苯并[α]芘对不同修复潜力羊茅属植物的根系分泌物中几种低分子量有机物的影响

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摘要 根系分泌物是植物与土壤间进行物质交换和信息传递的重要载体, 是植物响应外界胁迫的重要途径, 也是构成根际微生态特征的关键因素。根系分泌物与有机污染物的植物修复密切相关, 研究胁迫条件下不同修复潜力植物间根系分泌物的释放特征有助于揭示植物修复的内在机制。该文借助根际袋土培试验研究了苯并[α]芘(BaP)胁迫下5种羊茅属(*Festuca*)植物根系不同生长期(30–70天)几种低分子量有机物的分泌特征。结果表明: 1) BaP浓度在10.25–161.74 mg·kg⁻¹范围内时, 待试植物能有效地促进土壤中BaP的去除, 其修复潜力依次为苇状羊茅(*F. arundinacea*) > 草原羊茅(*F. chelungkiangnica*) ≥ 毛稈羊茅(*F. rubra* subsp. *arctica*) ≥ 贫芒羊茅(*F. sinomutica*) > 细芒羊茅(*F. stapfii*)。2) BaP胁迫增强了植物根系对可溶性糖的分泌: 随着胁迫强度的增大、胁迫期的延长, 其分泌量变化呈“先升后降”趋势。3) BaP胁迫促进了植物根系低分子量有机酸的释放, 植物的修复潜力越大, 有机酸高峰值出现时的胁迫浓度越高; 组成成分较稳定, 草酸、乙酸、乳酸和苹果酸为主要组分(>97.34%), 在修复潜力较强植物的根系分泌物中检测出微量的反丁烯二酸。4) BaP胁迫对氨基酸种类影响不大, 但对分泌量影响较大。其中, 苏氨酸、丝氨酸、甘氨酸、丙氨酸的分泌量随BaP胁迫强度的增强而剧增; 脯氨酸、羟脯氨酸和天冬氨酸近乎以加和效应甚至协同效应的形式参与植物对BaP胁迫的应激反应: 参与应激组分的分泌量随胁迫强度的增强而剧增, 植物的修复潜力越强, 参与的组分越多。可见BaP胁迫下, 5种羊茅属植物根系分泌物中几种低分子量有机物的释放特征与植物自身的修复潜力有关: 修复潜力越强, 释放量越多且成分也越复杂, 并呈现出较强的环境适应性及生理可塑性。

关键词 苯并[α]芘; 羊茅属植物; 根系分泌物; 低分子量有机物; 种间差异

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Effects of benzo [α] pyrene on the organic compounds of low molecule weight excreted by root systems in five *Festuca* species with different remediation potentials

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Abstract

Aims Root exudates have specialized roles in nutrient cycling and signal transduction between a root system and soil, as well as in plant responses to environmental stresses. They are the key regulators in the rhizosphere communications and can modify the biological and physical interactions between roots and soil organisms. Phytoremediation is an important measure to remove organic pollutants from contaminated soil, and root exudates are considered to be closely related to the mechanisms in the phytoremediation of soils contaminated by organic pollutants. This study was designed to determine the characteristics of root exudates in five *Festuca* species under the stress of benzo [α] pyrene (BaP) and to identify the effects of BaP on the organic compounds of low molecule weight in root exudates.

Methods Five *Festuca* species, which had been tested to be tolerant to the BaP stress, were used in this study. A soil-cultivating test, with rhizobag technique, was conducted to investigate the effect of BaP concentration on the organic compounds of low molecule weight in root exudates at different growth stages (30–70 days). The BaP

concentrations in the contaminated soils were set for 10.25 mg·kg⁻¹, 20.37 mg·kg⁻¹, 40.45 mg·kg⁻¹, 80.24 mg·kg⁻¹, and 161.74 mg·kg⁻¹ (denoted by T₁, T₂, T₃, T₄ and T₅, respectively).

Important findings The presence of vegetation enhanced the dissipation of BaP in soils. This effect was especially marked in treatment with *F. arundinacea*, followed sequentially by that of *F. chelungkiangnica*, *F. rubra* subsp. *arctica* and *F. sinomutica*; the dissipation of BaP in treatment with *F. stapfi* was lowest during the entire experiment. The contents of soluble sugars, organic acids, and amino acids in root exudates were all increased by the BaP treatments. The contents of soluble sugars in root exudates increased notably at relatively low BaP levels (T₁–T₃) or in earlier stress stages (30–40 days), and declined at relatively high BaP levels (T₄–T₅) or in later stress stages (40–70 days), with highest values always occurring in the T₃ treatments on day 50 of the experiments. In the five *Festuca* species, oxalic acid, acetic acid, lactic acid and malic acid are the main constituents of organic acids in root exudates, at greater than 97.34% in total in all treatments. However, there are traces of fumaric acid in the root exudates of *Festuca* species with stronger remediation potentials. When the contents of organic acids in root exudates reached the peak, the stronger the remediation potentials of plants were, the higher the concentrations of BaP would be to induce stress. Nineteen types of common amino acids were found in root exudates of *Festuca* and the proportion of total amino acids in root exudates remain stable under all the BaP stress treatments, albeit varying contents of the 19 types of amino acids under different BaP concentrations. The contents of all amino acid in root exudates increased with increasing BaP concentrations; especially, the contents of secreted threonine, serine, glycine and alanine increased significantly among the 19 types of amino acids and the differences were significant among all treatments with different BaP concentrations ($p < 0.05$). However, proline, hydroxyproline, and aspartic acid participated in the stress responses of plants almost in the form of additive or synergistic effects, and their contents in root exudates increased markedly with increasing BaP concentrations in soils; the differences among different treatments were significant ($p < 0.05$). The more constituents of amino acids there were in stress responses, the stronger the remediation potentials of plants would be. All these illustrate that the characteristics of root exudates in *Festuca* were closely related to their remediation potential under the BaP stress. The greater the remediation potentials were, the more organic compounds of low molecular weight there were and the more complex those compounds would be. Moreover, they also showed a stronger environment adaptability and physiological plasticity.

