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Research article

Targeting prokaryotic choline oxidase into chloroplasts enhance the potential of photosynthetic machinery of plants to withstand oxidative damage

P. Sharmila, M.L.V. Phanindra, Firoz Anwar, Kavita Singh, Shipra Gupta, P. Pardha Saradhi*

Department of Environmental Biology, University of Delhi, Delhi 110 007, India

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ABSTRACT

Chloroplasts from plants of transgenic lines expressing prokaryotic choline oxidase gene (the *codA$_{ps}$* gene; GenBank accession number-AY589052) and wild-type of chickpea and Indian mustard were evaluated for their efficacy to withstand photoinhibitory damage, by exposing them to high light intensity ($\sim$1200 µmol m^{-2} s^{-1} photon flux density) at 10 and 25 °C. Western analysis confirmed presence of choline oxidase in chloroplasts of only transgenic lines. The loss in PS II activity in chloroplasts of wild-type exposed to high light intensity was significantly higher than that in chloroplasts of transgenic chickpea as well as Indian mustard. Although, chloroplasts of both wild-type and transgenic chickpea as well as Indian mustard were more sensitive to photoinhibitory damage at 10 than at 25 °C, the damage recorded in chloroplasts harboring choline oxidase was significantly lower than those of wild-type. High light promotes H_2O_2 production in chloroplasts more significantly at low temperature (10 °C) than at 25 °C. We compared low temperature accelerated photoinhibition of chloroplasts with that caused due to exogenously applied H_2O_2. Although exogenous H_2O_2 accelerated high light intensity induced loss in PS II activity of chloroplasts of wild-type, it caused only a little alteration in PS II activity of chloroplasts from transgenic lines of both chickpea and Indian mustard, demonstrating that the chloroplasts harboring choline oxidase are better equipped to resist photoinhibition. We hypothesize that H_2O_2 produced by choline oxidase as a byproduct during synthesis of glycinebetaine is responsible for building stronger antioxidant system in chloroplasts of transgenic lines compared to that of wild-type.

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1. Introduction

Abiotic stresses in particular drought, frost/low temperature, salinity are amongst the significant and refractory constraints to productivity of crops like chickpea and Indian mustard [1–6]. Transgenic technology has become an inevitable component of modern agriculture for enhancing tolerance of crop plants to abiotic stresses. Advancement in genetic engineering has facilitated the introduction of desired gene(s) from vast variety of living systems into various plant species even across extreme sexual boundaries, which is otherwise impossible through conventional breeding [1,7]. This technology made it possible to enhance tolerance of crop plants such as Indian mustard, rice, tomato etc. against abiotic stresses by introducing bacterial/prokaryotic genes such as the *codA* gene from *Arthrobacter globiformis*, the *mtlD* and *CDH* genes from *Escherichia. coli* [1,8–14]. The *codA* gene from *A. globiformis* is most well tested amongst the genes that are known to enhance abiotic stress tolerance of crop plants. Amongst the four glycinebetaine biosynthetic pathways, the one mediated by choline oxidase is the simplest one as it is a single gene/enzyme mediated pathway. Choline oxidase, the translated product of the *codA* gene, is a FAD-dependent enzyme that catalyzes the four electron oxidation of choline into glycinebetaine in presence of O_2 [1,15]. Glycinebetaine is a compatible solute that protects cellular machinery which includes membranes, proteins, nucleic acids, electron transport complexes etc. against various abiotic factors [1,6,8,10,12].

Photosynthesis is the single most vital metabolic process that not only regulates plant growth and development, but also directly controls overall productivity. Photosynthetic machinery is highly sensitive to abiotic stresses such as low temperature, drought, salinity and high light intensity [1,6,8,10]. Crop plants such as chickpea and Indian mustard are prone to photoinhibition, especially under abiotic stresses in particular low temperatures [1,6]. Therefore, during present investigations we have tested if the chloroplasts of the transgenic lines of chickpea and Indian mustard developed by our research team possess any enhanced potential to withstand photoinhibitory damage compared to the chloroplasts of

* Corresponding author. Tel./fax: +91 11 27662387.
E-mail address: ppsaradhi@gmail.com (P. Pardha Saradhi).

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respective wild-type plants. In this communication, we are reporting for the first time that the H_2O_2 produced by choline oxidase as a byproduct during synthesis of glycinebetaine is responsible for the enhanced potential of the chloroplasts of *codA_{ps}* transgenic lines of both chickpea and Indian mustard to resist photoinhibition.

2. Results and discussion

2.1. Raising transgenic lines

Transgenic lines of both chickpea and Indian mustard were raised using *Agrobaterium tumefaciens* harboring pSG vector. pSG vector was constructed by cloning 2.5 kb $codA_{ps}$ gene cassette [consisting of the $codA_{ps}$ gene (AY589052) of *A. globiformis* joined to transit peptide sequence of small subunit of Rubisco of chickpea and poly A signal of nopaline synthase with CaMV 35S promoter] in pPZP200 vector having *npt II* gene cassette between the *loxP* sites [13].

