8 days old etiolated maize tissue

Ground to fine powder in presence of liquid nitrogen

Added extraction buffer in 1:3 ratio

Mixed thoroughly

Centrifuged at 15,000 rpm, at 4°C for 45 minutes

Pellet discarded

Supernatant ultra-centrifuged at 1,00,000 x g at 4°C for 1 hour

Pellet discarded

Supernatant given 0-40% ammonium sulfate cut and centrifuged at 10,000 x g for 30 minutes at 4°C

Pellet discarded

Supernatant given 40-50% ammonium sulfate cut and centrifuged at 10,000 x g for 30 minutes at 4°C

Supernatant discarded

Pellet re-suspended in extraction buffer and dialysed extensively

Proteins run on DEAE Sephacel column

Active fractions pooled

Active proteins run on CaM Sepharose column

Gel purification

Purified protein

FIG 1. Purification flow chart for ZmCCaMK: Flow chart showing different steps of purification of Ca\(^{2+}\)/CaM-dependent kinase from maize.
FIG 2. Protein elution profile and Ca\(^{2+}\)-stimulated activity from DEAE Sephacel column: Proteins bound to DEAE Sephacel column were eluted using 0-0.4M KCl gradient. Protein concentration and Ca\(^{2+}\)-dependent activity was checked in every alternate fraction. Inset, 10\% SDS-PAGE showing protein profile of the active fraction after CBB staining.
FIG 3. Protein elution profile and Ca\textsuperscript{2+}/CaM-stimulated activity from CaM Sepharose column: Proteins bound to CaM Sepharose column in a calcium-dependent were step eluted with 1-4 mM EGTA and protein concentration was estimated. *Fraction no. 1, 2* proteins eluted with 1mM EGTA, *Fraction no. 3, 4* proteins eluted with 2 mM EGTA, *Fraction no. 5, 6* proteins eluted with 3 mM EGTA and *Fraction no. 7, 8* proteins eluted with 4 mM EGTA. Inset, 12.5% SDS-PAGE showing protein profile of the active fraction after silver staining.
FIG 4. Gel electrophoretic profile at different steps of purification of ZmCCaMK:

