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叶面喷施二巯基丁二酸对晚稻籽粒镉及矿质元素含量的影响

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摘要:通过考察在水稻开花期叶面喷施一次重金属螯合剂2,3-二巯基丁二酸(DMSA)调控Cd经水稻韧皮部向籽粒中转运,评估将DMSA作为降Cd叶面调理剂的可行性。田间试验过程中,以DMSA为研究对象,采用小区试验法研究了DMSA对Cd及部分矿质元素在水稻不同器官中分布的影响,探讨了DMSA阻控Cd向水稻籽粒迁移转运的机制。结果表明:在晚稻开花期叶面喷施一次DMSA,可使晚稻籽粒中Cd含量降低15.84%~46.09%,穗轴中Cd含量降低10.03%~41.41%,穗颈中Cd含量降低9.13%~28.46%,顶端第一节中Cd含量降低18.30%~38.32%,对其他器官中Cd含量无显著影响。水稻籽粒Cd含量与DMSA喷施浓度在一定范围内呈现出明显的剂量效应关系,但是当喷施浓度超过 $4 \text{ mmol} \cdot \text{L}^{-1}$ 时籽粒中Cd含量则不再持续降低。喷施DMSA降低了籽粒中Mn含量,但是对其他矿质元素如K、Mg、Ca、Fe、Zn的含量则无显著影响。喷施DMSA显著降低了水稻成熟期旗叶Cd向顶端第一节的转移系数,同时Cd由穗颈向穗轴中的转移系数也表现出降低趋势。据以上结果推测,在水稻开花期叶面喷施DMSA,主要是通过与水稻叶片等组织中的Cd²⁺形成螯合物来降低Cd向籽粒中的转运。喷施DMSA同时降低晚稻籽粒中Mn含量,是否表明影响了Cd、Mn公用转运基因*Nramp5*的表达仍有待进一步研究。

关键词:水稻;镉;2,3-二巯基丁二酸;叶面喷施

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Foliar application of DMSA : Effects on Cd and other mineral elements in rice grains

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Abstract: Cd pollution in farmland soils has become one of the most important agricultural environmental problems in China. In this study, the feasibility of 2,3-dimercaptosuccinic acid (DMSA) as a foliar modulator for the reduction of rice grain Cd content was evaluated. After spraying DMSA on rice leaves during flowering, we monitored the transport of Cd from phloem to grain. Moreover, we analyzed the effects of DMSA on the distribution of Cd and some other mineral elements in different parts of the rice, and discussed the mechanism through which DMSA could prevent the migration of Cd to the rice grains. The results showed that the application of DMSA on the leaf surface of late rice reduced the rice grain Cd content by 15.84%~46.09%; additionally, the Cd content in the rachis, the first internode, and the first node decreased by 10.03%~41.41%, 9.13%~28.46%, and 18.30%~38.32%, respectively. No significant difference in Cd content was noted in the other organs of the plant. The rice grain Cd content and the concentration of sprayed DMSA showed an obvious dose-effect relationship within a certain range; however, once the spraying concentration exceeded $4 \text{ mmol} \cdot \text{L}^{-1}$, the rice grain Cd content did not decrease continuously anymore. The results of this study can be used to support agricultural production. We further noticed that the foliar application of DMSA reduced the rice grain Mn content, but did not have significant effects on the content of other mineral elements (e.g. K, Mg, Ca, Fe, and Zn). After the application of DMSA, the transfer coefficient of Cd from the flag leaf to the first node of the plant was significantly re-

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duced. The transfer coefficient of Cd from the first internode of the plants to the grains also showed a decreasing trend, but the reduction was not significant. According to the above results, the application of DMSA on rice leaves during flowering can reduce the transport of Cd to the grains, mainly through the chelation of Cd²⁺ in the leaves and other tissues of the plant. The Mn content reduction in late rice grains, following the application of DMSA, might indicate that this acid affected the expression of gene *Nramp5* (a transporter of Cd and Mn); nevertheless, this hypothesis needs to be confirmed through further studies. Here, we provide new theoretical bases for the development of a new type of modulator that can reduce rice grain Cd content. The application of this modulator might ensure the quality and safety of rice in Cd-polluted farmland soils.

