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## 控释氮肥对抗除草剂转基因水稻田 土壤甲烷排放的影响

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**摘要:**采用温室盆栽和静态箱-气相色谱法,研究了控释氮肥对抗除草剂转基因水稻和亲本常规水稻稻田土壤甲烷( $\text{CH}_4$ )排放的影响。供试土壤为灌育型水稻土,氮肥种类为尿素和控释氮肥。结果表明,与对照(尿素)相比,控释氮肥提高了水稻分蘖数、株高、生物量及产量。水稻品种对 $\text{CH}_4$ 季节性排放规律没有明显影响, $\text{CH}_4$ 排放通量基本表现为,自水稻移栽后逐渐升高,移栽后62—92 d出现峰值,而后逐渐降低至水稻收获。与对照相比,控释氮肥可显著降低 $\text{CH}_4$ 排放通量和全生育期累积排放量。抗除草剂转基因水稻稻田土壤 $\text{CH}_4$ 排放通量和累积排放量均显著低于亲本常规水稻。研究认为,一次性基施控释氮肥和种植抗除草剂转基因水稻对有效减缓稻田甲烷排放具有重要意义。

**关键词:**控释氮肥; 抗除草剂转基因水稻; 甲烷; 排放通量; 水稻土

### Effect of controlled-release nitrogen fertilizer on $\text{CH}_4$ emission in transgenic rice from a paddy soil

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**Abstract:** Nitrogen is one of essential nutrients in rice production. Although nitrogen supply increased productivity, nitrogen utilization efficiency was very low in rice production. Some researchers revealed that nearly two-fifths of nitrogen input was lost in different pathways. Excessive use of nitrogen fertilizer not only caused waste of resources but also brought harmful impacts on eco-environment, such as greenhouse effect and pollution to water body and soil. Rice paddies are regarded as one of major  $\text{CH}_4$  emission sources with annual estimates about 31 to 112 Tg, accounting for 5%—19% of global total  $\text{CH}_4$  emissions.  $\text{CH}_4$  emission was promoted by application of fresh organic fertilizer and significantly reduced by biogas fertilizer after fermentation treatment in rice paddies. Effects of chemical nitrogen fertilizer on  $\text{CH}_4$  emission from rice paddies were complicated, which were controlled by soil C/N ratio, fertilizer type, fertilization amount and mode, etc. Fertilization affected  $\text{CH}_4$  emission through influencing soil physicochemical properties, soil microbial community (methanogens and methanotrophs) and plant growth (e.g. development of aerenchyma, formation of root exudates). Controlled-release nitrogen fertilizer (CRNF), as eco-friendly fertilizer, is able to delay nitrogen release, provide a synchronous N supply for plant, thus reduce the accumulation of inorganic N in soil and the risk of N losses. Fewer reports are available regarding the effect of controlled-release N fertilizer on  $\text{CH}_4$  emission in rice paddies. Genetic transformation in rice has achieved rapid development since the first transgenic modification in 1988. Genes containing traits such as resistant

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to insects, diseases, and tolerant to herbicides, drought and salt have been effectively transferred into different rice varieties. Transgenic rice brought higher yield with less labor intensity, cost and use of pesticides and environmental pollution. However, it is still under argument about the safety of transgenic rice on eco-environment and human health under commercial cultivation. A pot experiment with rice cultivars was conducted to investigate the effect of nitrogen fertilizers on CH<sub>4</sub> emission from a paddy soil under greenhouse conditions. The experiment was designed with two fertilizer types, i.e. urea and controlled-release nitrogen fertilizer (CRNF), and two rice cultivars, i.e. herbicide-resistant transgenic rice (*japonica* line B2) and its parent conventional rice (*japonica* cv Xiushui 63), and performed at the Station of Agricultural Meteorology, Nanjing University of Information Science and Technology, Nanjing, China. CH<sub>4</sub> emission was determined by the closed chamber method at 10-day interval during rice growing period in a loamy clay paddy soil. The results indicated that, compared with control (urea), CRNF supply increased tiller number, plant height, biomass and yield in rice. CH<sub>4</sub> fluxes gradually increased from 22 d after transplanting, then reached the main peak at reproductive phase (62—92 d after transplanting), and sharply decreased until rice harvest. In comparison with control (urea), one-time basal application of CRNF significantly decreased CH<sub>4</sub> emission from the paddy soil. The total CH<sub>4</sub> emission was significantly lower from the transgenic rice cultivar than the conventional rice cultivar. It is suggested that one-time basal application of CRNF and planting herbicide-resistant transgenic rice are helpful in mitigating CH<sub>4</sub> emission from the paddy soil.

