Microbiological Research 199 (2017) 29–39

Contents lists available at ScienceDirect

Microbiological Research

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

Piriformospora indica promotes growth, seed yield and quality of *Brassica napus* L.

Zhen-zhu Su[a,b], Ting Wang[c], Neeraj Shrivastava[b,d], You-yuan Chen[b], Xiaoxi Liu[b], Chao Sun[b], Yufeng Yin[e], Qi-kang Gao[f], Bing-gan Lou[b,*]

[a] *State Key Laboratory for Rice Biology, Institute of Biotechnology, Zhejiang University, Hangzhou, China*
[b] *Institute of Biotechnology, Zhejiang University, Hangzhou, China*
[c] *Institute of Insect Science, Zhejiang University, Hangzhou, China*
[d] *Institute of Microbial Technology, Amity University, New Delhi, India*
[e] *Agricultural Technology Extension Center, Zhejiang University, Hangzhou, China*
[f] *Analysis Center of Agrobiology and Environmental Sciences, Zhejiang University, Hangzhou, China*

ARTICLE INFO

Article history:
Received 27 September 2016
Received in revised form 19 February 2017
Accepted 25 February 2017
Available online 27 February 2017

Keywords:
Piriformospora indica
Brassica *napus* L.
Growth promotion
Seed yield
Oil quality

ABSTRACT

In current scenario, crop productivity is being challenged by decreasing soil fertility. To cope up with this problem, different beneficial microbes are explored to increase the crop productivity with value additions. In this study, *Brassica napus* L., an important agricultural economic oilseed crop with rich source of nutritive qualities, was interacted with *Piriformospora indica*, a unique root colonizing fungus with wide host range and multifunctional aspects. The fungus-treated plants showed a significant increase in agronomic parameters with plant biomass, lodging-resistance, early bolting and flowering, oil yield and quality. Nutritional analysis revealed that plants treated by *P. indica* had reduced erucic acid and glucosinolates contents, and increased the accumulation of N, Ca, Mg, P, K, S, B, Fe and Zn elements. Low erucic acid and glucosinolates contents are important parameters for high quality oil, because oils high in erucic acid and glucosinolates are considered undesirable for human nutrition. Furthermore, the expression profiles of two encoding enzyme genes, *Bn-FAE1* and *BnECR*, which are responsible for regulating erucic acid biosynthesis, were down-regulated at mid- and late- life stages during seeds development in colonized plants. These results demonstrated that *P. indica* played an important role in enhancing plant growth, rapeseed yield and quality improvement of *B. napus*.

1. Introduction

The world-wide production of vegetable oil is approximately 87 million metric tons in total per year. During the last decade, worldwide consumption of vegetable oils has enhanced more than 50% with doubled price. *B. napus* is the third edible oil-producing crop source providing approximately 13% of the world's supply of vegetable oil after soybean and oil palm in the world. It is widely cultivated in countries like, China, India, Canada and other temperate regions. The annual planting acreage is about 8 MH (million hectare) in China, which accounts for a quarter of the world's total rapeseed production. China is the largest contributor for *B. napus* production worldwide (Hu et al., 2009; Lu et al., 2011; Zhao et al., 2012; Hua et al., 2012). Rapeseed oil typically has a fatty acid composition of 5% palmitic (C16:0), 1% stearic (C18:0), 15% oleic (C18:1), 14% linoleic (C18:2), 9% linolenic (C18:3) and 45% erucic acid (C22:1) (Ackman, 1990; Shahidi, 1990).

