

潮霉素作为筛选剂对大豆发状根诱导的影响

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摘要:筛选标记是转基因材料创制中转化细胞存活、生长和发育筛选的基本要素。选用4个不同的大豆基因型,分别以发根农杆菌诱导子叶节产生发状根的数量和根的伸长量为指标,研究了不同质量浓度潮霉素对发状根诱导和根伸长的影响,以期大豆遗传转化过程中筛选剂的多样性选择及抗潮霉素育种奠定基础。结果表明:潮霉素可以有效抑制非转化细胞的生长,能够用作大豆发状根转化系统中的筛选剂,但不同基因型大豆品种对潮霉素的敏感性存在较大差异。自贡冬豆、吉林小粒1号、吉育47和中黄30在发状根诱导阶段的潮霉素适宜筛选浓度分别为40,16,16,24 mg·L⁻¹;根伸长阶段分别为49,46,33,55 mg·L⁻¹。

关键词:大豆;基因型;发状根转化;潮霉素;适宜浓度

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Effect of Hygromycin as a Screening Agent on the Induction of Soybean Hairy Roots

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Abstract: Selectable marker genes perform a fundamental and essential role in plant transformation by providing the mechanism for the selective survival, growth and development of transformed cells. In the research, four soybean genotypes were selected, and the effects of different concentrations of hygromycin on the hairy root induction and the root elongation were investigated, with the indicators of number of the hairy roots induced by cotyledons which were infected with the *Agrobacterium rhizogenes* and the root elongation separately. The purpose was to lay the foundation for the diversity of choices of the selection agent in the soybean genetic transformation process and for the hygromycin resistance breeding. The results showed that, the hygromycin could effectively inhibit the growth of non-transformed cells, successfully act as a successful screening agent in the soybean hairy root transformation system. However, a great difference among different soybean genotypes was exhibited in the sensitivity to hygromycin. The suitable screening concentrations of hygromycin for Zigongdongdou, Jilinxiaoli 1, Jiyu 47 and Zhonghuang 30 at the hairy root induction stage were 40, 16, 16, 24 mg·L⁻¹; and the suitable screening concentrations at the root elongation stage were 49, 46, 33, 55 mg·L⁻¹, respectively.

Key words: Soybean; Genotype; Hairy root transformation technology; Hygromycin; Suitable concentration

大豆(*Glycine max* L. Merr.)是重要的粮油兼用作物,也是目前转基因品种种植面积最大的作物。2012年,全球转基因大豆的种植面积已达8 070万hm²,约占世界大豆种植总面积的81%,转基因技术已成为大豆新品种培育的重要手段^[1-3]。目前使用较为广泛的大豆转化体系,以根癌农杆菌介导的子叶节转化体系^[4]和基因枪介导的幼胚转化体系^[5]为主。但两个转化体系均存在转化周期较长、转化效率较低等问题^[6-7],不利于快速评估目标基因在大豆育种中的应用价值。近年来,随着基因功能分析研究需求的不断增加,发根农杆菌介导的大豆发状根转化体系越来越受到重视。该转化体系具有周期短、效率高、单细胞分化而来的发状根变异率低等突出优点^[8-9]。尽管目前尚不能用这种方法获得转基因再生植株,但已在固氮、抗病、耐盐

等与根系密切相关的基因功能分析和育种价值评价等方面发挥了重要作用^[10-16]。利用适宜的培养条件,还可将诱导出的发状根进一步诱导出愈伤组织,从而进一步拓展基因功能分析的范围^[17]。常用的大豆发状根转化体系,一般在转化过程中不使用筛选剂对转化体进行筛选,尽管能够产生较多的发状根并获得较高的转化率,但后期转基因材料的鉴定工作量较大,费时、费力^[18-19]。

筛选标记是转基因材料创制中转化细胞存活、生长和发育筛选的基本要素,在植物转基因研究中,选择一种适宜的筛选基因作为筛选标记基因,不但有利于提高转化效率,而且便于进行转化体的识别和鉴定^[20]。筛选标记基因以抗生素类、除草剂类基因为主^[21],其中,潮霉素(hygromycin)抗性基因是目前使用较多的标记基因之一,已在多种

