连作草莓根系分泌物自毒作用的模拟研究

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摘 要 草莓(*Fragaria ananassa*)根系分泌物的自毒作用是草莓连作病害发生机理研究的重要内容之一。应用组织培养技术提取草莓根系分泌物,并对其自毒作用进行了测定。结果表明,在含有根系分泌物的生根培养基中定植的草莓组培苗,其生根、根系生长均受到不同程度的抑制,生物量显著下降,而且根系分泌物对草莓幼苗根系生理活性具有抑制作用。主要表现为根系 TTC 还原活性下降、相对电导率增大、SOD 酶活性降低及 MDA 生成量增多等方面,并导致草莓幼苗生长发育不良、病害加重。说明草莓根系分泌物具有自毒作用,连作条件下田间根系分泌物逐年积累后产生的自毒作用,可能是草莓再植病害发生的重要原因。

关键词 草莓 根系分泌物 自毒 化感作用 组织培养

SIMULATION OF AUTOTOXICITY OF STRAWBERRY ROOT EXUDATES UNDER CONTINUOUS CROPPING

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Abstract Replant disease is a serious problem for the development of sustainable cropping systems of strawberry, *Fragaria ananassa*. Previous studies have shown that auto-toxicoids secreted from roots play an important role in replant disease of some crops, and autotoxicity of root exudates is a very important aspect of understanding replant disease mechanisms. In this paper, root exudates were extracted and their autotoxicity was studied by using tissue culture methods in order to avoid interferences from external factors.

Root tissue culture mediums (MS + BA 0.2 mg·L⁻¹), in which one generation of seedlings had been cultivated, were collected in a bio-clean cabinet and mixed with a new culture medium. Five treatments were set up in which the volume proportion of the new culture medium to the used culture medium were 10:30, 15:25, 20:20, 25:15 and 30:10. Tissue culture seedlings of the same age and with the same appearance were chosen and inoculated in the five culture medium treatments. Our results showed that on the 40th day after inoculation, development and growth of roots were inhibited in mixed tissue culture mediums, and the inhibitory effects were greater as the proportion of the used culture medium increased. As a result, increments of plant height and leaves, number of roots, length of roots, and weight of plants were reduced in mixed culture mediums as compared to the pure new culture medium treatment.

The vermiculite in which strawberry tissue culture seedlings had been cultivated for 60 days was collected and placed into 500 ml erlenmeyer flasks. The same quantity of distilled water was added to each flask and then shaken on a shaker for 48 hours. After centrifuging (2 670 × g) for 20 minutes, the supernatant was collected and evaporated on a rotary vaporizer at 42 °C to 1/10 volume, and then filtered through qualitative filter paper. The extract was diluted in a series of density solutions of 50%, 40%, 30%, 20% and 10% (V%). Strawberry seedlings were treated once every 5 days with 10 ml of the different solutions and the growth of seedlings measured after 40 days. Control seedlings were treated with distilled water. Results showed that root exudates of strawberry could inhibit the growth of strawberry, and the inhibitory effect became stronger with increasing concentrations of root exudates. Physiological disease occurred in treatments treated by root exudates, and the disease index was greater as the concentration of root exudates increased. At the same time, strawberry root activity was also restrained by root exudates. Physiological analysis showed that activities of 2,3,5-Triphenyl Tetrazolium Chloride (TTC) reduced and superoxide-dismutase (SOD) decreased, and root relative conductivity and malondialdehyde (MDA) contents increased. The greater the concentration of root exudates, the

more significant the effect on all of these physiological factors.

Our results showed that when root exudates accumulated in their growing medium, the growth and metabolism of strawberry roots were inhibited, which resulted in an increase in the percentage of electrolytes in cells, a decrease in the free radical scavenging activity of roots, and an increase in root lipid peroxidation. Hence, the growth and development of strawberry seedlings showed reduced vigor, which could lead to declines in root resistance to soil-borne disease, and replant disease of strawberry could become increasingly serious.

