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Short communication

Wide range of interacting partners of pea Gβ subunit of G-proteins suggests its multiple functions in cell signalling

Deepak Bhardwaj [a,b], Suman Lakhanpaul [a], Narendra Tuteja [b,*]

[a] Department of Botany, University of Delhi, India
[b] International Centre for Genetic Engineering and Biotechnology, Aruna Asaf Ali Marg, New Delhi 110067, India

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ABSTRACT

Climate change is a major concern especially in view of the increasing global population and food security. Plant scientists need to look for genetic tools whose appropriate usage can contribute to sustainable food availability. G-proteins have been identified as some of the potential genetic tools that could be useful for protecting plants from various stresses. Heterotrimeric G-proteins consisting of three subunits Gα, Gβ and Gγ are important components of a number of signalling pathways. Their structure and functions are already well studied in animals but their potential in plants is now gaining attention for their role in stress tolerance. Earlier we have reported that over expressing pea Gβ conferred heat tolerance in tobacco plants. Here we report the interacting partners (proteins) of Gβ subunit of *Pisum sativum* and their putative role in stress and development. Out of 90 transformants isolated from the yeast-two-hybrid (Y2H) screening, seven were chosen for further investigation due to their recurrence in multiple experiments. These interacting partners were confirmed using β-galactosidase colony filter lift and ONPG (O-nitrophenyl-β-D-galactopyranoside) assays. These partners include thioredoxin H, histidine-containing phosphotransfer protein 5-like, pathogenesis-related protein, glucan endo-beta-1, 3-glucosidase (acidic isoform), glycine rich RNA binding protein, cold and drought-regulated protein (*corA* gene) and soluble inorganic pyrophosphatase 1. This study suggests the role of pea Gβ subunit in stress signal transduction and development pathways owing to its capability to interact with a wide range of proteins of multiple functions.

1. Introduction

G-proteins are important signalling molecules in plants. They consist of three subunits Gα, Gβ and Gγ and the number of each subunit in various plants can vary [1]. G-proteins remain attached to G-protein coupled receptor (GCR1) or can act through regulator of G-protein signalling (RGS) and with the reception of the signal from elicitors the heterotrimer disassociates rendering the subunits to act independently and thus allowing Gα and Gβγ interactions with other cellular proteins [2–4]. With the recent discovery of Arabidopsis G-protein intractome, it is now evident that plant G-proteins are multifunctional and play a significant role in the development and environmental stresses which further need to be explored in the wake of global climate change [5].

The Gβ subunit, which contains WD40 domains is reported to be involved in many significant functions and processes of the plants such as development of leaves, gynoecium, stomata, and root and also in pathogen resistance and alleviating heat stress [6–11]. RGB1 mutants of rice Gβ are shorter in height and have small sterile seeds [12].

Recently, it has been shown that Arabidopsis has one more Gγ subunit called Gγ3 apart from Gγ1 and Gγ2; also these three Gγ's are equivalent to Gβ subunit as the triple Gγ1Gγ2Gγ3 mutant has an identical phenotype to the *agb1* mutant [13,14]. PsGβ subunit also has role in heat tolerance which has been earlier proved in over expressing lines of tobacco-transformed with 35S-PsGβ [11]. Moreover, AGB1 is also involved in the resistance against necrotrophic fungi [9]. Though, the effect of Gβ subunit is versatile and well known but the intricate pathway involved in heat tolerance and resistance against pathogens is yet to be deciphered. In order to address the question related to elucidation of the pathway involved in these processes, we used *Pisum sativum* Gβ subunit (PsGβ) and identified its interacting proteins (IPs). Here, we report the interacting partners of PsGβ cloned from the Y2H studies. From 90 IPs that we got initially, seven IPs were chosen for further investigation due to their recurrence in multiple experiments.

* Corresponding author. Tel.: +91 11 26742357; fax: +91 11 26742316.
E-mail address: narendra@icgeb.res.in (N. Tuteja).

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Selected interacting partners were confirmed using colony filter lift and ONPG assay.

2. Results and discussion

G-proteins are widely known to play a significant role in various biological processes in living organisms. However, the precise mechanism and their role in plants are far from understood. In order to isolate the interacting partners (IPs) of Gβ in pea, we performed Gal4 based yeast-two-hybrid (Y2H) screening assay. *P. sativum* cDNA library cloned in yeast pGADT7 vector was used as a prey and *P. sativum* Gβ cloned in pGBKT7 was used as bait. Both the gene and library were co-transformed into yeast strain AH109 and a total of about 90 positive colonies were observed in two-drop out (2 DO)-SD medium (Fig. 1A). These 90 colonies were randomly selected and were shifted to 3 DO-SD (Fig. 1B). Out of 90, few colonies that showed healthy growth even after 15 days were patched on 3 DO-SD+5 mM 3-AT [3-Amino- 1, 2, 4-triazole] (Fig. 1C). Seven colonies that showed healthy growth for 15 days exhibited similar growth pattern when patched on higher concentration of 3 DO-SD+30 mM 3-AT, thus confirming strong interaction. These experiments were repeated thrice to avoid any possibility of false negative (Fig. 1D). Subsequently, the same colonies were tested for β-galactosidase activity using colony filter lift assay and all of them showed the expected blue colour (Fig. 1E). No significant colony growth at this selection medium was detectable in negative control experiments, where the AH109 was transformed with empty vectors. The ONPG (O-nitrophenyl-β-D-galactopyranoside) assay was also performed for all the seven colonies for measuring strength of interaction following high-throughput quantitative β-galactosidase assay [15]. Significant β-gal activity was found in all the seven but colonies 35 and 39 showed higher activity then the other five (Fig. 2). No β-gal activity was observed in the negative controls co-expressing empty vectors. Interestingly, all IPs were found common in the three repetitive experiments. Sequences of these seven clones were analysed for homology and the accession numbers of the genes showing high similarity with them along with their putative functions are given in Table 1. These genes have been reported to play significant role in environmental stresses and plant development (Fig. 3). In fact, two out of seven interacting partners, namely, pathogenesis-related protein and glycine rich protein have been reported in Arabidopsis interactome [5]. All the experiments from Y2H to β-Gal (Colony filter lift assay and ONPG) were repeated three times and same interacting partners were found in these three replications.

