The application of secondary metabolites in the study of sorghum insect resistance

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Abstract. Insect attack is one of the main factors for limiting the production of rice and sorghum. To improve resistance to pests of rice and sorghum will be of great significance for meliorating their production and quality. However, the source and material of anti-pest was scarce. In this study, we will study on the expression patterns of hydrocyanic acid biosynthesis relative genes in sorghum firstly. And we will also genetically transform them into rice and sorghum by specific and constitutive promoters and verify their pest-resistant ability. Finally, high pest-resistant genetically modified new sorghum cultivars will be bred with favorable comprehensive agronomic traits.

1. Introduction

Insect attack is a predominant factor, which is hazardous to high and dependable yield for agriculture crops. The plantation area worldwide for sorghum crops was up to 40 million ha² in 1999 [1], and sorghum enjoyed its harvest area for more than 400 thousand hectares [2] worldwide in 2009. However, loss due to pest damage is up to one billion US dollar approximately each year. There are at least 150 kinds of hazardous insects to sorghum worldwide [3].

The hazardous insects to sorghum in China mainly include Ostrinia furnacalis (European corn borer), Chilo venosus (spotted sugarcane borer), Aphis sacchari (sorghum aphid), Leucania separate (armyworm), Phyllotreta nemorum (flea beetle) and Helicoverpa armigera (cotton bollworm) and the similar. These insects, as capable of propagating for several generations during one year, shall infect different organs of sorghum plants at different growth and development stages, and they also impact leave photosynthesis to make the stem and leaves so crooked and brittle that the plants are unable to ear or blossom or fruit, though earing is expected. Mild damage may result yield loss for 10-30%, while sometimes for 40-70% and even total crop failure in sever cases [3].

To cultivate resistant varieties is the most optimal approach, but there are some problems if conventional pest-resistant varieties are depended on solely, such as a long journey for new variety breeding and narrower hereditary basis of sorghum. Sorghum varieties with natural pest-resistance to borers or other insects have not been located so far home and abroad. When pollution-free production and green consumption are under great promotion nowadays, people are faced with a hot spot and the difficulty in plant protection, or even in molecular breeding studies on pest resistance. That is how to resolve conflicts between sorghum pest damage control and less or even no chemicals to be applied for such purposes.

Based on resistance gene sources, breeding technology for pest-resistant varieties of transgene includes the following plans. Firstly, BT gene whose source is from bacillus thuringiesis is the most
applied one [4]. Secondly, pest-resistance genes, whose source is from plants, such as lectin genes, proteinase inhibitor genes and the similar are mostly involved accordingly [5]. Finally there is a new strategy that autologous gene segments of an insect shall be utilized. Expression of key genes for growth and development or for biochemical metabolism in the insects shall be under intervention and inhibition by RNA as an indirect way of pest control.

2. Research progress in pest-resistance of secondary metabolites

Plants have developed their numerous adaptive mechanisms or defense mechanisms when they interact with their environment or compete with it for a long time. These mechanisms involve morphology and structure, physiological and biochemical metabolism and others. These characteristics have transformed into heritable properties to be reserved gradually, so this may regarded as evolution and development for plants during their adaption to environment. Secondary metabolites produced by plants against pest infection mostly include phenols, terpenoids, alkaloids and some signaling molecules related to defense mechanism, together with resistance to be improved by regulating secondary metabolism pathways.

Plants, when exposed to injury or damage, shall produce specific secondary metabolites to defense against insect attacks [6]. Several plants, including sorghum, are able to produce cyanogenetic glycoside [7], a defensive chemical resistant to herbivore and pests [8]. As capable of antioxidation, it also plays an important role in nitrogen turnover and storage [9]. For instance, upon artificial damage or taken by phytophagous insects, sorghum leave shall release toxic HCN to fight against pest attacks. As Tattersall and others reported in 2001, two CYP450 genes (tyrosine-N-hydroxylase (CYP79A1) and (CYP71E1)), which were responsible for catalyzing synthesis of cyanogentic glycosides, were transferred to arabidopsis thaliana. Then such transgenic arabidopsis thaliana synthesized dhurrin, a substance resistant to flea beetles, pests for Cruciferous plants [10].