**Key Words:** controlled-release nitrogen fertilizer; transgenic rice; methane; flux; paddy soil

水稻是主要粮食作物之一,人口增加和耕地减少是影响粮食供给的主要矛盾,增施化肥是提高粮食产量的有效措施。氮是肥料三要素之一,氮肥施用能促进农作物增产,但其肥料利用率仍普遍较低<sup>[1]</sup>。低肥效不但造成肥料资源的浪费,也会污染水体、土体和农产品等,对生态环境和食品安全带来不良影响<sup>[2-3]</sup>。

稻田是CH<sub>4</sub>重要的排放源,年均排放量约为31—112 Tg CH<sub>4</sub>,占全球CH<sub>4</sub>排放总量的5%—19%<sup>[4]</sup>。施肥是影响稻田CH<sub>4</sub>排放的重要因素之一。研究表明,添加新鲜有机肥能促进稻田CH<sub>4</sub>的排放<sup>[5]</sup>,而沼肥经发酵处理后可明显减少CH<sub>4</sub>的产生和排放<sup>[6]</sup>。化学氮肥对稻田CH<sub>4</sub>排放的影响较复杂,与土壤C/N比、肥料的种类、施肥量和施肥方式等有关<sup>[7-8]</sup>。

控释氮肥可延缓氮素释放速率,减少氮素损失并供植物持续吸收利用<sup>[9]</sup>,目前对水稻施控释氮肥的研究主要涉及氮的吸收利用、气态损失(如反硝化与氨挥发)和水稻产量等方面<sup>[10-12]</sup>,而对稻田甲烷排放方面的研究鲜有报道。转基因水稻可提高产量、减少劳动强度、降低成本,减少农药使用造成的环境污染,但其安全性尚不明确,商业化种植对生态环境及人体健康的影响备受关注和争议。因此,开展本研究对于进一步完善转基因水稻在施控释氮肥下生

态风险评价的内容具有重要的理论和实践意义。

## 1 材料与方法

### 1.1 盆栽试验与管理

盆栽试验于2010年5月—11月在南京信息工程大学农业气象试验站(32.0°N, 118.8°E)温室内进行。该站地处亚热带湿润气候区,年均降水量1100 mm,年均气温15.6 °C。供试土壤为潴育型水稻土,灰马肝土属,耕层土壤质地为壤质黏土,粘粒含量为26.1%,土壤pH值为6.2(1:1土水比),全碳、全氮的含量分别为19.4和1.45 g/kg。土壤经自然风干,除杂(石块和植物残体等),磨碎过10目筛,混匀备用。

该试验为水稻品种和肥料种类双因子试验,供试水稻为抗除草剂转基因水稻(B2)和亲本常规水稻(秀水63),供试肥料为控释氮肥(CRNF)和常规氮肥尿素(U)。试验采用完全区组设计,设4个处理:(1)常规水稻+常规施肥(PU);(2)转基因水稻+常规施肥(TU);(3)常规水稻+控释氮肥(PC);(4)转基因水稻+控释氮肥(TC)。每处理3次重复,共12个盆钵。

供试控释氮肥LPS100(N 40%)为树脂包膜类迟释型控释氮肥,施后第30天开始释放养分,形成S型释放曲线(CHISSO公司,日本)。供试常规肥料为尿素(N46%)、磷酸二氢钾(P 22.8%、K 28.7%)、氯

