Archives of Biochemistry and Biophysics 504 (2010) 123–131

Contents lists available at ScienceDirect

Archives of Biochemistry and Biophysics

journal homepage: www.elsevier.com/locate/yabbi

Carotenoid inhibitors reduce strigolactone production and *Striga hermonthica* infection in rice

Muhammad Jamil [a], Tatsiana Charnikhova [a], Francel Verstappen [a], Harro Bouwmeester [a,b,*]

[a] Laboratory of Plant Physiology, Wageningen University, Droevendaalsesteeg 1, 6708 PB Wageningen, The Netherlands
[b] Centre for Biosystems Genomics, P.O. Box 98, 6700 AB Wageningen, The Netherlands

ARTICLE INFO

Article history:
Available online 21 August 2010

Keywords:
Carotenoid inhibitors
Strigolactone
Rice
Parasitic plants
Striga hermonthica

ABSTRACT

The strigolactones are internal and rhizosphere signalling molecules in plants that are biosynthesised through carotenoid cleavage. They are secreted by host roots into the rhizosphere where they signal host-presence to the symbiotic arbuscular mycrorrhizal (AM) fungi and the parasitic plants of the *Orobanche*, *Phelipanche* and *Striga* genera. The seeds of these parasitic plants germinate after perceiving these signalling molecules. After attachment to the host root, the parasite negatively affects the host plant by withdrawing water, nutrients and assimilates through a direct connection with the host xylem. In many areas of the world these parasites are a threat to agriculture but so far very limited success has been achieved to minimize losses due to these parasitic weeds. Considering the carotenoid origin of the strigolactones, in the present study we investigated the possibilities to reduce strigolactone production in the roots of plants by blocking carotenoid biosynthesis using carotenoid inhibitors. Hereto the carotenoid inhibitors fluridone, norflurazon, clomazone and amitrole were applied to rice either through irrigation or through foliar spray. Irrigation application of all carotenoid inhibitors and spray application of amitrole significantly decreased strigolactone production, *Striga hermonthica* germination and *Striga* infection, also in concentrations too low to affect growth and development of the host plant. Hence, we demonstrate that the application of carotenoid inhibitors to plants can affect *S. hermonthica* germination and attachment indirectly by reducing the strigolactone concentration in the rhizosphere. This finding is useful for further studies on the relevance of the strigolactones in rhizosphere signalling. Since these inhibitors are available and accessible, they may represent an efficient technology for farmers, including poor subsistence farmers in the African continent, to control these harmful parasitic weeds.

Introduction

Rice is a cash crop for small to medium scale subsistence farmers of sub-Saharan Africa and during the past few decades is becoming an important and rapidly growing food source throughout the region [1]. Due to the increase in the population (4% per annum) and preferences of urban consumers for rice, domestic rice consumption is rising at a rate of 6% per annum [2,3]. To fulfil the demand, Africa is becoming a major importer of rice and its annual share in global import is 32% [4]. About 9 million tons of rice were imported during 2007, costing about US$ 2 billion per year [3]. Rice is the 5th cereal in Africa in terms of area harvested with about 9.5 million hectares (ha) during 2008 and has the 4th position in terms of production with about 23 million tons during 2008 [5]. The average yield of rice in sub-Saharan Africa is very low (2.4 tons ha^{-1}) and constitutes one of the main challenges of rice production [3]. In addition to other limiting factors, weed competition and especially infestation by parasitic weeds is one of the main causes of low rice yield in the region [2,6,7].

Striga hermonthica is an obligate hemiparasitic plant species that parasitizes grasses and major cereal crops – such as maize, sorghum and millet – in the African continent. It grows on the roots of its host and withdraws photosynthates, minerals and water from the host through a direct connection with the host xylem in an organ called the haustorium. These parasites cause much damage to the host plant and they are a major biotic constraint in cereal production in the African continent imposing a threat to food security [2]. The yield losses due to infection by *Striga* spp. range from 20% to 80% and severe infestation sometimes leads to complete crop failure [8]. In sub-Saharan Africa, an estimated 30–50 million hectares of cultivated area are infested with seeds of *Striga* spp. and this infestation is increasing day by day contributing to poverty of African people [9].

