

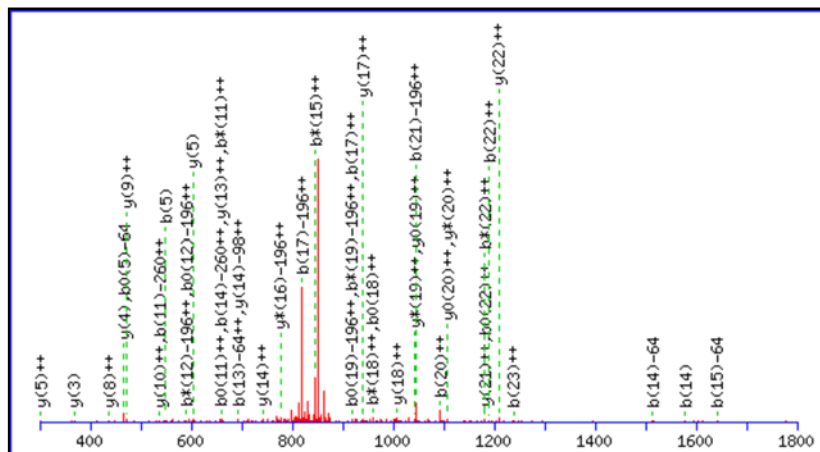
Mascot Search Results**Peptide View**MS/MS Fragmentation of **MSGQESHDISSGGEQAGVDHPPPR**Found in **AT5G64330.1** | Symbols: JK218, RPT3, NPH3 | NPH3 (NON-PHOTOTROPIC HYPOCOTYL 3); signal transducer | chr5:25744794-25747451 FORWARD

Match to Query 18: 2651.019723 from(884.680517,3+)

Title: PR1100_%.Salt.2749.2749.3.dta

Data file C:\Users\CooperB\Desktop\Ray\mgfs\MS2special.txt

Click mouse within plot area to zoom in by factor of two about that point

Or, 300 1800 Label all possible matches ☐ Label matches used for scoring ☒

Monoisotopic mass of neutral peptide Mr(calc): 2650.0156

Fixed modifications: Carbamidomethyl (C) (apply to specified residues or termini only)

Variable modifications:

M1 : Oxidation (M), with neutral losses 0.0000(shown in table), 63.9983

S10 : Phospho (ST), with neutral losses 0.0000(shown in table), 97.9769

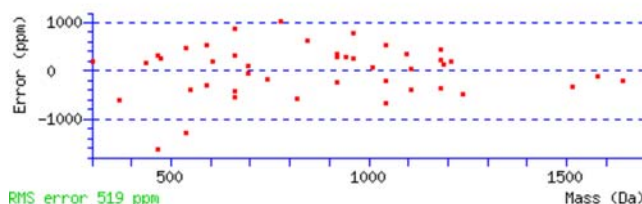
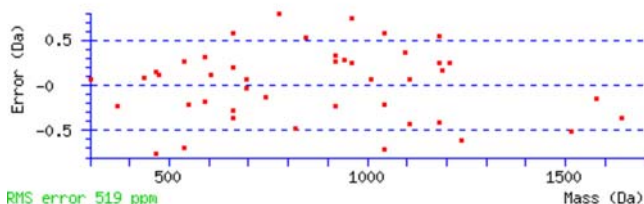
S11 : Phospho (ST), with neutral losses 0.0000(shown in table), 97.9769

Ions Score: 27 Expect: 0.045

Matches : 44/616 fragment ions using 57 most intense peaks ([help](#))

