期均高于 S10,苗期是根腐病的主要发病期。总之,在连作条件下,无论抗病品种还是感病品种大豆成熟期其根面和根际细菌减少,真菌和主要病原菌(镰孢霉 *Fusarium*)都会大量富集,表明根系分泌物对根面和根际的微生物种群有选择性地促进或抑制作用。

关键词：连作大豆；根面；根际；微生物区系；根腐病
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Change of microflora in the rhizoplane and rhizosphere of different disease resistance soybean cultivars

Ⅱ. Change of microflora in the rhizoplane and rhizosphere of soybean under continuous cropping condition

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Abstract: The mechanisms of soybean continuous cropping obstacle were complicated. Previous research found many factors might cause the obstacle, such as the change of physical and chemical properties in the soil, plant allelopathy and soil-borne disease, among which soil-borne disease was the dominant reason for replanting obstacle. The microflora in the rhizoplane and rhizosphere were affected by the root activity of the soybean in the pervious year, a case in point was that the root exudates changed the soybean rhizosphere microorgnism communities, as a result of which the biological environment in rhizosphere was changed. So root exudates was a significant factor leading to soil-borne disease of the soybean in the next year. Many studies had indicated that the soybean root rot (SRR) was one of the most serious replanting diseases. Our previous experiment showed that the microflora is different in the rhizoplane and rhizosphere between resistant (Suinong 10, S10) cultivar and susceptible cultivars (Hefeng 25, H25). A more pathogenic microbe (*Fusarium*) was observed in the rhizoplane and rhizosphere soil of susceptible cultivar at the mature stage. The aim of the experiment was to investigate how the root of resistant cultivars and susceptible cultivars resisting SRR further influence the microflora in the rhizoplane and rhizosphere soil. Seeds were sowed in the soil and leaves and roots was removed out after the harvest of soybean planted the last year.

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With pot experiment, two cultivars were sowed, no planting as the control (bulk). The experiment was conducted in a greenhouse. Sampling was taken at the trifoliate stage, beginning of flowering stage, early filling stage and harvest maturity stage, respectively. The microflora in the rhizoplane, rhizosphere of the two cultivars and bulk soil were investigated during the growth stage with plate culture method.

The results showed the numbers of colony forming units (CFU) of bacteria in the rhizoplane of the two cultivars decreased gradually with the growth of soybean indicating no significant difference in the rhizoplane and rhizosphere soil between the two cultivars. The numbers of CFU of bacteria in the rhizosphere of H25 decreased gradually with the growth of soybean, while that of S10 increased during the trifoliate stage to early filling stage, and then decreased dramatically at the mature stage. The root nodule weight of resistant cultivar S10 was significantly higher than that of the susceptible cultivar H25. It was observed that the number of CFU of fungi and *fusarium* in rhizoplane of H25 was much more than that of S10 at both trifoliate stage and mature stage. The seedling stage was the stage that soybean was easily infected of the root rot disease. In a word, the decrease in the number of rhizoplane and rhizosphere soil bacteria of both the resistant cultivar and susceptible soybean cultivars and the increase of fungi and *fusarium* at the mature stage indicated that soybean root exudates had certain selective acceleration or inhibition on the microflora in rhizoplane and rhizosphere soil.

Key words: soybean continuous cropping; rhizoplane; rhizosphere; microflora; root rot disease

大量研究表明,大豆连作障碍产生的原因比较复杂,是多种因素共同作用的结果。其中,土传病害是导致大豆连作减产的重要原因[1-3];而前茬大豆根系活动,特别是根系分泌物作用,改变了大豆根际微生物的组成[4],并引起根际生物学环境发生变化,是导致下茬大豆土传病害严重发生的主要因素。植物可在很大程度上改变根际微生物环境,促进一些微生物类群生长,而限制另一些微生物类群生长。不同植物种类或同一植物的不同基因型的根系分泌物在组成和数量上均存在差异。有研究表明,棉花根际土壤中的棉花黄萎病菌(*Verticillium dahliae*)数量随着连作抗病品种年限的增长而减少[5]。

