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·博士论文摘要·

利用生物技术提高三倍体狗牙根抗逆性

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狗牙根是一种重要的暖季型草坪草。三倍体矮生狗牙根(*Cynodon dactylon*×*C. transvaalensis* cv. TifEagle)已广泛应用。本文研究其抗旱性、建立组织培养与再生体系、体细胞无性系变异育种技术及抗逆基因工程等。主要结果和结论如下:

1. 抗旱性及化学调控

测定矮生狗牙根在停止浇水过程中叶片相对含水量、电导率、脯氨酸含量及抗氧化酶活性的变化;研究其抗旱性与抗氧化酶活性的关系;研究ABA等生长调节剂提高其抗旱性的效应和机理。

2. 建立组织培养再生体系

以亲本匍匐茎节为外植体,探讨胚性愈伤组织诱导、继代和再生的条件;建立快速增殖、高频组织培养的再生体系;借助光学组织切片和扫描电镜,揭示体细胞胚胎发生是TifEagle愈伤组织的再生方式。

3. 体细胞无性系变异体的筛选及鉴定

从连续悬浮继代愈伤组织再生的植株中,筛选出夏季和春季匍匐生长速度较快的株系;在不同年份越冬中多次获得评分较高的株系;研究愈伤组织进行长时间连续继代悬浮培养能有效地诱导体细胞变异,从再生植株中筛选有益的性状。

4. 矮化变异体

从再生植株中选出一株矮化变异体(TV4)。测定该变异体及其亲本的形态和抗逆性特征;矮化变异体具有矮化、密度大、颜色浓绿和根系发达等特点,其耐冷性略有提高,是一个很有应用潜力的新株系。

5. 耐盐变异体的筛选及生理鉴定

以0.3 mol/L的NaCl作为选择压力,采用间歇筛选法从悬浮继代18个月以上的愈伤组织中筛选耐盐的细胞系,并获得再生植株;耐盐性测定结果表明,50%变异体株系的耐盐性高于亲本;测定变异体和亲本在正常和盐胁迫条件下的Na^+/K^+比、脯氨酸含量、可溶性糖含量和部分抗氧化酶活性;探讨耐盐变异体的耐盐机理。结果表明,耐盐株系具有不同的耐盐机理,但在正常生长条件下的脯氨酸含量均高于亲本,而Na^+/K^+比则低于亲本。

6. 遗传转化、转基因检测及抗逆性鉴定

构建*BADH*和*CMO*的表达载体。采用基因枪转化狗牙根愈伤组织,获得PCR检测呈阳性的植株;对转*BADH*植株进行Southern杂交分析,出现多条杂交信号,证明基因以多拷贝方式整合到狗牙根基因组中;对部分转基因植株进行耐盐性和耐旱性分析,结果表明,一些转基因株系耐盐性和耐旱性明显提高。

关键词:狗牙根;生物技术;抗逆性;组织培养;体细胞变异;转基因
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Biotechnological Approaches to Improvement of Triploid Bermudagrass for Abiotic Stress Tolerance

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Objectives of our research were to investigate the improvement of triploid bermudagrass (*Cynodon dactylon* ×*C. transvaalensis* cv. TifEagle) for stress tolerance by biotechnological methods. The results were summarized in the following:

1. Drought tolerance and its chemical control in turfgrass species

The drought tolerance in 3 turfgrass species were comparatively studied by measuring relative water content (RWC), ion leakage, antioxidants enzyme activity during withholding irrigation. The results indicated triploid bermudagrass cultivar, Tifdwarf, had highest drought tolerance, followed by manilagrass and carpetgrass. ABA and S_{3307} treatments increased the drought tolerance of turfgrasses, which were associated with their induced antioxidants enzyme activity.

2. Establishment of tissue culture and regeneration in triploid bermudagrass

Sterilized node segments with 5 mm were used as explants and callus induction, suspension subculture, and regeneration were studied. Basal medium composition, proline, phytohormones were compared in the callus induction, suspension culture and regeneration. High effective tissue culture system was established in triploid bermudagrass. Somatic embryogenesis during regeneration in triploid bermudagrass was demonstrated by using scanning electron microscope and light microscope observations.

3. Somaclonal variations and selections for cold resistance in triploid bermudagrass

Morphological variations in leaf color, leaf length and width, stolons internodes length were observed among the regenerated plants from the initial 138 embryogenic calluses, which had been continuously subcultured for over 18 months by suspension culture. Several lines grew fast in the field during spring and summer. Partial lines maintained green color and continuous growth in the winter. The results indicated that somaclonal variations were induced during the long-term subculture of callus and among them useful variants could be selected. It is suggested that somaclonal variation may be used for triploid bermudagrass breeding.

4. A dwarf somaclonal variant in triploid bermudagrass

A dwarf somaclonal variant, TV4, and its parent TifEagle were comparatively studies in morphological characters and response to chilling stress. In comparison to TifEagle, TV4 showed significant morphological variation with decrease in stolons internodes length and leaf length, increase in leaf width. TV4 had extensive root system which would allow it higher drought tolerance, showed higher chilling resistance than TifEagle.

5. Selection of salt-tolerant lines in triploid bermudagrass and their physiological analysis

The salt-tolerant callus lines selected on the medium contained 0.3 mol/L NaCl were regenerated on the medium contained 0.1 mol/L NaCl. Half of the regenerated plants lines showed tolerance to 0.25 mol/L NaCl stress, compared to its parent Tifeagle. Na^+, K^+, Na^+/K^+, proline, soluble sugars and antioxidant enzyme activity were measured after the plants were subjected to salt stress. The results showed that the tolerant lines showed diversity in physiological index in response to salt stress. All the tolerant lines had much higher proline content and lower Na^+/K^+ ratio under non-stress condition.

6. Transgenic plants production, molecular analysis and their stress tolerance in triploid bermudagrass

Transgenic plants for betaine aldehyde dehydrogenase gene (*BADH*) under the control by Ubi promoter were produced by particle bombardment. PCR amplification and PCR-Southern blot analysis showed partial regenerated plants positive. Southern blot of genomic DNA filter demonstrated that BADH was integrated into the genomics in transgenic plants in a manner of multiple copies. Three transgenic lines showed greatly increased tolerance to salt and drought stress.

Key words: Triploid bermudagrass; Biotechnology; Stress tolerance; Tissue culture; Somaclonal variation; Transgenic plants

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