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植物的基因功能研究中得到了广泛应用^[22-23]。

在转化实验中,筛选剂浓度过低,难以抑制或杀死未转化细胞,会造成假阳性率过高,进而影响转化体的筛选和鉴定工作;而筛选浓度过高,会同时杀死转化细胞或影响其生长,难以获得转化材料。因此,进行受体材料筛选剂耐受性试验,对确定理想的筛选临界浓度,保证理想的筛选效果非常重要^[24]。现以不同质量浓度潮霉素处理对4个大豆品种发状根诱导和根伸长的影响进行研究,以期确定理想的筛选浓度,进一步提高大豆发状根转化体系的效率,降低转化体识别鉴定的工作量。

1 材料与方法

1.1 材料

供试大豆品种为自贡冬豆、吉林小粒1号、吉育47及中黄30,由本实验室保存提供;发根农杆菌(*Agrobacterium rhizogenes*) K599菌株由澳大利亚Queensland大学Peter Gresshoff教授惠赠,本实验室保存提供。潮霉素为Sigma公司产品,GUS染液为Biolab Co., Ltd.公司产品。

1.2 发根农杆菌的活化及发状根的诱导

参照Tao等^[25]的方法,对参试菌株进行活化,菌液的OD₆₀₀值达到0.6~0.8时用于大豆子叶节的侵染。发状根的诱导参照Cao等^[26]的方法,在1/2 MS诱导培养基中进行2 d恢复培养后,进行不同浓度潮霉素处理试验。试验中涉及到的所有培养基的配制均参照Huang等^[27]的方法进行。

1.3 发状根诱导阶段子叶对潮霉素的敏感性试验

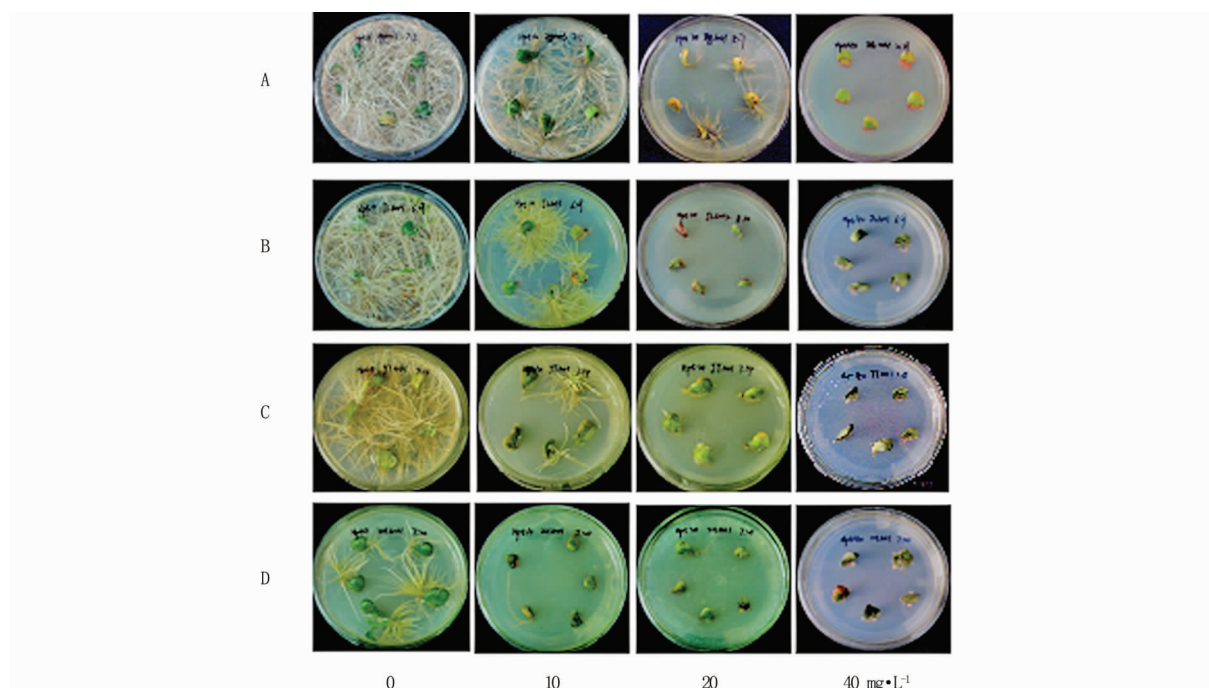
将经过2 d恢复培养的大豆子叶接种于含不同浓度潮霉素的1/2 MS诱导培养基上,22℃光照培养,25 d后,记录子叶诱导产生发状根的数量。先设置0,10,20,40 mg·L⁻¹潮霉素质量浓度处理对4个大豆品种进行初筛,再根据初筛结果针对每个品种设置不同细化浓度的处理,进一步摸索适宜的临界筛选浓度。每个浓度处理20个子叶外植体,分为4皿,每皿均匀插入5个子叶,重复3次,测定不同浓度下未诱导发状根的子叶数及子叶诱导出的发状根的数量。

