Study on cloning and expression analysis of cold tolerance genes in different fall dormant alfalfa

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Introduction During Chinese agricultural structural adjustment , the development of feed crops is one of the directions in which popularization first is clover . Clover industry should have more suitable species as guarantee . Alfalfa , which is called *the king of grazing*" , is one of the most important legumes in China . It has many merits such as adaptability , good quality , stress resistance , rate of growth , etc . It could also be used for environmental greening and food industry , especially the leaf protein of alfalfa is a good material for medical and health foodstuff . Low temperature is one of the mostly serious factors limiting development of an alfalfa industry and it is very easily happened about freezing injury and dying phenomenon in northern cold areas . Therefore it is urgently need to breed alfalfa species with capability of cold resistance . Cold resistance was one kind of genetic characters to adapt low temperature during a long period , cold resistant gene only expressed under conditions such as low temperature , short duration of sunshine , water stress and make the plants develop the capability of cold resistance .

Fall dormancy of alfalfa is one kind of variation about conformation and vitality because of brightness and climate changing . The growth of alfalfa should be at a standstill when fall and winter comes , and it would gradually enters into hibernate condition in order to resistant bad environment . Alfalfa did not grow until having suitable conditions .Fall dormancy of alfalfa had directed relationship to cold resistance and growth performance the grades were divided into nine according to the difference of cold tolerance and also was an index to identify breeds .

Materials and results Cold tolerance genes were primarily divided into two types including constitutional expression and inducing expression . Cold inducing gene expressed only under special condition and showed the ability of cold resistance . CAS was a cold applimation-specific gene family including CAS15A , CAS15B , CAS17 , CAS18 , Pmcig7 , Pmcig34 in alfalfa to resist cold which could express through low temperature induction , its metabolic pathway is not clear . Greatly obtaining the interest protein with the genetic engineering methods is important means in cold resistant breeding . Tobacco , as the plant transgenic model , has successfully expressed many exogenous gene including different original genes of plants , animals and microorganisms . In order to get transgenic plants greatly expressed , this research transferred exogenous cold tolerance gene of alfalfa into tobaccos mediated by Agrobacterium tumefaciens .

Cold tolerance was studied with three different fall dormancy alfalfa strains (Gongnong NO .2 , Beijixing , Meiguoxiong) . The specific primers were designed according to consensus amino acids sequence of alfalfa , and three CAS18 whole cDNA sequences of cold specific-accomplication gene from different alfalfa were obtained by RT-PCR to offer excellent genetic resources for investigation and application of alfalfa cold tolerance . The result showed that the three genes cloned did not matched with the sequence published in GeneBank , the length of three genes were 951bp、918bp、948bp .The predicted polypeptide was 134 amino acids with 1 .3 kDa and pI 5 .40 . The functional and transmembrane domain analysis showed that the gene cloned contained special functional domain in CAS family , it should be one new member in CAS family which was named as CAS19 . CAS19 is a matrix protein because there is no transmembrane domain and it maybe have no function of transmembrane transport .

In order to expand the scope of planting and application of white clover , the CAS19 gene was transferred into white clover to enhance the abilities of cold tolerance . However , we knew nothing about the effect and expresses mechanism of the CAS19 gene and whether it is expressed in white clover , CAS19 gene was transferred into tobacco and the expression pattern was analyzed . Three genes were cloned into expression vectors with 35S promoter mediated by Agrobacterium tumefaciens . Transgenic tobacco was tested . Forty tobaccos were selected from each types and the achievement ratio was 62 .5% ,57 .5% , 52% . The results proved that the CAS19 gene of three different fall dormancy alfalfa had been integrated into tobacco genome and expressed successfully .