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Variability of Carotenoid Biosynthesis in Meiotic Offspring of Fusarium Temperatum Strains

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Abstract

Fusarium temperatum is a new emerging species recognized as important and toxigenic pathogen of maize, prevalent in temperate region of northern hemisphere. The present study aimed to identify the variability of this species in terms of carotenoid biosynthesis under various light condition in relation to fungus mating type. Analysis of offspring subpopulation of 80 isolates obtained by crossing parental *Fusarium temperatum* strains indicated that light wavelength and fungal genotype significantly affected pigment yield. The highest levels of carotenoids were observed after incubation of isolates under blue light. Occurrence of the more extreme fungus phenotypes than either parent was stated in 20% to 42 % isolates depending on light condition.

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It means that transgressive segregation can significantly change fungus population from generation to generation and drive the species evolution. No phenotypic differences in carotenoid biosynthesis were found between MAT1-1 and MAT1-2 *F.temperatum* strains.

Keywords: Fusarium temperatum; variability.

1. Introduction

Carotenoids are a group of lipophilic pigments widely distributed in nature. More than 750 naturally occurring carotenoids of diverse structure and properties have been reported among plants, algae, bacteria and fungi. With respect of chemical structure carotenoids constitute a class of secondary metabolites characterized by the presence of a 40 carbons chain formed from 8 isoprenoid units. Its structure contains alternately single and double bounds which are responsible for bright carotenoids color [1]. The polyene chain of these compounds can cyclize at one or both sides as well as may undergo substitution with hydroxyl, keto, epoxy and aldehyde groups. Common for fungi carotenoid oxygenases can shortened carbon skeleton leading to uprising apocarotenoids. Carotenoids biosynthesized by fungi are complex of xnantophyls i.e. oxygenized carotenoids and to a minor extent carotens (unsaturated carotenoid). In fungi similarly to other eukaryota carotenoids are biosynthesized via mevalonate pathway. The immediate precursor for fungal carotenoids is phytoene, colorless compound formed by condensation of two geranylgeranyl diphosphate molecules. As highly lipophilic compounds they are suspended in lipid cell fractions i.e. oil droplets present in cytoplasm and cell wall structure [2] however precise knowledge regarding localization of these metabolites in fungal structure is still scanty. Biological properties of carotenoids are complex. They are recognized commonly as a crucial element of cell photoprotection system [3] as well as they are highly effective oxide ROS scavengers [4], some of carotenoids exhibited antimicrobial properties against *E.coli* and *C.ablicans* [5]. Fungi as carotenoids producers have been already reported in XIX century by Zopf [6] who pointed out on occurrence of yellow pigment probably lipochrome origin in some thallus parts (sporangia and spores) of *Pilobolus* and *Mucor* fungi. Species of the *Fusarium* genus due bright mycelium color are potent source of various pigments including carotenoids. Occurrence of these metabolites were recognized in *Fusarium oxysporum* [7], *Fusarium aquaeductuum* [8], and *Fusarium fujikuroi* [9], *Fusarium graminearum* [10] and *Fusarium verticillioides* [11]. At molecular level cluster of genes responsible for carotenoid synthesis were stated in *Fusarium incarnatum-equiseti* species complex [12]. *Fusarium fujikuroi* – species considered as carotenogenesis model was described as producer neurosporaxanthin together with numerous but at substantial minor amounts its precursors i.e. ζ-carotene, neurosporene, lycopene, γ-carotene, β-carotene and torulene. The general spectrum of known *Fusarium* carotenoids is much more wider, includes various phytoene derivatives and varied substantially among species[13]. Carotenoids, similarly to other secondary metabolites, are usually accumulated during the later stages of cultivation [14]. Several environmental stress factors can affect this process [15]. Carbon sources, nitrogen to carbon ratio, pH, temperature as well as light which significantly stimulated carotenoids biosynthesis. The role of light in fungal carotenogenesis was originally noticed by Schopfer and Jung (1935) during studies on carotenoids production by *Neurospora sitophila* [16]. Among *Fusaria* strong carotenoids accumulation after exposition of fungal cultures to light were noticed for *Fusarium aquaeductuum* [17]. Available data demonstrate also influence of mating type on secondary metabolites pathways, their direct effects