Total soluble proteins and active protein fractions after different steps of purification were run on 10% SDS-PAGE and stained with either CBB (crude extract, soluble proteins, 40-50% ammonium sulfate fraction and DEAE Sephacel elute) or silver stained (CaM Sepharose elute and purified protein). Molecular weight markers are shown in the last lane.
FIG 5. Gel electrophoretic purity of ZmCCaMK: lanes 1-3 show different concentrations of purified ZmCCaMK run on a 12.5% gel and silver stained, Lane 4 shows the marker.
FIG 6. Effect of various physiological factors on kinase activity with histone III S as a substrate: Histone III S was in vitro phosphorylated in presence of different factors using 50 ng purified ZmCCaMK. A, Autoradiogram showing phosphorylated histone III S under different conditions. B, Specific activity of ZmCCaMK under similar conditions.
FIG 7. Effect of various exogenous factors on kinase activity with syntide-2 as a substrate: Syntide-2 was *in vitro* phosphorylated in presence of different factors using 50 ng purified ZmCCaMK. A, Autoradiogram showing phosphorylated syntide-2 under different conditions. B, Specific activity of ZmCCaMK under similar conditions.
**FIG 8. Effect of pH on ZmCCaMK activity:** *In vitro* kinase assays were performed at different pH buffers with Histone III S as a substrate and 50 ng of purified ZmCCaMK. 0.25M citrate buffer pH 3-6 and 0.25 M Tris buffer pH 7-10 were used for assay. A, Autoradiogram showing histone III S phosphorylation at different pH values. B, Plot of specific activity values under similar conditions.
FIG 9. Determination of optimum values of various factors on ZmCCaMK activity with histone III S as substrate: Kinase assay was performed with A, different Mg$^{2+}$ concentration in presence of Ca$^{2+}$/CaM. B, different free Ca$^{2+}$ concentration C, different CaM concentration in presence of 1 µM free Ca$^{2+}$ and D, different CaBP concentration in presence of 1 µM free Ca$^{2+}$.
FIG 10. CaM specific stimulation of kinase activity: Histone III S was *in vitro* phosphorylated in presence of 0.5 mM EGTA, 10 μM Ca²⁺, 100 nM CaM, 100 nM *E. histolytica* CaBP and saturating amount of moss CaM antibodies using 50 ng purified ZmCCaMK. A, Autoradiogram showing Histone phosphorylation under different conditions and B, Bar diagram showing specific activity of ZmCCaMK under similar conditions.
FIG 11. CaM specific stimulation of kinase activity: Casein was *in vitro* phosphorylated in presence of 0.5 mM EGTA, 10 μM Ca²⁺, 100 nM CaM, 100 nM *E. histolytica* CaBP and saturating amount of moss CaM antibodies using 50 ng purified ZmCCaMK. A, Autoradiogram showing casein phosphorylation under various conditions and B, Bar diagram showing specific activity of ZmCCaMK.
FIG 12. CaM specific stimulation of kinase activity: Syntide-2 was in vitro phosphorylated in presence of 0.5 mM EGTA, 10 μM Ca^{2+}, 100 nM CaM, 100 nM *E. histolytica* CaBP and saturating amount of moss CaM antibodies using 50 ng purified ZmCCaMK. A, Autoradiogram showing syntide-2 phosphorylation under various conditions and B, Bar diagram showing specific activity of ZmCCaMK.
FIG 13. Direct binding of purified ZmCCaMK with $^{45}$Ca$^{2+}$: Purified ZmCCaMK (5 \( \mu \)g) was run on 10% SDS-PAGE and transferred on nitro-cellulose membrane. After incubation with buffer containing $^{45}$Ca$^{2+}$ the membrane was exposed for autoradiography. Lane A, Autoradiogram showing binding of $^{45}$Ca$^{2+}$ with purified protein (5 \( \mu \)g). Lane Mr, Molecular weight marker in kDa.
