Review

Biological research advancement in *Aloe*

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*Aloe*, a genus belonging to *Aloaceae* family, is distributed throughout the world and has rich medical ingredients. At present, *Aloe* has been widely applied in many aspects for human being. In this paper, we introduced briefly its basal biological characteristics, including germplasm resources, growth habit, active ingredients and biological functions, and discussed deeply its research development in cytology and molecular biology. This knowledge stated here is beneficial for understanding and exploring its potential medical and edible values more sufficiently.

Key words: *Aloe*, medical plant, cytology, molecular biology.

INTRODUCTION

Germplasm resources

*Aloe*, a genus belonging to *Aloaceae* family (www.ipni.org), belongs to perennial, evergreen and monocot crassulacean acid metabolism (CAM) plant. It is one of herbaceous or woody plants, and characterized by stemless, large, thick and fleshy leaves showing lance shaped, a sharp apex and a spiny margin. *Aloe* is widely distributed in the world (Steenkamp and Stewart, 2007). In southern Africa and Madagascar islands, it is cultivated as a popular economic plant (Zhu, 2007). In P. R. China, it is also planted widely in many provinces including Guangdong, Yunnan, Fujian and Hainan (Xiong, 2002). *Aloe* originated from Africa, includes at least 300 wild species and over 200 bred varieties according to previous literature (Zheng et al., 2005), however, only about ten of them are applicable to medicine and less than ten can be eaten safely (Xiong, 2002).

Growth habit

*Aloe* is fond of warmth and light, even being exposed in sunshine about two months, it will not die, only presents no continuous growing. On the contrary, it is afraid of cold, shady and humidity. It will be frozen and can not grow when environmental temperature is below -10℃; if it grows in ponding soil for long time, its roots and leaves will be harmed seriously and become necrotic gradually. More interestingly, *Aloe* shows strong resistance to drought, salt and poor soil, certainly, it can grow better in loose and fertile soil than in consolidated and nutrient deficient soil. Generally, *Aloe* can blossom after growing about 2 to 3 years; nevertheless, its proliferation is mainly accomplished by tillering in root due to pollen abortion (Lv et al., 2004).

Active ingredients

*Aloe* has complex chemical ingredients, from literature, there are over 100 secondary metabolites in leaf (Xiong, 2002). The main chemical components in *Aloes* can be classified into seven compounds, including anthraquinones, carbohydrates, amino acids, organic acids, minerals and microelements, active enzymes and vitamins, among which anthraquinones are the most important active ingredients and the four matters showing quite high medical values, including aloin, *Aloe* emodin, *Aloe* bitter and *Aloe* lectin, belong to anthraquinones (Wang, 2009). In general, the content of active ingredients is determined by variety and ecological condition (Wang et al., 2002; Ye et al., 2005). For example, in the experiment by Qiao et al. (2009), blue
light was beneficial to the accumulation of anthraquinones.

**Biological functions**

*Aloe* has important applications for human being in many fields, including medicine, food, cosmetic, health care and horticulture. Take medical value as an example, generally speaking, it plays important roles in many aspects (Grindlay and Reynolds, 1986), such as bacteriostasis, reducing blood lipid, resistance to life ageing and tumor forming. More specifically, it can provide curative effects to some extent in constipation, tuberculosis, skin disease, heart disease, diabetes and so on (Reynolds and Dweck, 1999; Kambizi and Afolayan, 2008). With the development of modern science and technology, many unknown functions in *Aloe* were gradually revealed. Owing to unique effective gradients and special functions, *Aloe* gained many reputations, such as these dynames “the champion among health care medicines”, “the best heath food in 21st century” and “new star in plant”. In Japan, it is known as “all-day service” and “doctor away”. Because of the ability of curing large number of diseases, *Aloe* is also reputed as “the standby medicine-chest”. In addition, it is named “raw cosmetic materials” and “natural beautician”, due to the important effect on face-painting. Furthermore, *Aloe* is also “air pollution detector” because the pollution extent of air is related to its growing status (Yang and Ma, 2000).

**CYTOLOGICAL RESEARCHES**

**Chromosome**

Many studies showed that the chromosome number for somatic cell of most *Aloe* is $2n = 14$, and the haploid set genome consists of three short chromosomes and four long ones (Brandham and Doherty, 1998; Ji et al., 2002; Alam and Khanam, 2005). Many studies demonstrated that most plants have *Arabidopsis*-type telomeres consisting of many repeat copies of the sequence 5'-TTAGGG-3' (Adams et al., 2000a), nevertheless, this kind of telomeres are not found in *Aloe*, similar to *Allium*, *Nothoscordum* and *Tulbaghia*. *Aloe* lacks *Arabidopsis*-type telomeric repeats, but it have vertebrate-like telomeric sequences ($T_2A_G_3$) according to the report by Weiss and Scherthan (2002). Tetraploid and hexaploid *Aloe* can be induced from diploid *Aloe* (Ren et al., 2007). In the report by Wang et al. (2001), the highest induction rate was up to 50% after treatment with 0.06% colchicines for 12 h. Compared to diploid, polyploid *Aloe* displayed thicker, larger and darker leaves, besides, this kind of *Aloe* presented more excellent physiological and economical characters, and stronger resistance to cold and disease is a typical example (Ren et al., 2007).