Key words benzo [α] pyrene; *Festuca*; root exudates; organic compounds of low molecule weight; interspecies difference

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多环芳烃(polycyclic aromatic hydrocarbons, PAHs)是环境中普遍存在的持久性有机污染物,多数具有致癌、致畸或致突变效应(Chen & Liao, 2006)。因其性质稳定、水溶性差,易于被土壤颗粒吸附,土壤中PAHs含量逐年上升(Tao *et al.*, 2004; Li *et al.*, 2006),严重威胁着土壤环境质量及农产品质量安全;土壤中PAHs的迁移转化行为及其环境效应已成为环境修复领域的研究热点(Mueller & Shann, 2006; Lakshmanan *et al.*, 2014)。

植物的存在能加快土壤中PAHs的去除(Joner & Leyval, 2003; Kim *et al.*, 2004; Gao *et al.*, 2006),根系分泌物(root exudates)营造的根际微生态特征则是影响PAHs快速消解的重要原因(Sumia *et al.*, 2013;

Tejeda-Agredano *et al.*, 2013)。植物修复过程中,根系分泌物在改善土壤属性、提高污染物的生物可利用性、缓解环境胁迫等方面具有重要作用(Bertin & Yang, 2003; Phillips *et al.*, 2012; Shahzad *et al.*, 2015)。一方面,根系分泌的某些胞外酶能够直接参与PAHs的降解过程;另一方面,分泌物中的可溶性糖、低分子量有机酸、氨基酸等物质,为根际微生物提供充足的营养,增加根际微生物的数量、提高微生物活性,进而加速PAHs降解(Susarla *et al.*, 2002; Xie *et al.*, 2012; Lakshmanan *et al.*, 2014)。同时,根系分泌物还会直接影响PAHs的固定和活化,进而影响PAHs在土壤-植物系统中的迁移转化行为、促进PAHs去除(Marschner *et al.*, 2004; Abhilash *et al.*,

2012); PAHs的环数越多, 根际效益越明显(Sun *et al.*, 2010)。不同植物间, 因根系分泌物的组成成分及其释放特征常存在一定差异, 对根际微生物的区系结构、代谢活性的影响也不一样, 进而表现出不同的修复潜力(Kirk *et al.*, 2005; Luo *et al.*, 2006; Hunter *et al.*, 2014)。因此, 探讨PAHs胁迫下不同修复潜力植物间根系分泌物中主要组分的释放特征及其种间差异, 对于研究植物修复的生物学机制、揭示不同植物间修复潜力的种间差异具有重要意义。鉴于此, 本研究以对PAHs污染土壤具有一定修复潜力的羊茅属(*Festuca*)植物为试验对象, 以苯并[α]芘(BaP)为PAHs代表物, 借助根际袋土培法, 对比研究PAHs胁迫下不同修复潜力的5种羊茅属植物在不同生长期时根系分泌物中几种低分子量有机物的释放特征, 为植物修复理论的研究与实践提供依据。

1 材料和方法

1.1 试验材料

1.1.1 PAHs代表物

BaP是PAHs类污染物中分布最广、致癌性最强的烃类物质, 其来源广泛、稳定性强, 且与其他PAHs含量有一定相关性。本研究选择BaP (Aldrich, 纯度> 97%)为PAHs代表物。

1.1.2 供试植物

供试植物为5种羊茅属植物: 苇状羊茅(*F. arundinacea*, Fa)、草原羊茅(*F. chelungkiangnica*, Fc)、毛稈羊茅(*F. rubra* subsp. *arctica*, Fr)、贫芒羊茅(*F. sinomutica*, Fm)、细芒羊茅(*F. stapfii*, Fs), 种子购于四川省草原研究所。种子经3% H_2O_2 溶液消毒20 min、蒸馏水冲洗干净后于烧杯中浸泡吸胀24 h, 置于培养皿中恒温(35 $^{\circ}\text{C}$)催芽15 h后, 播种于根际袋。

1.1.3 供试土壤

供试土壤为中性紫色土, 采自成都大学试验农场表层土(5–20 cm, 44.6 $\text{pg}\cdot\text{L}^{-1}$ 检出限下未检测到BaP)。土壤理化性质: pH 7.19, 有机质含量 $2.14 \times 10^4 \text{ mg}\cdot\text{kg}^{-1}$, 阳离子交换量(CEC) 20.43 $\text{cmol}\cdot\text{kg}^{-1}$, 速效N、P、K分别为31.62、24.74、94.81 $\text{mg}\cdot\text{kg}^{-1}$; 砂粒、粉粒和黏粒的质量分数分别为71.27%、9.59%和19.14%。土壤风干后过2 mm筛, 备用。

1.2 试验方法

土培试验采用根际袋试验装置在智能温室内

进行(许超等, 2010), 涉及3个处理、5个水平(5个BaP胁迫水平下的5种植物、5个生长期), 重复3次。具体如下:

按照 150 $\text{mg}\cdot\text{kg}^{-1}$ $\text{CO}(\text{NH}_2)_2$ 、50 $\text{mg}\cdot\text{kg}^{-1}$ $\text{Ca}(\text{H}_2\text{PO}_4)\cdot 2\text{H}_2\text{O}$ 和100 $\text{mg}\cdot\text{kg}^{-1}$ K_2SO_4 比例补充底肥后, 取适量土壤过2 mm筛, 加入BaP丙酮溶液; 待丙酮挥发后, 用未污染土壤不断稀释、搅拌, 过2 mm筛、混匀, 结合农田土壤中PAHs的背景值, 获得5个污染水平。50%田间持水量(WHC)、室温状态下平衡7天(Pan *et al.*, 2008; Wei & Pan, 2010)后, 测得土样中BaP的初始含量分别为: 10.25 $\text{mg}\cdot\text{kg}^{-1}$ (T_1)、20.37 $\text{mg}\cdot\text{kg}^{-1}$ (T_2)、40.45 $\text{mg}\cdot\text{kg}^{-1}$ (T_3)、80.24 $\text{mg}\cdot\text{kg}^{-1}$ (T_4)和161.74 $\text{mg}\cdot\text{kg}^{-1}$ (T_5)。取污染土样2 kg装入20 cm $\times$ 15 cm的塑料盆钵中, 其中的400 g装在300目尼龙网袋中; 尼龙网袋放在盆中央, 四周及底部均为同质土样, 保持袋内外土壤高度相同。每种污染水平分装75盆, 50% WHC、室温状态下平衡5天(Pan *et al.*, 2008; Wei & Pan, 2010); 选取籽粒饱满、大小均匀的催芽后种子单播于袋内土壤中, 每种植物播种15盆, 每盆10颗, 置于温室中; 另设一组没有播种的污染土样作为对照, 每个处理重复3次。试验期间, 日间室温为25 $^{\circ}\text{C}$ 、夜间为18 $^{\circ}\text{C}$, 自然光照条件; 每2天随机交换盆钵在温室中的位置, WHC维持在50% (称重补水法)。播种日记为0天, 在植物生长30、40、50、60和70天时, 分别对不同胁迫强度的供试植物、土样破坏性取样: 连同根际袋小心取出植物幼苗、尽可能不伤及其根系, 轻轻抖落根际土壤后收集其根系分泌物; 混匀根际土壤、非根际土壤后, 四分法取样, 装袋、冷冻干燥, 过1 mm筛后低温(–20 $^{\circ}\text{C}$)保存、分析BaP残留量。

1.3 根系分泌物收集

取出根际袋, 用自来水将根洗净、去离子水冲洗3遍后, 浸于盛有适量Milli-Q超纯水的烧杯中(锡纸包裹杯壁, 确保根部避光; 液面高度以漫过根部为宜), 自然光照下收集4 h, 用少量高纯水冲洗根系3次, 冲洗液与收集液合并、慢速滤过0.45 μm 微孔滤膜(Tu *et al.*, 2004; 许超等, 2010)。将滤液定容至50 mL, –20 $^{\circ}\text{C}$ 以下储存, 待分析。取10 mL滤液真空冷冻干燥至干, 加1 mL Milli-Q超纯水溶解, –20 $^{\circ}\text{C}$ 以下储存, 用于分析低分子量有机酸; 取20 mL滤液真空冷冻干燥至干, 加2 mL Milli-Q超纯水溶解, –20 $^{\circ}\text{C}$ 以下储存, 用于分析氨基酸(许超等,

2010)。

1.4 分析方法

1.4.1 可溶性糖测定

使用蒽酮比色法。可溶性糖含量以每kg根(干质量)分泌的可溶性糖计量, 单位为 $\text{mg}\cdot\text{kg}^{-1}$ 。

1.4.2 低分子量有机酸测定

使用高效液相色谱(HPLC)测定。检测条件: Agilent 1100色谱仪(Agilent Technologies Palo Alto, USA), XB-C₁₈色谱柱、紫外检测器($\lambda = 210 \text{ nm}$); 流动相为 $0.025 \text{ mol}\cdot\text{L}^{-1}$ 的 $\text{H}_3\text{PO}_4\text{-KH}_2\text{PO}_4$ 缓冲液(pH值2.3), 流速为 $0.7 \text{ mL}\cdot\text{min}^{-1}$, 进样量为 $20 \mu\text{L}$, 柱温 30°C 。所用样品3个重复。低分子量有机酸释放量以每kg根(干质量)分泌的有机酸总量计量, 单位为 $\text{mg}\cdot\text{kg}^{-1}$ 。

1.4.3 氨基酸测定

使用日立L-8900型氨基酸自动分析仪(Hitachi, Tokyo, Japan)测定。检测条件: 离子交换柱规格 $2.6 \text{ mm} \times 150 \text{ mm}$, 交换树型号: NO 2619 (52051), 柱温 53°C , 泵流速 $0.225 \text{ mL}\cdot\text{min}^{-1}$, 进样量 $50 \mu\text{L}$, 分析时间 72 min 。在检测波长 $\lambda = 440 \text{ nm}$ 条件下, 检出限为 $2.2 \times 10^{-9} \text{ mol}$, 保留时间重现性变异系数(CV) 1%以下、峰面积重现性CV 1.5%以下。所用样品3个重复。氨基酸量以每kg根(干质量)分泌的有机酸总量计量, 单位为 $\text{mg}\cdot\text{kg}^{-1}$ 。

