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不同生长期转 Bt 基因水稻秸杆还土对淹水土壤酶活性的影响

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摘要：在实验室条件下通过秸杆还土试验比较了不同生长期转 Bt 基因克螟稻及其亲本稻秸杆对淹水土壤酶活性的影响。研究结果表明，与同一生长期的亲本稻秸杆相比，孕穗期和成熟期克螟稻秸杆对淹水土壤磷酸酶活性的影响较小；相反，对淹水土壤脱氢酶活性的影响非常显著，并且孕穗期秸杆与成熟期秸杆的添加对淹水土壤脱氢酶活性的影响趋势也存在较大差异。推测造成淹水土壤脱氢酶活性的显著性差异的主要原因可能是由于 Bt 插入基因表达的多效性所致。结果认为土壤脱氢酶活性可作为转 Bt 基因水稻生态安全风险性评价的潜在指标。

关键词：转 Bt 基因克螟稻；秸杆；土壤脱氢酶；土壤磷酸酶

Enzyme activities variation in flooded soils amended with Bt transgenic rice straws at different stages of plant development

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Abstract: The transformation of genes from *Bacillus thuringiensis* (Bt) that code for the production of insecticidal toxins into crops reduces the pollution associated with the application and run-off of chemical pesticides, because the toxins are produced continuously within these plants. However, there is increasing concern that these crops may pose risks to natural and agricultural ecosystems. So far, most of the research has focused on upland crops, such as Bt-corn, and Bt-potato. No studies have been carried out on the effect of Bt transgenic rice on the biochemical processes in flooded paddy soil.

In practice, rice straw is usually incorporated into soil to enhance soil fertility. As a result, toxins as well as other expressed foreign gene products may accumulate in soil and constitute a hazard to soil ecological processes. Soil enzyme activity has often been proposed to be an early and sensitive indicator of anthropogenic effects on soil ecology both in natural and agroecosystems. In the present study, we assessed the possible influences of Bt-transgenic rice straw, from cultivar KMD, on dehydrogenase and neutral phosphatase activities in flooded paddy soil under laboratory conditions. Cultivar KMD was derived from a commercial Chinese *Oryza japonica* rice variety Xiushu 11, transformed with a synthetic *cry*1Ab

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gene from *B. thuringiensis* and *gusA*, *hph* and *nptII* genes under the control of a maize spelling promoter. The experiments were conducted in plastic pots each containing 1500g air-dried fluvio marine yellow loamy soil amended either with 3% (w/w) Bt-transgenic rice straw or 3% (w/w) parental rice straw at the booting stage or at the maturing stage. Nine hundred mL sterile distilled water was added to submerge the soil. The flooded soils were then incubated in the dark at 28±1℃. Soil samples were taken from each pot at defined intervals over the incubation to assay for enzyme activity. Results showed that there were few significant differences in neutral phosphatase activity over the course of the incubation between soils amended with Bt-transgenic rice straw and parental rice straw either at the booting stage or at the maturing stage. However, significant differences in dehydrogenase activities between soils amended with Bt-transgenic rice straw and the parental rice straw were observed both at the booting stage and at the maturing stage. In addition, the trends of the changes of soil dehydrogenase activities over incubation time also varied. When soil amended with Bt-transgenic rice straw at the booting stage, dehydrogenase activities were significantly higher on sampling days 7 and 14, and lower on sampling days 21, 30, 35 and 49 than those in soil that had parental rice straw added at the same stages, but there were no significant differences on sampling days 70 and 84. When treated with straw at the maturing stage, dehydrogenase activities in soil amended with Bt-transgenic rice straw were significantly lower among sampling days 7 to 49, and higher on sampling days 70 and 84 than those in soil amended with parental rice straw. Soil dehydrogenase rice activity could be a potential index for risk assessment on the release of Bt-transgenic rice in the environment.