化钾(K 52.4%)。本试验各处理施用的氮磷钾总量养分相等,氮(以N计)、磷(以P<sub>2</sub>O<sub>5</sub>计)、钾(以K<sub>2</sub>O计)肥料施用量分别为150、150和150 kg/hm<sup>2</sup>。常规氮肥50%作基肥,50%分别于分蘖和孕穗期追肥。控释氮肥及各盆钵的磷钾肥全部基施。水稻种子经消毒处理后,于5月29日播种育苗,6月30日移栽,11月9日收获。每盆钵定苗2株,盆钵直径20 cm,高30 cm,每盆装入土壤5 kg,试验期间各处理盆钵土面始终保持约5 cm的水层。病虫害防治依据实际情况进行。

## 1.2 测定方法

气体样品的采集与分析采用静态箱-气相色谱法。在水稻生长期,采样间隔为10 d,采样时间为当日9:00—11:00。PVC静态箱底面半径为8.5 cm,箱高120 cm,箱体直径与盆钵内径相吻合。采样时将PVC静态箱与盆钵相扣,通过淹水层液封保证静态箱气密性。封箱后分别于0、15、30 min,用带有三通阀的针筒采集50 mL气样,将所采气样注入事先抽成真空的气袋中。同时,记录箱温、气温及土温(5 cm和10 cm)。所采气样用带有氢火焰离子检测器(FID)的气相色谱仪(GC-9890,上海)检测CH<sub>4</sub>浓度。CH<sub>4</sub>排放通量计算公式如下:

$$F = \rho \times H \times T / (T+t) \times 60 \times dc/dt$$

式中,F为CH<sub>4</sub>排放通量(mg m<sup>-2</sup> h<sup>-1</sup>);ρ为标准状态下CH<sub>4</sub>气体密度(0.714 kg/m<sup>3</sup>);H为采样箱气室高度;T为理想气体标准状态下的空气气温273.15 K;t为采样时箱内平均温度;dc/dt为箱内目标气体浓度随时间变化的回归曲线斜率。积分求得不同生育阶段和全生育期CH<sub>4</sub>累积排放量。

## 1.3 数据统计分析

试验数据用Excel 2003进行整理与绘图,用统计软件SPSS 13.0进行差异显著性检验和重复测量的多因素方差分析。

## 2 结果

### 2.1 控释氮肥对水稻生长的影响

表1表明,与等氮尿素分施相比,一次性基施控释氮肥提高了水稻分蘖数、株高、生物量及产量,转基因水稻增产23.99%、亲本常规水稻增产9.66%。水稻生长要素的交互分析表明,水稻品种对分蘖数及株高的影响达显著水平,肥料种类对株高、生物量和产量的影响达显著水平,水稻品种×肥料种类的互作效应对株高和地上部生物量达显著水平(*P*<0.05)。

表1 控释氮肥对水稻生长及产量的影响

Table 1 Effect of CRNF (controlled-release nitrogen fertilizer) on rice growth parameters and yield under greenhouse conditions

处理 Treatment	分蘖数 Tiller number	株高/cm Plant height	生物量 Biomass/kg		产量/kg Yield
			地上部 Straw weight	地下部 Root weight	
TU	16±1.73	103.23±2.84	29.72±2.62	23.28±0.45	18.13±0.67
PU	22±3.60	96.93±1.63	34.96±3.94	23.95±2.15	18.40±0.55
TC	17±3.46	111.20±1.44	44.28±1.67	28.31±5.93	22.48±1.95
PC	23±1.00	98.03±1.71	36.30±2.74	25.34±3.83	20.18±0.10

方差分析 ANOVA					
品种 Cultivar	*	*	ns	ns	ns
肥料 Fertilizer	ns	*	*	*	*
品种×肥料 Cultivar×Fertilizer	ns	*	*	ns	ns

水稻分蘖数测定在最大分蘖期;株高和产量测定在完熟期;地上部和根系干重测定在抽穗期;表中数据为3次重复平均值±标准误差;\*表明与对照相比达到显著水平(*P*<0.05);TU:转基因水稻+常规施肥 Transgenic rice + urea;PU:常规水稻+常规施肥 Parental rice + urea;TC:转基因水稻+控释氮肥 Transgenic rice + CRNF;PC:常规水稻+控释氮肥 Parental rice + CRNF