大豆根腐病是连作的主要病害之一。先前试验表明,根腐病的抗病和感病品种根面和根际微生物区系存在差异,种植一季后感病品种根面和根际镰孢霉(*Fusarium*)数量更多[6]。为了进一步探索根腐病不同抗性品种根系分泌物在连作大豆过程中对根面和根际微生物种群的影响,选用种植一季大豆后的土壤(剔除根和叶片等残茬)作为供试土壤,种植根腐病的抗感品种两个,通过检测绥农 10 号(抗病)和合丰 25(感病)两个品种生育期间根面和根际微生物区系的动态变化,以探讨连作条件下不同大豆品种对根面和根际微生物区系的影响。

1 材料与方法

1.1 试验方法

试验选用种植一季大豆后的哈尔滨地区的黑土,经过筛、混匀、尽量剔除根系和叶片等,以避免残茬造成的试验误差。供试的两个品种为易感大豆根腐病的合丰 25(H25)和抗根腐病的绥农 10 号(S10)。

盆栽试验为每盆装土 1.5kg,所用肥料及用量为: N[$Ca(NO_3)_2$] 150 mg/kg, P(K_2HPO_4) 150mg/kg, K (KCl) 100 mg/kg, Mg ($MgSO_4 \cdot 7H_2O$) 50 mg/kg, Zn ($ZnSO_4 \cdot 5H_2O$) 5 mg/kg, Cu ($CuSO_4 \cdot 5H_2O$) 5 mg/kg。大豆出苗后每盆定苗 3 株,以不种大豆作为土体对照。分别在 4 个不同生育期取样,每次 3 个重复。试验在网室中进行,控制土壤含水量为 18%左右。

1.2 样品采集和制备方法

分别在大豆三叶期(V3)、初花期(R1)、鼓粒初期(R5)和成熟期(R8),即出苗后 14、28、42 和 84d 取土样。土体土(Bulk)从不种大豆的盆中采集,土样混匀后取 10g 加入 90mL 无菌水置于三角瓶中;土体土悬液、根际土悬液和根面悬液的制备方法参见参考文献[6]。

1.3 可培养微生物的分离培养和计数

方法参见参考文献[6]。

2 结果与分析

2.1 不同大豆品种根面及根际可培养细菌的动态变化

两个品种大豆根面细菌随生育期呈递减趋势,品种间无差异(图 1)。但种植两个大豆品种的根际细菌显著高于未种大豆土体中的细菌,说明根系分泌物有利于细菌在根际的定殖和扩繁。土体细菌随生育期基本无明显变化; H25 根际细菌随生育期也

逐渐减少，S10从三叶期到鼓粒初期根际细菌逐渐增加，鼓粒初期达到最高值，而后急剧递减(图2)。三叶期时，H25根际细菌高于S10，到鼓粒初期和成熟期H25根际细菌低于S10。

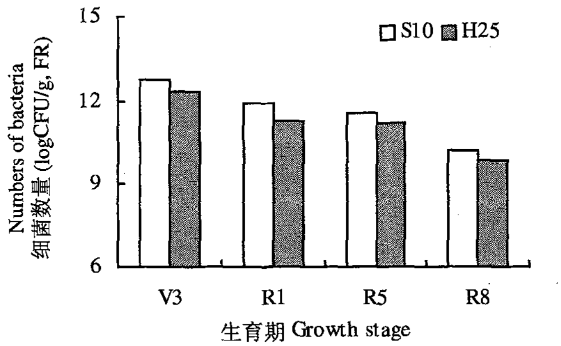

图1 不同品种大豆根面细菌数量

Fig.1 Numbers of bacteria in rhizosplane of soybean cultivars

(V3: 三叶期 trifoliate stage; R1: 初花期 Beginning of flowering; R5: 鼓粒初期 Early grain filling; R8: 成熟期 Maturity harvest; FR: 鲜根 Fresh root; 下同 Same as follows.)