Explants of chickpea consisting of single cotyledon with half embryonal axis {as detailed in Anwar et al. [16]} were incubated on MS medium supplemented with 2 μM kinetin, 10 μM 2-iP and 4 μM TDZ for 6 days and then co-cultivated with *Agrobacterium* harboring pSG vector for 48 h. After washing with 250 mg/l cefotaxime these explants were transferred to MS medium supplemented 20 mg/l kanamycin and 250 mg/l cefotaxime. After 14 days, healthy adventitious shoots arising from the explants were transferred to fresh MS medium supplemented with 2 μM kinetin, 5 μM 2-iP and 50 mg/l kanamycin. Healthy shoots arising from different portions of the same explant or from different explants were carefully marked as independent putative transgenic lines and were sub-cultured on MS medium with 2 μM kinetin and 5 μM 2-iP at regular interval of 14 d by gradually increasing the concentration of kanamycin to 100 mg/l. Strong shoots of independent putative transgenic lines were rooted by exposing their cut ends to 10 s pulse treatment with 100 μmol/ml IBA as detailed in Anwar et al. [16] in presence of 50 mg/l kanamycin. A number of putative transgenic lines obtained through this protocol were evaluated for the presence of the *codA* gene through PCR and Western analysis. Through this transformation protocol 21 independent and healthy transgenic lines were selected. Southern analysis indicated that seven of these transgenic lines possessed single insert of the *codA* gene. By advancing these transgenic lines to T3 and T4 generations, it was possible to select the homozygous lines of each of the independent transgenic lines. One of the seven independent homozygous transgenic lines at T4 generation, with relatively superior features like over all growth and superior seed yield compared to other lines, was chosen for the present study. Details of the way transgenic lines of Indian mustard were raised are given in Gupta et al. [13]. One of the 15 independent homozygous transgenic lines of Indian mustard (at T4 generation) with single insert of the *codA* gene that was relatively superior to other lines in terms of over all growth was chosen for present studies.

2.2. Localization of choline oxidase in chloroplast

Immunoblot analysis of crude enzyme extract of the leaves of transgenic lines of both chickpea and Indian mustard showed the presence of choline oxidase, whereas the extracts from respective wild-type plants lacked choline oxidase (Fig. 1). This confirmed that wild-type chickpea and Indian mustard do not possess choline oxidase or any protein/polypeptide that could cross react with the antibodies against choline oxidase. As expected no band of choline oxidase was observed when the crude enzyme extract was treated with proteinase K. Intact chloroplasts isolated from leaves of transgenic lines showed the presence of choline oxidase even if they were treated with proteinase K (Fig. 1). However, disrupted chloroplasts treated with proteinase K failed to show any choline oxidase band (Fig. 1). These results convincingly demonstrated the presence of choline oxidase within the isolated chloroplasts.

2.3. Chloroplasts of transgenic lines resist high light intensity induced photoinhibition

The chloroplasts isolated from the leaves of wild-type and transgenic lines of both chickpea and Indian mustard were subjected to

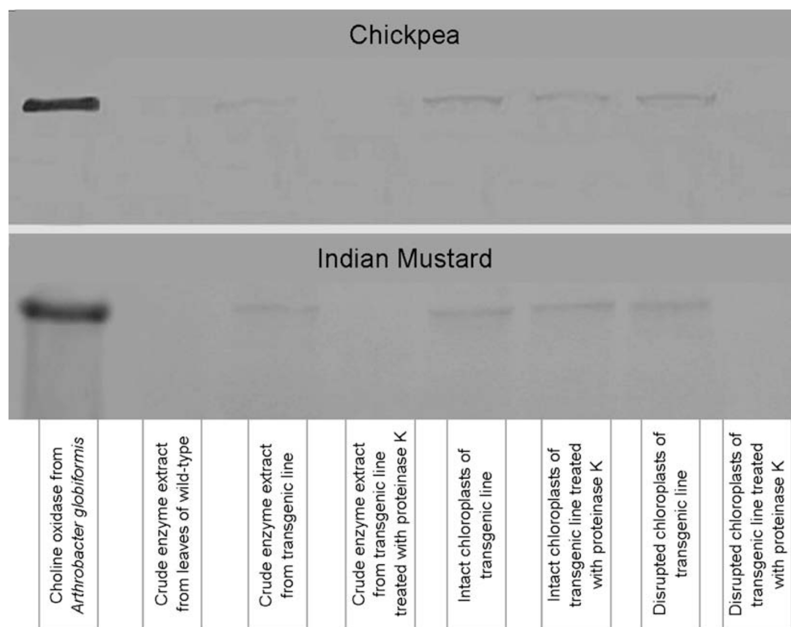

Fig. 1. Western blot analysis to detect the presence of choline oxidase in the chloroplasts of transgenic lines of chickpea and Indian mustard. Please note the presence of choline oxidase in intact chloroplast, intact chloroplasts treated with proteinase K, and disrupted chloroplasts. These observations along with the absence of choline oxidase in disrupted chloroplasts treated with proteinase K, demonstrate the localization of choline oxidase in the chloroplasts.

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