Over the past few decades, the global scientific community has done its best to help to find solutions to get rid of this noxious weed and to improve the life of poor African farmers, but so far with only limited success. To date, not even a single efficient and economically viable *Striga* control method is available [10].

* Corresponding author at: Droevendaalsesteeg 1, 6708 PB Wageningen, The Netherlands. Fax: +31 317 418094.
E-mail address: harro.bouwmeester@wur.nl (H. Bouwmeester).

0003-9861/$ - see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.abb.2010.08.005

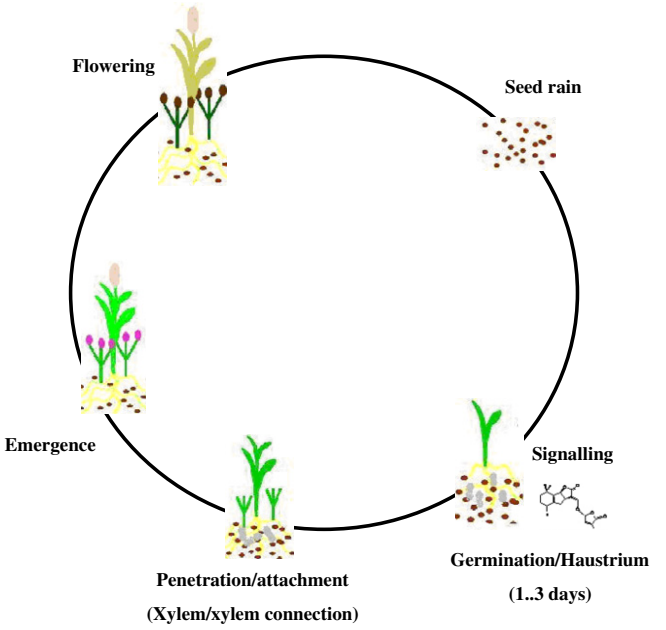

Fig. 1. The life cycle of *Striga hermonthica* (adapted from [53]). After conditioning and perceiving strigolactones, *Striga* seeds can germinate. The radicle grows towards the host root, attaches and penetrates the host root using a specialized feeding structure called haustorium. Developing *Striga* plants grow underground for 4–7 weeks prior to emergence. Much of the damage to the host already occurs at this stage. Then it forms a shoot, emerges above the soil, flowers and produces seeds after which the lifecycle can start again.

Integrated *Striga* management has been given due attention in recent years, but the results are limited. The necessity to control *Striga* before emergence, the production of large numbers of *Striga* seeds by even a single plant, the long seed viability and the complicated life cycle of *Striga* spp. are some of the factors responsible for this failure [11]. Nevertheless, knowledge of the *Striga* lifecycle (Fig. 1) should lay the foundation for *Striga* control. We postulate that control during the earlier stages of the lifecycle may prove most successful instead of at later stages even simply because much of the damage to the host already occurs during the early stages of attachment, prior to *Striga* emergence [12]. The first interaction between host and parasite is the induction of germination of the parasite by the host-root secreted germination stimulants [13,14]. *Striga* seeds will not germinate without perceiving these signals from the host and these signalling molecules could be a potential target to develop a *Striga* control strategy [13].

Compounds from several different secondary metabolite classes such as dihydrosorgoleone, an isoflavanone, sesquiterpene lactones and strigolactones, have been reported to be secreted by the host root and have germination stimulant activity [13–15]. However, there is more and more evidence that the strigolactones are the major class of germination stimulants [14]. In addition to being host-finding factors for parasitic plants, it was reported some years ago that strigolactones also act as host detection signal for arbuscular mycorrhizal (AM)[1] fungi [15]. Plants can establish a symbiotic relationship with AM fungi in which the fungus helps the plant to take up nutrients from the soil, whereas the plant in return delivers carbohydrates to the fungus. The strigolactones hence have a double role as germination stimulant for parasitic plants and as branching factors for AM fungi [14]. Very recently strigolactones were also reported to have an internal, hormonal, signalling function as tillering or shoot branching inhibitors [16].

