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Silencing GA 20-oxidase Gene by RNAi for breeding dwarf perennial ryegrass

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Key Words : perennial ryegrass , plasmid construction , RNAi , hpRNA , genetic transformation , Dwarf mutant

Introduction Perennial ryegrass (*Lolium perenne* L) , is one of the most widely distributed grasses in the temperate regions for forage and turf . For turf usage , lawn needs mowing frequently because of its fast growth , which amounts to much more labor . GA 20-oxidase is a crucial enzyme in Gibberellin (GA) biosynthesis . RNA interference (RNAi) is a powerful tool for gene silencing , so in this study , we used RNAi and genetic transformation techniques to create dwarf perennial ryegrass .

Materials and methods According to GA 20-oxidase gene sequence of perennial ryegrass , two pairs of specific primers containing special restriction enzyme sites were designed . The target fragments , forward and reverse , were amplified and inserted into both sides of an intron of an intermediated vector pSK-int respectively . Then the target fragment about 700 bp was cut by *Bam*H I and *Sac* I from recombinant-intermediated vector , and was cloned into a binary plasmid p2355 to construct the plant expression plasmid p23Rg723 (Tan et al , 2007) . And p23Rg723 was introduced into *Agrobacterium tumefaciens* EHA105 and then used to transform perennial ryegrass calli . Transformed calli were selected by 50 or 100 mg/L paromomycin and regenerated in differentiation medium . The resistant plants were identified by PCR and GUS histochemical assay . The height of the transgenic plants was evaluated after 3 month growing in green house . Data were analyzed using a SPSS 16.0 , General ANOVA model .

Results The plasmid p23Rg723 , with inverted repeat DNA fragment of GA 20-oxidase gene , was successfully constructed and transformed the calli of perennial ryegrass mediated by *Agrobacterium tumefaciens* . After the calli were selected , regenerated , identified by PCR and GUS histochemical assay , eighteen transgenic plants were obtained . Among them , 11 were dwarf plants . There were significant differences between wide types and dwarf mutants . The average height of dwarf plant was 10.8 cm , compared to that of wide type which was 21.7 cm after 3 months growing in green house (Table 1) . The result is similar to that found in tomato (J Xiao et al , 2006) .

Table 1 The plant height of wide types and dwarf mutants .

Number	Height of plant (cm)											Average Height (cm)
	1	2	3	4	5	6	7	8	9	10	11	
Wide types	23.3	22.1	22.0	19.5	19.8	20.6	21.4	24.0	21.6	22.5	21.4	21.7
Dwarf mutants	13.5	12.3	11.5	10.4	8.6	9.8	10.0	9.7	11.1	7.9	14.0	10.8

LSD P<0.01

Conclusions The transcriptional products of the foreign inverted repeat DNA fragment can form hairpin RNA (hpRNA) to interfere with GA 20-oxidase gene expression and resulted in dwarf phenotype of transgenic perennial ryegrass . The results showed we can use RNAi and genetic transformation techniques to create and breed dwarf ryegrass for turf usage . This also demonstrated that GA 20-oxidase gene plays an important role in plant growth .

Reference

J Xiao , H Li , J Zhang , R Chen , Y Zhang , B Ouyang , T Wang , Z Ye (2006) . Dissection of GA 20-oxidase members affecting tomato morphology by RNAi-mediated silencing . *Plant Growth Regulation* , 50 :179-189 .