Key words Strawberry, Root exudates, Autotoxicity, Allelopathy, Tissue culture

自 20 世纪 70 年代以来,作物连作病害中化感作用的研究在国内外屡见报道(Israel, 1973; Sewell, 1979; 杨兴洪等, 1991; 孙文浩等, 1992; 高子勤等, 1998; 阎飞等, 2000)。大多数学者认为,证明化感作用的存在、描述现象并对其影响程度进行定量化,是作物连作病害中化感作用研究的第一步工作(Rice, 1984; 孔垂华, 1998)。而用适当的方法提取作物的化感物质,并在尽可能排除土壤、微生物以及其它外界干扰因子影响的条件下,对化感物质的生物活性进行测定,又是其中最基础、最重要的部分(杜英君等, 1999)。根系分泌是得到化感物质的主要途径之一,作物根系分泌物中含有化感物质,尤其是自毒物质。对根系分泌物自毒作用研究,是作物连作病害机理研究中十分重要的内容(Chou, 1976; Yu & Matsui, 1994; 周志红等, 1998; 林思祖等, 1999; 喻景权等, 1999)。本研究应用组织培养技术,在尽可能排除外界因素干扰的条件下提取草莓(*Fragaria ananassa*) 根系分泌物,并对它们的自毒作用进行了测定,旨在明确草莓根系分泌物对自身生长发育的不利影响及其对草莓连作病害发生的重要性。

1 材料和方法

1.1 供试植物材料

草莓组培苗,品种为“全明星”。

1.2 草莓根系分泌物自毒作用的测定

1.2.1 生根培养基中的根系分泌物对二茬草莓组培苗生长影响的测定

取 40 ml MS 生根培养基(MS 培养基,附加 BA $0.2\text{ mg}\cdot\text{L}^{-1}$),置于 100 ml 三角瓶中,每瓶接种 4 株长势基本相同草莓组培小植株,于光照度 4 000 lx、光周期 14 h、温度 20 ~ 27 ℃ 条件下培养时间 30 d。无菌条件下收集上述培养过一代草莓生根苗的培养基,50 ℃ 水浴中融化,然后补充一定量新生根培养基,制成混合培养基(设 5 个处理: $G_{10:30}$ 、 $G_{15:25}$ 、 $G_{20:20}$ 、 $G_{25:15}$ 和 $G_{30:10}$,其中旧、新培养基体积比为: 10:30、15:25、20:20、25:15、30:10 ml)。取长势基本相同的草莓组培小植株,分别接种于以上培养基、新

MS 生根培养基(CK_1)、1/2 MS 生根培养基($CK_{1/2}$),每瓶 4 株,每处理重复 8 次。定期调查草莓生长及生根情况,40 d 后收获生根幼苗,测定各部分鲜重。

1.2.2 草莓根系分泌物的提取

将经组培获得的草莓生根幼苗定植于直径为 6 cm 的营养钵中,营养钵中基质为蛭石,营养钵底的小孔用多层滤纸封住,22 ~ 28 ℃ 的温室中培养 60 d,定期浇灌清水,每 30 d 浇灌 1 次营养液(郝素琴, 1999)。然后收集营养钵中的蛭石,分置于 500 ml 三角瓶中,在三角瓶中加入与蛭石体积相等的蒸馏水,摇床震荡 48 h,再以 $2\ 670\times g$ 离心 20 min,取上清液旋转蒸发(42 ℃)至体积的 1/10,定性滤纸过滤后,作为根系分泌物母液备用。

1.2.3 根系分泌物对草莓幼苗生长及根系生理活性影响的测定

将长势基本相同的草莓组培幼苗定植于直径为 6 cm 的营养钵中(基质为蛭石),缓苗 15 d 后,分别定期浇灌 50%、40%、30%、20%、10% ($V\%$) 的根系分泌物溶液,每 5 d 浇灌 1 次,每次每株浇灌 10 ml,对照处理浇清水,每处理设 3 组重复,每组 5 株,40 d 后取苗测定植株生长指标、地上部病级及根系 TTC 还原能力、离子渗漏、丙二醛生成量、超氧化物歧化酶(SOD)活性等生理指标(白宝璋等, 1994; 邹琪, 1995)。