FIG 14. Gel mobility shift caused by binding of calcium with the purified ZmCCaMK: Purified ZmCCaMK (1 μg) was incubated with sample buffer containing Ca$^{2+}$ (5 mM) or Ca$^{2+}$ and EGTA (5 mM), run on a 7% SDS-PAGE and silver stained. Figure shows a shift in mobility of ZmCCaMK due to binding of calcium. C and Mr represent control without any additives and molecular weight markers in kDa respectively.
FIG 15. Kinetic studies with ZmCCaMK using histone III S as substrate: *In vitro* kinase assay was performed with varying histone III S concentration and 50 ng of purified ZmCCaMK. Figure shows plot of ZmCCaMK specific activity values with varying histone III S concentrations. Km and Vmax values were determined using Lineweaver-Burk plot (Inset).
FIG 16. Kinetic studies with ZmCCaMK using syntide-2 as substrate: In vitro kinase assay was performed with varying syntide-2 concentration and 50 ng of purified ZmCCaMK. Figure shows plot of ZmCCaMK specific activity with varying syntide-2 concentrations. Km and Vmax values were determined using Lineweaver-Burk Plot (Inset).
FIG 17. Kinetic studies with ZmCCaMK using de-phosphorylated casein as substrate: \textit{In vitro} kinase assay was performed with varying casein concentration and 50 ng of purified ZmCCaMK. Figure shows plot of ZmCCaMK specific activity with varying casein concentrations. \(K_m\) and \(V_{\text{max}}\) values were determined using Lineweaver-Burk plot (Inset).
FIG 18. Kinetic studies with ZmCCaMK using \( \gamma^{\text{32}}\text{P} \text{ATP} \) as substrate: In vitro kinase assay was performed with varying \( \gamma^{\text{32}}\text{P} \text{ATP} \) concentration and 50 ng of purified ZmCCaMK. Figure shows plot of ZmCCaMK specific activity with varying \( \gamma^{\text{32}}\text{P} \text{ATP} \) concentrations. Km and Vmax values were determined using Lineweaver-Burk plot (Inset).
FIG 19. Effect of different inhibitors on ZmCCaMK activity using histone III S as substrate: In vitro kinase assay was performed in presence of different calcium and CaM antagonists and kinase inhibitors (2 mM EGTA, 100 µM each of CPZ, TFP, KN-62 and W7, 10 µg 48/80, 5 nM staurosporine, 100 µM H7) with histone III S as substrate and 50 ng of purified ZmCCaMK. A, Autoradiogram showing Histone III S phosphorylation. B, Specific activity of ZmCCaMK under similar conditions.
FIG 20. Effect of different inhibitors on ZmCCaMK activity using syntide-2 as a substrate: *In vitro* kinase assay was performed in presence of different calcium and CaM antagonists and kinase inhibitors (2 mM EGTA, 100 μM each of CPZ, TFP, KN-62 and W7, 10 μg 48/80, 5 nM stauroaporine, 100 μM H7) with syntide-2 as substrate and 50 ng of purified ZmCCaMK. A, Autoradiogram showing syntide-2 phosphorylation. B, Specific activity of ZmCCaMK under similar conditions.
FIG 21. Concentration kinetics and determination of IC50 values of different kinase inhibitors on ZmCCaMK activity with histone III S as substrate: In vitro kinase assay was performed with varying inhibitor concentration and 50 ng of purified ZmCCaMK with histone III S as substrate. Figure shows plot of ZmCCaMK specific activity value in presence of varying concentrations of different CaM antagonists/kinase inhibitors. IC 50 values for the inhibitors was calculated from these plots.