1.4 根伸长阶段离体发状根对潮霉素的敏感性试验

选取未经筛选、伸长到5~6 cm的发状根,保留根尖,切取约1 cm的根段,置于含不同质量浓度潮霉素的1/2 MS诱导培养基上,标定生长基线,22℃光照培养,15 d后,记录离体发状根的伸长量。先设置0,30,40,50 mg·L⁻¹潮霉素质量浓度处理对4个大豆品种进行初筛,再根据初筛结果针对每个品种设置不同细化浓度的处理,进一步摸索适宜的临界筛选浓度。每个浓度处理40条发状根,分为4皿,每皿10条发状根,均匀置于标定的生长基线两侧,重复3次,测定不同浓度下未伸长的离体发状根数及发状根的伸长量。

1.5 数据分析

使用Excel2003及SAS9.2软件对不同浓度潮霉素处理所获数据的差异显著性进行分析。



A: 自贡冬豆; B: 吉林小粒1号; C: 吉育47; D: 中黄30

A: Zigongdongdou; B: Jilinxiaoli 1; C: Jiyu 47; D: Zhonghuang 30

图1 发状根诱导阶段不同品种对潮霉素的耐受性

Fig. 1 The tolerance of different varieties to the hygromycin agent at the hairy root induction stage

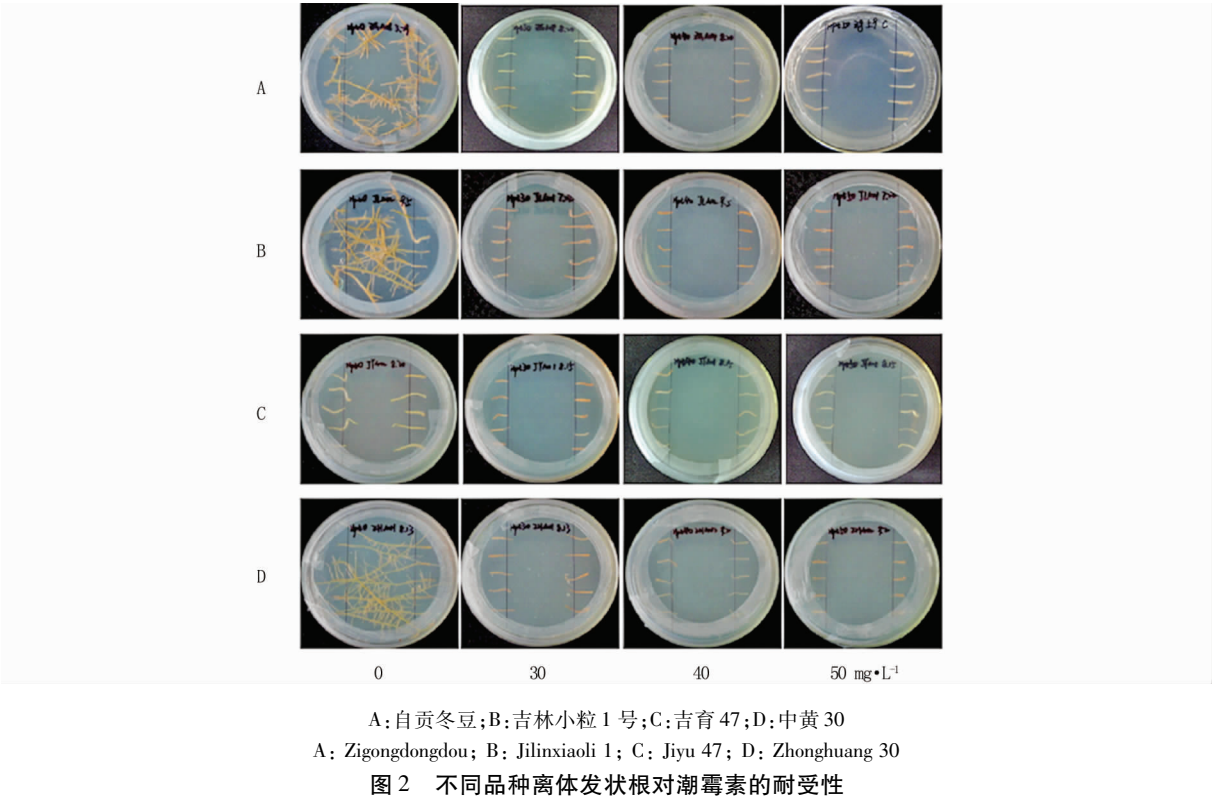
2 结果与分析

2.1 不同品种对潮霉素耐受性的初步筛选

首先针对不同品种对潮霉素的耐受性进行初步筛选,通过发状根诱导、生长状态,初步分析 4 个大豆品种在发状根诱导阶段(图 1)和根伸长阶段

(图 2)对潮霉素的耐受性。初步确定可能的筛选临界浓度范围。