on carotenoids biosynthesis is not clear and unequivocal. The MATa *Saccharomyces cerevisiae* strain has been recognized as more suitable producer of epicedrol than MAT α strain [18], moreover genes alone which determine the mating-type thalus can also act as regulators of metabolic pathways. Most recently *Penicillium chrysogenum* MAT1-1-1-1 was found to be penicillin cluster genes regulator [19]. Regarding carotenoids biosynthesis, MATα *Saccharomyces cerevisie* genotypes achieved 12–15 % higher lycopene yield than opposite MATa type strains [20]. In *Fusarium verticillioides* MAT1–2-1 mating-type gene was found as upregulator of photo-inducible carotenoid biosynthesis. On the other hand [21] Rico and his colleagues (2010) did not observed significant mating types effect on linalool production. The key objectives of this work was analysis of carotenoids produced by *Fusarium temperatum* meiotic offspring under various light condition in relation to mating type. This newly described and commonly occurring in temperate areas species was recognized as important maize pathogen [22]. The taxon has been documented as various secondary metabolites producer however its phytoene derivatives has not been studied so far.

2. Materials and Methods

2.1 Fungal strains

Fungal isolates used in this study constituted offspring subpopulation generated by crossing parental *Fusarium temperatum* strains KFn 777 x KFn 666, of opposite mating type Mat1-1 , Mat1-2 respectively. The single ascospore isolates were derived from asci obtained from peritetia which arised on medium surface. To avoid the risk of clonally copies among offspring strains the only one spore per ascus from fruit bodies was taken. Mating assays were performed on carrot agar medium according to procedure described by [23]Leslie and Summerell (2006). All the strains were deposited in fungal collection of Department of Plant Pathology, being the part of WAUF Herbarium.

2.2 Mating type genotyping

Determination of *F.temperatum* mating types was performed in multiplex PCR assay [24]. For Mat1-1, Mat1-2 idiomorph detection recommended primers: Gfmat1a(5′-GTTCATCAAAGGGCAAGCG-3'), Gfmat1b(5′-AAGCGCCCTCTTAACGCCTTC-3′) and Gfmat2c(5′-AGCGTCATTATTCGATCAAG-3′),Gfmat2d (5′-CTACGTTGG AGCTGTACAG-3′) were used respectively. Slight modification regarding amplification scheme was proposed, and the applied program was as follows: one cycle at 94°C for 1 min, followed by 35 cycles at 94°C for 60 s, 58°C for 60 s, 72°C for 60 s and 35 cycles with a final elongation step for 10 min at 72°C. The amplification result was visualized in 1,3% agarose gel (containing 0.1 mg/ml ethidium bromide) after electrophoresis in 1.0× TBE (Tris-Borate Buffer). The images of the banding patterns were acquired under UV light using a INGenius LHR2 gel imaging system (SynGene)

2.3 Carotenoids biosynthesis, condition, extraction and total contents determination

F. temperatum strains were seeded on potato dextrose agar (PDA) in petri plates. Following inoculation, all the cultures were incubated at 20˚C and held in different light condition i.e. darkness or exposed 20 cm beloved the source of white, blue (440 nm) and red (660 nm) light emitting diode with the light intensity at the level 200 ±