1.4.4 BaP测定

参照Pan等(2008)的方法, 略作改进: 取 2.0 g 土样于离心管内, 加入 2.0 g 无水 Na_2SO_4 , 混匀; 加入

10 mL 二氯甲烷(CH_2Cl_2), 40°C 下超声萃取 1 h ; $4000 \text{ r}\cdot\text{min}^{-1}$ 下离心。取 3 mL 上清液过Fisher Pasteur玻璃管硅胶柱, $1:1$ 二氯甲烷和正己烷洗脱, 40°C 下浓缩至干, 用甲醇定容到 2 mL , 过 $0.22 \mu\text{m}$ 微孔滤膜后进行HPLC (Waters 600, Waters China, Hong Kong, China)分析。检测条件: DAD检测器, $\phi 4.6 \times 250 \text{ mm}$ C₁₈反相色谱柱; 甲醇:水(V/V, 83:17)为流动相, $\lambda = 290 \text{ nm}$ 。在此条件下, BaP检测限为 $44.6 \text{ pg}\cdot\text{L}^{-1}$, 加标回收率为 94.36% ($n = 7$, 相对标准差(RSD) $< 5.94\%$)。

1.5 数据分析

数据经Microsoft Excel 2003处理后, 借助SPSS 11.5软件最小显著差异(LSD)法分析不同处理间差异显著性。

2 结果和分析

2.1 BaP胁迫对待试植物生物量的影响

图1为BaP胁迫下5种羊茅属植物在50天时的生长状况。可以看出, 轻度($T_1\text{-}T_2$)或中度($T_3\text{-}T_4$)胁迫刺激了植物生长, 5种植物生物量(单株干质量)变化呈现 $T_4 \geq T_3 \geq T_2 \geq T_1 \geq \text{CK}$ 的趋势, T_4 水平时达到峰值, 且 $T_4 > T_1$ 、 $T_4 > \text{CK}$ ($p < 0.05$); 细芒羊茅的生物量变化虽然呈现 $T_4 \geq T_3 \geq T_2 \geq T_1 \geq \text{CK}$ 的趋势, 但各胁迫水平间差异不显著($p > 0.05$)。重度(T_5)胁迫抑制了植物生长, 其生物量明显低于其他胁迫水平($p < 0.05$)。植物的根冠比也呈现与生物量

图1 苯并[α]芘(BaP)胁迫对5种羊茅属植物生物量、根冠比的影响(平均值±标准误差)。CK、 T_1 、 T_2 、 T_3 、 T_4 、 T_5 , BaP的初始含量分别为0、10.25、20.37、40.45、80.24和 $161.74 \text{ mg}\cdot\text{kg}^{-1}$ 。不同小写字母表示相同胁迫条件下差异显著($p < 0.05$)。

Fig. 1 Biomass and root: shoot ratio of plants in five *Festuca* species growing in soils contaminated with different concentrations of benzo [α] pyrene (BaP) (mean $\pm$ SE). CK, T_1 , T_2 , T_3 , T_4 , and T_5 designate the treatments with initial concentrations of BaP at 0, 10.25, 20.37, 40.45, 80.24 and $161.74 \text{ mg}\cdot\text{kg}^{-1}$, respectively. Fa, *F. arundinacea*; Fc, *F. chelungkiangnica*; Fr, *F. rubra* subsp. *arctica*; Fm, *F. sinomutica*; Fs, *F. stapfii*. Different lowercase letters indicate significant differences ($p < 0.05$) under the same stress conditions.

类似的变化,但细芒羊茅的峰值(0.0695 ± 0.0006)出现在T₃水平,之后则逐渐降低。相同胁迫强度下,除T₄、T₅水平的细芒羊茅外,5种羊茅属植物的生物量间无明显差异($p > 0.05$)。总体上,BaP胁迫对细芒羊茅的生长影响较大:相同胁迫强度下,其生物量、根冠比都低于其他植物,胁迫强度越大、趋势越明显;T₃–T₅水平时,差异达到显著水平($p < 0.05$)。

试验期间,BaP胁迫下5种羊茅属植物在其他时段的生长状态与50天时类似。总体上,待试植物除生物量略有变化外,无其他表现受害症状(叶脉周围出现褐色或黄褐色坏死斑点,叶缘褪绿、叶尖干枯,叶片下垂;叶色发黄、根毛减少),均显示出较强的BaP污染耐受能力。

2.2 待试植物对BaP污染土壤修复潜力的种间差异

图2为不同生长期(30–70天)内5种羊茅属植物对BaP污染土壤($10.25\text{--}161.74\text{ mg}\cdot\text{kg}^{-1}$)修复效果的种间差异。可以看出,植物的存在促进了土壤中BaP的去除:相同污染水平下,种植植物土壤中BaP的去除率远高于对照组($n = 5, p < 0.05$),修复期越长,差异越显著;植物的种类不同,对土壤中BaP的去除效果也不一样。其间,苇状羊茅表现出较强的修复潜力,草原羊茅、毛稈羊茅、贫芒羊茅次之,细芒羊茅最差。相同污染条件下,5种羊茅属植物对BaP污染土壤的修复潜力依次为:苇状羊茅>草原羊茅≥毛稈羊茅≥贫芒羊茅>细芒羊茅,但草原羊

茅与毛稈羊茅间、毛稈羊茅和贫芒羊茅间差异不甚显著($p > 0.05$)。

不同修复期(生长期)内,植物的修复潜力也不一样。修复前期(30–40天)、后期(60–70天)均低于修复中期(40–60天)($p < 0.05$),呈现明显出“S”形变化曲线,曲线拐点出现在修复期的第50天前后。