FIG 22. Concentration kinetics and determination of IC50 values of different calcium and CaM antagonists on ZmCCaMK activity with histone III S as substrate: *In vitro* kinase assay was performed with varying inhibitor concentration and 50 ng of purified ZmCCaMK with histone III S as substrate. Figure shows plot of ZmCCaMK specific activity value in presence of varying concentrations of different Ca and CaM antagonists. IC 50 values for the inhibitors was calculated from these plots.
FIG 23. Autophosphorylation of purified ZmCCaMK: Protein (5μg) was autophosphorylated in presence of 10 μM Ca²⁺, run on a 10% SDS-PAGE, dried and exposed for autoradiography. Figure shows autoradiogram showing autophosphorylation of purified protein.
Fig 24. Time kinetics of ZmCCaMK autophosphorylation and substrate phosphorylation: The reaction was started by addition of $\gamma P^{32}$ ATP and stopped at different time periods by applying small aliquots on P81 paper.
FIG 25. Effect of different physiological factors on autophosphorylation of ZmCCaMK: Purified ZmCCaMK (5μg) was autophosphorylated in presence of different factors (2 mM EGTA, 10 μM calcium, 100 nM CaM, 5 μM PS, 100 nM PMA), run on 10% SDS-PAGE and exposed for autoradiography. A, Autoradiogram showing autophosphorylated ZmCCaMK under different conditions. B, Specific activity of ZmCCaMK under similar conditions.
FIG 26. Effect of pH on ZmCCaMK autophosphorylation: Purified ZmCCaMK (5 μg) was autophosphorylated at different pH buffers (0.25 M citrate buffer for pH 3-6 and 0.25 M Tris buffer for pH 7-10). A, Autoradiogram showing autophosphorylation at different pH values. B, Plot of specific activity values under similar conditions.
FIG 27. Effect of different inhibitors on autophosphorylation activity of ZmCCaMK: Purified ZmCCaMK (5 µg) was autophosphorylated in presence of different calcium and CaM antagonists and kinase inhibitors (2 mM EGTA, 100 µM each of CPZ, TFP, KN-62 and W7, 10 µg 48/80, 5 nM staurosporine, 100 µM H7). A, Autoradiogram showing auto phosphorylation. B, Specific activity of ZmCCaMK under similar conditions.
FIG 28. Autophosphorylation of purified ZmCCaMK and comparison with substrate phosphorylation: Purified ZmCCaMK (5 μg) was autophosphorylated in presence of 2 mM EGTA (1), 10 μM Ca$^{2+}$ (2), and 10 μM Ca$^{2+}$+100 nM CaM (3). Autophosphorylated protein was run on 10% SDS-PAGE and exposed for autoradiography. Histone phosphorylation was also performed under similar conditions using 50 ng of purified ZmCCaMK. Figure shows autoradiogram of A, autophosphorylation of ZmCCaMK and B, histone phosphorylation.
FIG 29. Effect of autophosphorylation on substrate phosphorylation activity of ZmCCaMK: *In vitro* kinase assay was performed with histone III S as substrate in presence EGTA (2 mM), using non-autophosphorylated (A) and autophosphorylated (B) ZmCCaMK (1 µg). Figure shows autoradiogram depicting calcium-independent histone phosphorylation (B). Mr is protein molecular weight markers in kDa.
FIG 30. Degradation pattern of purified ZmCCaMK: Purified ZmCCaMK was stored at -20°C for different time periods and then autophosphorylated. All the degraded peptides show autophosphorylation.
FIG 31. Phosphoaminoacid analysis of phosphorylated proteins: Phosphorylated histone (1), syntide-2 (2) and ZmCCaMK (3), were run on paper chromatogram after digestion with 6N HCl and separated on a paper chromatogram along with phosphoamino acid standards phosphoserine (S), phosphothreonine (T) and phosphotyrosine (Y). Chromatogram containing phosphorylated samples was exposed for autoradiography. A, A paper chromatogram showing phosphoamino acid standards B, Autoradiogram showing showing phosphoamino acids of phosphorylated proteins.
8 Weeks Old New Zealand White Rabbit