Key words: Bt-transgenic KMD; rice straw; soil dehydrogenase activity; soil phosphatase activity

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水稻是世界上最主要的粮食作物之一,其生产受稻螟虫和稻纵卷叶螟等鳞翅目害虫的严重威胁。据统计,全世界 1994 年水稻产量因虫害的损失高达 27%,损失额达数亿美元[1];目前,水稻生产主要依靠化学杀虫剂控制螟虫和稻纵卷叶螟等。化学杀虫剂的大量使用不仅引发了严重的环境污染问题,而且导致了二化螟、三化螟等害虫对多种杀虫剂产生明显的抗性,有些治螟药剂如三唑磷等甚至能促进稻飞虱产卵,引起甚至促进稻飞虱为害。转基因技术的发展,将外源 Bt 基因导入水稻,使之产生杀虫活性,为水稻螟害的防治开辟了全新的途径。然而,转 Bt 基因作物的外源基因所表达的产物在环境中的释放和积累可能引发环境污染和导致潜在的生态风险性等问题[2,3]。美国的 Stotzky[4,5]和 Donegan[6]研究小组先后对转 Bt 棉花和转 Bt 玉米所产生的杀虫蛋白的环境行为进行了研究,发现转 Bt 玉米和转 Bt 棉花的表达产物杀虫蛋白能迅速和紧密地与粘土矿物和腐殖酸及土壤表面活性颗粒等结合,其在土壤中的持留期可达 180d 以上,且仍保留相当高的杀虫活性(56%±11.9%和 68%±11.9%)[7]。由于在生产实践中,水稻秸杆以及生长发育阶段的残枝败叶、根系分泌物等通常'还田',因此转 Bt 基因"克螟稻"表达产物可以经秸杆还田和根系分泌等途径,释放到农田生态系统中去。若这些转基因表达产物在环境中形成积累,则有可能导致土壤中对植物生长发育和抗病特性相关的有益微生物种群结构和功能以及土壤肥力造成负面影响。目前,对转 Bt 基因农作物的生态风险性评价研究对象主要集中在转 Bt 玉米、Bt 棉花以及转 Bt 马铃薯等旱田作物[5,6,8,9]。至今未见有关转 Bt 基因水稻对淹水条件下土壤生物学活性的影响的研究报道。

本文以转 Bt 基因克螟稻(KMD)及其亲本稻秸杆为试材,在实验室条件下研究了转基因克螟稻不同时期秸杆还土对淹水土壤脱氢酶和磷酸酶活性影响。

1 材料与方法

1.1 材料

1.1.1 供试水稻秸杆及其处理 试验秸杆来源于转 Bt 基因"克螟稻"RT11 及其亲本水稻(秀水 11)。"克螟稻"RT11 为浙江大学核农所与加拿大渥太华大学合作采用农杆菌介导法培育而成的第 11 代转 Bt *cry1Ab* 基因纯合株系[10]。经喂虫试验表明转基因克螟稻对 8 种鳞翅目害虫具有 100%的致死率[11]。试验采用水稻生长的孕穗期和成熟期秸杆。新鲜秸杆经常温风干,旋风捣碎器磨碎,60 目过筛后置 4℃冰箱存放备用。其基本理化性状如表 1 所示。

表 1　供试水稻秸杆的基本理化性状

Table 1　General characteristics of rice straws used*

样品名称 Straws	水解性氮 (g/kg) Available N	速效磷(g/kg) Available P	速效钾 (g/kg) Available K	全碳(g/kg) Total carbon
孕穗期亲本稻秸杆 Parental rice straw at the booting stage	15.6(±0.01)	2.75(±0.06)	11.06(±0.27)	334.14(±3.45)
孕穗期克螟稻秸杆 KMD straw at the booting stage	18.7(±0.02)	2.91(±0.03)	12.38(±0.00)	317.07(±10.35)
成熟期亲本稻秸杆 Parental rice straw at the maturing stage	8.0(±0.01)	1.26(±0.04)	9.60(±0.00)	353.70(±3.45)
成熟期克螟稻秸杆 KMD straw at the maturing stage	8.9(±0.06)	1.72(±0.05)	4.90(±0.20)	326.80(±3.45)

* 样品用浓 H_2SO_4-H_2O_2 消煮,水解性氮用碱解扩散法测定;速效磷用钼锑抗比色法测定;速效钾用火焰光度法测定;全碳用 $K_2Cr_2O_7$ 氧化法测定

1.1.2　供试土壤及其处理　供试土壤为具代表性的黄松土,采自浙江大学华家池校区实验农场未种植过转基因克螟稻的水稻田表层土(0～15 cm)。土样经室温风干,碾碎,过筛(2 mm)去杂后置 4℃冰箱保存备用。供试土壤基本理化性状为:有机质 14.1 g/kg,水解性氮 115.5 mg/kg,速效磷 25.2 mg/kg,速效钾 58.5 mg/kg,pH(H_2O)为 7.0。