Keywords: rice; cadmium; 2,3-dimercaptosuccinic acid; foliar application

镉(Cd)是一种对人体毒性较高的有害重金属元素^[1]。由工、农业生产导致的环境污染,已经使我国有大约 $2.786\times10^5\text{ hm}^2$ 的农用地受到Cd污染^[2],耕地Cd污染点位超标率高达7%^[3],农田Cd污染已成为重要的农业环境问题。水稻籽粒中的Cd主要来源于土壤^[4],土壤轻度Cd污染即可导致稻米Cd含量超过国家食品安全限量标准^[5],由食用稻米导致的Cd摄入量占我国人群Cd摄入量的56%^[6]。由于土壤Cd污染的隐蔽性、累积性和长期性以及Cd在水稻中累积受其自身形态^[7-9]、作物品种及生育期^[10-11]等多种因素影响,我国大面积稻田Cd污染治理技术难度大,国内外至今仍缺乏成熟且能被大面积推广应用的修复技术^[12-14]。因此,研发环境友好、农户可接受且易于大面积推广的农艺生产措施,对保障水稻安全生产具有重要意义。

Cd向水稻籽粒迁移转运的机制十分复杂。水稻通过木质部从根向茎转运Cd的能力是决定水稻茎秆中Cd积累浓度的主要影响因素^[15]。Cd进入水稻茎、叶等组织后则主要通过韧皮部向水稻籽粒中转运,几乎100%的籽粒Cd来源于韧皮部运输^[16-17]。在水稻开花期调控Cd经韧皮部向籽粒中转运是一种降低水稻籽粒Cd含量的重要途径。近年来,大量研究表明在水稻开花期喷施Si^[18-19]、Se^[20]、Zn^[21]、Mn^[22]等元素,可以显著降低Cd在水稻籽粒中的累积量。此外,在水稻开花期喷施小分子酸类物质也可以显著降低水稻籽粒中的Cd含量^[23]。当前,在水稻开花期喷施叶面调理剂已成为一种降低水稻籽粒Cd含量的重要农艺措施,具有广泛的应用前景。

重金属螯合剂2,3-二巯基丁二酸(DMSA)分子中含有两个巯基,可以与多种有毒重金属如Cd²⁺、Pb²⁺、Hg²⁺等形成稳定的螯合物^[24],在医疗上可以作为重金属中毒的解毒剂。该化合物具有水溶性较好、毒性低的特点,在全球范围内得到广泛应用^[25]。本研究于水稻开花期叶面喷施一次DMSA以探索喷施

DMSA调控水稻韧皮部Cd转运降低水稻籽粒中Cd含量的可行性,并探讨DMSA降低水稻籽粒中Cd含量的潜在机制。

1 材料与方法

1.1 试验材料与试验地点

在湖南省湘阴县鹤龙湖镇黄花岭村选择土壤和稻米Cd含量均超标的水稻田进行试验。试验田土壤类型为水稻土,其基本理化性质为:pH 6.21,有机质40.83 g·kg⁻¹,全氮0.173%,全磷0.019%,全钾1.3%,速效钾83.87 mg·kg⁻¹,速效磷16.2 mg·kg⁻¹,阳离子交换量18.92 cmol·kg⁻¹,Cd 0.71 mg·kg⁻¹,Mn 360.71 mg·kg⁻¹,Zn 172.71 mg·kg⁻¹。

以当地主栽品种黄花占为试验材料,种子购于当地种子公司。2,3-二巯基丁二酸(DMSA)购于国药集团,分析纯。

1.2 试验方法

田间小区面积设定为10 m²(5 m×2 m)。试验小区共分1个对照处理组和3个试验处理组,每个处理组重复4次。将适量DMSA溶于1 mol·L⁻¹的KOH溶液中,用田间灌溉水稀释至2 L,用0.5 mol·L⁻¹的HCl调节pH为8.0~8.5,分别配制成2、3、4、5 mmol·L⁻¹的DMSA水溶液。于水稻开花期向叶面手动均匀喷施处理液1次。对照组喷施2 L pH为8.0~8.5的田间灌溉水。

水稻采用旱育秧方式,于2018年7月18日移栽。施肥方法依照水稻测土配方施肥技术,每公顷施用尿素398 kg、钾肥210 kg,其中基肥占总施肥量的62%,分蘖肥占总量的29%,穗粒肥占总量的9%。整个生育期无显著病虫害发生。

1.3 样品的采集与处理

于水稻成熟期,选取小区中心长3.0 m、宽1.5 m处喷施较为均匀部分,用铁锹每小区随机连根挖取3株水稻植株,装入网袋。常温自然风干后手动将水稻植株分为籽粒、穗轴、穗颈、旗叶、顶端第一节、顶端第

