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Production of dragon's blood in *Dracaena cochinchinensis* plants by inoculation of *Fusarium proliferatum*

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ABSTRACT

Dragon's blood is a traditional medicine widely used in the world from ancient times. However, little is known about its formation mechanism. This work aimed to gain some insights into its formation mechanism and to control its production. The results demonstrate that wounding plus causal fungal infection and keeping the wound moist are essential for efficient dragon's blood formation in *Dracaena cochinchinensis*. Two fungal isolates YM-266 and YM-71213 of *Fusarium proliferatum* increased the yield of dragon's blood in *D. cochinchinensis* trees by 2.7- and 3.3-times compared to that of the control (wounding alone and keeping the wound moist), respectively. The fungal induced dragon's blood had almost identical chemical constituents to that of the natural dragon's blood with a higher loureirins a and b content as analyzed by TLC and HPLC. In addition, the induced dragon's blood had similar antimicrobial activity and similar or higher antioxidant activity than that of the natural dragon's blood. The novel biological technology developed here for the production of dragon's blood is safe, repeatable, practical, and feasible for the farmers, enabling the production of dragon's blood in a sustainable way without destroying the endangered trees and environment.

1. Introduction

Dragon's blood is a deep red resin, which has been used as a precious traditional medicine and artistic material by many civilizations since ancient times [1]. It is originally and mainly obtained from trees of *Dracaena* spp., with trees of *Daemonorops* spp., *Croton* spp. and *Pterocarpus* spp. as alternative resources. *Dracaena cochinchinensis* (Lour.) S.C. Chen was found in Yunnan Province, China in 1970s [2]. Since then it has been developed as the main source of dragon's blood substituting for the imported dragon's blood from Africa obtained from *Dracaena cinnabari*. Oral administration of dragon's blood could stimulate blood circulation and relieve pain, mainly for the treatment of coronary heart disease, cerebral infarction and other thrombotic diseases; external therapeutics of it could stop bleeding, promote wound healing, mainly used for various skin or mucosal diseases. Recent research shows that dragon's blood has antimicrobial, antioxidant, antiviral, antitumor and cytotoxic activities as well [1].

Commercial (pharmaceutical) dragon's blood is the alcohol extract of the resinous wood of *Dracaena* trees. The main components of dragon's blood are flavonoids and stilbenoids (phenolics) [3–6]. These compounds are biosynthesized via the phenylpropanoid pathway (Fig. 1). However, the major effective constituents of dragon's blood are still unclear. In China, the content of loureirin b is arbitrarily used for the evaluation of the quality of dragon's blood. A recent work shows that loureirin b restrains the formation of thrombus in rat, reduces the viscosity of whole blood and blood plasma and prolongs the time of thromboplastin in the model of acute blood stasis rats [7]. Loureirin b also takes part in the analgesic effects of dragon's blood in rats [8]. The chemical structure and biosynthesis pathway of loureirins a and b are presented in Fig. 1.

D. cochinchinensis grows extremely slow with very low and unstable dragon's blood yield [9]. Only trees with 30–50-year-old have the possibility to produce dragon's blood. In particular, dragon's blood is produced mainly by the xylem parenchyma cells of the trunk. There is no secretory tissue to secrete dragon's blood [6], so that it is impossible to collect dragon's blood without destroying the tree by incision of a tube like collecting rubber latex. To obtain a few pieces of dragon's blood material, a tree with hundreds of years, even thousands of years old is often destroyed, resulting in the resource of dragon's blood less and less. On the other hand, the clinical demand of it is ever increasing [2,4,10].