试验设置 4 个处理,分别为 1)1.5 kg 含 3% (w/w) 孕穗期亲本稻秸杆(NBTS1)的风干土,2)1.5 kg 含 3% (w/w) 孕穗期克螟稻秸杆(BTS1)的风干土,3)1.5 kg 含 3% (w/w) 成熟期亲本稻秸杆(NBTS2)的风干土和 4)1.5 kg 含 3% (w/w) 成熟期克螟稻秸杆(BTS2)的风干土。带秸杆的土样经滚筒充分混匀 30 min 后,分别置于直径 15 cm,高 13 cm 的盆钵中。加蒸馏水 900 ml 淹水约 1 cm,28±1℃恒温室避光培养。定期取样分析土壤酶活性。采用玻璃管(Φ25 mm)3 点垂直采样,经充分混匀后备用。每次取样后均补充等量蒸馏水以维持相同的淹水状态。各试验处理均设置 3 次重复。

1.2　土壤酶活性的测定

土壤脱氢酶活性的测定采用 TTC 还原法[12];土壤磷酸酶活性的测定用苯磷酸二钠为基质,培养后比色法测定酚的生成量[13]。脱氢酶试验使用新鲜培养土,磷酸酶试验使用风干土。

1.3　数据处理

试验处理间数据的差异性分析采用 DPS 数据处理软件[14]中的 Duncan's 新复极差测定方法进行(在 $p=0.05$ 水平上进行比较)。

2　结果与讨论

2.1　克螟稻不同生长期秸杆还土对淹水土壤磷酸酶活性的影响

转 Bt 基因克螟稻及其亲本稻不同生长期秸杆的还土试验结果表明:孕穗期克螟稻秸杆的添加对淹水土壤磷酸酶活性没有显著影响(图 1);成熟期克螟稻秸杆的添加在培养初期(7～14d)对淹水土壤磷酸酶活性略有影响,其磷酸酶活性略高于亲本对照,培养 22d 后,两者之间的酶活性无明显差异(图 2)。造成培养初期添加成熟期转基因克螟稻秸杆处理土壤磷酸酶活性

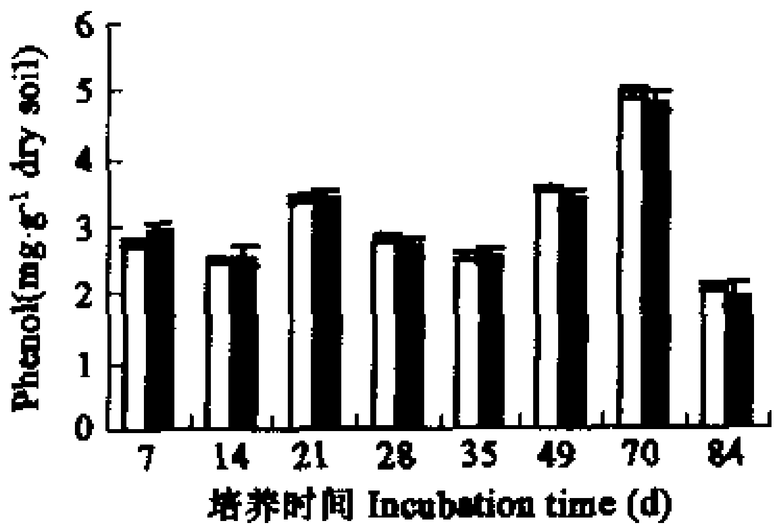

图 1　孕穗期克螟稻及其亲本稻秸杆对淹水土壤磷酸酶活性影响的差异

Fig. 1　Variations of KMD and its parental rice straws at booting stage on the phosphatase activities in flooded soil

□NBTS2　■BTS2

大于亲本稻秸杆的原因有可能是由于两种秸秆中速效磷含量的差异。由表1可见，成熟期克螟稻秸秆中的速效磷含量与相应的亲本稻秸秆相比差异显著，速效磷含量比亲本稻秸秆高出36.57%，而孕穗期克螟稻秸秆中速效磷的含量仅比亲本稻高5.82%。