二节、顶端第二叶、顶端第二节间、基部茎叶、根,共计10个部分。用蒸馏水漂洗3次后于70℃烘干72 h,冷却至室温后将各部分磨粉,分别收集到自封袋中,以备消解。

1.4 Cd及6种水稻矿质营养元素的测定方法

参照Liu等^[10]的方法,分别称取磨碎后的植株样品约0.25 g于消解管中,加入7 mL浓硝酸,摇匀,室温下静置12 h。将消解管放入电热消解仪上进行消解,110℃加热2.5 h后冷却至室温,再向消解管中加入1 mL H₂O₂摇匀,110℃继续加热1.5 h。将消解管内的液体于170℃下赶酸至0.5 mL以内,再将消解液稀释并转移至25 mL容量瓶内定容,用ICP-MS测定样品中Cd、K、Ca、Mg、Fe、Mn、Zn含量。本方法对7种元素的回收率为95%~105%,检出限为0.3~5.5 μg·kg⁻¹。

1.5 数据统计及分析

采用Microsoft Excel进行相关数据的计算、统计与处理。利用SPSS 17.0软件进行统计分析,新复极差法(Ducan's)进行多重比较、差异显著性检验。利用Origin 2018作图。

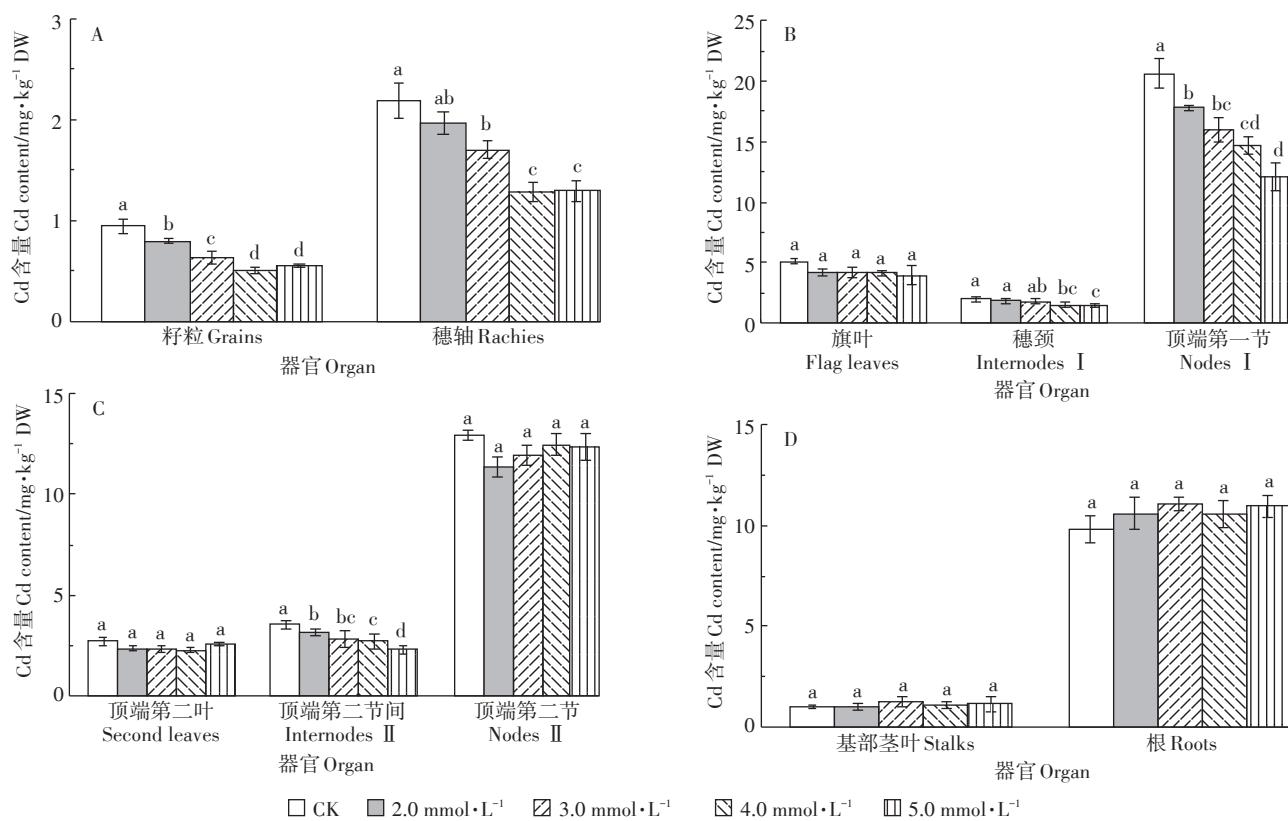
2 结果与分析

2.1 DMSA对水稻不同器官Cd含量的影响

由图1A可见,籽粒中Cd含量随着DMSA喷施浓度增加表现出显著的降低趋势,与对照相比籽粒中Cd含量降低幅度为15.84%~46.09%。但当DMSA使用浓度高于4 mol·L⁻¹时,籽粒中Cd含量未表现出持续下降,表明继续增加DMSA用量已不能起到持续降低籽粒Cd含量的效果。穗轴Cd含量也表现出显著下降趋势,不同处理Cd降低幅度为10.03%~41.41%,同样当DMSA喷施浓度高于4 mol·L⁻¹时穗轴中Cd含量也未出现持续降低现象。

由图1B可见,喷施DMSA后不同处理间旗叶中Cd含量与对照相比未出现显著差异,但是穗颈和顶端第一节中Cd含量出现显著降低,两种器官中Cd含量与对照相比的降低幅度为9.13%~28.46%和18.30~38.32%。