从图 1 可以看出,在发状根诱导阶段,不同大豆品种对潮霉素的耐受性存在一定差异,初步确定自贡冬豆、吉林小粒 1 号、吉育 47 及中黄 30 在该阶段的潮霉素临界筛选浓度分别约为 40,20,20,20 $\text{mg}\cdot\text{L}^{-1}$ 。



从图 2 可以看出,自贡冬豆、吉林小粒 1 号、吉育 47 及中黄 30 离体发状根对潮霉素的耐受性也存在明显差异,初步确定在离体发状根根伸长阶段,其潮霉素临界筛选浓度分别约为 50,40,30,50 $\text{mg}\cdot\text{L}^{-1}$ 。

2.2 发状根诱导阶段子叶对潮霉素的耐受性

根据初筛结果,针对每个品种设置不同细化浓度的处理,进一步分析不同大豆品种在发状根诱导阶段对潮霉素的耐受性。

从表 1 可以看出,在发状根诱导阶段,通过潮霉素筛选能够明显降低诱导出发状根的数量及能够产生发状根的子叶数量。不同品种对潮霉素的耐受性存在一定差异,当潮霉素浓度达到 16 $\text{mg}\cdot\text{L}^{-1}$ 左右

时,就能有效抑制吉林小粒 1 号和吉育 47 产生发状根;而中黄 30 和自贡冬豆则需要使用 24 $\text{mg}\cdot\text{L}^{-1}$ 和 40 $\text{mg}\cdot\text{L}^{-1}$ 左右的潮霉素才能达到类似的效果。在发状根诱导阶段,通过使用一定浓度的潮霉素筛选,可以达到对非转化发状根诱导的抑制作用,从而在转化过程中减少非转化发状根的形成,达到筛选效果。在维持子叶具有较好的生长状态条件下,尽可能降低非转化发状根的诱导数量,试验确定自贡冬豆、吉林小粒 1 号、吉育 47、中黄 30 在其发状根诱导阶段适宜的潮霉素筛选临界浓度分别为 40, 16, 16, 24 $\text{mg}\cdot\text{L}^{-1}$ 。