地上部病级按 5 级划分。0 级:无病斑;1 级:病斑面积占叶部表面积 1/4 以下;2 级:病斑面积占 1/4 ~ 1/2;3 级:病斑面积占 1/2 ~ 3/4;4 级:病斑面积占 3/4 以上。

病情指数:

$$DI = \frac{\sum_{i=0}^n X_i \cdot S_i}{\sum_{i=0}^n X_i \cdot S_{\max}} \times 100$$

式中, X_i 为某级病根数; S_i 为病级; $S_{\max}$ 为最高病级。

2 实验结果

2.1 根系分泌物对二茬草莓组培苗生长的影响

在草莓旧生根培养基中,留有前茬生根组培苗的根系分泌物。因此,混合培养基中旧生根培养基

比例越大,根系分泌物含量也就越高。从表 1 中可以看出,混合培养基对二茬草莓的生根、根系生长、茎叶生长均有一定的抑制作用,而且随着旧生根培养基比例的增大,抑制程度也越为明显。定植 40 d 后,CK₁ 处理的株高增量、叶片增量、生根数、根长,分别为混合培养基处理的 3.7 ~ 6.2 倍、1.7 ~ 3.0 倍、2.6 ~ 3.0 倍、1.1 ~ 1.2 倍;茎叶和根系的鲜重分别为混合培养基各处理的 2.1 ~ 3.0 倍和 1.5 ~ 1.9 倍。另外,比较表 1 中 1/2 MS 生根培养基处理、CK₁ 处理和混合培养基处理的各项生长指标发现,CK_{1/2} 处理植株生长状况虽略逊于 CK₁ 处理,但多项指标明显优于各混合培养基处理,说明新旧培养基中营

养成分含量,不是造成不同处理组培植株生长差异的主要原因。

2.2 根系分泌物对草莓幼苗生长及根系生理活性的影响

2.2.1 根系分泌物对草莓幼苗生长的影响

表 2 是用不同浓度的根系分泌物定期浇灌草莓幼苗 40 d 后测定的生长发育指标及地上部病情指数。可以看出,草莓根系分泌物对幼苗的生长有一定的抑制作用,而且随着浇灌根系分泌物浓度的增加,草莓幼苗生长受抑制程度逐渐增加。主要表现为株高增量、根系鲜重、茎叶鲜重随分泌物浓度的增大而减小。由于根系分泌物的自毒作用,使浇灌分

表 1 不同培养基对草莓组培苗生长的影响
Table 1 The effect of different mediums on the growth of strawberry tissue culture seedlings