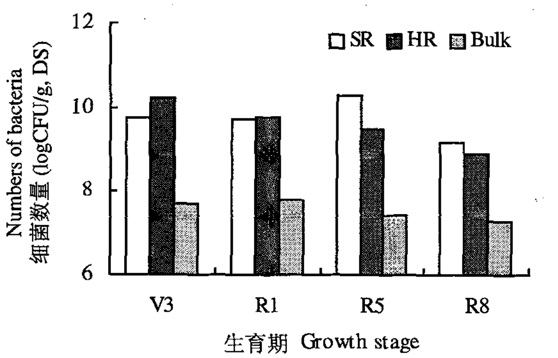

图2 不同品种大豆根际和土体细菌数量

Fig.2 Numbers of bacteria in rhizosphere and bulk soil of soybean cultivars

(HR: H25根际 Rhizosphere soil of H25; SR: S10根际 Rhizosphere soil of S10; DS: 干土 Dry soil; 下同 Same as follow)

2.2 不同品种大豆根面可培养真菌和镰孢霉(*Fusarium*)的动态变化

大豆三叶期和成熟期H25根面的真菌数和镰孢霉数高于S10(图3，图4)。苗期是大豆根腐病的高发期。三叶期感病品种H25根面比抗病品种S10根面定殖较多有害菌镰孢霉，成为根腐病的初侵染源。

通过统计各个生育期镰孢霉占真菌数的百分比，发现除H25的初花期和S10的鼓粒初期外，其它各生育期镰孢霉均是根面真菌的优势菌(比例大于30%)，而根际真菌只有到成熟期时镰孢霉才成为优势菌，土体真菌中镰孢霉所占比例最大时仅为17.7%(表1)。

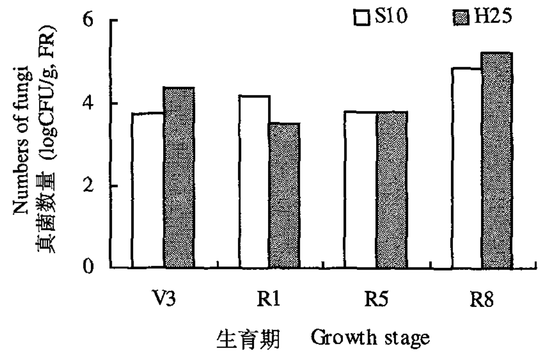

图3 不同品种大豆根面真菌数量比较

Fig.3 Numbers of fungi in rhizosplane of soybean cultivars

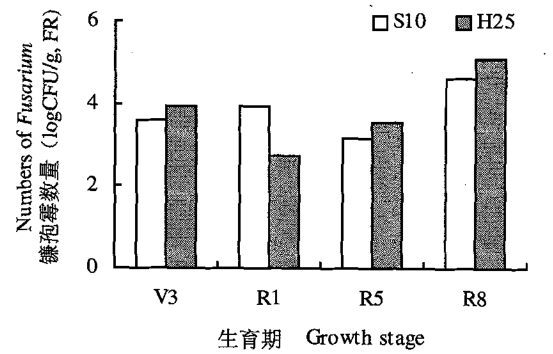

图4 不同品种大豆根面镰孢霉数量比较

Fig.4 Numbers of *Fusarium* in rhizosplane of soybean cultivar

表1 镰孢霉占真菌的百分比(%)

Table 1 The percentage of *Fusarium* sp. in fungi

项目 Item	生育期 Growth stage V3	R1	R5	R8
H25根面 Rhizosplane	37.1	17.0	57.1	74.9
S10根面 Rhizosplane	69.9	65.4	27.3	53.2
H25根际 Rhizosphere	8.7	7.7	2.9	66.2
S10根际 Rhizosphere	9.3	6.0	3.2	45.9
土体 Bulk	2.6	4.1	6.2	17.7

2.3 不同品种大豆根际和土体可培养真菌和镰孢霉(*Fusarium*)的动态变化

大豆根际真菌数在各个生育期均大于土体真菌数(图5)，除鼓粒初期外，根际镰孢霉数也高于土体的(图6)，说明连作条件下大豆根系分泌物对根际真菌数量和种类有进一步的选择作用，使有害菌镰

孢霉不断积累。抗感两个品种在三叶期和初花期根际真菌总数无差异,鼓粒初期和成熟期抗病品种根际真菌数高于感病品种 H25。两品种大豆根际的镰孢霉数在整个生育期均无差异。