20 µmol m-2 s-1. After 7 days of fungal growth, talus of the colonies were collected from medium surface, transferred to ependorf tubes, and vacuum freeze-dried for 24 h. To extract carotenoids lyophilized samples were grinding by micro pestles using quartz sand. After destruction of the fungal cell wall, the pigments were extracted with absolute ethanol for 24 h at 35°C while shaking at 100 rpm. The probes were centrifuged at 5000×g for 10 min, obtained supernatant was dried under stream N_2 and the residue was resuspended in 1ml ethanol. Standard calibration curve was constructed by dissolving 10 mg of β Carotene (Sigma C9750) in 1:10 solution of DMSO:EtOH (50 ml) in 50-mL volumetric flask. From these stock solution 11 ethanol dilutions from 200 to 0 ppm were prepared in 10 mL volumetric flask with arithmetic (20 ppm) progression of the concentration. Each concentration of β Carotene standard sample was measured with spectrometer microplate reader (TECAN Nanoquant Infinite 200 Pro multimode) at a range of 400–520 nm UV-vis spectra. Calibration curve (Figure 1) were prepared for absorption of each solution measured at 455 nm . Total carotenoids contents was determined spectrophotometrically using β carotene standard curve equation f(x) = 0,0097x - 0,0292 where x means β carotene concentration, f(x) – absorbance value and determination coefficient R^2=0.992. Absorption measurements were carried out in the micro-titrate plate at the maximal absorption spectra of β carotene in EtOH (λ= 455 nm); using 100 µl of assayed solution in each well. Four repetition per probe (strain carotenoids extract) were applied.

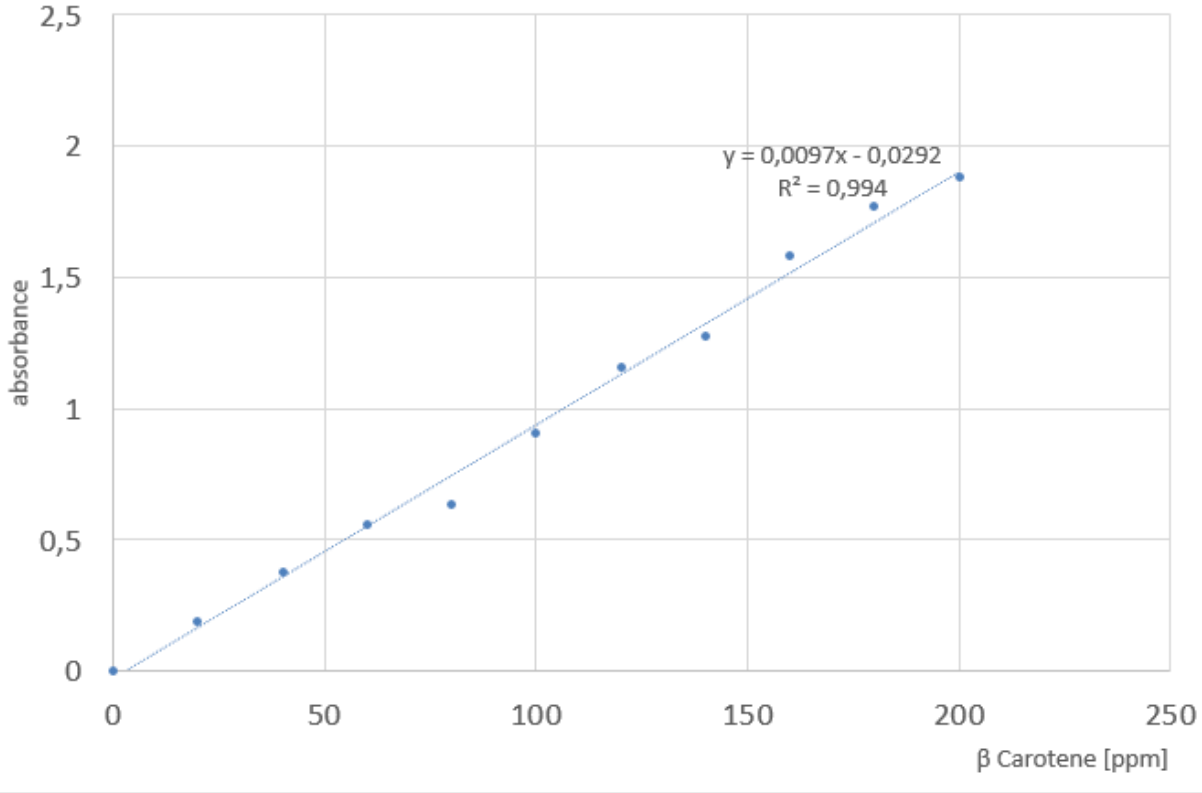

Figure 1: The calibration curve of β Carotene

2.4 Statistics

The data were analyzed by one-way analysis of variance. The Tukey's test were applied to evaluate significant

differences ($P \leq 0.05$) among means.

