The occurrence of tocopherols and tocotrienols in Annatto (Bixa orellana L.) has attracted so much interest because these compounds are well recognized for their antioxidative effect. Thus, any information regarding the levels of tocopherols and tocotrienols in annatto seeds should be addressed. In the present study, $\gamma$-tocopherol and $\gamma$- and $\delta$-tocotrienol were evaluated in 11 accesses from APTA germplasm bank located in Pindorama, SP. The tocotrienols used as analytical standard were isolated from palm oil commercially named TOCOMIN. The analytical method involved the whole seeds saponification with KOH, liquid-liquid extraction with diethyl ether: petroleum ether: ethyl acetate (60:30:05) and determination by high performance liquid chromatography using fluorescence detection. A silica 60 column (250 x 4.6 mm d.i.) and a mobile phase composed of n-hexane: ethyl acetate: acetic acid (97.6:1.8:0.6, v/v/v) at a flow rate of 1.5 mL min$^{-1}$, was used to separate the compounds. The mean concentrations of $\gamma$-tocopherol varied between 5 and 12 mg 100g$^{-1}$. Levels of tocotrienols ranged from 71-243 mg 100g$^{-1}$ and 201-1375 mg 100g$^{-1}$ for $\gamma$ and $\delta$-tocotrienol, respectively. The $\delta$-tocotrienol isomer was the most representative one, corresponding to 92% of all compounds analysed.