Erucic acid, a member of Very-Long-Chain-Fatty-Acids (VLCFAs) which is specifically accumulated in oilseed, and glucosinolates, both are important parameters of seed quality. Diet rich in erucic acid was associated with fibrotic myocardium and increased blood cholesterol levels (de Wildt and Speijers, 1984). Therefore, erucic acid content directly influences the quality of oil seed. Erucic acid is synthesized in a particular elongation pathway in cytosol in plant seeds. This elongation process is presumed to be a four-step process, catalyzed by 3-ketoacyl-CoA synthase (KCS), 3-ketoacyl-CoA reductase (KCR), 3-hydroxyacyl-CoA dehydratase (HCD) and *trans*-2, 3-enoyl-CoA reductase (ECR) enzymes (Harwood 1996). *Bn-FAE1* gene (which encodes the 3-ketoacyl-CoA synthase) encoding KCS and *BnECR* gene encoding ECR have been all characterized

* Corresponding author.
E-mail address: bglou@zju.edu.cn (B.-g. Lou).

http://dx.doi.org/10.1016/j.micres.2017.02.006
0944-5013/

(Barret et al., 1998; Puyaubert et al., 2005). Transcript levels of *BnECR* (trans-2, 3-enoyl-CoA reductase) at mid- and late stages of seed development in low erucic acid rapeseed cultivars were found to be lower than that in high erucic acid rapeseed cultivars, suggesting that *BnECR* was involved in the biosynthesis of erucic acid (Ni et al., 2011). The function of *Bn-kcr1* and *Bn-kcr2* isoforms (two 3-ketoacyl-CoA reductase cDNA isoforms) were identified by heterologous complementation in yeast by restoring elongase activities (Puyaubert et al., 2005).

Glucosinolates are sulphur-rich plant secondary metabolites, largely found in the order Brassicales which includes the economically and nutritionally important crops, such as *B. napus*, *B. oleracea* and *Arabidopsis thaliana* (Halkier and Gershenzon, 2006). *B. napus* contains a high level of glucosinolates, ranging from 100 to180 mmol/g in defatted seed meal, which affects livestock and human health, and have been harnessed for food and other end-uses (Griffiths et al., 1998). However, glucosinolates and their hydrolyzed products have a wide range of positive attributes in plant resistance to insects and pathogens. Glucosinolates play an excellent role in plant defense against pathogens (Clay et al., 2009) and generalist herbivores from different feeding guilds (Mewis et al., 2006; Gols et al., 2008; Kim et al., 2008; Müller et al., 2010). Ally-isothiocyanate, as a hydrolyzed product of glucosinolates, is used as a natural preservative to inhibit the microbial growth (Mari et al., 2002; Rhee et al., 2003; Bednarek et al., 2009). Isothiocyanates hydrolyzed from some glucosinolates by myrosianse can also be utilized as bio-fumigants for soil- borne pests (Brown and Morra, 2005; Bellostas et al., 2007). Moreover, glucosinolates are also in response to non-pathogenic microbes, which in turn affect resistance against pathogens and generalist herbivores (van de Mortel et al., 2012). For example, the root colonization of a non-pathogenic rhizobacteria *Pseudomonas fluorescens* stimulated the biosynthesis of indolyl and aliphatic glucosinolates in *A. thaliana*, which finally induced systemic resistance against several bacterial pathogens, such as *P. syringae* pv *maculicola*, *P. alisalensis*, and *P. viridiflava*, and the pest *Spodoptera exigua* (van de Mortel et al., 2012).

P. indica is a well known and most studied endophytic fungus for vegetative growth and resistance to plants since past decades. The fungus was isolated from desert of Rajasthan, India, forming an association with roots of many plant species (Verma et al., 1998; Varma et al., 1999; Waller et al., 2005, 2008; Oelmüller et al., 2009; Franken, 2012). It has been reported that more than 12 families and 24 species can be colonized by this fungus (Lou et al., 2007; Franken, 2012). *P. indica* is able to stimulate growth, increase biomass and seed yield, improve the product quality by value additions in host plant, and confer resistance against various abiotic and biotic stresses (Peškan-Berghofer et al., 2004; Baltruschat et al., 2008; Sherameti et al., 2008; Stein et al., 2008; Oelmüller et al., 2009; Sun et al., 2010; Bagde et al., 2011; Dolatabadi et al., 2011; Gosal et al., 2011; Das et al., 2012; Franken, 2012).