表 1 潮霉素对不同大豆品种发状根诱导的影响

潮霉素质量浓度 Concentration/ $\text{mg}\cdot\text{L}^{-1}$	未诱导出发状根的子叶平均数 Mean of cotyledons with no induction of hairy roots				诱导出发状根的平均数 Mean of hairy roots			
	A	B	C	D	A	B	C	D
0	1.7 a	7.7 a	4.3 a	2.3 a	2.4 a	0.7 a	0.9 a	1.8 a
14	—	15.7 b	16.7 b	—	—	0.3 b	0.3 b	—
16	—	18.7 b	18.3 b	—	—	0.1 c	0.1 c	—
18	—	19.7 b	19.7 b	—	—	0.1 c	0.1 c	—
20	—	20.0 c	20.0 c	—	—	0 c	0 c	—

续表 1

潮霉素质量浓度 Concentration/mg·L ⁻¹	未诱导出发状根的子叶平均数 Mean of cotyledons with no induction of hairy roots				诱导出发状根的平均数 Mean of hairy roots			
	A	B	C	D	A	B	C	D
22	—	20.0 c	20.0 c	5.7 b	—	0 c	0 c	0.7 b
24	—	20.0 c	20.0 c	17.0 c	—	0 c	0 c	0.1 c
26	—	20.0 c	20.0 c	18.7 c	—	0 c	0 c	0.1 c
28	—	20.0 c	20.0 c	18.3 c	—	0 c	0 c	0.1 c
30	—	—	—	19.3 c	—	—	—	0.1 c
32	—	—	—	20.0 d	—	—	—	0 c
34	—	—	—	20.0 d	—	—	—	0 c
36	10.7 b	—	—	20.0 d	0.6 b	—	—	0 c
38	14.3 b	—	—	—	0.4 c	—	—	—
40	19.3 b	—	—	—	0.1 d	—	—	—
42	19.3 b	—	—	—	0.1 d	—	—	—
44	19.7 b	—	—	—	0.1 d	—	—	—
46	20.0 c	—	—	—	0 d	—	—	—
48	20.0 c	—	—	—	0 d	—	—	—
50	20.0 c	—	—	—	0 d	—	—	—

每个处理 20 个子叶外植体,重复 3 次;A:自贡冬豆;B:吉林小粒 1 号;C:吉育 47;D:中黄 30。

Twenty cotyledons explants were for each treatment, with three repeats. A: Zigongdongdou; B: Jilinxiaoli 1; C: JiYu 47; D: Zhonghuang 30.

2.3 离体发状根对潮霉素的耐受性

度的处理,进一步分析不同大豆品种的离体发状根

根据初筛结果,针对每个品种设置不同细化浓

对潮霉素的耐受性(表 2)。

表 2 潮霉素对不同大豆品种离体发状根伸长量的影响

Table 2 Effect of Hygromycin agent on theelongation of the vitro hairy roots of different varieties

潮霉素质量浓度 Concentration/mg·L ⁻¹	未伸长的离体发状根的平均数 Mean of the vitro hairy roots with noelongation				发状根平均伸长量 Mean of the elongation of hairy roots/mm			
	A	B	C	D	A	B	C	D
0	3.7 a	5.3 a	2.7 a	5.7 a	10.0 a	8.2 a	12.1 a	10.6 a
23	—	—	16.3 b	—	—	—	2.4 b	—
28	—	—	19.0 b	—	—	—	1.3 c	—
33	—	—	19.3 b	—	—	—	0.5 d	—
37	—	17.0 b	—	—	—	2.1 b	—	—
38	—	—	19.7 b	—	—	—	0.5 d	—
40	16.7 b	16.7 b	—	17.0 b	2.7 b	1.6 c	—	2.2 b
43	17.3 b	18.3 b	21.7 b	—	1.5 c	1.1 d	0.4 d	—
45	—	—	—	17.7 b	—	—	—	1.7 c
46	18.7 b	18.7 b	—	—	0.8 d	0.5 e	—	—
48	—	—	24.7 c	—	—	—	0.4 d	—
49	19.7 b	21.3 b	—	—	0.5 e	0.4 e	—	—
50	—	—	—	18.3 b	—	—	—	1.2 d
52	24.0 c	24.3 c	—	—	0.4 e	0.4 e	—	—
55	24.3 c	—	—	19.7 b	0.4 e	—	—	0.6 e
60	—	—	—	19.3 b	—	—	—	0.5 e
65	—	—	—	25.3 c	—	—	—	0.4 e

