

Fig. 1 A proposed model of photosynthetic carbon flow in *Arabidopsis thaliana* overexpressing *FbβCA3*. The cytosolic *FbβCA3* having low K_m for CO_2 increase the hydration of CO_2 . The dashed arrows indicate the diffusion of CO_2 and HCO_3^- within the cytosol and the chloroplast. The relatively higher amounts of CO_2 and HCO_3^- present in the mesophyll cells of overexpressor likely to increase the CO_2 diffusion gradient into the chloroplast during day time. Utilization of diffused CO_2 by *rubisco* to make 3-phosphoglycerate would accelerate the carbon reduction cycle favoring the carboxylation activity. *FbβCA3* overexpression increased the flux of the carboxylic acid to the tricarboxylic acid cycle (TCA) in mitochondria to play an anaplerotic role to synthesize higher amounts of total amino acids and proteins that contribute to increase photosynthetic efficiency and biomass.

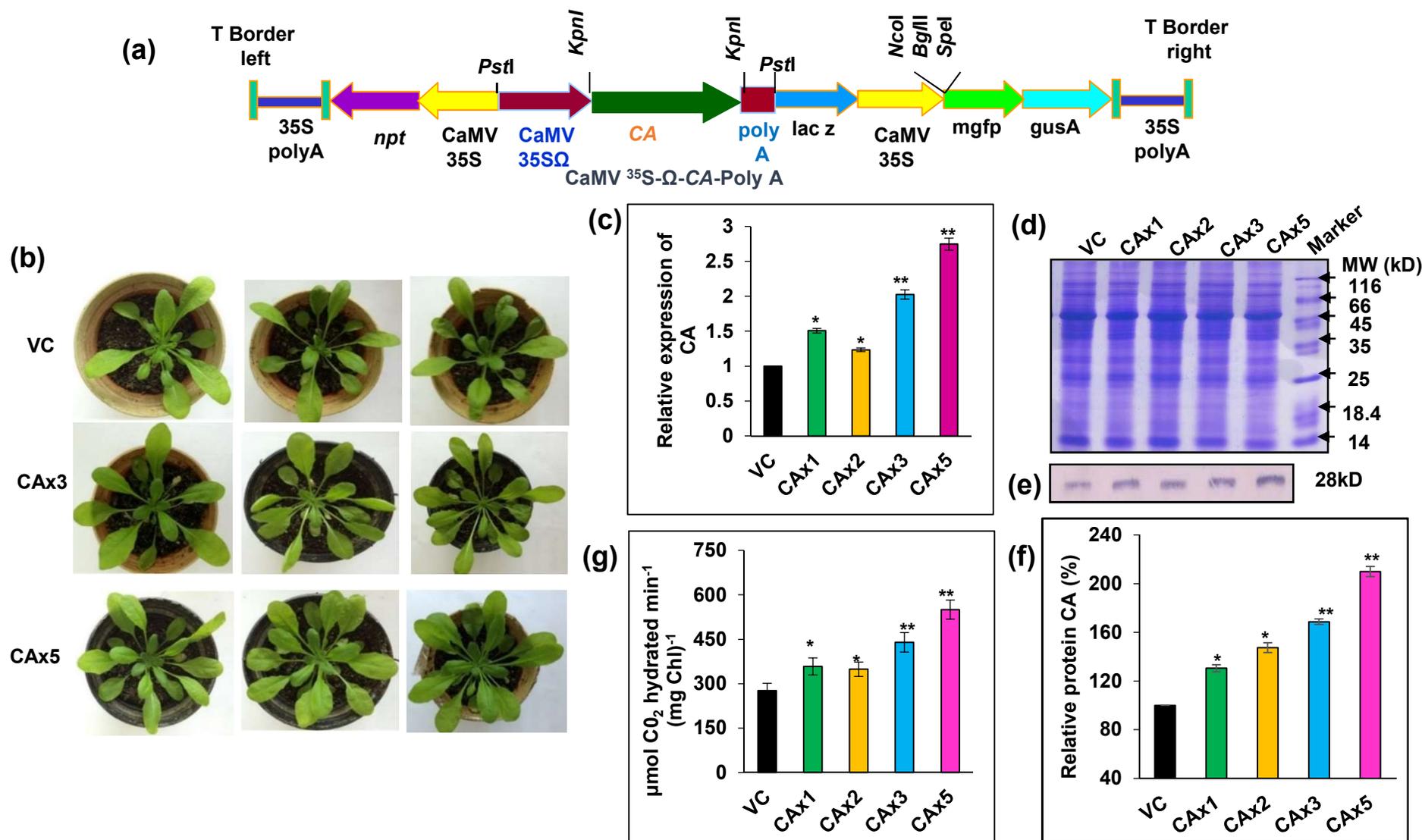


Fig. 2 A schematic representation of the transgene used for *Arabidopsis* transformation, photographs and conformation of *Flaveria bidentis* CA overexpressed in *Arabidopsis*. (a): *Flaveria bidentis* βCA3 cloned to pCAMBIA1304 vector having CaMV35S- Ω -poly A promoter cassette; CaMV35S-npt, coding region of neomycin phosphotransferase gene with CaMV35S promoter; CaMV35S Ω , CaMV 35S promoter with omega (Ω) enhancer; *FbβCA3* cDNA, coding region of *FbCA* gene; Poly A, Poly A tail; (b): *Arabidopsis* vector control (VC) and CAX (CAX3 & CAX5) plants grown at 21°C under 14h L / 10h D photoperiod in cool-white-fluorescent light ($100 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) for 4 weeks in pots; (c): qRT-PCR of *FbβCA3*—relative gene expression of CA in VC and transgenic lines; (d): 15% SDS-PAGE- twenty