3. Results

Light as well as the fungal strains were principal components affecting carotenoids yield. The both factors influenced the scope of the analyzed feature and average value of total carotenoids content. The highest concentration of these metabolites was detected in thallus of the *F.temperatum* strains exposed to blue light (35,1 ppm) and the lowest when the fungus were incubated in darkness (8,9 ppm). Intermediate effect was observed after culture exposition to white (11,8 ppm) and red (15,1ppm) light. (Figure 2).

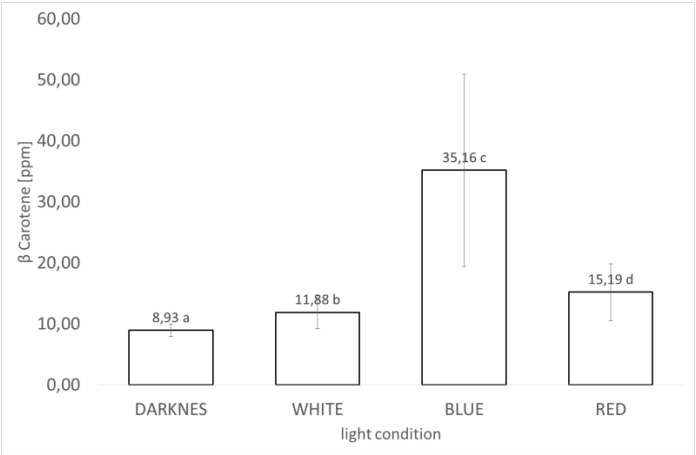

Figure 2: The carotenoids concentration in thallus of *F.temperatum* strains incubated in different light condition.

The carotenoids concentration was fungal strain depended. Carotenoids produced by progenies strains of *F.temperatum* cross exhibited typically for qualitative characters continuous distribution. The level of carotenoids biosynthesized in culture during their cultivation in darkness ranged from 7,4 to 12,9 ppm while the pigments content after culture incubation under white light from 7,8 to 22,4 ppm. The total carotenoids content in the same set of *F.temperatum* strains was extended from 7,7 to 27,7 ppm in culture exposed to red and from 7,5 to 84,3 ppm after exposition to blue light (Figure 3).

In all applied incubation light conditions some of the progeny had more extreme phenotypes than either parent. The traits of transgressive segregants exhibited 34, 16, 19 and 23 isolates incubated in darkness, white, blue and red light respectively out of 80 fungus progenies (Figure 3). The mean level of metabolites biosynthesized by the transgressive segregants of the fungus was significantly different than average carotenoid concentration in thallus of the strains with intermediate characteristic between parental isolates. Such relationship occurred in all light regimes

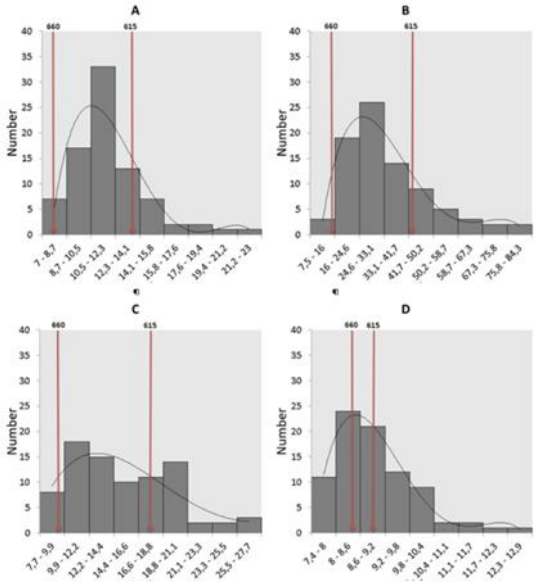

Figure 3: Distribution of carotenoids concentration in *F.temperatum* strains incubated in darkness (D) and after exposition to white (A), blue (B) and red (C) light.