每个处理 40 条根,重复 3 次;A:自贡冬豆;B:吉林小粒 1 号;C:吉育 47;D:中黄 30。

Forty lines of roots were for each treatment, with three repeats; A: Zigongdongdou; B: Jilinxiaoli 1; C: JiYu 47; D: Zhonghuang 30.

从表 2 可以看出,在离体发状根伸长阶段,潮霉素的筛选能够明显抑制离体发状根的伸长。在此阶段,不同大豆品种对潮霉素的耐受性同样存在着较大差异,当潮霉素浓度达到 $33 \text{ mg} \cdot \text{L}^{-1}$ 左右时,就能有效抑制吉育 47 离体发状根的伸长,而在达到类似的筛选效果下,提升潮霉素的处理浓度会造成试剂的浪费;而自贡冬豆、吉林小粒 1 号和中黄 30 则需要使用 $46 \text{ mg} \cdot \text{L}^{-1}$ 以上,甚至达到 $55 \text{ mg} \cdot \text{L}^{-1}$ 的潮霉素才能获得类似的效果。在离体发状根伸长阶段,通过使用适宜临界浓度的潮霉素对离体发状根进行筛选,可以达到抑制其伸长的作用,从而在转化过程中减少非转化离体发状根的形成,达到筛选效果,同时有助于在其诱导愈伤组织的试验中,降低后续对愈伤组织进行阳性鉴定的工作量。在维持离体发状根具有较好的生长状态下,尽可能降低非转化离体发状根的数量,试验确定自贡冬豆、吉林小粒 1 号、吉育 47、中黄 30 在其离体发状根伸长阶段适宜的潮霉素筛选临界浓度分别为 49, 46, 33, 55 $\text{mg} \cdot \text{L}^{-1}$ 。

3 结论与讨论

在发根农杆菌介导的大豆发状根转化体系中,若不添加任何筛选剂,会诱导产生大量的发状根,但其中转基因发状根的比率较低,造成后期转基因材料筛选鉴定的工作量加大^[26],因此进行受体材料筛选剂耐受性试验,对确定理想的筛选临界浓度,保证理想的筛选效果是非常重要的。目前关于发根农杆菌介导的发状根转化体系的研究报道中,对如何确定筛选试剂及其理想使用浓度,尚缺乏系统的研究^[19]。摸索筛选剂的选择和使用浓度,有助于降低筛选鉴定工作量,提高相关后续研究的效率^[28]。

潮霉素可干扰植物细胞叶绿体、线粒体中的核糖体与延长因子 EF-2 的结合,从而抑制肽链的延长。当潮霉素进入非转化细胞中,可与细胞内的 30S 核糖体结合,使细胞中蛋白质合成受阻,导致细胞逐渐褐化死亡^[29]。近年来,该筛选剂已在大麦^[21,30]、大豆^[31]、水稻^[32]、甘蔗^[33]等多种植物中得到了广泛的应用,分别研究了潮霉素对不同受体材料的适宜筛选浓度,提高了相应转化体系的筛选效率。另外,潮霉素作为一种筛选标记已成功应用于多种植物组织^[34-35]、霉菌类转化^[36-37]的筛选试验中。

不同物种及同一物种不同基因型均对潮霉素的耐受性存在一定差异,本研究通过系统比对试验,明确了 4 个大豆品种自贡冬豆、吉林小粒 1 号、

吉育 47 及中黄 30 在其发状根诱导阶段和离体发状根伸长阶段对潮霉素的耐受性,确定了较为理想的临界筛选浓度,为后续在转化实验中的应用提供了依据。同时,也证实了不同大豆品种对潮霉素的耐受性存在较大差异,以潮霉素作为筛选标记时,应根据基因型的不同,确定适宜的筛选浓度。

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