five μg protein was loaded in each lane and SDS-PAGE was run to check equal loading; (e): Western blot- protein samples from the gel were transferred to nitrocellulose membrane and immunoblot analysis of CA protein was made using *Flaveria bidentis* CA antibodies; (f): Quantification of CA blot- relative expression of CA in transgenic lines; (g): CA enzymatic activity- the activity of CA in VC was $276 \mu\text{mol CO}_2$ hydrated $(\text{mg Chl})^{-1} \text{min}^{-1}$. CA activity ranged from 348 to $550 \mu\text{mol CO}_2$ hydrated $(\text{mg Chl})^{-1} \text{min}^{-1}$ in different transgenic lines (CAX1, CAX2, CAX3 and CAX5); VC- vector control plants containing the null vector pCAMBIA1304). Each data point is the average of five replicates, and error bars represents $\pm\text{SD}$; asterisks indicate significant differences determined by t test (* $P < 0.05$)

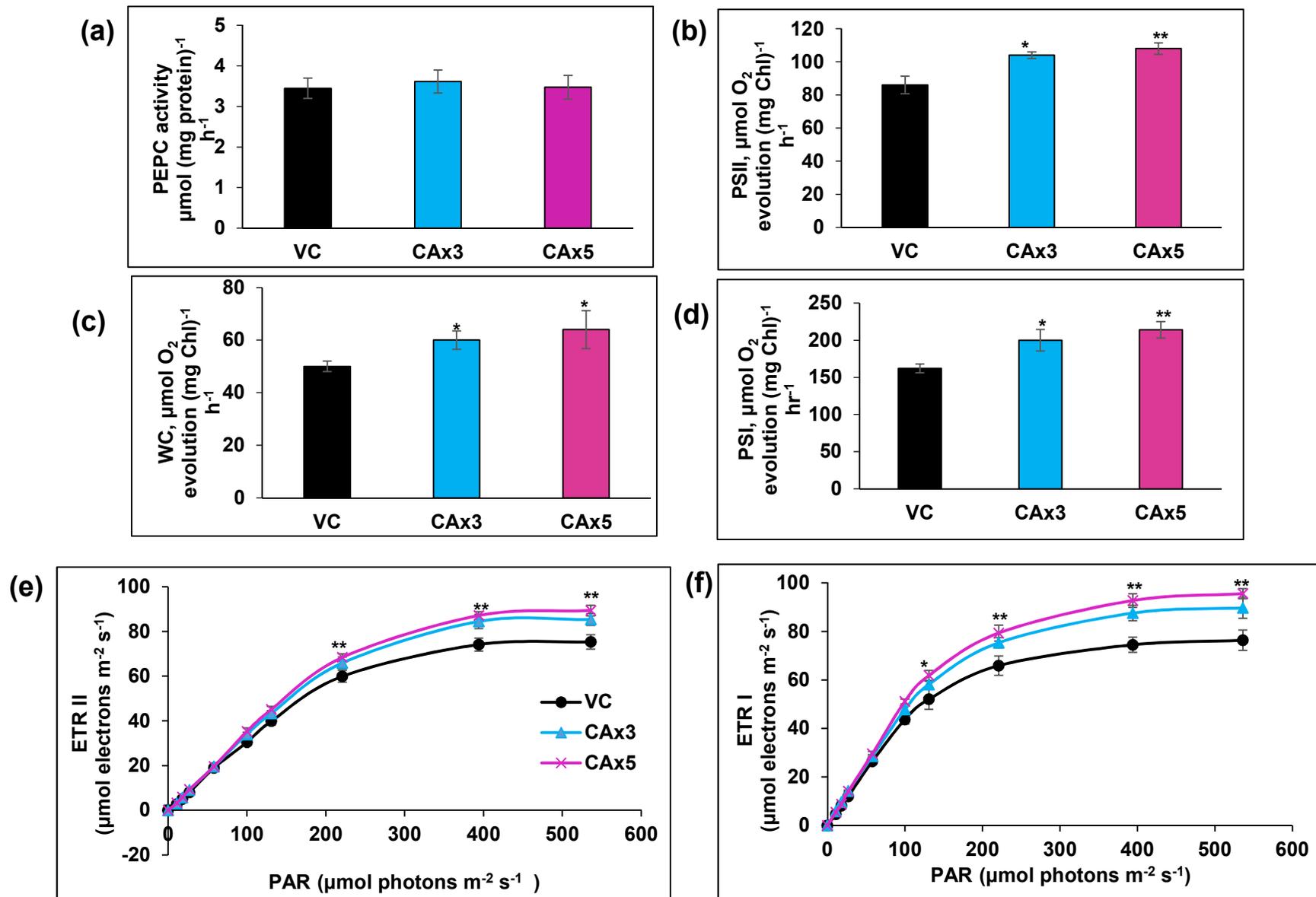


Fig. 3 PEPC enzymatic activity in-vitro, electron transport reactions in isolated thylakoid membrane and electron transport rates (ETR II & ETR I) in the intact leaves. (a): PEPC enzymatic activity- the activity of PEPC in transgenics was similar to vector control plants ($\sim 3 \mu\text{mol/mg protein/hr}$); **(b):** Electron transport through PSII (oxygen evolution; water to PD); **(c):** whole chain (water to MV; oxygen uptake); **(d):** PSI (ascorbate to MV; oxygen uptake) was measured polarographically, as described under “Materials and Methods”; **(e):** ETR II; **(f):** ETR I, VC- vector control; and 2 different transgenic lines, CAX3 and CAX5. Each data point is the average of five replicates and the error bars represent \pm SE; asterisks indicate significant differences determined by t test (* $P < 0.05$, ** $P < 0.001$)