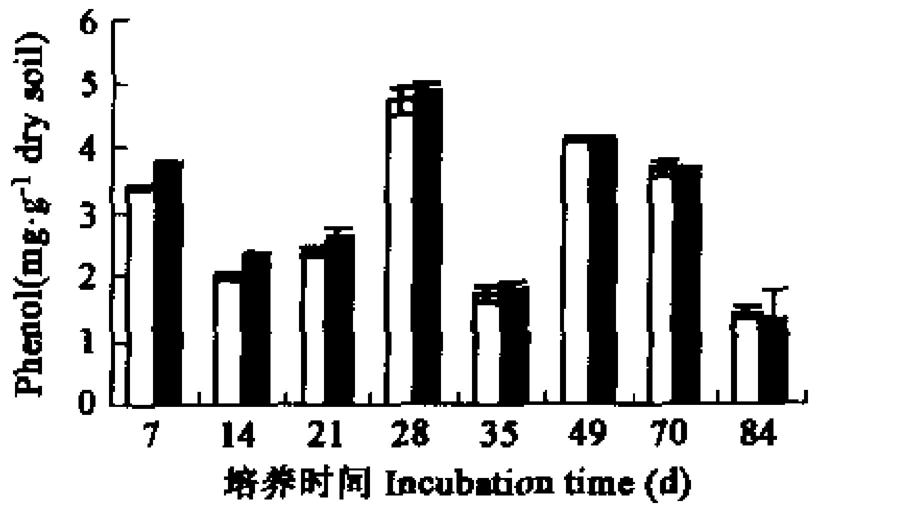

图2 成熟期转基因水稻及其亲本稻稻秸秆对淹水土壤磷酸酶影响的差异

Fig. 2 Variations of KMD and its parental straws at the maturing stage on the neutral phosphatase activity in flooded soil

□NBTS2 ■BTS2

2.2 克螟稻不同生长期秸秆还土对淹水土壤脱氢酶活性的影响

不同生长期的克螟稻秸秆还土对淹水土壤脱氢酶活性的影响见图3和图4。试验结果表明淹水土壤脱氢酶活性对转基因克螟稻不同时期秸秆的添加反应极其敏感。从图3可见，与亲本对照相比，孕穗期克螟稻秸秆的添加在培养初期(前两周)显著地促进了淹水土壤脱氢酶的活性；但随着培养时间的延长，却表现出较明显抑制作用。然而这种抑制性并不持续，培养第70天和第84天的结果分析表明两种孕穗期秸秆对淹水土壤脱氢酶活性的影响基本相似。相比之下，淹水土壤脱氢酶活性对成熟期克螟稻秸秆的反应更为敏感。由图4可见，在整个培养过程中，添加成熟期克螟稻秸秆的淹水土壤脱氢酶活性与亲本对照土壤脱氢酶活性之间的差异极其显著。在培养的前56d，成熟期转基因水稻秸秆还土对土壤脱氢酶活性具有强烈的抑制作用，其酶活仅为亲本对照组的32.48%～67.46%；然而，培养的第70天和第84天的取样分析表明成熟期转基因克螟稻还土对土壤脱氢酶活性具有显著的促进作用，此时的克螟稻秸秆处理土样中脱氢酶的活性分别为亲本对照的2.475倍和4.129倍。

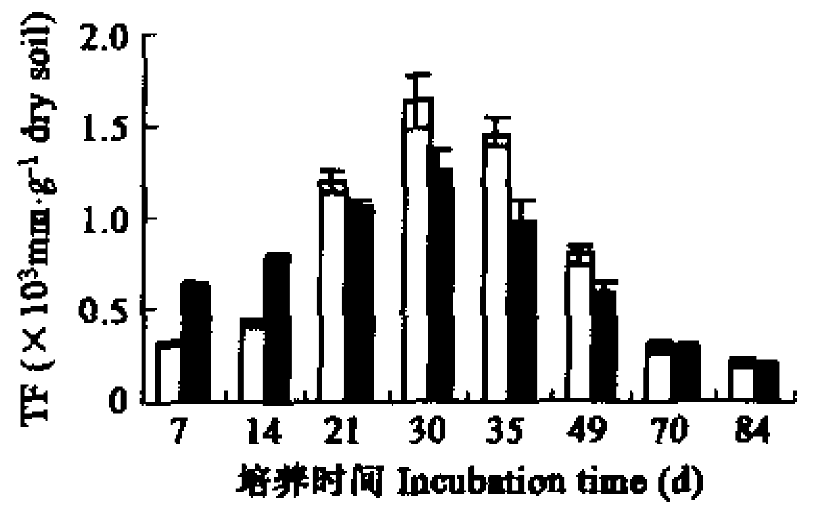

图3 孕穗期克螟稻及其亲本稻秸秆对淹水土壤脱氢酶活性影响的差异

Fig. 3 Variations of KMD and its parental rice straws on the dehydrogenase activities in the paddy soil