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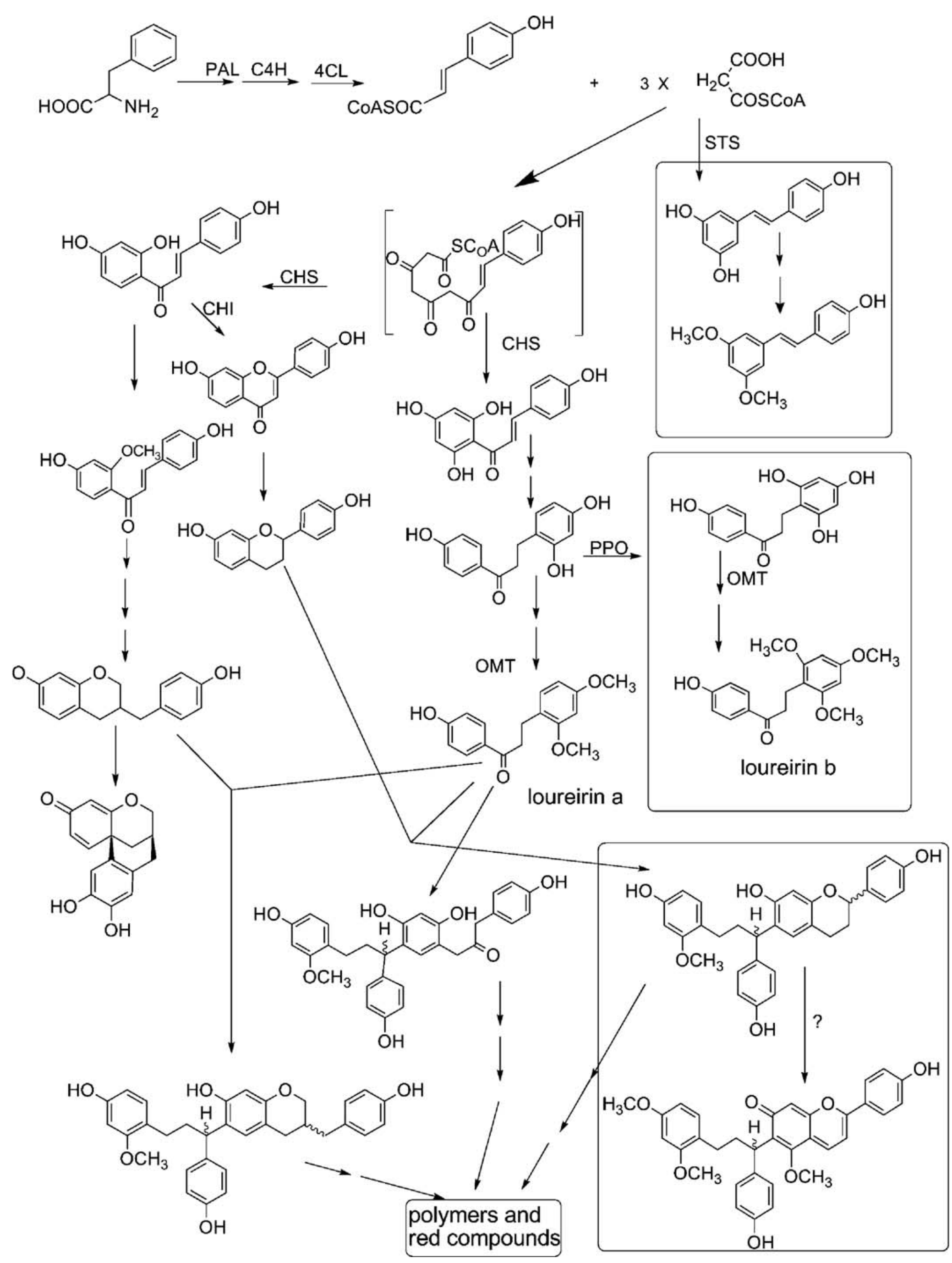

Fig. 1. Scheme of the phenylpropanoid pathway showing the biosynthesis of flavonoids, phenolic compounds and stilbenoids of dragon's blood (adapted from [26] and [9]). The formula and biosynthesis pathway of loureirins a and b are included. Enzymes in the pathway: PAL, phenylalanine ammonia-lyase; C4H, cinnamate-4-hydroxylase; 4CL, 4-coumaryl: CoA-ligase; CHS, chalcone synthase; OMT, *O*-methyltransferase; CHI, chalcone isomerase; STS, stilbene synthase; ?, no enzymes reported (perhaps as chalcone polymerase, CHP); PPO, polyphenol oxidase.

Presently, *D. cochinchinensis* has been one of China's endangered species listed in the national third protection group [11]. Owing to world wide overexploitation, other *Dracaena* spp. that produce dragon's blood such as *D. draco* [12] and *D. cinnabari* [13] have been included in the *IUCN* (IUCN-International Union for the Conservation of Nature and Natural Resources) *Red List of Threatened Species* (www.iucnredlist.org) as well. Fortunately the artificial propagation and cultivation of *D. cochinchinensis* via seeds and cuttings are very easy. Presently the plantation scale of *D. cochinchinensis* in Yunan Province is about 1500 ha. It is estimated that to satisfy the domestic demand in China the plantation area has to reach 17,000 ha.

Most of the research work on *Dracaena* spp. focuses on the analyses of their chemical constituents as well as the bioactivities and therapeutic applications of dragon's blood [1,3,4,14]. Little is known about the formation mechanism on dragon's blood; there is no efficient method to promote or induce dragon's blood formation. At what time a tree will produce dragon's blood and how much will be produced are uncertainty. Without an efficient induction method the young planted trees could not produce dragon's blood to release the current demand stress; people have to continue to collect the product from the wild trees. Without an induction method an investor could not benefit from the plantation of the trees within 30–50 years. This might mean that an investor cannot