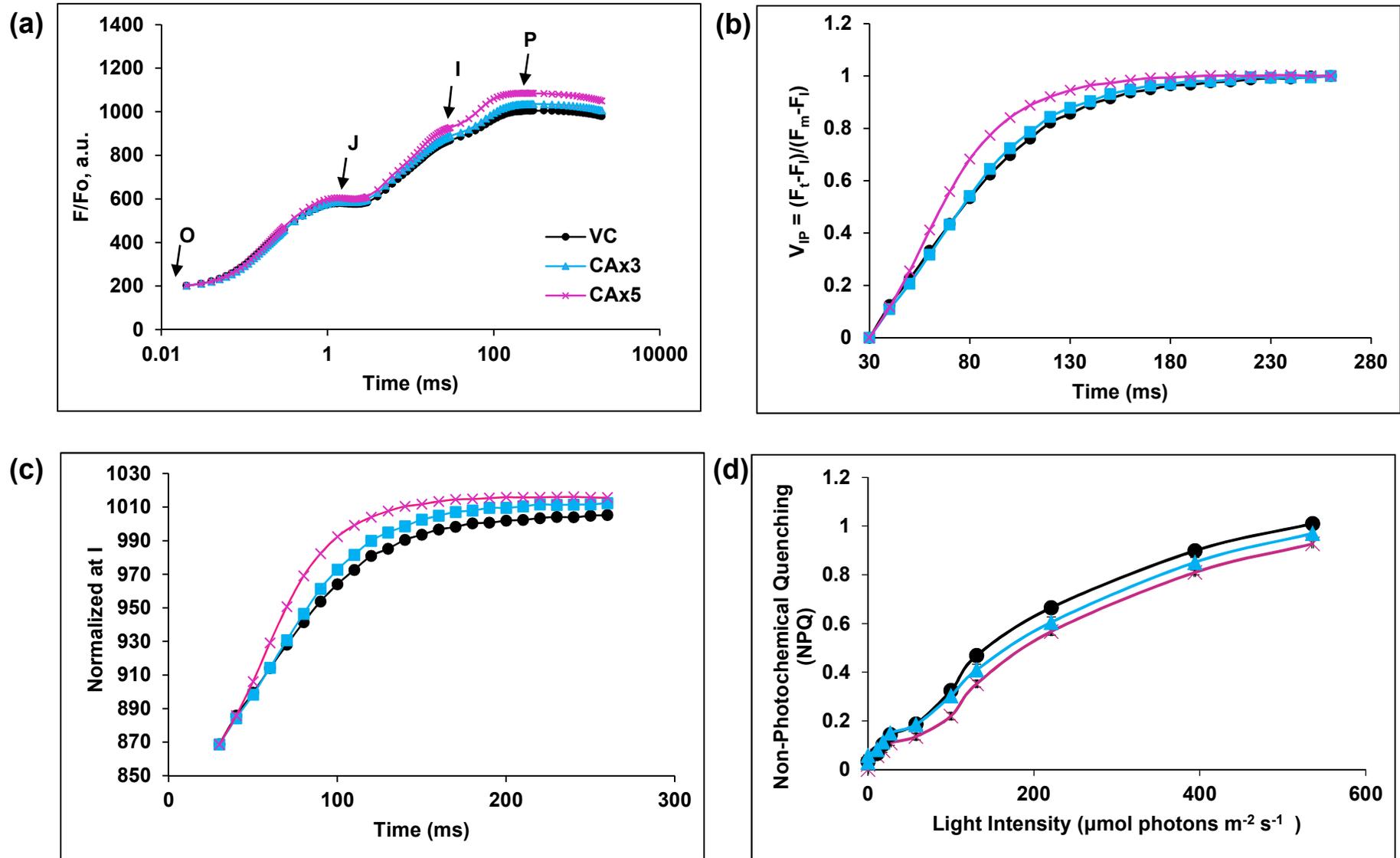


Fig. 4 The OJIP curve of chlorophyll *a* fluorescence and the non-photochemical quenching (NPQ) of the excited state of chlorophyll *a* of the vector control and *FbβCA3x* plants grown in soil. (a): Chl *a* fluorescence transients, the OJIP curves normalized at the O level; (b): Variable fluorescence transients from the I to the P—double normalized between I (F_I) and P (F_P): $V_{IP} = (F_t - F_I)/(F_P - F_I)$; (c): Variable fluorescence transients from the I—single normalization; F_t in the diagram, stands for fluorescence at time t (F_t), and F_0 is for fluorescence at the O level; (d): NPQ of the excited state of chlorophyll at different light intensities. Each data point is an average of 8 replicates and error bars represent \pm SE; asterisks indicate significant differences determined by t test ($*P < 0.05$)