测定项目 Item	处理 Treatment	测定时间 Measuring time (植苗后天数 Days after planting , DAP)				
		0 d	10 d	20 d	30 d	40 d
株高 Shoot height (cm)	CK ₁	3.18 ^a	4.24 ^a	5.16 ^a	6.44 ^a	7.78 ^a
	CK _{1/2}	3.16 ^a	3.94 ^{ab}	5.14 ^a	6.53 ^a	7.86 ^a
	G _{10:30}	3.16 ^a	3.68 ^b	3.86 ^b	4.10 ^b	4.40 ^b
	G _{15:25}	3.16 ^a	3.56 ^{bc}	3.70 ^b	3.90 ^b	4.14 ^{bc}
	G _{20:20}	3.18 ^a	3.26 ^c	3.42 ^c	3.48 ^c	4.00 ^c
	G _{25:15}	3.2 ^a	3.24 ^c	3.32 ^c	3.44 ^c	3.82 ^c
	G _{30:10}	3.16 ^a	3.22 ^c	3.24 ^c	3.34 ^c	3.90 ^c
	CK ₁	3.6 ^a	5.0 ^a	6.2 ^a	7.2 ^a	8.6 ^a
叶片数(个) Number of leaves	CK _{1/2}	3.5 ^a	4.6 ^{ab}	5.4 ^b	6.0 ^b	7.8 ^b
	G _{10:30}	3.5 ^a	4.0 ^{bcd}	4.2 ^{de}	5.4 ^c	6.0 ^d
	G _{15:25}	3.5 ^a	3.8 ^{cd}	4.0 ^{ef}	4.8 ^d	5.8 ^d
	G _{20:20}	3.6 ^a	4.0 ^{bcd}	4.4 ^d	5.2 ^{cd}	5.4 ^e
	G _{25:15}	3.6 ^a	4.2 ^{bc}	5.0 ^c	5.6 ^c	6.6 ^c
	G _{30:10}	3.4 ^a	3.4 ^d	3.8 ^f	4.8 ^d	6.0 ^d
	CK ₁	—	5.0 ^a	8.2 ^a	12.0 ^a	14.0 ^a
	CK _{1/2}	—	4.0 ^b	6.2 ^b	11.0 ^b	12.0 ^b
根数(条) Number of roots	G _{10:30}	—	3.2 ^{bc}	4.2 ^c	4.4 ^d	5.2 ^c
	G _{15:25}	—	2.6 ^c	4.8 ^c	5.2 ^{cd}	5.2 ^c
	G _{20:20}	—	1.6 ^d	2.6 ^d	3.6 ^c	4.6 ^c
	G _{25:15}	—	1.4 ^d	2.4 ^d	4.4 ^d	5.4 ^c
	G _{30:10}	—	1.0 ^d	2.0 ^d	4.2 ^{de}	5.2 ^c
	CK ₁	—	—	1.46 ^a	3.04 ^a	5.92 ^a
	CK _{1/2}	—	—	1.30 ^{abc}	2.77 ^c	5.72 ^a
	G _{10:30}	—	—	1.02 ^d	2.77 ^c	5.52 ^{ab}
根长 Length of roots (cm)	G _{15:25}	—	—	1.18 ^{cd}	2.98 ^{ab}	5.08 ^{cd}
	G _{20:20}	—	—	1.40 ^{ab}	3.06 ^a	5.30 ^{bc}
	G _{25:15}	—	—	1.34 ^{abc}	2.90 ^{ab}	4.80 ^d
	G _{30:10}	—	—	1.24 ^{bc}	3.08 ^a	4.94 ^{cd}
	CK ₁	—	—	—	—	0.897(0.218 ^a)
	CK _{1/2}	—	—	—	—	0.756(0.216 ^a)
	G _{10:30}	—	—	—	—	0.429(0.147 ^b)
	G _{15:25}	—	—	—	—	0.394(0.143 ^b)
茎叶(根) 鲜重 Fresh weight of stem and leaf (Root) (g)	G _{20:20}	—	—	—	—	0.364 ^d (0.139 ^b)
	G _{25:15}	—	—	—	—	0.320 ^{ef} (0.132 ^{bc})
	G _{30:10}	—	—	—	—	0.302(0.116 ^c)
	CK ₁	—	—	—	—	—
	CK _{1/2}	—	—	—	—	—
	G _{10:30}	—	—	—	—	—
	G _{15:25}	—	—	—	—	—
	G _{20:20}	—	—	—	—	—

表中同项同列数据后相同字母表示在 $p = 0.05$ 水平上无显著差异 Data followed by same letters are not significantly different at $p = 0.05$ level in a line

表 2 不同浓度的草莓根系分泌物对草莓幼苗生长的影响
Table 2 Effect of different concentration of root exudates on growth of strawberry seedlings

处理 Treatment	株高增量 (cm) Increment of shoot height	新增叶片数 (个) Increment of leaves	根系鲜重 (g) Fresh weight of root	茎叶鲜重 (g) Fresh weight of stem and leaf	病情指数 (%) Disease index
根系分泌物 Root exudates	50%	0.37 ^e	1.5 ^{ab}	0.67 ^e	0.88 ^c
	40%	0.65 ^d	1.4 ^b	0.72 ^b	1.28 ^b
	30%	0.69 ^d	1.5 ^{ab}	0.76 ^b	1.30 ^b
	20%	0.79 ^c	1.6 ^a	1.10 ^a	1.39 ^{ab}
	10%	1.07 ^b	1.7 ^a	1.07 ^a	1.49 ^a
对照 (CK)	1.54 ^a	1.5 ^{ab}	1.11 ^a	1.33 ^{ab}	7.2 ^e

a、b、c、d、e 同表 1 See Table 1

泌物的各处理产生生理性病害 地上部出现病斑 ,且病情指数随分泌物浓度的增大而增大。浓度最大的处理 (50%) 生长发育受抑制程度最为明显 ,与对照相比其株高增量、根系鲜重、茎叶鲜重分别下降了 76.0%、39.6%、33.8% ;而病情指数却是对照的 6.5 倍。