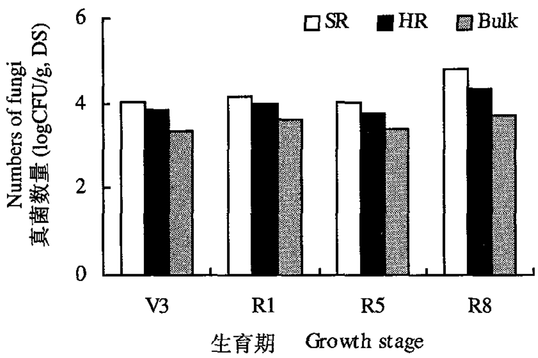

图 5 不同品种大豆根际和土体真菌数量

Fig.5 Numbers of fungi in rhizosphere and bulk soil of soybean cultivars

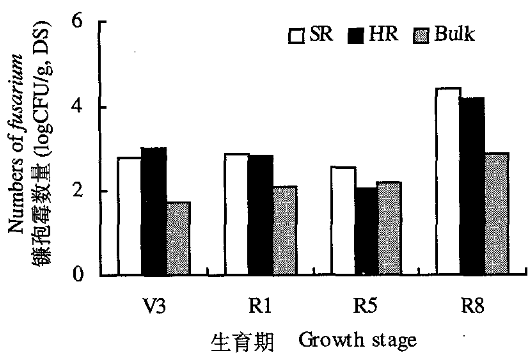

图 6 不同品种大豆根际和土体镰孢霉数量

Fig.6 Numbers of *Fusarium* in rhizosphere and bulk soil of soybean cultivars

2.4 不同品种大豆根瘤重的比较

在大豆的初花期和鼓粒初期,分别测定两品种根瘤重,抗病品种 S10 根瘤重均显著高于感病品种 H25(表 2)。

表 2 不同品种大豆根瘤重比较

Table 2 Weights of root nodule of different soybean cultivars

品种 Cultivars	根瘤重 (mg/g,FR) Weights of root nodule	
	初花期 Beginning of flowering	鼓粒期 Early grain filling
S10	2.48 a	17.86 a
H25	0.15 b	2.04 b

注:不同字母表示差异达 5%显著水平。

Note: Different letters mean significant at 5% level.

3 讨论

以前试验表明[6],种植一季后感染根腐病的大豆品种根面和根际积累的病原生物镰孢霉明显高于抗病品种,感病品种大豆的根瘤重明显低于抗病品种。除去残茬后的土壤继续种植后,在大豆各个生育期根面和根际真菌种类均较单一,优势菌为镰孢霉 *Fusarium* sp.或青霉菌 *Penicillium* sp.;而土体相对丰富,没有明显的优势菌,主要种类有:镰孢霉(*Fusarium*)、青霉(*Penicillium*)、黑根霉(*Rhizopus*)、轮枝孢(*Verticillium*)、曲霉(*Aspergillus*)和木霉(*Trichoderma*)等。

本试验结果表明,两个品种大豆根面细菌随生育期呈递减趋势,品种间无差异。除个别生育期外,这两个品种根面的主要优势菌为镰孢霉,成熟期根际的优势菌也是镰孢霉。而且分离到的两株优势镰孢霉为尖孢镰刀菌(*Fusarium oxysporm* Schlecht.)和茄腐镰刀菌[*Fusarium solani* (Mart.) Sacc.],是大豆根腐病的主要致病菌[7-8]。在大豆初花期和鼓粒初期,抗病品种根瘤重均高于感病品种,与正茬具有相同的趋势。可能是由于根分泌物的组成和数量的差异,引起根际环境的变化,根系发育不良,进而使结瘤能力下降[9]。

徐瑞富等[10]研究不同连作年限的花生田土壤微生物群落,表明连作可导致土壤中某些病原真菌(如引起花生根腐病的镰孢霉 *Fusarium*)的富集。本试验结果表明,在连作的条件下,无论是抗病品种 S10 还是感病品种 H25,均造成根面、根际真菌种类单一化,根面细菌数量的减少和病原菌(镰孢霉)数量的积累。表明大豆根系分泌物在正茬条件下对根际微生物的选择性促进或抑制的基础之上[6],在连作条件下对根际微生物有更进一步的选择作用。因此,大豆根分泌物在连作障碍中起着不可忽视的作用。

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