由图1C可见,喷施DMSA后不同处理间顶端第二节叶和顶端第二节中Cd含量与对照相比未出现显著



图中不同小写字母表示处理间差异达到5%显著水平($n=4$)。下同

Different lowercase letters indicate significant differences between different treatments at 5% level($n=4$). The same below.

图1 喷施DMSA对水稻各器官中Cd含量的影响

Figure 1 Effects of foliar application of DMSA on Cd content in rice organs

差异,但是顶端第二节间 Cd 含量则呈现出随 DMSA 喷施浓度增加而显著降低的趋势,降低幅度为 11.00%~34.76%。

由图 1D 可见,喷施 DMSA 后不同处理间基部茎叶和根中 Cd 含量无显著差异。

2.2 喷施 DMSA 对水稻 Cd 转运系数的影响

Cd 在水稻不同器官中的浓度分布反映了其在水稻器官间的迁移能力,一般用转移系数来表示。转移系数为相邻器官间 Cd 含量的比值:

转移系数($TF_{a/b}$)=a 器官 Cd 含量/b 器官 Cd 含量。

由图 2A 可见,在水稻开花期叶面喷施一次 DMSA 后,Cd 由穗颈向穗轴的转移系数($TF_{\text{穗轴}/\text{穗颈}}$)随着 DMSA 喷施浓度的增加表现出逐渐降低的趋势,但不同处理间转移系数的差异尚未达到显著水平。由图 2B 可见,当喷施 DMSA 后,Cd 由旗叶向顶端第一节的转移系数发生了显著变化,随着 DMSA 喷施浓度的增加,Cd 由旗叶向顶端第一节的转移系数($TF_{\text{顶端第一节}/\text{旗叶}}$)呈显著降低趋势,表明喷施 DMSA 减少了 Cd 由旗叶向顶端第一节的转运。开花期喷施 DMSA 对 Cd 在其他器官间的转移系数没有显著改变,表明喷施 DMSA 对 Cd 在其他器官间的迁移转运