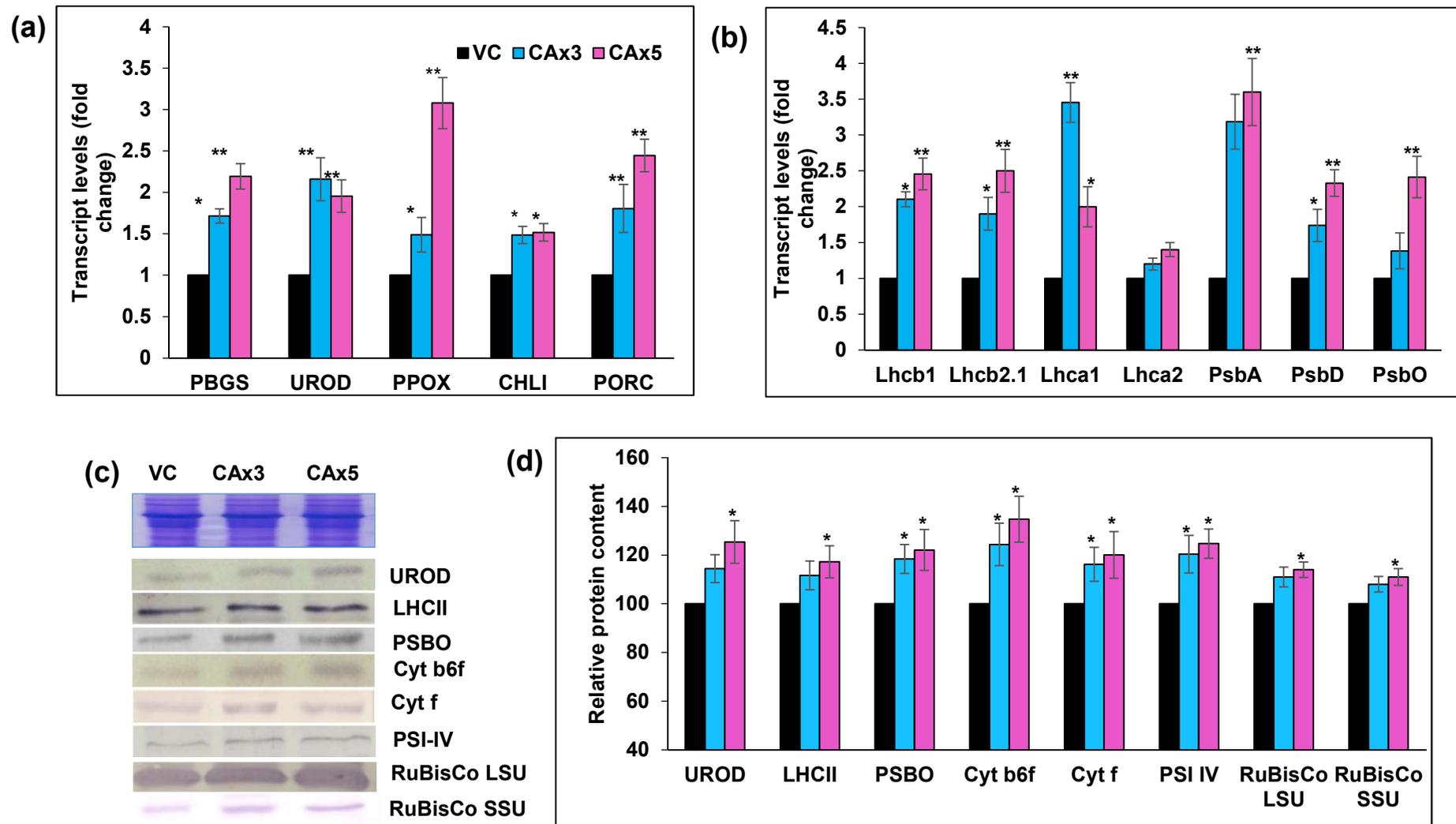


Fig. 5 Relative gene expression and immunoblot analysis. Relative expression of genes related to (a): chlorophyll biosynthesis; (b): photosynthesis; (c): SDS-PAGE (12%) of protein (20 μ g) isolated from VC and transgenic plants to check equal loading and the immunoblot to check the abundance of electron transport chain components; (d): Quantification of western blot by densitometry analysis using Alpha Ease FC software.; *PBGS*- porphobilinogen synthase; *UROD*- encoding uroporphyrinogen decarboxylase; *PPOX1*- encoding protoporphyrinogen oxidase; *CHLI*- encoding protoporphyrin-IX Mg-chelatase; *PORC*-encoding the light-inducible protochlorophyllide oxidoreductase; *Lhcb1* and *Lhcb2*, encoding components of the light harvesting complex associated with PSII; *Lhca1* and *Lhca2*, encoding components of the light harvesting