□NBTS2 ■BTS2

脱氢酶存在于所有活生物细胞。在土壤中，脱氢酶活性是土壤微生物种群及其活性的重要敏感性指标[15,16]，已被广泛用于土壤微生物活性和农用化学物质的对土壤微生物影响的直接测定[17,18]。导致不同生长期克螟稻秸秆对土壤脱氢酶活性显著差异的主要原因可能是外源基因在水稻染色体中的插入引发基因表达的多效性效应，进而引起水稻秸秆化学组成的变化所致。克螟稻大田生长试验表明其农艺性状也发生了不同于非转基因亲本的明显变化，如植株高度降低、分蘖力增强、穗长、穗粒数、千粒重明显下降等[19]。由表1可见，亲本稻秀水11中导入苏云金杆菌杀虫蛋白基因(*cry1Ab*)等外源基因后，水稻秸秆中的某些化学物质含量发生了较大的变化，如秸秆含碳量和速效钾含量的下降以及氮和磷含量的上升等。营养基质含量的较大差异可能导致淹水条件下土壤微生物种群数量、种群结构和组成的变化，从而导致土壤脱氢酶活性的显著差异。Donegan[9]和Stotzky[20]等人先后采用转Bt棉花和Bt玉米为试材，研究了Bt蛋白对旱田土壤微生物的影响，结果表明纯化的Bt蛋白对土壤微生物没

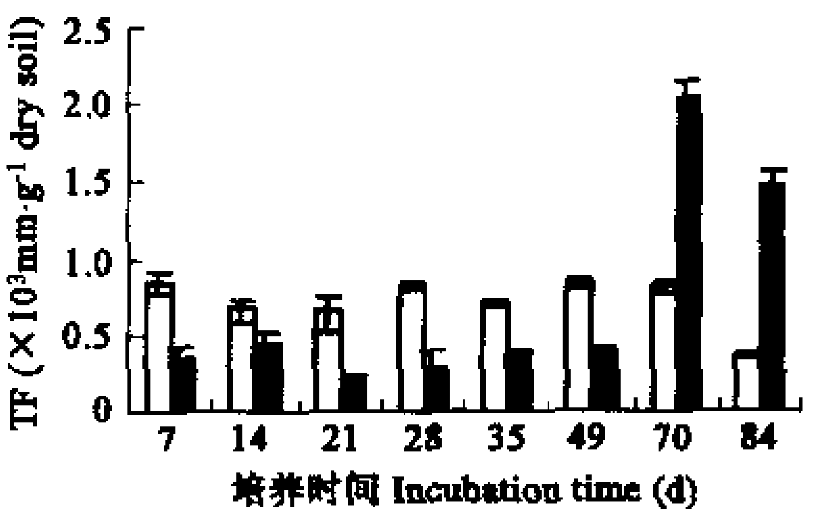

图4 成熟期克螟稻及其亲本稻秸秆对淹水土壤脱氢酶活性影响的差异

Fig. 4 Variations of KMD and its parental rice straws at the maturing stage on the dehydrogenase activity in flooded soil

□NBTS2 ■BTS2

有直接的影响,认为转基因植物对土壤微生物的影响是由于遗传操作、组培或基因表达的多效性效应导致植物生化特性的变化所致。此外,吴刚[21]等在对 *cry1Ab* 基因在转基因"克螟稻"后代表达的时空特性研究中发现,Bt 蛋白在叶片中的含量在不同代数水稻间表现出一致的变化规律,即孕穗期>分蘖期>成熟期>苗期>抽穗期。本研究中不同生长期克螟稻秸秆的添加对淹水土壤脱氢酶活性影响趋势的明显不同(图 3～图 4)也表明淹水土壤脱氢酶活性变化可能不是由于转基因水稻克螟稻秸秆中转入的 Bt 基因表达产物蛋白的直接作用结果。尽管如此,鉴于土壤脱氢酶活性对转基因克螟稻秸秆添加的高度敏感性,作者认为土壤脱氢酶活性的测定可以作为转基因水稻生物安全性评价的潜在性指标。

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