## 2.2 控释氮肥对水稻CH<sub>4</sub>排放通量的影响

由图1可见,与对照相比,一次性基施控释氮肥下稻田CH<sub>4</sub>排放的季节性变化并不相同。在水稻生育期内,各处理CH<sub>4</sub>排放通量的变化趋势均表现为,自水稻移栽后,逐渐升高,在生殖生长期(62—92 d)

出现峰值,然后逐渐降低至水稻收获,说明一次性基施控释氮肥未改变CH<sub>4</sub>排放通量的季节性变化趋势。但是,不同水稻品种稻田CH<sub>4</sub>排放通量峰值出现的时间和大小存在差异。转基因水稻的主峰值低于亲本常规水稻,出现在移栽后的72—82 d,而亲本

常规水稻则出现在移栽后的 82—92 d。分蘖期后(62—111 d),一次性基施控释氮肥处理的  $\text{CH}_4$  排放

通量显著低于等氮尿素分施处理( $P<0.05$ )。

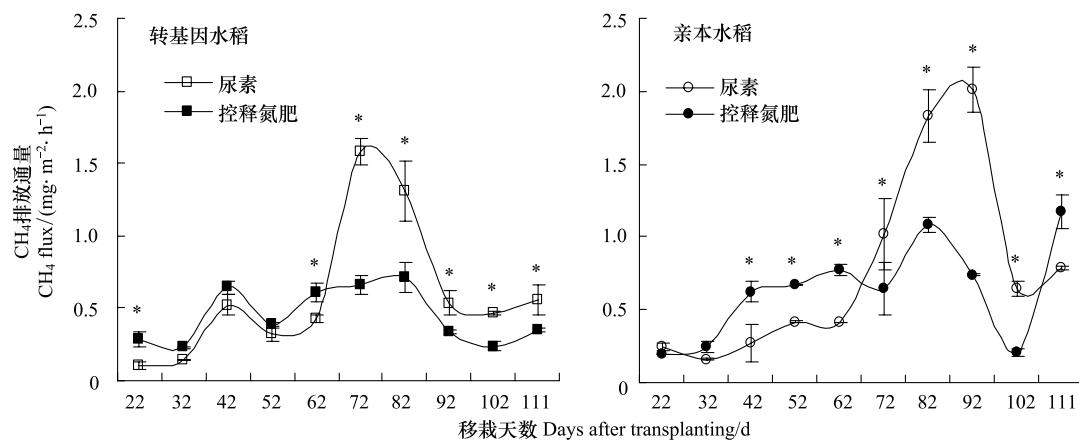


图 1 控释氮肥对  $\text{CH}_4$  季节性排放的影响

Fig.1 Effect of CRNF (controlled-release nitrogen fertilizer) on seasonal variation of  $\text{CH}_4$  flux under greenhouse conditions

对试验数据进行重复测量多因素方差分析,其中 Mauchly 球形检验  $P = 0.000$ ,采用 Greenhouse-Geisser 校正。方差分析结果表明,不同采样时间之间达显著性差异( $F = 194.071, P = 0.000$ ),时间与肥料种类( $F = 76.102, P = 0.000$ )、时间与水稻品种( $F = 47.166, P = 0.000$ ),以及时间与肥料种类、水稻品种之间( $F = 32.303, P = 0.000$ )均存在交互效应。组间效应的检验结果也说明,肥料种类、水稻品种、及两者之间的互作效应均有统计学意义( $P<0.05$ )。由此可见,稻田甲烷排放随时间的变化,以及时间因素作用随肥料种类、水稻品种和肥料种类×水稻品种的互作效应的不同而不同,肥料种类、水稻品种及两者的