**Karyotype and band pattern**

Although, most *Aloe* has same chromosome number, different species display differences in karyotype. The karyotype of *Aloe* consists of near-to-terminal and near-to-middle centromere chromosomes (Ji et al., 2002; Zheng et al., 2005). According to the classification criterion reported by Stebbins (1971), the karyotypes of *Aloe* belong to ‘3B’, ‘4B’, ‘3C’ or ‘4C’ (Zheng et al., 2005). For example, $K(2n) = 45 + 10s$ of *Aloe ferox Miller* and $K(2n) = 45 + 10s$ of *Aloe arborescens* were belonged to 4C and 3B, respectively (Liang and Bo, 2001). At present, the studies on chromosome band pattern of *Aloe* were hardly ever reported, only *Aloe yuanjiangensis* from P. R. China was analyzed by Li et al. (2003), which pattern formula is $2n = 14 = 4st + 6sm + 4m$, and the result obtained by Giemsa C-bands showed that the short arms of chromosomes 3 and 4 presented whole bands, while the other chromosomes just displayed centromeric bands.

**Meiosis and pollen abortion**

As is well-known, *Aloe* shares a conserved bimodal karyotype with a basic genome of four large and three small submetacentric oracrocentric chromosomes. But due to deviant chromosomal conjugating of pollen mother cells, the genetic materials of their daughter cells behave heterogeneous frequently (Brandham, 1990). In addition, some abnormal phenomena can be found during meiosis for pollen mother cells, such as univalent, multivalent, chromosome bridge, lagged chromosome and micronuclei (Lv et al., 2005). And thus, the pollens descended from pollen mother cells are often sterile (Spare, 1975), this can explain high self-incompatibility of *Aloe*. But interestingly, its stigmas located on the top of gynoeica have high receptivity ability in pollination (Tie et al., 2004).

**Tissue culture**

*Aloe* will bloom and fruit after planting for years, nevertheless, its seeds is often lacking due to great interval between male and female flowering time and high-frequency pollen sterileness. Therefore, natural and artificial propagation are mainly depended on tillering in root and cuttage, respectively. Comparatively, tissue culture can accelerate propagating of *Aloe* and improve its partial economic traits (Qin and Luo, 2003; Bedini et al., 2009). At present, there are many studies on *Aloe* tissue culture in literature (Qu et al., 2004; Hashemabadi...
and Kaviani, 2008). With respect to the effects of plant regulators on tissue culture in Aloe, You (2001) reported 6-BA (6-Benzylaminopurine) was the most important phytohormone for callus induction on MS basal media, followed by IBA (indole-4-butyric acid), then 2,4-D (2,4-dichlorophenoxyacetic acid), and NAA (1-naphthylacetic acid) was the worst. Additionally, Xiao et al. (2006) reported the perfect combination of plant growth regulators for callus induction was “4.0 mg/L 6-BA + 0.25 mg/L NAA + 3.0 mg/L 2, 4-D + 0.5 mg/L IBA”. As for Aloe tissue culture, different parts of plant possess different callus induction rate and regeneration ability, and stem segment is considered as the best explant (Long et al., 2007). For example, in the result as described by Yuan et al. (2001), the stems of Aloe barbadensis Mill could be induced by callus and differentiated into plantlets, but under same experimental condition, the leaf can hardly be differentiated.

MOLECULAR RESEARCH

Gene cloning and expression

Aloe is a CAM plant which possesses phosphoenolpyruvate carboxylase (PEPCase) and NADP-malic enzyme (NADP-ME). Till date, both of the two enzyme genes had successfully been cloned from Aloe (Honda et al., 1996; Houda et al., 2000). According to the report by Honda (1996), the circadian rhythm for PEPCase activity may be regulated at post-translation level. As for Aloe NADP-ME, Honda et al. (2000) reported three major isoforms, including 65 kDa protein with a pI of 5.5 in leaf, 65 kDa protein with a pI of 5.6 in roots, and 72 kDa protein in leaf and root simultaneously. Further, Sun et al. (2003a) pointed out the expression of NADP-ME gene can be induced by high salt, dehydration, and exogenous plant regulator abscisic acid, but not by cold treatment, and the further experiment indicated that its protein expression level would gradually increase with the prolonging of treat time.

