**Fig.S1** Pearson correlation coefficients between different scoring dates and replications of the IBM population scored for the Ca-deficiency phenotype observed when lines were grown in pots amended with 13.3g osmocote. Rep1.1, Rep1.2, Rep1.3, Rep1.4, Rep 1.5, Rep 1.6, Rep 1.7 represent the phenotype on November 26, 28, 30, December 3, 5, 7, 10 of the first replication; Rep2.1, Rep2.2, Rep2.3, Rep2.4 represent the phenotype on January 7, 9, 11, 14. WA1 and WA2 represent "weighted average" of the two replications respectively. LSMEAN is Line least-square means of two replications.

**Fig. S2-S5** QTL Plots of LOD (upper graph) and additive value (lower graph) for the Ca-deficiency phenotype in the IBM RIL population for chromosomes 1, 2, 3, and 6. WA1= the WA of the first replication, WA2= the WA of the second replication, LSMEAN=Line least-square means of two replications.

**Supplemental Table legends**

**Table S1.** List of the lines used in this study

**Table S2.** Elemental profiles of B73 maize in the control and treatment. Results of ionomic analysis of B73 maize grown without fertilizer amendment (CT) or with the addition of 13.3g Osmocote per pot (TR); presented as mean content of each element (mg/kg), standard deviation (SD) and significance of the difference between the CT and TR (Sig). Rep I is the first repetition, Ret II is the second repetition.

**Table S3.** Frequency of Ca-deficiency phenotype of B73 and Mo17 seedlings grown with different combinations of ammonium, nitrate, and calcium. The different amounts of Osmocote, NH4NO3, Ca(NO3)2 in each treatment solution are indicated, expressed as grams per liter as well as equivalent molar concentrations of ions of interest. Maize seedlings of lines B73 and Mo17 were assessed for presence of the Ca-deficiency phenotype at. 3d and 6d of treatment. The experiment was repeated, with 5 seedlings in each of 2 repetitions.

**Table S4** The Ca-deficiency phenotype of 276 RIL population in Greenhouse. "Rep" is the replication. Rep1.1, Rep1.2, Rep1.3, Rep1.4, Rep 1.5, Rep 1.6, Rep 1.7 represent the phenotype on November 26, 28, 30, December 3, 5, 7, 10 of first replication expressed as the proportion of plants showing the Ca-deficiency phenotype. Rep2.1, Rep2.2, Rep2.3, Rep2.4 represent the phenotype on January 7, 9, 11, 14. WA1 and WA2 represent "the weighted average value" of two repetitions respectively. "LSMEAN" represents "Line least-square means" of two replications. NA represents missing data.

**Table S5** Candidate genes located in the QTL on all chromosome and gene annotations. These 6 genes were selected from among 316 predicted to be encoded in the identified QTL intervals. "Pos" represents the physical position of candidate gene based on the B73 V4 map.