complex associated with PSI; *PsbA* and *PsbD*, Core proteins, encoding photosystem II D1 and D2 proteins; *PsbO*, encoding for OEC33, the oxygen-evolving complex; LHCII- light-harvesting chlorophyll-binding proteins; Cytb6f and Cytf, psaE- PSIIIV; rubisco LSU and SSU- rubisco large and small subunit. Western blot data is an average of three independent replicates. qRT-PCR data are expressed as the mean \pm SE of three independent experiments performed in triplicate. Asterisks indicate significant differences determined by Student's t-test compared to control (* P < 0.05, ** P < 0.01)

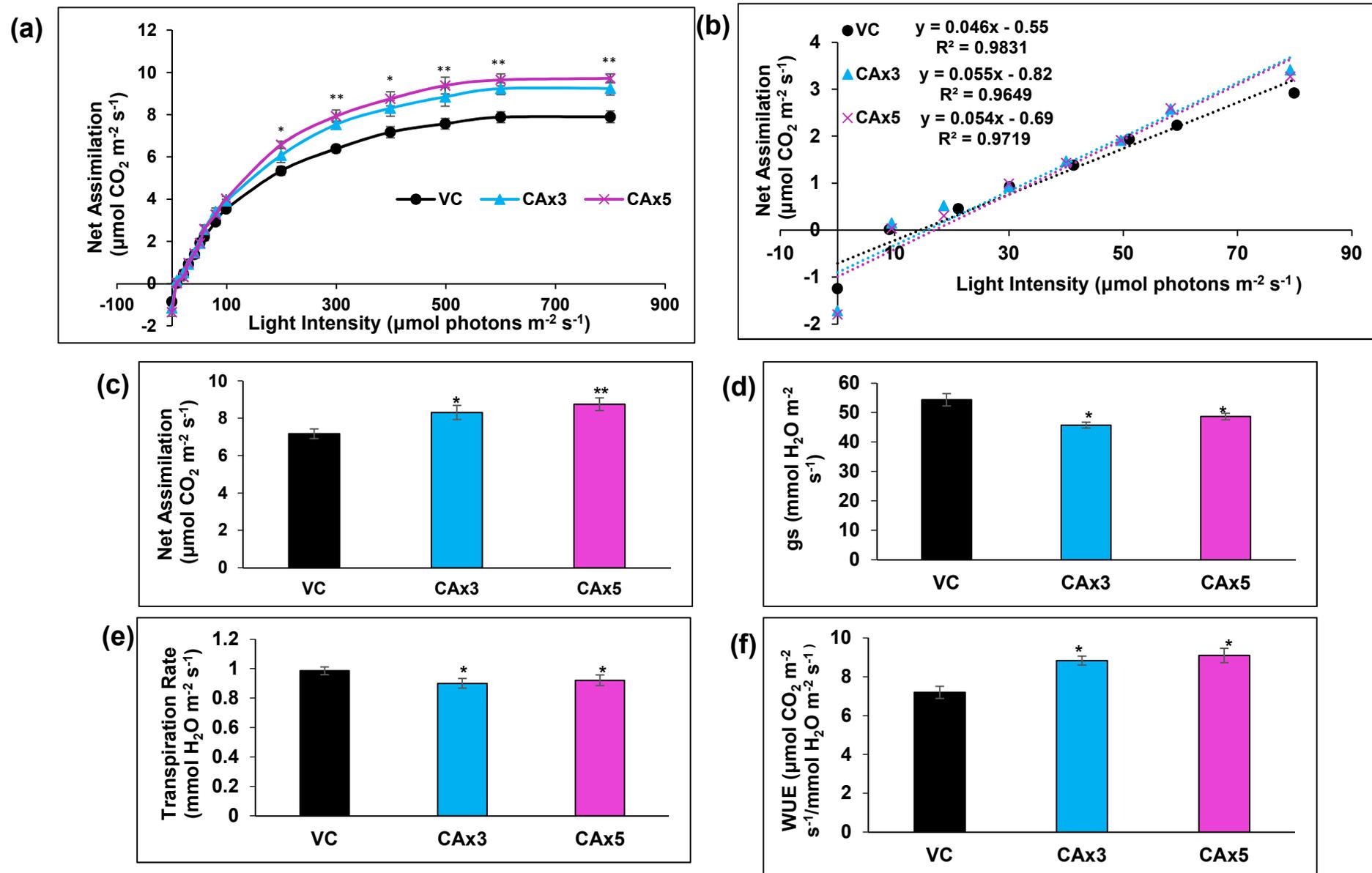


Fig. 6 Photosynthesis (net CO₂ assimilation rate) light response curve. (a): Net CO₂ assimilation rates of vector control and CAx plants were monitored by IRGA (LiCor-6400/XT) in ambient CO₂ at 400 μmol photons m⁻² s⁻¹ at 21°C; (b): Net CO₂ assimilation rates up to 80 μmol photons m⁻² s⁻¹; (c): Net CO₂ assimilation rates; (d): Stomatal conductance (gs); (e): Transpiration rate; (f): Water use efficiency (WUE) of vector control and transgenic *Arabidopsis* plants. Each data point is an average of five replicates and error bars represent SE. Asterisks indicate significant differences determined by t test (*P<0.05).

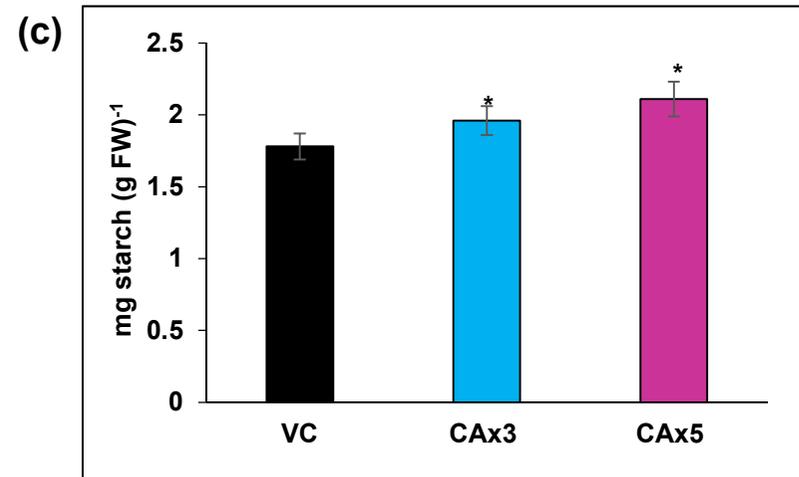
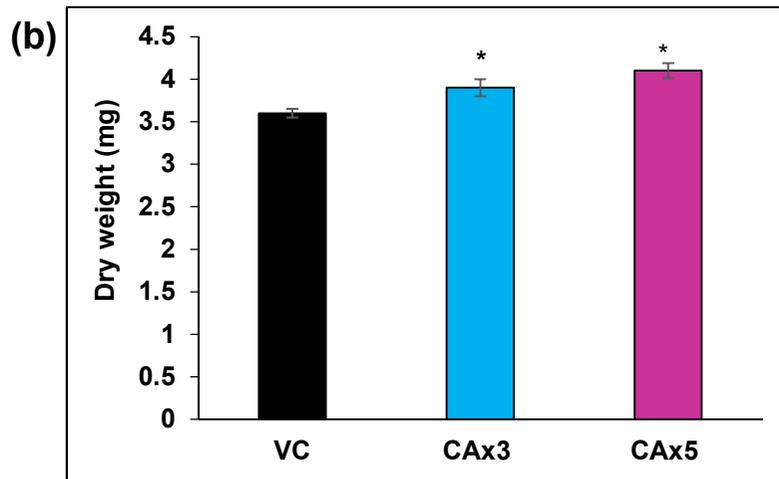
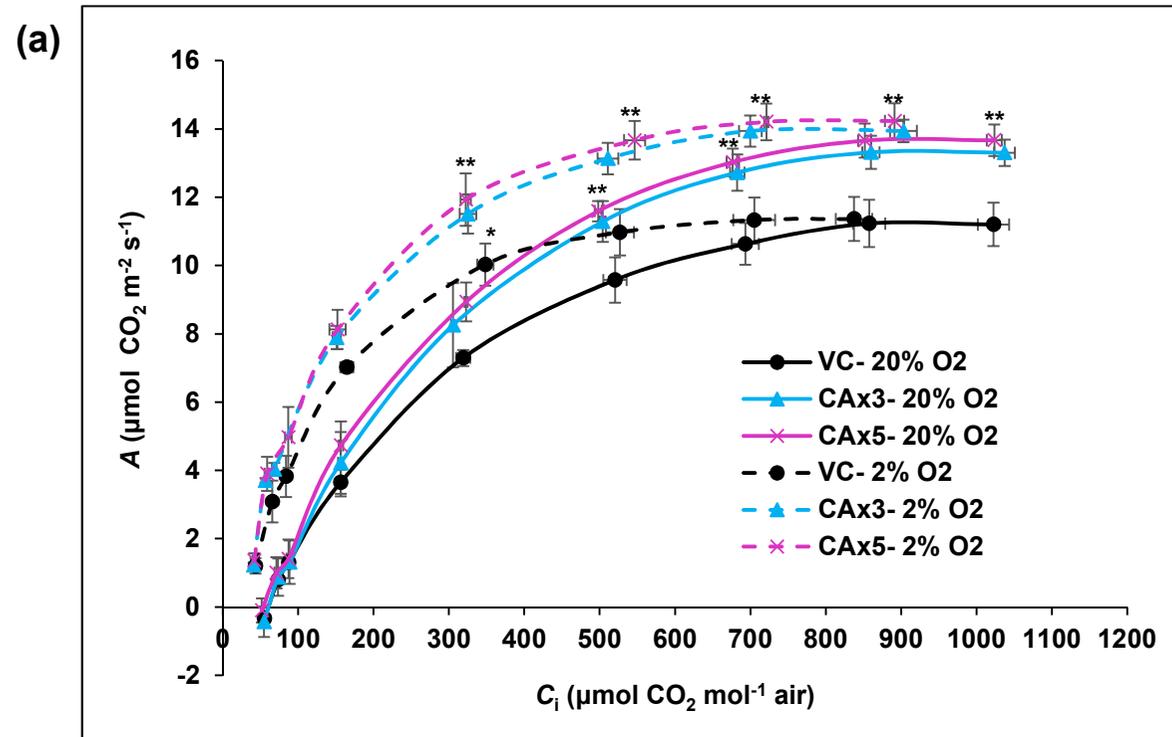


Fig. 7 Photosynthetic carbon fixation rate as a function of increasing intercellular [CO₂] at 21% O₂ and 2% O₂. (a): A/C_i curve; dashed lines represents to at 2% O₂; (b): Dry weight; (c): Starch content, per g FW. Each data point is an average of five replicates and error bars represent SE. Asterisks indicate significant differences determined by t test (* $P < 0.05$).