Additionally, some resistance genes to environmental stress were also cloned from Aloe. A new cold-induced dehydration-responsive element-binding (DREB) gene was cloned by Wang and He (2007), named Aloe DREB1 and encoding an AP2/ethylene response element binding protein transcription factor. Afterwards, another gene encoding DREB protein was isolated by Zhang et al. (2009a), the gene named AIDREB2 is an active regulator and its expression is involved in dehydration tolerance and cold acclimation in Aloe. Besides, some other genes were successfully also isolated from Aloe, including a novel aldo-keto reductase gene by Mortita et al. (2007) and three novel type III polyketide synthase genes (PKS3, PKS4 and PKS5) by Mizuuchi et al. (2009). Besides these, Sun et al. (2003b) constructed a cDNA subtractive library by the method of suppression subtractive hybridization and Zhang et al. (2007) obtained some expressed sequence tags derived from the sixth chromosome of Aloe.

Genetic transformation

Although, Aloe has strong resistance to drought and many pathogens in plant, it is afraid of cold (Zhang et al., 2009b), and thus, particular attentions in transgenic study have been focused on cold stress. In order to improve the resistance to cold stress of Aloe plant, Chen et al. (2005) first introduced otsA gene into its genome via microprojectile bombardment, and obtained some transgenic plants. Subsequently, Chen et al. (2007) transformed Aloe with otsA gene mediated by Agrobacterium, and the transgenic plants presented more content of trehalose than untransformed plants as control. Lately, Zhao et al. (2009) introduced TaDREB gene isolated from wheat into Aloe by Agrobacterium, and the results demonstrated that this transformation could improve the resistant ability of Aloe to low temperature.

As for the influence factors on Aloe transformation, many experimental results showed that acetosyringone was necessary in improving transformation rate mediated by Agrobacterium (Chen et al., 2004; Pan et al., 2005). In addition, Pan et al. (2005) reported pretreatment time of the Agrobacterium liquid and the pH value could significantly influence genetic transformation. The optimum infection time was 12 to 18 min, and the best temperature and cocultivation time were 25°C and 5 days, respectively. With respect to host bacteria, EHA105 was more efficient than LBA4404, AGL1 and C58C1 in the genetic transformation (Pan et al., 2005). As for antibiotics, Chen (2004) pointed out that untransformed Aloe was resistant to cefotaxime and carbenicillin, while susceptible to kanamycin and hygromycin. As far as concrete procedure of microprojectile bombardment transformation, Chen (2005) talked about that pre-culture, hypertonicity treatment and postponing screening could contribute to successful transformation.

Genetic diversity

To this day, only few studies on genetic diversity of Aloe species were reported in literature, Adams et al. (2000b) constructed a phylogenetic tree using 28 Aloe species by sequence analysis of the internal transcribed spacer (ITS) of 18S-5.8S-26S rDNA, and found the distribution of 18S-5.8S-26S rDNA was variable in number, location and size in different Aloe species. Afterwards, Hou et al. (2001); Li et al. (2002); Shioda et al. (2003) investigated the phylogenetic relationship for different varieties of Aloe using random amplified polymorphic DNA technology.
PROBLEMS AND PROSPECTS

Despite a large number of reports on studying Aloe, there still exist some fundamental problems which need to be worked out. To begin with, systemic classification of Aloe is disputed. Most researchers deem that Aloe belongs to Aloeaceae family, but some scholars proposed that the genus should be included in Liliaceae or Xanthorrhoeaceae family (http://www.ars-grin.gov/cgi-bin/npgs/html/taxon.pl?311403). Secondly, although Aloe extracts have been widely applied in phytomedicine, its many noxious ingredients are still not ascertained (Steenkamp et al., 2007), for example, Rabe et al. (2005) reported the first case of acute hepatitis induced by Aloe preparation, but the specific noxious ingredients were not clear. In fact, only few Aloe varieties are safe enough to be eaten. Additionally, though more and more evidences suggest Aloe extracts can suppress the forming and growth of tumor to some extent, the obtained results only were based on experimental rat, not human (Lee et al., 2001; Park et al., 2009; Wang et al., 2010).

At present, the active ingredients and biological function of Aloe have attracted more and more researchers (Shen et al., 2001; Zhang et al., 2009b), but the study at molecular level has just started, many fields need to be further explored by means of modern experimental technology. We believe that this paper can provide some useful information for the biologic research of Aloe, of course, we also confirm that Aloe as “the best health food in 21st century” will bring enormous economic and social benefits in future.

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