There are three major types of toxic glycosides in plant food: cyanogenic glycosides, hioglycosides and saponins. The chemical name for dhurrin is listed as P - hydroxy - (S) - phenethanol nitroxsyl - beta - D – glucoside. It is the cyanogentic glycoside in sorghum plants and produced by tyrosine. Dhurrin is able to disturb β-glucosidase hydrolysis in the leaf tissue and release hydrogen cyanide (HCN) and p-hydroxybenzene. Such a procedure is called cyanogenic procedure for plants [11]. Cyanogenic glycosides do not show toxicity themselves, but if fodder with cyanogenic glycosides is fed, it will become toxic when cyanogenic glycosides produce HCN by means of hydrolysis under some favorable conditions [12]. Then it is also observed that dhurrin enjoyed higher concentration in seedling stage and younger leaf growth stage than it did at maturity stage [13]. As some literature reported [13], related genes to yamide synthesis (CYP79A1 and CYP71E1) enjoyed their highest expression at 1-2d of sorghum emergence, at 2d in particular, and later both concentration and activity declined. As a result, HCN concentration was the highest at 5d of sorghum emergence, and later it tended to decline.

As what we have perceived, HCN expression was rather obvious at sorghum seedling stage, and full-length genomes of CYP79A1 [14], CYP71E1 [15], UGT85B1 and dhurrinase [16] were cloned from sorghum plants. Also there are a great many reports on pest resistance, but situations remain unchanged, and no studies have been carried out on pest resistance for sorghum or other commercial crops so far. The major reasons are discussed as the following two aspects: Firstly, genetic transformation studies on sorghum are falling far behind those on cereal crops, paddy rice for instance, though sorghum is a kind of vital multi-purpose crops. However, there are some successful cases during these years. Girijashankar and others (2005) adopted particle bombardment to transfer cry1Ac, the insecticidal protein gene into grain sorghum[17]. The genes are practically actuated by mpiC1, the wound induced promoter, cry1Ac protein accumulation in the damage fresh leaf tissues amounted to 1-8ng/g leaf tissue for transgenic sorghum plants at their T1 generation. Lu and others (2009) employed agrobacterium-mediated transformation with successful transfer results for sorghum, and the transformation frequency was up to 0.7%[18]. Therefore, there are no transgenic varieties of sorghum at the market as a fact, but biological technology shall be applied as a crucial means for pest control, and it is capable of increasing sorghum yield accordingly. Secondly, HCN, as a sort of metabolites in the plants, shall be produced via pyrolysis of cyanogenetic glycoside or hydrocyanic ether. Then there is another pathway, namely ACC (1-Aminocyclopropane-1-Carboxylic Acid) which is able to produces
ethylene, as well oxidase at the same time upon ACC oxidases are catalyzed. Therefore, cyanides are widespread in plant tissues, and they could be apparently produced in edible seed germination in particular. Cyanides not only prove to be toxic for pests, but also potentially hazardous to safety for other beneficial organisms or human being [19-20].

3. Conclusion

Then we shall have to consider safety, so we plan to use specific promoters to transfer genes of cyanide synthesis pathway to cause its high expression at some development stage for plants. Then cyanides shall be only toxic to pests, while harmless to human being and cattle. Furthermore, transgenic crops with high pest-resistance shall be applied to other secondary industries. At present we have successfully cloned A and B genes in sorghum plants through Gateway clone technology, and the constructed pM DC141 expression vector was imported into NNB EH A105. The PCR and sequencing results showed that the constructed vectors and the obtained engineered bacteria were identical with the expected. Next, we plan to carry out genetic transformation for arabidopsis thaliana, maize and sorghum plants in order to verify these genes function associated with fending off insect attack.

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