2.3 待试植物根系分泌物中几种低分子量组分的释放特征

2.3.1 可溶性糖的释放特征

图3显示,BaP胁迫不同程度地促进了根系分泌物中可溶性糖的分泌,并随着胁迫强度增大(图3A)、胁迫期延长(图3B)呈现“先升后降”变化。相同胁迫期(图3A: 50天)内,各处理组根系可溶性糖的分泌量均高于对照组(CK), T₃胁迫水平下达到峰值; T₂、T₃、T₄处理与CK组间差异显著($p < 0.05$),但T₁、T₅处理与CK组间差异不显著($p > 0.05$)。相同胁迫水平(图3B: T₃)下,不同胁迫期内根系可溶性糖分泌量也不一样,40天时最多,且明显多于其他时期($p < 0.05$),但在30、60和70天时可溶性糖释放量差异不大($p > 0.05$)。可见,BaP胁迫能促进植物根系分泌更多的可溶性糖,在一定胁迫强度、胁迫期内,可溶性糖分泌量随胁迫强度的增强而增多;超越此范围,分泌量开始减少。

2.3.2 低分子量有机酸的释放特征

5种羊茅属植物根系分泌物中,草酸($83.76\% \pm$

图2 待试植物对BaP污染土壤修复潜力的种间差异(平均值±标准误差)。T₁、T₂、T₃、T₄、T₅, BaP的初始含量分别为10.25、20.37、40.45、80.24和161.74 $\text{mg}\cdot\text{kg}^{-1}$ 。数据源右侧的不同小写字母表示相同胁迫条件下差异显著($p < 0.05$)。

Fig. 2 Differences in phytoremediation potentials of benzo [a] pyrene (BaP)-contaminated soils among five *Festuca* species (mean $\pm$ SE). T₁, T₂, T₃, T₄, and T₅ designate the treatments with initial concentrations of BaP at 10.25, 20.37, 40.45, 80.24 and 161.74 $\text{mg}\cdot\text{kg}^{-1}$, respectively. Fa, *F. arundinacea*; Fc, *F. chelungkiangnica*; Fr, *F. rubra* subsp. *arctica*; Fm, *F. sinomutica*; Fs, *F. stapfii*. Different lowercase letters next to the right of data points indicate significant differences ($p < 0.05$) under the same stress conditions.

图3 苯并[α]芘(BaP)的胁迫强度(A)、T₃处理下胁迫期(B)对5种羊茅属植物根系分泌物中可溶性糖含量的影响(平均值±标准误差)。CK、T₁、T₂、T₃、T₄、T₅、BaP的初始含量分别为0、10.25、20.37、40.45、80.24和161.74 mg·kg⁻¹。不同小写字母表示相同胁迫条件下差异显著($p < 0.05$)。

Fig. 3 The effects of benzo [α] pyrene (BaP) stress level (A) and stress stage under T₃ treatment (B) on the total amount of soluble sugars in root exudates of five *Festuca* species (mean ± SE). CK, T₁, T₂, T₃, T₄, and T₅ designate the treatments with initial concentrations of BaP at 0, 10.25, 20.37, 40.45, 80.24 and 161.74 mg·kg⁻¹, respectively. Fa, *F. arundinacea*; Fc, *F. chelungkiangnica*; Fr, *F. rubra* subsp. *arctica*; Fm, *F. sinomutica*; Fs, *F. stapfii*. Different lowercase letters indicate significant differences ($p < 0.05$) under the same stress conditions.

0.05%)、乙酸(6.34% ± 0.03%)、乳酸(4.95% ± 0.03%)和苹果酸(2.29% ± 0.02%)的质量分数之和超过了低分子量有机酸总量的97.34%。苇状羊茅的根系分泌物中检测出微量的顺丁烯二酸(0.27% ± 0.01%)、反丁烯二酸(0.18% ± 0.01%)，草原羊茅、毛稈羊茅、贫芒羊茅的根系分泌物中检测出反顺丁烯二酸(0.43% ± 0.02%)，细芒羊茅的根系分泌物中仅检测出微量顺丁烯二酸(0.16% ± 0.01%)；不同污染水平下同种植物根系分泌物中低分子量有机酸的组成成分基本不变。图4为待试植物根系在不同胁迫期内释放的低分子量有机酸总量的变化趋势。

图4A显示，相同胁迫期(50天)内，苇状羊茅根系分泌的有机酸总高于相同污染水平下的其他植物，污染水平越高、有机酸分泌量越多，呈现T₅ ≥ T₄ > T₃ > T₂ > T₁ > CK趋势；除T₄与T₅间外，各污染水平间差异显著($p < 0.05$)。草原羊茅、毛稈羊茅、贫芒羊茅根系分泌的有机酸总量以T₄水平最高，呈现T₄ > T₃ ≥ T₅ > T₂ > T₁ > CK趋势；除T₃与T₅间外，各污染水平间差异显著($p < 0.05$)。细芒羊茅以T₃水平最高，呈现T₃ > T₄ ≥ T₂ > T₅ ≥ T₁ > CK趋势，但T₄与T₂、T₅与T₁间差异不显著($p > 0.05$)。

图4B显示，相同胁迫水平(T₃)下，植物根系在不同胁迫期释放的有机酸总量也不一样。修复潜力最强的苇状羊茅在30天时释放量最多，呈现30天 > 40天 > 50天 > 60天 ≥ 70天趋势；除第60、70天外，其

余各时期间差异显著($p < 0.05$)。修复潜力较强的草原羊茅、毛稈羊茅、贫芒羊茅在30天时的释放量最多($p < 0.05$)，其余各时期间差异不大($p > 0.05$)；修复潜力最差的细芒羊茅在各个时期的释放量无明显差异($p > 0.05$)。