互作效应对稻田甲烷排放的影响显著。

### 2.3 控释氮肥对水稻不同生育期 $\text{CH}_4$ 累积排放量的影响

从水稻不同生育期稻田  $\text{CH}_4$  累积排放量来看(表 2),以水稻灌浆至完熟期的累积排放量较高,占全生育期累积排放量的 26.21%—53.94%,但不同处理间存在差异。与等氮尿素分施相比,一次性基施控释氮肥显著降低抽穗—扬花期、灌浆—完熟期及全生育期稻田  $\text{CH}_4$  累积排放量( $P<0.05$ ),但转基因水稻分蘖期和亲本水稻分蘖期与拔节—孕穗期除外。

表 2 控释氮肥下水稻不同生育期  $\text{CH}_4$  累积排放量的变化

Table 2 Sub-total amount of  $\text{CH}_4$  emission during different rice growth stages fertilized with CRNF (controlled-release nitrogen fertilizer)

Treatment	分蘖期 Tillering		拔节—孕穗期 Jointing to booting		抽穗—扬花期 Heading to flowering		灌浆—完熟期 Grain-filling to maturity		全生育期 Whole growth period	
	排放量/ ( $\text{mg}/\text{m}^2$ )	占比/ (%)	排放量/ ( $\text{mg}/\text{m}^2$ )	占比/ (%)	排放量/ ( $\text{mg}/\text{m}^2$ )	占比/ (%)	排放量/ ( $\text{mg}/\text{m}^2$ )	占比/ (%)	排放量/ ( $\text{mg}/\text{m}^2$ )	
TU	209.91±10.31a	15.68	331.10±21.89b	24.73	346.33±14.15c	25.86	451.73±1.03b	33.73	1339.07±8.14b	
PU	184.85±16.60a	10.67	270.86±29.80a	15.63	342.39±7.92c	19.76	934.54±28.13c	53.94	1732.64±73.41c	
TC	290.81±4.64b	29.48	272.15±19.29a	27.58	165.07±28.55a	16.73	258.61±24.49a	26.21	986.64±29.11a	
PC	313.13±28.06b	23.28	344.89±0.31b	25.64	207.76±4.29b	15.44	479.50±5.97b	35.64	1345.28±20.49b	

表中数据为平均值±标准误差;同列中标记不同字母者代表处理间差异达显著水平( $P<0.05$ );TU:转基因水稻+常规施肥 Transgenic rice + urea; PU:常规水稻+常规施肥 Parental rice + urea; TC:转基因水稻+控释氮肥 Transgenic rice + CRNF; PC:常规水稻+控释氮肥 Parental rice + CRNF

不同水稻品种对各生育期  $\text{CH}_4$  累积排放量有一

定影响(表 2)。一次性基施控释氮肥下,各生育期

转基因水稻的  $\text{CH}_4$  累积排放量低于常规水稻,以拔节—孕穗期、抽穗—扬花期和灌浆—完熟期以及全生育期较为明显,差异达显著水平( $P<0.05$ )。

### 3 讨论

稻田  $\text{CH}_4$  的排放具有明显的季节性变化,但因水分状况、温度和肥料类型及其相互作用而异。本试验中,水稻移栽后(生长初期)的  $\text{CH}_4$  排放通量较低,原因在于盆栽用土经过筛去除了前作根茬等有机底物,使产  $\text{CH}_4$  菌缺少赖以生存和产生  $\text{CH}_4$  的物质基础,且水稻植株较小,根系及植株通气组织均不发达,  $\text{CH}_4$  排放的植株传输效率较低所致。随着水稻生长,根系分泌物和脱落物逐渐增加,水稻土产  $\text{CH}_4$  潜力不断提高,导致  $\text{CH}_4$  排放通量逐渐升高并出现峰值。

