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Functional loss of GABA transaminase (GABA-T) expressed early leaf senescence under various stress conditions in *Arabidopsis thaliana*☆

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ABSTRACT

GABA-transaminase (GABA-T) involved in carbon and nitrogen metabolism during the plant development process via GABA shunt and GABA-T mutant, which is defective in GABA catabolism, is ideal model to examine the role of GABA-T in plant development and leaf senescence of plant. We have characterized GABA transaminase knock out mutant *pop2-1* that is transition and *pop2-3* which is T-DNA insertion mutant of *Arabidopsis thaliana* during various stress conditions.The GABA-T knockout mutant plants displayed precocious leaf senescence, which was accompanied by the assays of physiological parameters of leaf senescence during various stress conditions. Furthermore, our physiological evidence indicates that *pop2-1* and *pop2-3* mutations rapidly decreased the efficiency of leaf photosynthesis, chlorophyll content, GABA content, GABA-T, and glutamate decarboxylase (GAD) activity and on the other hand increases membrane ion leakage, malondialdehyde (MDA) level in stress induced leaves. However, cell viability assay by trypan blue and *insitu* Hydrogen peroxidation assay by 3,3-diaminobenzidine (DAB) in stress induced leaves also display that *pop2-1* and *pop2-3* mutant leaves show oversensitivity in response to different stress conditions as compared to wild type. These results strongly indicate that the loss-of-function of GABA transaminase gene induces early leaf senescence in *Arabidopsis thaliana* during various stress conditions.

1. Introduction

Senescence is a genetically and developmentally synchronized event; it not only promotes the remobilization of the nutrients to developing parts of the plant, but also involved the consecutive changes in the function or structure of various cellular components, along with modification in the expression of majority of the genes[1–5]. Many Senescence-associated genes (*SAGs*) were isolated and identified from a variety of plant species such as tomato, rice, maize and Arabidopsis to elucidate the molecular mechanisms underlying leaf senescence [6–12]. However, in vivo function of very small number of *SAGs* has been established till now. Despite the facts, the information available to understand the molecular mechanism of leaf senescence in plants is still insufficient.

During leaf senescence GABA shunt plays an integral role in carbon/nitrogen metabolism and it is also important for plant growth and development [13–15]. GABA is one of the key component of GABA shunt and in mammalian central nervous system act as inhibitory neurotransmitter [16]. In plants, GABA play role as metabolites as well as signalling molecule in many mechanism, it accumulate during various stress conditions in plant cell including leaf senescence process [13,17,18]. It has been studied that GABA shunt pathway under abiotic stress can potentially regulate GABA metabolism [12].

Protein degradation is the most consequential breakdown process during the leaf senescence, which is followed by decrease in photosynthetic activities, disassembles of cellular components, degradation of macromolecules and remobilization of the nutrients to developing part of the plants [2,4,19–21]. In the leaf senescence process, catabolism of proteins and nucleic acids takes place that released ammonia, which was used to converted glutamate into glutamine by the enzyme glutamine synthetase (GS). During leaf senescence, in GABA shunt pathway, some amino acids such as alanine and aspartate released from proteolysis were involved in the proteolytic activities and transamination reactions that increased

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the amount of glutamate. Conversion of some part of glutamate into GABA using the enzyme glutamate decarboxylase (GAD) was enhanced due to the increased availability of the glutamate substrate for GAD. Further, the conversion of GABA into succinic semialdehyde was takes place in mitochondria using the enzyme GABA transaminase. Then succinic semialdehyde is converted into succinate with the help of an enzyme succinic semialdehyde dehydrogenase. Thus the metabolism of GABA and glutamate via GABA shunt assimilate carbon and nitrogen skeletons that enters into tricarcoxylic acid cycle (TCA cycle) [15,22]. It has been also investigated that Osl2 gene encoded GABA pyruvate transaminase play a key role in carbon/nitrogen metabolism during rice leaf senescence [22].

Arabidopsis thaliana is belongs to the Brassicaceae family, it is a typical monocarpic plant with relatively short life cycle, readily distinguishable developmental stages, and a well-defined and reproducible senescence program. Dissimilar to numerous monocarpic species, *Arabidopsis* shows a remarkable disconnection between reproductive development and longevity of leaf tissues. In this respect, the mutants of *Arabidopsis* are an excellent model for studying the role of GABA transaminase in oxidative stress tolerance and longevity control in plants. In this study we have analysed *Arabidopsis thaliana* knock out mutant plants *pop2-1, pop2-3* of GABA–transaminase gene. Since the relation between *GABA Transaminase* mutant and leaf senescence is completely unknown, no attention has been given to *pop2-1* and *pop2-3* mutants and leaf senescence, we aim to explore the effects of GABA-T defection on leaf senescence and other stress conditions to elaborate its potential mechanism.