2.3.3 氨基酸的释放特征

除色氨酸外，其他19种常见氨基酸均能在待试植物的根系分泌物中检测到。总体上，BaP胁迫促进了氨基酸的分泌，胁迫强度越大、分泌量越多。相同胁迫期(50天；图5A)内，苇状羊茅根系的分泌量总高于相同污染水平下的其他植物($p < 0.05$)，不同污染水平间差异显著($p < 0.05$)；草原羊茅、毛稈羊茅、贫芒羊茅根系的分泌量呈现T₅ ≥ T₄ > T₃ > T₂ > T₁ > CK趋势，T₅、T₄间差异不显著($p > 0.05$)；细芒羊茅根系的分泌量虽随着污染水平的升高而增多，但T₅、T₄、T₃间差异不显著($p > 0.05$)。相同胁迫强度(T₃；图5B)下，苇状羊茅根系的分泌量总多于其他植物($p < 0.05$)，30天时的分泌量明显低于其他各时期($p < 0.05$)；自40天起趋于稳定，各时期释放量无显著差异($p > 0.05$)。草原羊茅、毛稈羊茅、贫芒羊茅根系的释放量呈现30天 < 40天 < 50天 ≤ 60天 ≤ 70天趋势，30、40、50天间差异显著($p < 0.05$)；自50天起趋于稳定，各时期释放量差异不大($p > 0.05$)。细芒羊茅根系的释放量虽随胁迫时期的延长而增多，但60、70天时的差异不显著($p > 0.05$)。

图4 苯并[α]芘(BaP)的胁迫强度(A)、T₃处理下胁迫期(B)对5种羊茅属植物根系分泌物中有机酸总量的影响(平均值±标准误差)。CK、T₁、T₂、T₃、T₄、T₅, BaP的初始含量分别为0、10.25、20.37、40.45、80.24和161.74 mg·kg⁻¹。不同小写字母表示相同胁迫条件下差异显著($p < 0.05$)。

Fig. 4 The effects of benzo [α] pyrene (BaP) stress level (A) and stress stage under T₃ treatment (B) on the total amount of organic acids in root exudates of five *Festuca* species (mean ± SE). CK, T₁, T₂, T₃, T₄, and T₅ designate the treatments with initial concentrations of BaP at 0, 10.25, 20.37, 40.45, 80.24 and 161.74 mg·kg⁻¹, respectively. Fa, *F. arundinacea*; Fc, *F. chelungkiangnica*; Fr, *F. rubra* subsp. *arctica*; Fm, *F. sinomutica*; Fs, *F. stapfii*. Different lowercase letters indicate significant differences ($p < 0.05$) under the same stress conditions.

图5 苯并[α]芘(BaP)的胁迫强度(A)、T₃处理下胁迫期(B)对5种羊茅属植物根系分泌物中氨基酸总量的影响(平均值±标准误差)。CK、T₁、T₂、T₃、T₄、T₅, BaP的初始含量分别为0、10.25、20.37、40.45、80.24和161.74 mg·kg⁻¹。不同小写字母表示相同胁迫条件下差异显著($p < 0.05$)。

Fig. 5 The effects of benzo [α] pyrene (BaP) stress level (A) and stress stage under T₃ treatment (B) on the total amount of amino acids in root exudates of five *Festuca* species (mean ± SE). CK, T₁, T₂, T₃, T₄, and T₅ designate the treatments with initial concentrations of BaP at 0, 10.25, 20.37, 40.45, 80.24 and 161.74 mg·kg⁻¹, respectively. Fa, *F. arundinacea*; Fc, *F. chelungkiangnica*; Fr, *F. rubra* subsp. *arctica*; Fm, *F. sinomutica*; Fs, *F. stapfii*. Different lowercase letters indicate significant differences ($p < 0.05$) under the same stress conditions.

试验期间, BaP胁迫对根系分泌物中氨基酸的种类影响不大, 但对分泌量的影响较大, 且影响程度大小不一。相比之下, 苏氨酸、丝氨酸、甘氨酸、丙氨酸、脯氨酸和羟脯氨酸的释放特征较为突出。

与CK组相比, BaP胁迫促进了植物根系对苏氨酸、丝氨酸、甘氨酸、丙氨酸的释放活动。试验浓度(T₁–T₅)范围内, 其分泌量随着胁迫强度的增大而

剧增, 不同胁迫水平间差异显著($p < 0.05$); 脯氨酸、羟脯氨酸和天冬氨酸在苇状羊茅根系也呈现类似的释放特征, 但在细芒羊茅根系的释放量却忽高忽低、多少不一(表1)。其间, 草原羊茅根系分泌的脯氨酸和天冬氨酸、毛稈羊茅根系分泌的脯氨酸和羟脯氨酸、贫芒羊茅根系分泌的天冬氨酸均随着BaP胁迫强度的增大而剧增, 不同水平间差异显著

表1 苯并[α]芘(BaP)胁迫50天时5种羊茅属植物根系分泌物中7种氨基酸释放量(mg·kg⁻¹)Table 1 Amount of seven amino acids released in root exudates of five *Festuca* species tested on day 50 under benzo [α] pyrene (BaP) stress (mg·kg⁻¹)