近年来,有关控释肥对水稻生长的影响已有较多报道。研究表明,一次性基施控释氮肥能提高水稻分蘖期生物量<sup>[13]</sup>、生育后期的根干重、根长和根系吸收面积<sup>[14]</sup>、以及通过增加单位面积有效穗和每穗结实粒数实现水稻增产<sup>[15]</sup>。本试验中,与尿素相比,控释氮肥可提高水稻分蘖数、生物量和产量(表1),但控释氮肥显著降低了水稻土  $\text{CH}_4$  的排放通量和累积排放量(图1,表2)。这一发现与一般认识相左,即水稻生物量、分蘖数与水稻土甲烷排放量一般呈正相关<sup>[16]</sup>。有研究表明,控释氮肥能提高水稻生育中、后期功能叶和根系中的 SOD ( superoxide dismutase )、POD ( peroxidase ) 等活性氧清除酶的活性,降低 MDA ( malonaldehyde ) 含量,从而延缓叶、根的衰老,提高水稻产量<sup>[14,17]</sup>。SOD、POD 等酶具有清除生物体内氧自由基的功能,保护生物体内免受自由基损害<sup>[18]</sup>。Keppler 等<sup>[19]</sup> 报道,有别于传统甲烷生物源,在有氧条件下很多植物本身可产生并释放甲烷。活性氧自由基( reactive oxygen species, ROS ) 可能在植物源甲烷的生成中起着关键作用。有报道指出,凡是引起 ROS 累积的胁迫条件(UV 辐射、干旱、营养缺乏等)都可能刺激其与果胶等物质中的甲氧基作用,继而产生甲烷<sup>[18,20-21]</sup>。本研究中,推测控释氮肥提高了水稻体内的活性氧清除酶的活性,清除了 ROS 的累积,减少了植株源甲烷产生的可能。稻田甲烷排放取决于其产生、氧化和传输的综合效应。根系能将来自叶片吸收和光合作用产生的氧气

释放到土壤中,形成根际微域“氧化圈”。而控释氮肥能改善根系形态和延缓根系衰老<sup>[14]</sup>,由此推测本研究中控释氮肥处理下良好的根系形态和活性提高了水稻根系的泌氧能力;而厌氧环境里,根系泌氧的减少诱发反硝化、铁硫还原反应导致根系发育不良甚至腐烂,又为甲烷的产生提供了丰富的底物。所以,尿素处理下水稻根系泌氧的“此消”和根系衰老腐烂的“彼长”导致控释氮肥下的水稻甲烷排放弱于普通尿素处理。另外,控释氮肥持续良好的供氮能力,促进了抽穗后光合产物的形成和抽穗前叶鞘和茎秆中临时性贮存的碳水化合物向穗部的转移<sup>[22]</sup>,使更多光合产物流向有机物积累,降低甲烷的排放。有关控释氮肥降低甲烷排放的机制,还需要通过试验进一步研究。

水稻品种在稻田甲烷排放中起着非常重要的作用<sup>[23]</sup>。本研究中,转基因水稻甲烷排放量显著低于亲本常规水稻(表2),说明种植抗除草剂转基因水稻对于减缓稻田甲烷排放有积极作用。其原因在于,一方面与转基因水稻的抗逆性和生长能力不同于亲本常规水稻有关<sup>[24]</sup>;另一方面转基因及其亲本不同基因型水稻的根际微生态环境有所不同。有研究发现,高产水稻因其根系发达,通气组织发育好,相应的根系径向泌氧量 ROL 和根际土壤氧气含量高于低产水稻<sup>[25]</sup>。3 种不同基因型水稻水培试验中,通过<sup>15</sup>N 稀释技术和 10  $\mu\text{m}$  的氧电极原位测定,发现高产水稻品种根际硝化强度和根表氧气含量显著高于低产品种<sup>[26]</sup>。不同的根系分泌物组成和数量、不同的根系泌氧能力和通气组织影响并形成了转基因及其亲本常规水稻稻田甲烷的排放差异。

因此,在施控释氮肥下,通过进一步试验探明水稻根系分泌物的数量和组成,检测水稻根际土壤产甲烷菌和甲烷氧化菌的变化,将有助于进一步阐明施控释氮肥降低水稻土甲烷排放,以及抗除草剂转基因水稻降低甲烷排放量的原因。研究认为,施用控释氮肥和种植抗除草剂转基因水稻对减少水稻土甲烷排放有积极意义。

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