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THE PUTATIVE COMMON BEAN KINASE *COK-4* PLAYS A ROLE IN PLANT DEVELOPMENT

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INTRODUCTION

Several anthracnose resistance loci have been identified in common bean (*Phaseolus vulgaris* L.), including the broad-based resistance locus *Co-4* (Melotto et al., 2004). *Co-4* contains several paralogs of the *COK-4* gene that is predicted to code for a protein kinase. The predicted COK-4 protein is highly similar to FERONIA (FER), a membrane-localized receptor-like kinase of Arabidopsis that has been implicated in the regulation of plant growth. We observed that the *fer-5* kinase null mutant has some developmental defects as it accumulates lower levels of fresh and dry weight than the wild type plants. Interestingly, genetic complementation of *fer-5* with *COK-4* partially rescued the wild type phenotype. Altogether, these data provide evidence that COK-4 is a structural and functional ortholog of FER kinase domain and these proteins may be involved in the control of growth-defense balance in plants.

MATERIAL AND METHODS

Genetically complemented *fer-5* mutant with the *COK-4* gene isolated from the resistant bean genotype SEL1308 was obtained using the floral-dip method (Clough and Bent, 1998). Transgenic plants were selected by spraying a solution of BASTA (0.0114% of glufosinate ammonium) supplemented with 0.005% of Silwet. Col-0 and *fer-5* plants containing the empty vector (35S-*GFP*), and the *fer-5* expressing *COK-4* (35S-*GFP*::*COK-4*) lines, were grown at 22-24^{o}C, 60±5% relative humidity, and 12 h photoperiod. Three sets of 10 four- to five-week old plants were collected to evaluated fresh and dry weights. The rosette of each genotype was kept at 70ºC during 72 hours to measure dry weight. The fresh weight was obtained immediately after removing the rosette from the soil. In both cases, the plant weight was obtained using analytical balance (OHAUS AV114 Adventurer Pro Analytical Balance) according with Abe et al. (2003).

RESULTS AND DISCUSSION

The *fer-5* mutant has lower fresh and dry weights than the Col-0 wild type plants (Fig. 1a and b). Phenotypic analysis showed that *fer-5* complemented with *COK-4* plants have significant increase in both fresh and dry weight when compared to the mutant lacking the kinase domain of FER (Fig. 1c and d). Nevertheless, the complemented lines still presented lower measurements than the Col-0 expressing empty vector (Col-0/GFP). These results suggest that COK-4 may not only be involved in common bean resistance (Oblessuc et al., 2015), but also have an additional function in the regulation of plant growth similar to FER. In addition, we demonstrate that the domain kinase of FER is required for protein function, reinforcing the importance of COK-4 as its functional ortholog. FER participates in several processes in the plant, such as growth, development and reproduction (Lindner et al, 2012; Wolf and Hofte, 2014). Additionally, FER has important roles in the signal transduction pathways of several hormones, including auxin and brassinosteroid that control plant growth (Duan et al., 2010; Deslauriers and Larsen, 2010). Further investigation of the possible role of COK-4 in these plant responses would add valuable information on common bean biological processes.

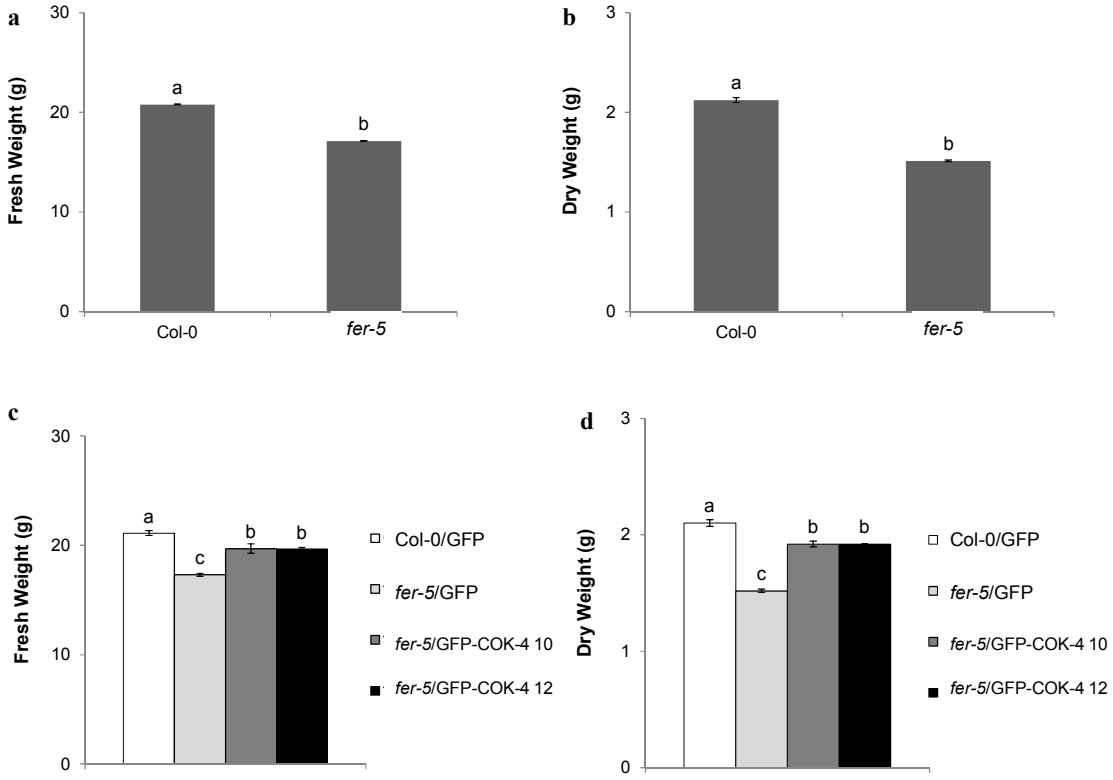

Fig. 1 (a) *fer-5* mutant has lower fresh and (b) dry weight than the Col-0 wild type plants. (c) *fer-5* expressing *COK-4* shows increased fresh weight as compared to *fer-5*. (d) *fer-5* complemented with *COK-4* lines has higher dry weight when compared with *fer-5* mutant. All data points are shown as average (n=30) ± standard deviation (SD) and statistical significance between the means was calculated with Tukey´s test at 5% of probability

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