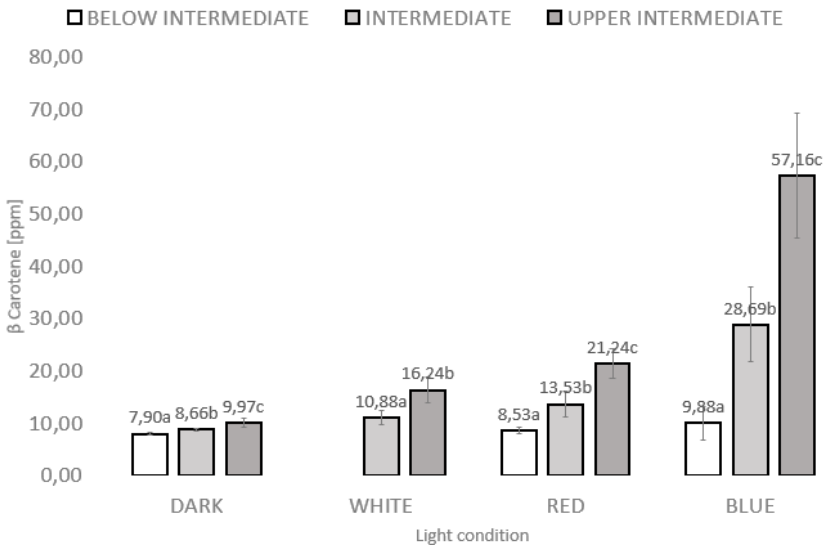

Figure 4: The mean level of carotenoids in *F.temperatum thallus* of the transgressive segregants

Fusarium temperatum mating types were not found to have a significant effect on carotenoid biosynthesis. Strains of the fungus representing MAT1-1 (55%) and MAT1-2 (45%) subpopulation biosynthesized equal carotenoids level in darkness as well as after exposition to white, red and blue light (Figure 5)

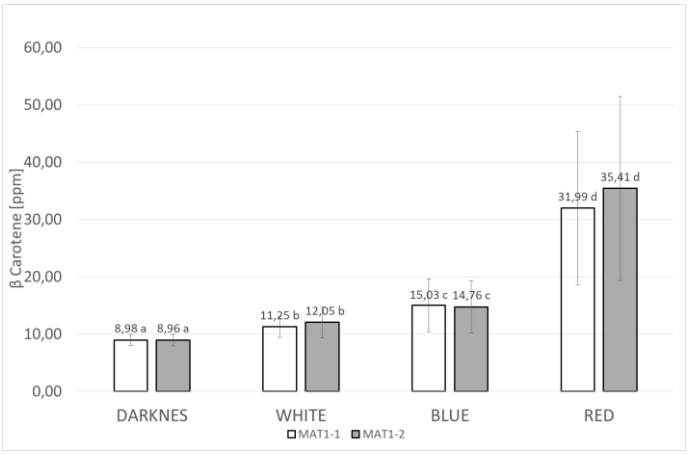

Figure 5: The mean level of carotenoids in thallus of Mat1-1 and Mat1-2 mating types of *F.temperatum* strains