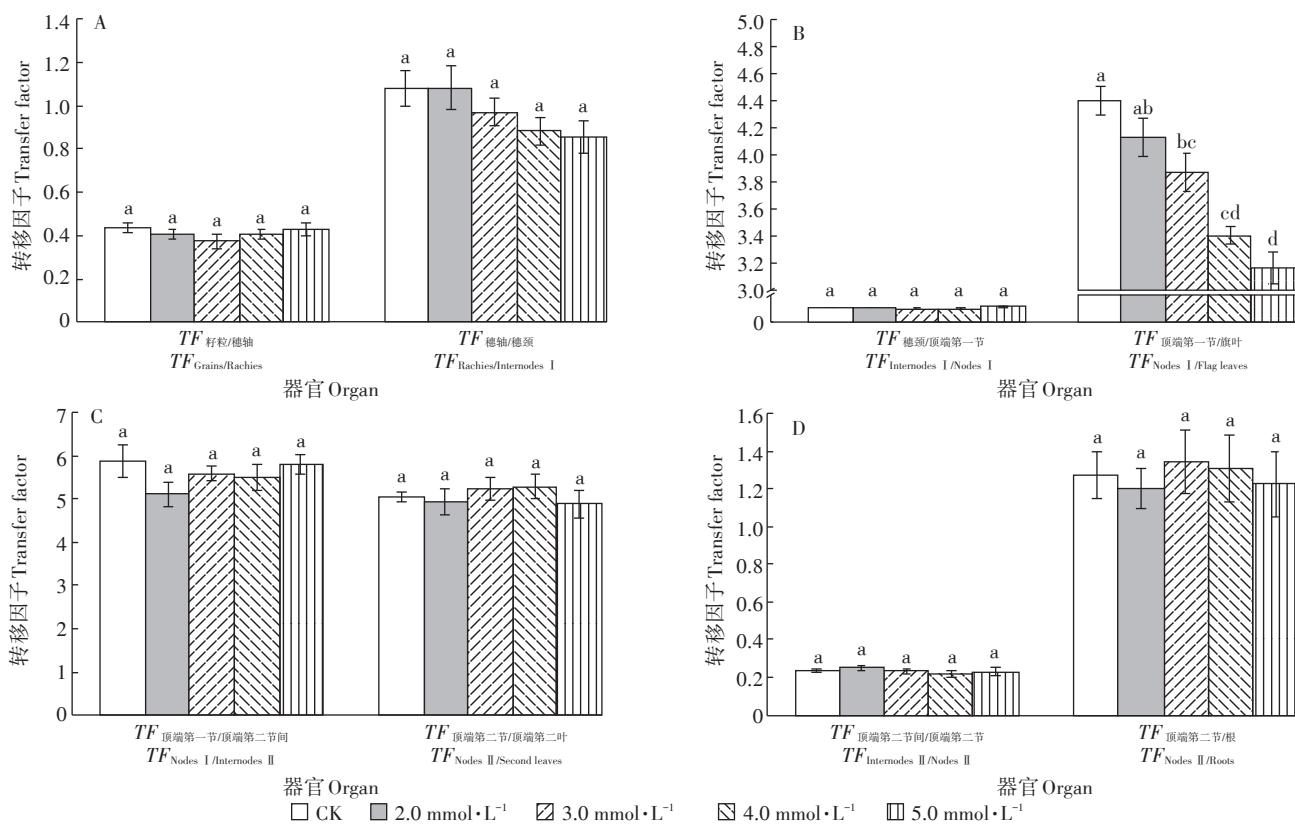
没有显著影响。

2.3 喷施 DMSA 对籽粒和穗轴中 6 种矿质元素含量的影响

水稻开花期叶面喷施一次 DMSA 对水稻籽粒和穗轴中的矿质元素含量影响如图 3 所示。由图 3A 至图 3E 可见,喷施 DMSA 对水稻籽粒和穗轴中的 K、Mg、Ca、Fe、Zn 含量的影响不大,4 个处理中矿质元素含量与对照相比未形成显著差异。但是,喷施高浓度 DMSA 对籽粒和穗轴中的 Mn 含量造成显著影响(图 3F),与对照组相比喷施 DMSA 导致籽粒和穗轴中 Mn 含量分别降低 5.79%~17.87% 和 13.51%~24.21%,表明水稻开花期叶面喷施 DMSA 影响了 Mn 向水稻籽粒中的转运。

3 讨论

动物实验表明,DMSA 在体内通过分子中含有的两个巯基与 Cd 形成稳定的螯合物,最终以螯合态排出体外从而达到降低 Cd 毒性的目的^[24~25]。有研究表明,Cd 在水稻韧皮部伤流液中大部分与蛋白类、植物螯合素等物质以结合态存在,小部分以离子形式存在^[26]。蛋白螯合态的 Cd 并非一种稳定形态,当遇到蛋白激酶后又会重新释放出 Cd²⁺。当 DMSA 进入水