Quite a few different strigolactones have already been identified in host and some non host plant species [17]. Strigol and strigyl acetate were identified in the false host cotton [18,19] and strigol was also detected in maize, pearl millet and sorghum [18,19]. Other strigolactones such as 5-deoxystrigol, sorgolactone and an isomer of strigol, named sorgomol were identified in sorghum [20,21]. Similarly, orobanchol has been described in red clover, tomato and rice [22], alectrol in cowpea [23] and 2′-epi-5-deoxystrigol and solanacol were recently identified in tobacco [21,24]. Classically the strigolactones have been described to belong to the sesquiterpene lactones by many authors [15,22,25]. However, recently it was shown that the strigolactones are derived from the carotenoid biosynthesis pathway [26]. Indeed, it was recently demonstrated that two carotenoid cleavage dioxygenases, CCD7 and CCD8, are required for strigolactone biosynthesis [16]. Hence, as postulated by Matusova et al. carotenoid cleavage is required for strigolactone biosynthesis, which classifies them as apocarotenoids [26].

In the study by Matusova et al. the use of the carotenoid inhibitor fluridone was one of the tools to prove the carotenoid origin of the strigolactones [26]. We postulated that this finding may have wider application and hypothesized that the use of carotenoid inhibitors such as fluridone, norflurazon, clomazone and amitrole, in very low concentrations, could be useful as a tool to reduce strigolactone production and ultimately *Striga* seed germination and *Striga* infection [27]. These inhibitors are used – in high concentrations – as herbicides that prevent the formation of carotenoids which leads to photo-bleaching of chlorophyll and hence kills weeds [28] (Fig. 2). Fluridone and norflurazon block phytoene desaturase that catalyzes the conversion of phytoene to phytoflu-

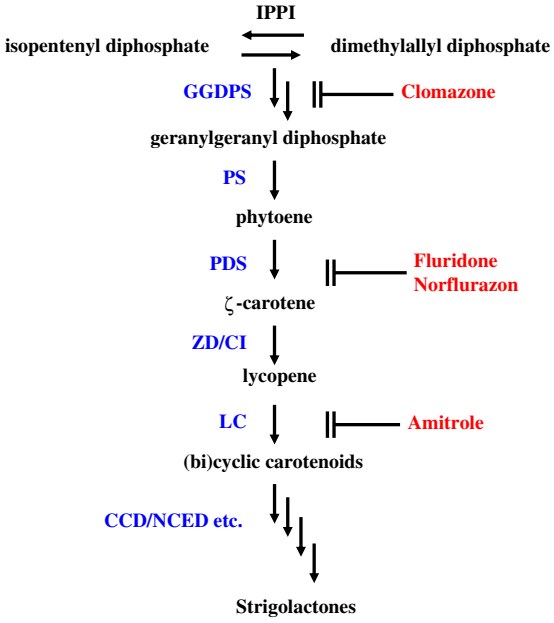

Fig. 2. Schematic diagram showing site of action of various carotenoid inhibitors at various steps in the carotenoid biosynthesis pathway. The arrows represent enzymatic steps. IPPI, isopentenyl diphosphate isomerase; GGDPS, geranylgeranyl diphosphate synthase; PS, phytoene synthase; PDS, phytoene desaturase; ZD, ζ-carotene desaturase; LC, lycopene cyclase; CCD, carotenoid cleavage dioxygenase; NCED, 9-*cis*-epoxycarotenoid dioxygenase; Fluridone and norflurazon block phytoene desaturase (PDS). Clomazone probably inhibits two key enzymes (GGDPS and IPPI). Amitrole interferes with lycopene cyclase.

[1] *Abbreviations used*: AM, arbuscular mycrorrhizal; LC–MS, liquid chromatography mass spectrometry; UPLC, ultra performance liquid chromatography; ESI, electro spray ionization; CE, collision energy; MRM, multiple reaction monitoring; CCD, carotenoid cleavage dioxygenase; NCED, 9-*cis*-epoxycarotenoid dioxygenase; *a.i.*, active ingredient; ANOVA, analysis of variance; LSD, least significant difference; NS, non-significant.