There is an increasing need to focus rapeseed crop improvement on optimizing performance characteristics such as fatty acid composition, seed oil content and yield. More generally, crop improvement is dependent on genetic breeding, in order to make significant advances in key traits. However, to date, little is known about whether exists other different strategies to improve the rapeseed yield and quality. Thus, this paper attempted to investigate the interaction between *P. indica* and *B. napus*, explore the potential in the pursuit of agronomical attributes for crop improvement and to provide more information available on the microbe-plant symbiosis. The understanding of interaction between *P. indica* and *B. napus* will aid in the development of suitable tools for altering seed development and seed quality traits.

2. Materials and methods

2.1. Fungal culture, co-culture in vitro, root staining and microscopy

Strain *P. indica* was cultivated on modified Kaefer medium (Hill and Kaefer, 2001) for 5 days in the dark at 25 °C, during which time conidia formation started. The margin of the culture was then sliced out. Scanning electronic microscopy was conducted using cryo-SEM (Hitachi S-3000N microscope, Japan), operating between 10 and 15 kV on samples containing a thin layer of gold sputter coating.

P. indica was grown on modified Kaefer medium at 25 °C for 5 days in dark. Then one fungal plug (5 mm each) was picked out and inoculated into a glass petri dish (90 mm × 90 mm) which contained 50 ml of modified plant nutrient medium (PNM) (Vadassery et al., 2009). The fungus was incubated at 25 °C for 7 days under continuous illumination (80 μM m^{-2} s^{-1}).

B. napus ( ) seeds were surface-sterilized in 70% ethanol for 2 min, in 2% sodium hypochlorite solution (5% active chlorine) for 10 min, rinsed several times in sterile water, and then planted in Murashige & Skoog solid medium (Murashige and Skoog, 1962) at 4 °C for 48 h. Then, the seedlings were transferred and treated at 22 °Cwith a 16-h-light (100 μM m-2 s-1)/8-h-dark photoperiod for 6 days. Later, they were transferred to the PNM mentioned above and placed at a distance of 1 cm from the mycelium plug for 15 days. The seedlings inoculated with sterile plug were mock-treated.

The roots of 15-day-old seedlings were collected, and washed thoroughly with distilled water, cut into 1-cm-long pieces and treated with 10% KOH solution at room temperature overnight. The roots were rinsed repeatedly using sterile water, dipped in 1% HCl solution for 1 min and then stained with 0.05% trypan blue for 3–5 min, finally rinsed several times in sterile water. The stained roots were observed under an Olympus confocal microscope BX51(Tokyo, Japan).

2.2. Co-culture in pots

After co-culture for 15 days on dishes, *P. indica*- and mock-inoculated seedlings were transplanted into plastic pots (250 mm × 240 mm, one seedling per pot, three biological replicates of 10 plants per replicate) filled with a 4: 2: 1 mixture of sphagnum: vermiculite: perlite. Plants were grown in greenhouse at 22/16 °C with a 16-h-light/8-h-dark photoperiod.

Control plants as well as plants colonized with *P. indica* were estimated after 80 days in pots, to assess growth parameters including root and shoot numbers and lengths, fresh and dry weights of roots, and so on. For the determination of the dry weight, the materials were dried overnight in an oven at 40 °C.

2.3. Field experiment

Broth cultures were prepared in 1L flasks containing 500 ml of autoclaved Kaefer liquid medium through inoculation with 5 fungal plugs (5 mm each) from 10-day-old agar culture of *P. indica*. Flasks were kept on a shaker (140 rpm) at the 25 ± 1 °C for 15 days till dense mycelial suspensions were generated.

Field experiment was conducted following a completely randomized design in 2015. Seeds were surface-sterilized as mentioned in 2.1, and placed in sterile perlite for germination. After 25 days the seedlings were transplanted into the field soil. For inoculation, inoculum (1% w/v) of crushed mycelium of *P. indica* was irrigated around the seedlings roots. The controls were also maintained without inocula and only with sterile distilled water. The parameter data was collected at different developing stage.