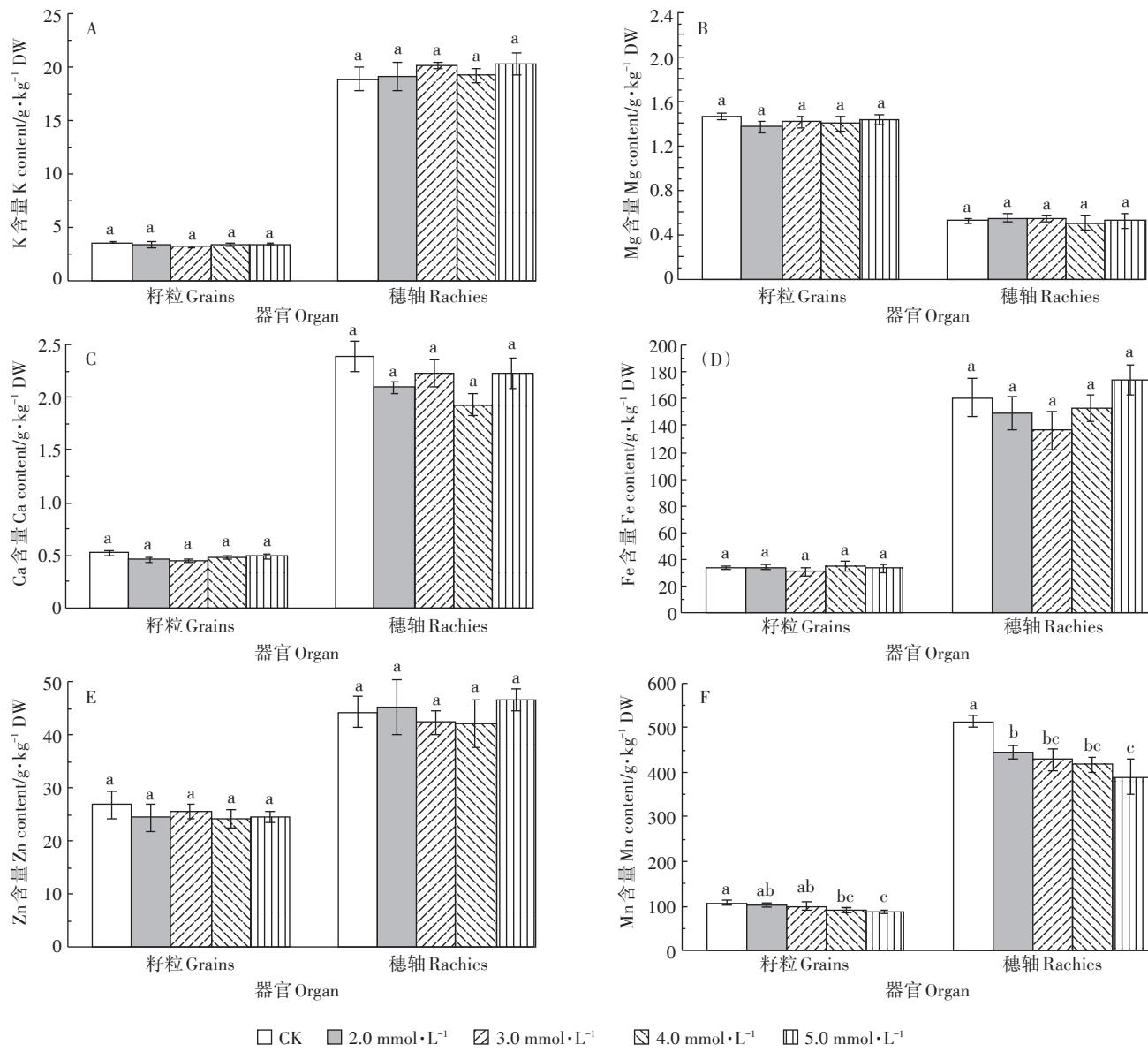


Figure 3 Effects of foliar application of DMSA on mineral elements concentration in grains and rachises

稻体内后可与Cd²⁺竞争性结合形成螯合物。植物自身也可合成多种含有巯基的解毒化合物如PCs^[27]、谷胱甘肽等^[28],它们都可以与Cd²⁺形成稳定的化合物并储存在植物液泡等器官中^[29],不仅降低了Cd对植物的胁迫效应,而且在一定程度上也降低了Cd向地上部的转运。据此推断,在水稻开花期叶面喷施DMSA主要是通过与水稻叶片等组织中的Cd²⁺形成螯合物来降低Cd向籽粒中的转运。可见,喷施DMSA降低籽粒中Cd含量的机制与喷施Zn²⁺^[21]、Mn²⁺^[22]等通过离子拮抗降低Cd向水稻籽粒中转运导致籽粒中Cd含量降低的机制有所不同,也与喷施Si^[18]增加水稻茎和叶片细胞壁固持Cd能力从而降低Cd向籽粒转运的