7 days

Collected Pre-Immune Serum

15 days

First Injection with 250 µg Purified Protein

15 days

First Booster with 100 µg Purified Protein

15 days

Second Booster with 100 µg Purified Protein

4 days

First Test Bleed and ELISA for Checking the Titre

15 days

Third Booster with 100 µg Purified Protein

4 days

Bleeding and Serum Collection

FIG 32. Immunization protocol of rabbit for production of ZmCCaMK antibodies.
FIG 33. Titration of ZmCCaMK antibodies with different protein concentrations: Total protein extract of maize coleoptiles (10-100 μg) was separated on SDS-PAGE and transferred on nitro-cellulose membrane. Western blotting was done with indicated dilutions of ZmCCaMK antibodies. Figure shows western blot showing no cross reactivity with pre-immune serum and a single band of 72 KDa at all the protein concentrations and all the dilutions of antibodies, verifying the monospecificity of antibodies.
FIG 34. Cross reactivity of ZmCCaMK antibodies with purified ZmCCaMK: Purified ZmCCaMK (1 μg) was run on a 10% SDS-PAGE, transferred on nitro-cellulose membrane and probed with 1:20,000 dilution of ZmCCaMK antibodies. Figure shows a single band of ZmCCaMK cross reacting with the ZmCCaMK antibodies.
FIG 35. Immunoprecipitation of ZmCCaMK activity: *In vitro* kinase assay was performed with histone III S as substrate in presence of 0-25 µl of ZmCCaMK antibodies. Antibodies precipitated down the activity in a concentration dependent manner. A, Autoradiogram showing histone III S phosphorylation in presence of increasing concentration of ZmCCaMK antibodies. B, Specific activity under similar conditions.
FIG 36. Specific Immunoprecipitation by ZmCaMK antibodies: *In Vitro* was performed with histone IIIS as substrate in presence of 0-25 μl of ZmCaMK antibodies, 0-25 μl of pre-immune serum and 0-25 μl of Brassica glyoxalase I antibodies. Only ZmCaMK antibodies could precipitate the kinase activity.
FIG 37. Immunoprecipitation of ZmCCaMK autophosphorylation activity: Purified ZmCCaMK (5 µg) was autophosphorylated in presence of 0-25 µl of ZmCCaMK antibodies. A, Autoradiogram showing autophosphorylation in presence of increasing concentration of ZmCCaMK antibodies. B, Specific activity under similar conditions.
FIG 38. Precipitation of rat brain CaM-kinase activity with ZmCCaMK antibodies:
Total rat brain extract (20 μg) was *in vitro* phosphorylated in presence of 5 mM EGTA (1), 100 μM Ca$^{2+}$ + 100 nM CaM (2), 5 μl of moss CaM antibodies (3), 5 μl moss CaM antibodies + 100 nM CaM (4) and 5 μl ZmCCaMK antibodies (5). Figure shows autoradiogram of phosphorylated samples under different conditions.
FIG 39. Presence of immunohomologues of ZmCCaMK in different plant species:
Total protein of different plants separated on 10% SDS-PAGE and western blot of similar protein samples with 1:20,000 dilution of ZmCCaMK antibodies. A single band of 72 KDa could be seen in all the plant samples tested, showing its ubiquitous presence.
FIG 40. Immuno-detection studies in different plant parts with ZmCCaMK antibodies: Total protein (25 μg) of different plant parts separated on 10% SDS-PAGE and western blot of similar protein samples with 1: 20,000 dilution of ZmCCaMK antibodies. ZmCCaMK is not specified to any particular plant part but roots have a very low expression compared to leaf.
FIG 41. Immuno-detection of ZmCCaMK during different stages of development in maize: Total protein (25 µg) of different day old maize plants separated on 10% SDS-PAGE and western blot of similar protein samples with 1: 20,000 dilution of ZmCCaMK antibodies. The kinase shows a development-dependent expression. ZmCCaMK level is very low in embryo and shows an increase up to 5 days. The level remains constant thereafter.
FIG 42. Effect of different stress treatments: Total protein (25 μg) of maize coleoptiles, given different stress treatments, separated on 10% SDS-PAGE and western blot of similar protein samples with 1:20,000 dilution of ZmCCaMK antibodies. 8 day old, etiolated, water grown plants served as control.
FIG 43. Effect of different light radiation on ZmCCaMK: Total protein (25 μg) of maize coleoptiles, given different light treatments for indicated time period, separated on 10% SDS-PAGE and western blot of similar protein samples with 1: 20,000 dilution of ZmCCaMK antibodies. D-Dark, R-Red light, FR-Far-red light, W-white light. Dark grown plants served as controls. Red light negatively regulates the level of kinase but it is not under phytochrome control.
TABLE 4. Time kinetics of red light treatment: Total protein (25 μg) of maize coleoptiles, given red light treatments for indicated time periods, separated on 10% SDS-PAGE and western blot of similar protein samples with 1: 20,000 dilution of ZmCCaMK antibodies. D-Dark, R-Red light. Dark grown plants served as controls.
FIG 45. Time kinetics of blue light treatment: Total protein (25 µg) of maize coleoptiles, given blue light treatments for indicated time periods, separated on 10% SDS-PAGE and western blot of similar protein samples with 1: 20,000 dilution of ZmCCaMK antibodies. D-Dark, B-Blue light. Dark grown plants served as controls. No effect of blue light could be seen on the level of ZmCCaMK level.
FIG 46. **Effect of different hormone treatments**: Total protein (25 μg) of maize coleoptiles, given different hormone treatments (10⁻⁴ M) via roots, separated on 10% SDS-PAGE and western blot of similar protein samples with 1: 20,000 dilution of ZmCCaMK antibodies. 8 day old, etiolated, water grown plants served as control.
FIG 47. Cross reactivity of bacterial host cells with ZmCCaMK antibodies: Western blot showing cross-reactivity of bacterial (phage host cells) proteins with ZmCCaMK antibodies before (A) and after (B) treatment with bacterial cell lysate. Maize total protein extract (C) served as control.
FIG 48. Screening of Sorghum cDNA library with ZmCCaMK antibodies: $3.8 \times 10^8$ plaques were screened from sorghum cDNA library constructed in λZapII. Plaques were transferred to nitro-cellulose membrane and probed with ZmCCaMK antibodies. Figure shows filters showing positive signals at the primary (1), secondary (2), tertiary (3) and quaternary (4) round of screening.
FIG 49. Excision of *pShSPI* clone: Gel showing excised and EcoRI/Xho I digested *Sorghum* clone (*pShSPI*) along with markers: A 0.8 kb insert was obtained. Uncut *pShSPI* was run as control.
FIG 50. Complete nucleotide sequence of pSbSpI clone.
At the nucleotide level:

**Homo sapiens calcium-activated potassium channel**

Query: 42 CAGCAGCTCTCGTGCAGTACTCACCCAGGCTGCTTCTCTCTCTCTCTGACTGGG 94

Sbjct: 2342 CAGCAGCTCTGTCTGCTGATCATCAGGAGGCCTCGGGGCTGAGGTGTCAGCTGG 2394

At the protein level:

**Calcium binding protein from Dictyostelium discoideum**

Query: 32 PECPAAPGSLHPEPASLRSTGTTGKP 109

Sbjct: 48 PQQPGAPGSNLPYTPYQQPGAPGAP 73

Query: 182 PQWPGGFPGCSLPQIP 226

Sbjct: 86 PQQPGAPGQYQQPGQGQQ 100

Query: 284 PQRVGSCG*LPFGQGK 331

Sbjct: 128 PQQPGAPGQYQQPGQ 143

**Serine/threonine-specific protein kinase minibrain homolog (HP86) (dyrk) [Homo sapiens]**

Query: 564 QQQ*LERCSGNCSCHHHHY 617

Sbjct: 594 QQNALHHHGNSSHHH 611

Query: 603 HHHHYSQIGREPL 641

Sbjct: 612 HHHHHHHHHGQAL 624

**A protein kinase dyrk [Rattus norvegicus]**

Query: 564 QQQ*LERCSGNCSCHHHHY 617

Sbjct: 594 QQNALHHHGNSSHHH 611

Query: 603 HHHHYSQIGREPL 641

Sbjct: 612 HHHHHHHHHGQAL 624

FIG 51. Blast analysis of pSbSP1 clone.
FIG 52. Screening of maize cDNA library with ZmCCaMK antibodies: $4 \times 10^8$ plaques were screened from maize cDNA library constructed in λZapII. Plaques were transferred to nitro-cellulose membrane and probed with ZmCCaMK antibodies. Figure shows filters showing positive signals at the primary (1), secondary (2), tertiary (3) and quaternary (4) round of screening.
Bluescript II SK+/- (Multiple cloning site)