2.2.2 根系分泌物对草莓幼苗根系生理活性的影响

图 1 表示了不同浓度根系分泌物对草莓幼苗根系 TTC 还原活性的影响。植物组织还原 TTC 的能力是细胞呼吸电子传递链活性的一种体现。它指示着根生长和代谢旺盛程度。从图 1 中可以看出 ,随着浇灌根系分泌物浓度的增大 ,草莓根系 TTC 还原活性呈明显下降趋势。说明如果根系分泌物在草莓根系生长的介质中积累到一定量 ,可抑制草莓根系的生长和代谢 ,使植株生长发育不良 ,抗病性降低。图 2 则表明 ,随着浇灌根系分泌物浓度的增大 ,草莓幼苗根系相对电导率逐渐增大 ,根系分泌物可增加草莓根系通过原生质膜由细胞内向细胞外的离子渗漏。因此 ,连作条件下土壤中积累的草莓根系分泌物会导致根系中养分离子的渗漏 ,自毒物质也更容易进入根组织而产生抑制作用。

图 1 根系分泌物对草莓根系 TTC 还原活性的影响
Fig.1 Effect of strawberry root exudates on root activity in TTC reduction

图 3 和图 4 表示了根系分泌物对草莓幼苗根系 SOD 酶活性和丙二醛生成量的影响。图中显示 ,浇

灌根系分泌物能抑制草莓根系 SOD 酶活性 ,增加丙二醛生成量 ,且随着浇灌根系分泌物浓度的增大 ,SOD 酶活性下降和 MDA 量增多表现得越突出。表明根系分泌物抑制了草莓根系清除自由基的能力 ,使自由基量增多 ,根系内细胞膜质过氧化水平上升。SOD 酶活性降低还可能导致根系抗病能力下降。在

图 2 根系分泌物对草莓根系离子渗漏的影响
Fig.2 Effect of strawberry root exudates on root electrolyte leakage

图 3 根系分泌物对草莓幼苗根系 SOD 活性的影响
Fig.3 Effect of strawberry root exudates on root SOD activities

图 4 根系分泌物对草莓根系组织脂质过氧化的影响
Fig.4 Effect of strawberry root exudates on root lipid peroxidation

田间这意味着可能因此导致连作草莓土传根部病害更易发生和加重。

3 讨 论

引起作物连作病害的因素是相当复杂的,对于作物连作病害发生的原初原因,至今还有一定的争议。自毒作用在作物再植病害中的重要性已经得到广泛的认同。本文利用组织培养技术,在排除土壤及土壤微生物干扰的条件下,提取草莓根系分泌物,并对根系分泌物的自毒作用进行了研究,发现草莓根系分泌物对二茬草莓根系活性具有抑制作用,导致植株生长发育不良,抗病力下降。前期研究表明,由 *Fusarium oxysporum* sp. *fragariae*、*Rhizoctonia solani*、*Verticillium dahliae* 等引起的土传根部病害加重,是草莓连作病害的主要表现(甄文超, 2003)。这和根系分泌物的自毒作用使植株抗病力下降有密切关系。生产中连作年限越长,土壤中累积的根系分泌物浓度越高,对连作草莓的生长发育的抑制作用也越强,连作病害发生也会越严重。

应用植物外植体组织培养技术,可获得与植物母体遗传性状完全相同的小植株,它们的次生代谢产物也应与母体相同,通过试管苗的交叉接种能直接观察到这些产物是否能产生自毒作用。另外,采用组织培养技术可不受季节、环境条件的限制,还可在排除土壤化学因素、土壤微生物因素及同一环境下其它植物对研究的干扰的情况下提取根系分泌物,并在短时间内随时鉴定其自毒作用,所得结论较能客观反映植物根系分泌的自毒作用。

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