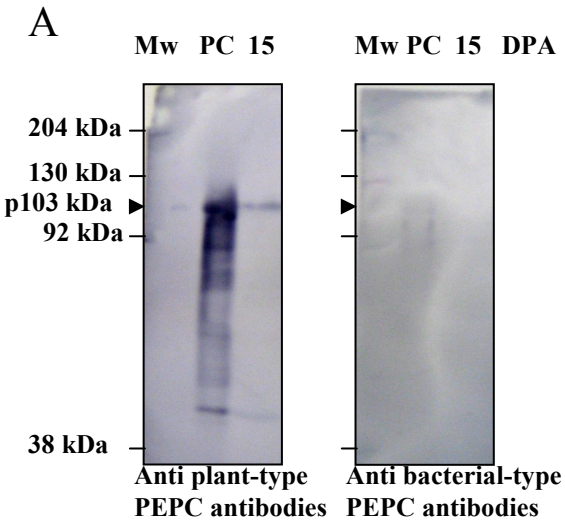
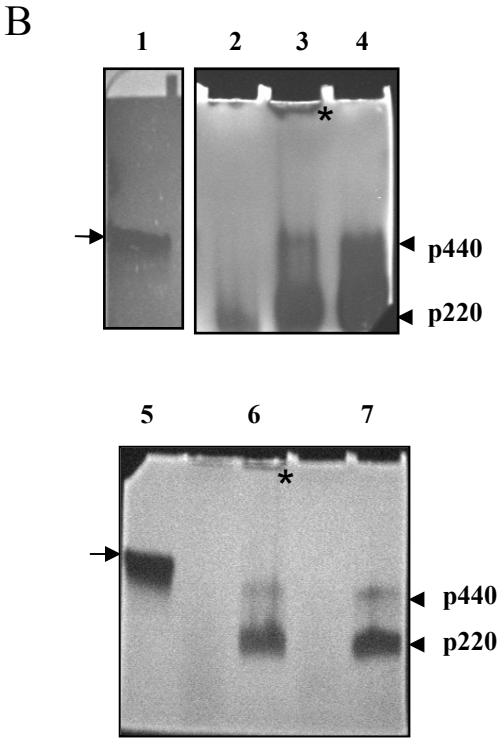
Fig. S1


A
Mw PC 15
Mw PC 15 DPA
204 kDa
130 kDa
p103 kDa
92 kDa
38 kDa
Anti plant-type PEPC antibodies
Anti bacterial-type PEPC antibodies


B
1
2
3
4
*
p440
p220
5
6
7
*
p440
p220

Suplemental Figure 1. A) Immunocharacterization of PEPC from 15 DPA seeds. Protein extracts from whole seed were prepared by the protocol described in Meimoun et al., (2007) using denaturing conditions (trichloroacetic acid (TCA). Soluble proteins (200 μg) were separated by SDS-PAGE (10% acrylamide), transferred onto nitrocellulose, and probed with polyclonal antibodies raised against sorghum leaf PEPC (30 μg per 20 mL of incubation medium), or antibacterial-type PEPC4 IgGs from *Arabidopsis thaliana* (20 μg per 20 mL of incubation medium). Immunolabeled proteins were detected by a horseradish peroxidase assay. The arrows indicate 103 kDa PEPC subunits (p103). PC, 0.1 U of purified PEPC from sorghum leaves. B) PEPC in barley seed is mainly present as a tetrameric/dimeric form. Proteins from seed (16 whole seed) were extracted as described in Materials and Methods section, in the absence (lanes 2, 3 and 4) or presence (lanes 6 and 7) of thiol reducing agents. The samples were submitted to non-denaturing polyacrylamide (5% acrylamide without staking gel) gels at 8 °C. After protein migration, the gels were treated and revealed for PEPC activity as described in Rivoal et al., 2002. Lanes 1 and 5 are semipurified PEPC from sorghum leaf (0.67 and 1.2 U respectively). Lanes 2, 15 DPA seed (0.12 U/110 μg of proteins); lanes 3 and 6, dry seed ((0.24 U/195 μg of proteins); line 4, 6 h imbibed seeds (0.27 U/200 μg of proteins) and line 7, 48 h-imbibed seed (0.312 U/120 μg of proteins. The reaction was performed during 5 or 10 min for lanes 5 to 7 or 1 to 4 respectively.