Role of Gβ subunit of G-proteins has been shown in many cellular and physiological responses of plants. Our results suggest the involvement of Gβ in defence and other signals with regard to stress related pathway. Two *P. sativum* thioredoxin proteins PsTRXh1 and PsTRXh2 have been reported to be involved in redox-regulation by interacting with different targets proteins [16]. Interestingly, it has already been shown that one of target of thioredoxin during seed germination in case of model legume *Medicago truncatula* was Gβ subunit along with other proteins such as UDP-Glc pyrophosphorylase and glycine rich RNA binding protein [17]. Other IP namely, histidine-containing phosphotransfer protein is an important component of cytokinin signalling pathway. Stimulation of GCR1 in response to cytokinin has been suggested earlier but was not pursued further due to the lack of evidence [18]. Cytokinin signalling is mediated by a HIS-ASP phosphorelay pathway. This pathway consists of a two-component system with histidine kinase as receptor for perceiving signal and a regulator which has conserved receiver domain that gets phosphorylated by histidine kinase [19]. Since, cytokinin is known to be associated with many developmental and phenotypic effects which are similar

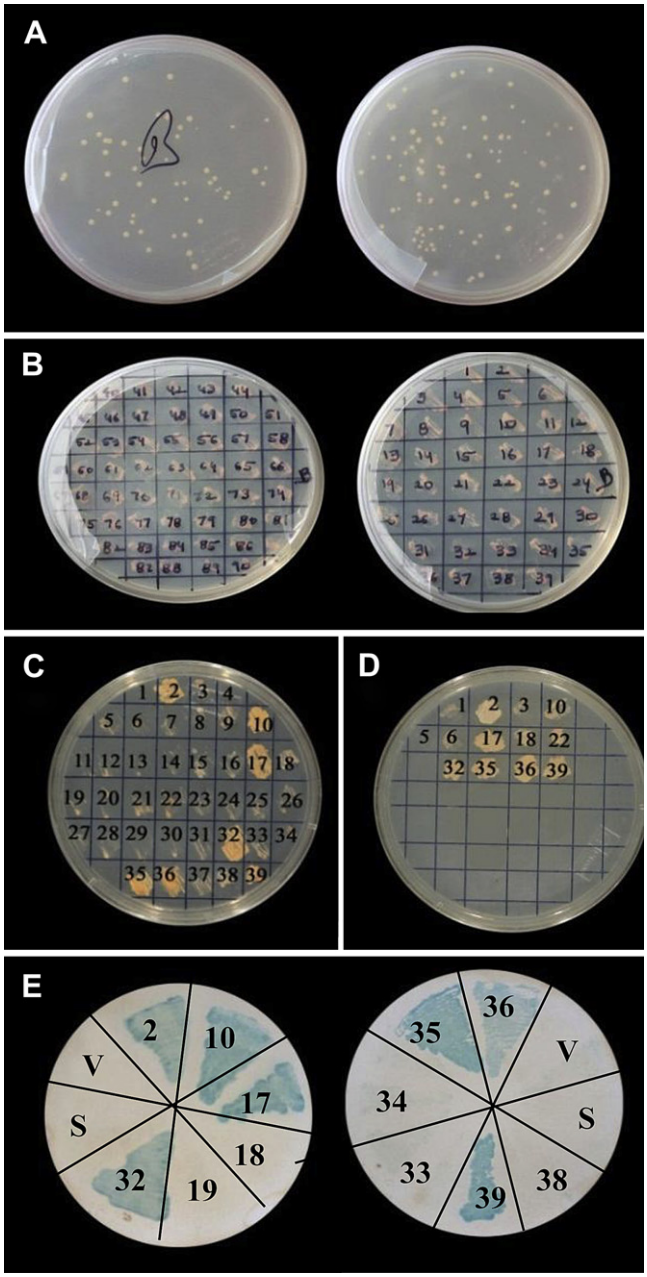

Fig. 1. Yeast-two-hybrid system-based interaction between PsGβ and stress induced cDNA library cloned in pGADT7 vector. A–B: Colonies grown on a synthetic dextrose plate lacking tryptophan and leucine (2-Drop out; 2DO); C–D: Colonies grown on synthetic dextrose plate lacking tryptophan, leucine and histidine(3-Drop out; 3DO); E–F: Colonies grown on a synthetic dextrose plate lacking leucine, tryptophan and histidine having 5 and 30 mM 3-AT(3-Amino-1, 2, 4-triazole); G–H: Same colonies were analysed for the β-galactosidase filter lift assay.Colonies 2,10,17, 32,35,36 and 39 are the clones that grew on the 30 mM 3-AT, whereas V(vector control); S(strain control) 18, 19, 33,34 and 38 were used as negative control.

to the functions of G-proteins such as cell division, root development, shoot growth and senescence. The cross talk between G-protein and cytokinin mediated pathway through the involvement of histidine-containing phosphotransfer protein is hereby proposed. Similarly, defensin and glucan endo-beta-1, 3-glucosidase (acidic isoform) belong to the family of pathogenesis-related protein (PR proteins) which are involved in wide range of physiological and developmental signalling processes [20]. Despite the presence of defensin genes in case of *agb1* mutants, their