4. Discussion

For many microorganisms the light spectral composition and luminance constitute basic source of information about environment. It is also true for fungi in case of which several genes expression is light depended thereby number of life processes is regulated by this environmental factor. Light responses in fungi include regulation of fruit bodies morphogenesis, sporulation, mitotic and meiotic morph induction as well as primary and secondary metabolism together with carotenoids biosynthesis. In accomplished studies, however carotenoids were accumulated in all applied light condition i.e. in darkness, white, blue and red illumination, spectrum of the light significantly affected biosynthesis of these pigment group. The highest concentration of carotenoids was stated after culture treatment with blue light and ranged from 7,5 to 84,3 ppm with the mean metabolite concentration for all progenies at the level 35,1ppm. Enhancement of carotenoids biosynthesis in response to blue light spectrum (400-500 nm) was reported also for *Neurospora crassa* [25], *Phycomyces blakesleeanus* [26], *Mucor spp including M. circinelloides* [27], *M.hiemalis* [28] and many other fungal species [29]. At molecular level, the tightly close relationship between exposition to blue light and carotenoids biosynthesis in fungi is explained by White Collar (WC) proteins presence which mediate the pigment accumulation. WC proteins (WC1 and WC2) originally described in *N. crassa* are element of WC complex which after stimulation by light initiates gene or genes transcription whose expression is light depended. In fungi the blue light spectrum was recognized as factor playing regulatory role in phototropism, ascomata and conidiomata development [30]. Most recently Trzaska and his colleagues [31]reported that blue light causes photoexcitation of endogenous porphyrins, resulting in the production of Reactive Oxygen Species what may be the reason of blue light fungistatic properties exhibited against *Scedosporium prolificans* and *Fusarium solani* after 60 min treatment at luminescence 216 J/cm^2. Performed analysis of progenies subpopulation generated by crossing parental *Fusarium temperatum* strains indicate on wider phenotypic offspring variation in carotene biosynthesis than their parents. Generally such phenomenon is called as transgressive segregation. Various mechanism could contributed to its occurrence like: an elevated mutation rate, reduced developmental stability, non-additive epistatic effects between alleles, over dominance, unmasking of rare recessive alleles, chromosome number variation, and complementary action of additive alleles [32]. Transgression seems to be common for living organism and has been described in animals [33], plants [34] and also in fungi [35] but its biological

significance is not unequivocally predicted. In agriculture, especially plant transgressive genotypes are commonly used in breeding strategy for various traits. In contrary, among fungi the phenomenon is poorly characterized, what was inspiration of the present research which showed that recombination may significantly change population of the fungus regarding carotene biosynthesis. In environment such sergeants with extreme (different) phenotypes could promote the colonization of new niches, most recently transgression has been also proposed as a potential mechanism for speciation. In filamentous fungi occurrence of transgressive segregation was proved in *G.zeae* for aggressiveness and DON production and two transgressive segregants were found in crosses of highly aggressive parents of this pathogen [36]. Many progeny of *Zymoseptoria tritici* after sexual crosses indicating transgressive segregation for melanin biosynthesis were obtained [37]. The aim of these paper was also analysis of mating types on carotenoids biosynthesis. However MAT are those genes which regulate sex in fungi, some of the paper reported that the alleles may also affect other traits like pathogenic properties [38,39] or secondary metabolites production [11] by opposite mating types. MAT1-2-1 mating-type gene was found as upregulator of photo-inducible carotenoid biosynthesis, despite that in present studies at any of the wavelengths, MAT1-1 and MAT1-2 genotypes did not exhibit significant difference regarding total level of carotenoids.

5. Conclusion

Carotenoids are isoprenoids pigments common to many groups of living organisms. In fungi their occurrence is tightly bound with inner surface of plasma membarne and their function is related with photoprotection and ROS defense systems. The carotenoids biosynthesis is often response to environmental stresses, including light what underline results of these studies. The significantly highest concentration of these metabolites (an average 35,16 ppm) was noted after exposure of *F.temperatum* cultures to blue light. The variability of the pigments level among offspring subpopulation of the fungus strains ranged from 7,5 ppm to 84,3 ppm and considerable percentage of the progenies (20% to 43%) had more extreme phenotypes than either parent. This finding stress that recombination may significantly change population of the fungus however *F.temperatum* is cryptic sex species i.e. poses ability to sexual reproduction but in nature it leads asexual lifestyle. In fungi mating process is governed by MAT idiomorphes which in case of some species also affect other fungal characteristics like morphology, asexual sporulation or secondary metabolites production. Performed studies did not support influence of MAT idiomorphes on carotene phenotype of *F.temperatum*. The opposite mating type strains of the fungus MAT1-1 and MAT1-2 produced statistically equal level of carotenoids. Comprehensive approach seems to be needed to elucidate non mating role of MAT idiomorphs among cryptic sex fungi.

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