FIG 53. Excision and restriction enzyme mapping of *pZmSP9* clone: Gel showing excised and Xho I RI digested Maize clone (*pZmSP9*) along with markers: A 2.5 kb insert was obtained. Uncut *pZmSp9* was run as control. For restriction mapping plasmid DNA was digested with different restriction enzymes and separated on a 0.8% agarose gel along with markers.
FIG 54. A representative autoradiogram showing sequence of pZmSP9 clone.
FIG 55. Partial nucleotide sequence of pZmSP9 clone using T3 and T7 primers.
emb|X67711|OSHSC70A  O. sativa hsp70 gene for heat shock protein 70
Length = 4794  Score = 783 bits (395), Expect = 0.0
Identities = 450/462 (97%), Positives = 450/462 (97%), Gaps = 5/462 (1%)
dbjiD21283|RICAD622 Rice mRNA for heat shock protein 70 (gene name AD622), partial cds
Length = 389  Score = 511 bits (258), Expect = e-143
Identities = 284/289 (98%), Positives = 284/289 (98%), Gaps = 3/289 (1%)
emb|X73472|ZMHSP70A Z. mays hsp 70-1 gene for heat shock protein 70
Length = 630  Score = 404 bits (204), Expect = e-110
Identities = 394/451 (87%), Positives = 394/451 (87%), Gaps = 5/451 (1%)
emb|X54030|LEHSC270 Lycopersicon esculentum hsc-2 mRNA for heat shock protein cognate 70
Length = 2117  Score = 289 bits (146), Expect = 4e-76
Identities = 376/449 (83%), Positives = 376/449 (83%), Gaps = 3/449 (0%)
gb|L26243|SPIC70A Spinacia oleracea heat shock C70 protein mRNA, complete cds.
Length = 2194  Score = 266 bits (134), Expect = 6e-69
Identities = 347/413 (84%), Positives = 347/413 (84%), Gaps = 4/413 (0%)
gb|AF034618|AF034618 Spinacia oleracea cytosolic heat shock 70 protein (HSC70-1) gene,
Length = 4349  Score = 266 bits (134), Expect = 6e-69
Identities = 347/413 (84%), Positives = 347/413 (84%), Gaps = 4/413 (0%)
emb|X74604|ATHHSC701 A. thaliana hsc70 mRNA for heat shock cognate protein
Length = 2209  Score = 256 bits (129), Expect = 6e-66
Identities = 343/412 (83%), Positives = 343/412 (83%), Gaps = 2/412 (0%)
emb|X77199|ATHHSC701 A. thaliana hsc70-1 gene
Length = 4297  Score = 254 bits (128), Expect = 2e-65
Identities = 355/427 (83%), Positives = 355/427 (83%), Gaps = 3/427 (0%)

gb|L41253|TOMHSC70A Lycopersicon esculentum Hsc70 gene, complete cds
Length = 3573  Score = 248 bits (125), Expect = 1e-63
Identities = 315/376 (83%), Positives = 315/376 (83%), Gaps = 2/376 (0%)

gb|AF002667|SCAF002667 Solanum commersonii heat shock cognate protein (SCHSP70) mRNA, complete cds
Length = 1149  Score = 234 bits (118), Expect = 2e-59  Identities = 289/345 (83%), Positives = 289/345 (83%), Gaps = 1/345 (0%)

FIG 56. Blast analysis of pZmSp9 clone with available databases.
FIG 57. HPLC profile of trypsin digested purified ZmCCaMK.
FIG 58. Tryptic peptide used for sequencing.
Query: 1 TTPSYVAFTDSER 13 Heat shock cognate 70-1 Arabidopsis thaliana
    TTPSYVAFTDSER
Sbjct: 26 TTPSYVAFTDSER 38

Query: 1 TTPSYVAFTDSER 13 Heat shock protein 70 Paralichthys olivaceus
    TTPSYVAFTDSER
Sbjct: 37 TTPSYVAFTDSER 49

Query: 1 TTPSYVAFTDSER 13 Heat shock 70 protein Spinacia oleracea
    TTPSYVAFTDSER
Sbjct: 40 TTPSYVAFTDSER 52

Query: 1 TTPSYVAFTDSER 13 Heat-shock protein Hsp70 Asbestopluma hypogeae
    TTPSYVAFTDSER
Sbjct: 3 TTPSYVAFTDSER 15

Query: 1 TTPSYVAFTDSER 13 Heat shock cognate protein HSC70 Brassica napus
    TTPSYVAFTDSER
Sbjct: 40 TTPSYVAFTDSER 52

Query: 1 TTPSYVAFTDSER 13 PsHSC71.0 protein - garden pea Pisum sativum
    TTPSYVAFTDSER
Sbjct: 40 TTPSYVAFTDSER 52

FIG 59. Blast analysis of tryptic peptide sequence.