2. Materials and method

2.1. Plant materials and growth conditions

Arabidopsis thaliana Landsberg *erecta* ecotype (L*er*) was use as wild type. Seeds of the *pop2-1 and pop2-3* mutants of GABA-Transaminase were kindly provided by Arabidopsis Biological Resource Centre (ABRC), Ohio State University, Columbia, USA. Plants were grown at 22°C for long day condition (16 h light/8 h dark cycle) aseptically or on soil. For soil growth, seeds were sown in Bio-Mix Potting Substratum, Soilrite (Keltech Energies Ltd. India.) and placed at 4 °C for 4 days in dark to break seed dormancy and later transferred to normal growth conditions. GABA transaminase *Arabidopsis thaliana* knock-out mutant plants *pop2-1* mutant has transition G → C, and *pop2-3* T-DNA insertion mutant. The homozygous *Arabidopsis thaliana* T- DNA mutant *pop2-3* were find out by using PCR with primer from left and right border of T-DNA and primer from flanking region. *pop2-1* were screened by dCAPS method [23]. Homozygous plants were used for further analysis.

2.2. Treatment of detached leaves with abiotic stresses induced leaf senescence

Rosette leaf 6 (counted from bottom) was isolated from a 23-d-old *Arabidopsis* wild type and knock out mutant plants. Under growth conditions, leaf 6 at the time of sampling was fully expanded; these fully expanded leaves do not start senescing until three d later. Five leaves from wild type and knock out mutant plants were used for each treatment. For wounding treatment, leaves of wild type and mutants plants were wounded by needle and were floated on 3 mm MES [2-(N-morpholino)ethane sulfonic acid] buffer (pH 5.8) and incubated for 24 h in growth chambers, without wounded leaves were used as control. For dehydration treatment, we detached whole *Arabidopsis* rosette, we then weighed them and put on filter paper to dry for 2 h under dim light. After 2 h, plants were weighed again to estimate water loss. For control, leaves were put under the normal condition and tried to prevent leaves from losing water. For dark treatment, leaves were floated on 3 mm MES buffer (pH 5.8) in petri plates that was covered with black paper and kept in growth chamber for 3 days or for control with proper light condition. For cold treatment, leaves were floated on 3 mm MES buffer (pH 5.8) and incubated for 2 h at 0 °C and for control kept at optimum temperature (22–25 °C) in growth chamber.

2.3. Treatment of detached leaves with senescence inducing agents

All experiments on detached leaves were performed with the sixth rosette leaves at 23 day after leaf emergence, when the leaves were just fully developed. Leaf disc were acquired by cutting leaves at the surmised centre of the petioles with a sharp tool, to minimize injuring effects. The detached leaves were floated abaxial side up in a buffer (pH 5.8) containing 3 mM MES, containing appropriate concentrations of the various senescence-inducing agents at 22 °C under continuous light for 5 days. 10 mM concentration of H_2O_2, 500 mM concentration of Mannitol, 40 μM ABA and different concentration of sucrose (0, 1, 2, 4, 6%) were used to induce oxidative stress, as indicated. As a control for all treatment, the detached leaves were floated on 3 mM MES buffer.

2.4. Measurement of pigment and membrane ion leakage

Determination of physiological parameters such as total chlorophyll content and membrane ion leakage of leaf senescence induced by abiotic and oxidative stresses was done. For chlorophyll determination, fresh leaf material was extracted with 80% (v/v) acetone, and the absorption of the extracts was measured at 663 and 645 nm using Schimadzu UV- 1800 spectrophotometer [24]. For ion leakage leaf disc are cut from each treated leaves and placed in closed vials containing 10 ml double distilled water followed by incubation on rotary shaker for 24 h, after which the electrical conductivity of solution (EC1) was determined by conductivity meter, then the samples were kept at 90 °C for 1 h, the electrical conductivity was measured again (EC2) after the solution was cooled at room temperature. The electrolyte leakage (ion leakage) was defined as EC1/EC2 × 100 and expressed as percentage [25].

2.5. Lipid peroxidation

The levels of lipid peroxidation was estimated with the thiobarbituric acid (TBA) assay by determining the concentration of malondialdehyde (MDA) as the end product of lipid peroxidation [26]. Fresh leaf sample was homogenized in 0.1% (w/v) trichloroacetic acid (TCA) solution. The homogenate was centrifuged at 15,000*g* for 10 min and the obtained supernatant was added to 0.5% (w/v) TBA in 20% (w/v) TCA. The mixture was then incubated at 90 °C for 30 min, and the reaction tubes were placed in an ice water bath. Samples were centrifuged at 10,000*g* for 5 min, and the absorbance of the supernatant was read at 532 nm.

2.6. Determination of GABA

GABA was extracted and determined from frozen tissues of the treated leaves of wild type, *pop2-1* and *pop2-3* mutants [27]. Harvested leaves were ground separately in microfuge tubes in liquid nitrogen until a fine powder was obtained. Methanol was added to each tube and the samples were mixed for 10 min. Liquid from the samples was removed by vacuum drying, and 70 mM lanthanum chloride was added to each tube. The tubes were shaken for 15 min