氨基酸 Acids	苇状羊茅 Fa	草原羊茅 Fc	毛稈羊茅 Fr	贫芒羊茅 Fm	细芒羊茅 Fs
苏氨酸 Thr	7.28, 21.29 (10.31–31.53)*	6.74, 16.81 (8.42–26.35)*	7.12, 17.36 (9.24–26.39)*	6.52, 18.81 (8.78–29.03)*	4.55, 12.18 (6.36–20.95)*
丝氨酸 Ser	29.36, 79.94 (40.95–116.42)*	22.24, 60.12 (34.25–81.27)*	22.07, 65.74 (38.42–76.05)*	19.08, 42.11 (27.24–64.31)*	19.60, 44.27 (25.63–62.48)*
甘氨酸 Gly	3.72, 11.62 (5.43–18.11)*	3.17, 8.84 (4.47–15.91)*	2.62, 9.56 (3.93–16.07)*	2.72, 9.51 (4.11–16.42)*	2.12, 6.75 (3.35–12.67)*
丙氨酸 Ala	5.63, 17.09 (8.16–26.14)*	4.57, 14.79 (6.94–24.07)*	4.09, 14.91 (5.81–21.47)*	3.95, 10.17 (5.74–22.41)*	3.42, 10.98 (5.14–19.35)*
脯氨酸 Pro	10.73, 31.45 (16.73–47.24)*	8.56, 25.33 (12.12–39.56)*	6.64, 21.37 (8.42–33.07)*	7.82, 18.17 (8.57–28.44)†	6.90, 12.78 (4.28–20.07) ^{††}
羟脯氨酸 Hyp	12.50, 35.42 (17.87–52.94)*	11.06, 11.57 (11.34–11.72)†	8.16, 27.08 (12.84–46.87)*	9.21, 16.74 (9.91–31.17)†	8.10, 15.97 (7.49–20.12) ^{††}
天冬氨酸 Asp	14.20, 39.36 (20.08–58.62)*	9.86, 18.87 (12.07–31.48)*	8.77, 9.65 (9.02–10.13)†	8.15, 24.32 (11.47–38.25)*	9.27, 17.07 (8.24–26.17) ^{††}

表中数据“A, B (C–D)”所表示的氨基酸释放量分别为对照组含量(A)、BaP处理的平均值(B)、最低值(C)及最高值(D)。↑*表示该氨基酸释放量随着土壤中BaP胁迫强度的增大而剧增, 不同处理间差异显著($p < 0.05$); ↑表示该氨基酸释放量随着BaP胁迫强度的增大而增加, 不同处理间差异不显著($p > 0.05$); ††表示该氨基酸释放量与BaP胁迫强度无明显相关性。

The data “A, B (C–D)” in each cell of this table indicate the amount of amino acids in root exudates in control (A), and the average (B), minimum (C) and maximum (D) among of amino acids under BaP treatments, respectively. ↑* indicate that the amount of amino acids released in root exudates markedly increased with increasing BaP concentrations in soils, and the differences were significant among treatments ($p < 0.05$); ↑ indicate that the amount of amino acids released increased with increasing BaP concentrations, but the differences were not significant among treatments ($p > 0.05$); †† indicate that the amount of amino acids released in root exudates has no apparent correlation with the concentrations of BaP in soils. Fa, *F. arundinacea*; Fc, *F. chelungkiangnica*; Fr, *F. rubra* subsp. *arctica*; Fm, *F. sinomutica*; Fs, *F. stapfii*.

($p < 0.05$); 但草原羊茅根系分泌的羟脯氨酸、毛稈羊茅根系分泌的天冬氨酸, 以及贫芒羊茅根系分泌的脯氨酸、羟脯氨酸虽随着胁迫强度的增大而增多, 但不同水平间差异不显著($p > 0.05$) (表1)。

3 讨论

植物根际对PAHs的降解效果与修复植物的种类有关。Yi和Crowley (2007)研究43种植物根部组织对PAHs的降解作用发现, 有些植物对PAHs降解有明显的促进作用, 有些则无作用或有抑制作用。根系分泌物所营造的根际环境及其所呈现的根际效应是植物响应外界胁迫的重要途径, 也是影响修复效果的关键因素(Gao *et al.*, 2010; 吴林坤等, 2014)。相同胁迫条件下, 根系分泌物的释放特征及其主要组分的种间差异主要取决于植物自身基因型的特异性及其生理状态的个体差异(Zhu *et al.*, 2009; D’Orazio *et al.*, 2013)。本研究以亲缘关系较近的5种羊茅属植物为实验对象, 其基因型差异相对较小, 在一定程度上能增加研究结果的可比性。尽管待试植物在无污染(CK)、轻度或中度污染(T₁–T₄)时的生物量, 以及被植物积累的BaP量(0.08%–0.21%, 平均(0.15 ± 0.02)%差异甚微, 但它们对BaP污染土壤的修复潜力却存在显著的种间差异。可见, 植物修复过程中, 即使是遗传关系很近的同属植物间, 修复潜力的种间差异仍然存在。

植物生长指标的变化是植物受到不同程度胁

迫伤害的直观表现症状(王海翠等, 2013)。本试验中, 低度(T₁–T₂)或中度(T₃–T₄) BaP胁迫刺激了植物生长, T₄水平时生物量出现峰值, 重度(T₅)胁迫则抑制了植物生长。通常, 多数植物对PAHs类环境胁迫具有一定的耐受域值, 而这种域值与PAHs的理化性质、植物种类和环境状况(Howsam *et al.*, 2000; Line *et al.*, 2003; 田晓雪等, 2008)以及PAHs进入细胞后的累积或转化机制等因素有关(刘玉和周璐璐, 2011; 刘静等, 2015)。一定域值内, 植物生长受到的抑制影响并不显著, 甚至能刺激植物生长; 超过这一域值, 植物生长就会受到抑制。Maliszewska-Kordybach和Smreczak (2000)报道, 由茛、葱、芘和蒽4种PAHs组成的混合物, 在浓度不超过10 mg·kg⁻¹时促进了小麦(*Triticum aestivum*)、玉米(*Zea mays*)及燕麦(*Avena sativa*)的生长, 高浓度时抑制生长; 刘泓等(2008)研究了不同浓度的荧蒽培养基对拟南芥(*Arabidopsis thaliana*)生长的影响, 发现不同浓度的荧蒽胁迫30天后拟南芥生长量明显受到不同程度的抑制。尽管这些研究都表明, 高浓度PAHs胁迫抑制植物的生长, 但抑制作用机制尚不明确; 低浓度的PAHs对植物生长的促进作用, 有学者推测是因为PAHs具有与植物生长激素类似的作用(陈世军等, 2012), 但也仅仅局限于推测, 还没有被试验和实践证实。