机制不同。为降低水稻籽粒中Cd含量,育种学家引入了基因编辑技术。当敲除负责调控水稻转运Cd和Mn的*Nramp5*基因^[30]后,无论是粳稻还是籼稻籽粒中Cd和Mn的浓度都出现大幅度降低,其中Mn浓度可下降80%以上^[31-32]。在本研究中,喷施DMSA对水稻籽粒和穗轴中必需营养元素K、Mg、Ca、Fe、Zn的含量没有显著影响,但是在降低籽粒中Cd含量的同时也显著降低了籽粒中Mn的浓度。喷施DMSA是否影响了*Nramp5*等基因的表达仍有待进一步研究。

关于水稻籽粒中Cd的来源日本学者给出了相互矛盾的结论。Fujimaki等^[33]利用¹⁰⁷Cd同位素示踪技术的研究结果表明,在灌浆期水稻从土壤中吸收的

Cd被运送到籽粒;Kashiwagi等^[34]的研究结果表明水稻籽粒中Cd主要来自于抽穗前累积在叶片和茎秆中的Cd,抽穗后从根运送到茎秆和穗部的Cd不影响糙米中Cd的累积。喻华等^[11]进一步研究表明,在田间水稻齐穗后土壤中有效Cd含量较低时,籽粒中的Cd同时来自于土壤和水稻体内在齐穗前累积的Cd。叶片是向籽粒净转移Cd的主要器官,叶片衰老期Cd通过韧皮部向籽粒转移是Cd再分配的主要过程^[35]。在本研究中,随着DMSA喷施浓度增加水稻籽粒中Cd含量呈现出显著降低趋势,最高降幅达到46.09%(图1A),而旗叶中Cd含量则未出现显著降低(图1B),表明喷施DMSA降低了旗叶中Cd经顶端第一节向籽粒的转运,对水稻籽粒Cd含量的降低具有较大贡献。Feng等^[36]的研究表明,水稻的根和节是Cd进入水稻地上部的主要障碍,这两个部位的Cd含量最高,当外界有效Cd含量较低时,进入水稻体内的大部分Cd被固定到这两个部位,这时水稻器官中Cd的再转运成为籽粒中Cd的主要来源。在本研究中开花期喷施DMSA显著降低了顶端第一节中Cd的含量(图1B),此现象与喷施纳米硅^[19]降低籽粒Cd含量的现象一致,表明顶端第一节中Cd含量的降低对籽粒Cd降低也具有较大贡献。

当开花期叶面喷施DMSA后,显著降低了Cd由旗叶向顶端第一节的转移系数(图2B)。同时Cd由穗颈向穗轴的转移系数(图2A)也出现降低趋势但并未达到显著程度,其他器官中Cd的转移系数并未见发生显著变化。此结果与喷施DMSA后水稻叶片中Cd含量未见显著变化相互印证。说明喷施DMSA影响籽粒中Cd含量降低的主要因素是降低了叶片Cd向籽粒的输送。

DMSA对人体的毒性较低,可以在医疗上作为人体Cd、As、Pb、Hg等^[24-25]重金属中毒后的解毒剂,因此其对人体的安全性很高。目前,利用DMSA阻控Cd向水稻籽粒中转运的研究鲜有报道,同时也未见DMSA是否对植物生长有负面影响的报道。本研究中同时开展了喷施DMSA对水稻产量影响的研究,稻谷产量与对照相比未见显著差异(本文未提供具体数据)。当DMSA的喷施浓度超过4 mmol·L⁻¹时水稻籽粒和穗轴中Cd含量并未出现持续降低(图1A),此结果为农业生产实践中DMSA的实际用量提供了指导。

4 结论

(1)在水稻开花期叶面喷施DMSA可以显著降低

籽粒中Cd含量。田间试验表明,喷施一次DMSA使籽粒中Cd最高降幅可达46.09%。

(2)旗叶Cd向顶端第一节中的迁移率降低对籽粒Cd含量降低有较大贡献。喷施DMSA显著降低了Cd由旗叶向顶端第一节中的迁移系数,表明与对照相比旗叶中Cd向籽粒中转运量显著减少。

(3)巯基化合物DMSA是一种潜在的降Cd叶面调理剂,具有较好的应用前景。

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