在土壤中, 根系分泌物与根际微生物间的相互作用是一个十分重要的过程: 根系通过分泌各种次

生代谢物质对根际微生物的种类、数量和分布产生影响,对根际微生物群落结构有选择塑造作用,进而影响PAHs的降解(Abhilash *et al.*, 2012; Edwards *et al.*, 2015)。本研究显示,胁迫环境中植物根系可溶性糖、低分子量有机酸、氨基酸等组分的释放特征与植物自身的修复潜力有关:修复潜力较强的苇状羊茅所分泌的可溶性糖、低分子量有机酸、氨基酸总多于其他几种植物;修复潜力越强,其分泌量、组成成分的变化幅度越大。相同胁迫条件下,修复潜力较强的植物对BaP的胁迫响应总表现出更强的环境适应性、生理活性上的可塑性,修复潜力越强,趋势越明显(图3–5)。可以推测,根系分泌物的释放特征势必影响根际微生物群落结构,并产生选择性塑造作用,但这种释放特征是植物为减轻BaP毒害而进行的自我生理调节还是BaP毒害破坏了根系正常的生理活动而造成细胞内有机物质的渗漏尚待进一步研究。

环境胁迫可能会影响根系分泌物的种类和数量(沈宏等, 2002; Wu *et al.*, 2014)。本试验在修复潜力较强植物的根系分泌物中检测到一种低分子量有机酸——反丁烯二酸,它可能与植物对BaP类有害物质的应激反应有关:植物在应激反应过程中所产生的破坏性氧自由基(reactive oxygen species, ROS)容易与细胞内的生物大分子发生反应,对线粒体、核糖体、溶酶体、过氧化物体、微粒体等细胞器以及核酸、细胞骨架蛋白等生物大分子的结构和功能产生损伤或毒害作用(Ramos & Garcia, 2007),反丁烯二酸类抗氧化助剂对活性氧自由基较敏感,能及时捕获并清除胁迫过程中产生的各种活性氧自由基、缓解环境胁迫的损害。但为什么仅在修复潜力极强的苇状羊茅、极差的细芒羊茅的根系分泌物中检测到顺丁烯二酸,尚待进一步研究。

根系分泌物的释放特征是植物根系胁迫反应时的集中表现(Jennifer *et al.*, 2015)。BaP胁迫促进了5种羊茅属植物对苏氨酸、丝氨酸、甘氨酸、丙氨酸的释放,许超等(2010)、谢晓梅等(2011)等在研究砒胁迫对玉米、黑麦草根分泌物影响时也得出了类似结论。本试验中,根系分泌物中脯氨酸、羟脯氨酸和天冬氨酸等组分对BaP的胁迫响应更近乎以加和效应(additivity)甚至协同效应(synergism)形式参与植物的修复过程,参与应激反应的组分越多,修复潜力越强:修复潜力最强的苇状羊茅根系以上3

种组分的释放量均随胁迫强度的增强而剧增;修复潜力次强的草原羊茅、毛稈羊茅根系上述组分中某2种的释放量随胁迫强度的增强而剧增;修复潜力次差的贫芒羊茅根系仅上述组分中某1种的释放量随胁迫强度的增强而剧增;修复潜力最差的细芒羊茅根系上述组分的释放量与BaP胁迫强度无明显相关性。但该响应模式是否具有普遍性,尚待进一步验证。

4 结论

(1) 试验浓度范围内,待试植物对BaP胁迫具有较强的耐受能力,其修复潜力依次为:苇状羊茅>草原羊茅≥毛稈羊茅≥贫芒羊茅>细芒羊茅,但草原羊茅与毛稈羊茅、毛稈羊茅与贫芒羊茅间差异不显著($p > 0.05$)。

(2) BaP胁迫促进了植物根系对可溶性糖的分泌,呈现“先升后降”波动:一定胁迫强度、胁迫时期内,单位根质量分泌的可溶性糖随胁迫强度的增大而增加;超过此范围,其分泌量逐渐降低。

(3) BaP胁迫促进了根系对低分子量有机酸的释放。修复潜力越强,其高峰值出现的胁迫强度越大、不同胁迫期内的释放量差异也越显著;胁迫期越长,释放量越低。其中,草酸、乙酸、乳酸和苹果酸是主要组分,其质量分数超过了有机酸总量的97.34%,在修复潜力较强的植物根系的分泌物中还检测到反丁烯二酸。

(4) BaP胁迫对根系分泌物中氨基酸的种类影响不大,但对分泌量影响较大。苏氨酸、丝氨酸、甘氨酸、丙氨酸的释放量随着胁迫强度的增加而剧增;脯氨酸、羟脯氨酸和天冬氨酸则近乎以加和效应甚至协同效应的形式参与植物对BaP胁迫的应激反应,参与响应的组分越多,表现出的修复潜力越强。

总体上, BaP胁迫环境中, 5种羊茅属植物根系的几种低分子量有机物的释放特征与植物的修复潜力有关,修复潜力越强,释放量越多且成分越复杂,并表现出更强的环境适应性及生理可塑性。

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