#	b	b ⁺⁺	b [*]	b ^{*++}	b ⁰	b ⁰⁺⁺	Seq.	y	y ⁺⁺	y [*]	y ^{*++}	y ⁰	y ⁰⁺⁺	#
1	148.0427	74.5250					M							24
2	235.0747	118.0410			217.0641	109.0357	S	2503.9875	1252.4974	2486.9610	1243.9841	2485.9770	1243.4921	23
3	292.0962	146.5517			274.0856	137.5464	G	2416.9555	1208.9814	2399.9289	1200.4681	2398.9449	1199.9761	22
4	420.1547	210.5810	403.1282	202.0677	402.1442	201.5757	Q	2359.9340	1180.4707	2342.9075	1171.9574	2341.9235	1171.4654	21
5	549.1973	275.1023	532.1708	266.5890	531.1868	266.0970	E	2231.8754	1116.4414	2214.8489	1107.9281	2213.8649	1107.4361	20
6	636.2294	318.6183	619.2028	310.1050	618.2188	309.6130	S	2102.8329	1051.9201	2085.8063	1043.4068	2084.8223	1042.9148	19
7	773.2883	387.1478	756.2617	378.6345	755.2777	378.1425	H	2015.8008	1008.4041	1998.7743	999.8908	1997.7903	999.3988	18
8	888.3152	444.6612	871.2887	436.1480	870.3047	435.6560	D	1878.7419	939.8746	1861.7154	931.3613	1860.7314	930.8693	17
9	1001.3993	501.2033	984.3727	492.6900	983.3887	492.1980	I	1763.7150	882.3611	1746.6884	873.8478	1745.7044	873.3558	16
10	1168.3976	584.7025	1151.3711	576.1892	1150.3871	575.6972	S	1650.6309	825.8191	1633.6044	817.3058	1632.6203	816.8138	15
11	1335.3960	668.2016	1318.3695	659.6884	1317.3854	659.1964	S	1483.6325	742.3199	1466.6060	733.8066	1465.6220	733.3146	14
12	1392.4175	696.7124	1375.3909	688.1991	1374.4069	687.7071	G	1316.6342	658.8207	1299.6076	650.3075	1298.6236	649.8155	13
13	1449.4389	725.2231	1432.4124	716.7098	1431.4284	716.2178	G	1259.6127	630.3100	1242.5862	621.7967	1241.6022	621.3047	12
14	1578.4815	789.7444	1561.4550	781.2311	1560.4710	780.7391	E	1202.5913	601.7993	1185.5647	593.2860	1184.5807	592.7940	11
15	1706.5401	853.7737	1689.5136	845.2604	1688.5295	844.7684	Q	1073.5487	537.2780	1056.5221	528.7647	1055.5381	528.2727	10
16	1777.5772	889.2922	1760.5507	880.7790	1759.5667	880.2870	A	945.4901	473.2487	928.4635	464.7354	927.4795	464.2434	9
17	1834.5987	917.8030	1817.5721	909.2897	1816.5881	908.7977	G	874.4530	437.7301	857.4264	429.2169	856.4424	428.7248	8
18	1933.6671	967.3372	1916.6405	958.8239	1915.6565	958.3319	V	817.4315	409.2194	800.4050	400.7061	799.4209	400.2141	7
19	2048.6940	1024.8507	2031.6675	1016.3374	2030.6835	1015.8454	D	718.3631	359.6852	701.3366	351.1719	700.3525	350.6799	6
20	2185.7530	1093.3801	2168.7264	1084.8668	2167.7424	1084.3748	H	603.3362	302.1717	586.3096	293.6584			5

21	2282.8057	1141.9065	2265.7792	1133.3932	2264.7951	1132.9012	P	466.2772	233.6423	449.2507	225.1290			4
22	2379.8585	1190.4329	2362.8319	1181.9196	2361.8479	1181.4276	P	369.2245	185.1159	352.1979	176.6026			3
23	2476.9112	1238.9593	2459.8847	1230.4460	2458.9007	1229.9540	P	272.1717	136.5895	255.1452	128.0762			2
24							R	175.1190	88.0631	158.0924	79.5498			1



NCBI **BLAST** search of [MSGQESHDISSGGEQAGVDHPPPR](#)
 (Parameters: blastp, nr protein database, expect=20000, no filter, PAM30)
 Other BLAST [web gateways](#)

All matches to this query

Score	Mr(calc):	Delta	Sequence
26.7	2650.0156	1.0041	MSGQESHDISSGGEQAGVDHPPPR
26.2	2650.0156	1.0041	MSGQESHDISSGGEQAGVDHPPPR
22.2	2650.0156	1.0041	MSGQESHDISSGGEQAGVDHPPPR
17.1	2650.0156	1.0041	MSGQESHDISSGGEQAGVDHPPPR
6.0	2650.0156	1.0041	MSGQESHDISSGGEQAGVDHPPPR
5.5	2651.0345	-0.0148	MVRSNCTNVGAVVMADVQSVSR
5.1	2649.9900	1.0297	HNCLSLSGSAEEICSSDCSHLK
5.1	2649.9900	1.0297	HNCLSLSGSAEEICSSDCSHLK
4.0	2650.0424	0.9773	ETGASVMTKLPFTGTGTEQGQDR
3.5	2650.0022	1.0175	ESGKSSVLM DIKPWDDETDMK

no -98 neutral loss, S10 S11 +80
 no -98 neutral loss, S2, S6 +80
 no -98 neutral loss, S6, S10 +80
 no -98 neutral loss, S6, S11 +80
 -98 neutral loss, S2, S11 -18

MS2: Top score 26.7 with probability of match 95% but 2nd best score similar. S10 [+80] and S11 [+80] are deduced, but +3 charge and supporting MS3 with site determining ion for S10 [-18]. High position confidence.

Mascot: <http://www.matrixscience.com/>

Mascot Search Results**Peptide View**MS/MS Fragmentation of **MSGQESHDISSGGEQAGVDHPPPR**Found in **AT5G64330.1** | Symbols: JK218, RPT3, NPH3 | NPH3 (NON-PHOTOTROPIC HYPOCOTYL 3); signal transducer | chr5:25744794-25747451 FORWARD

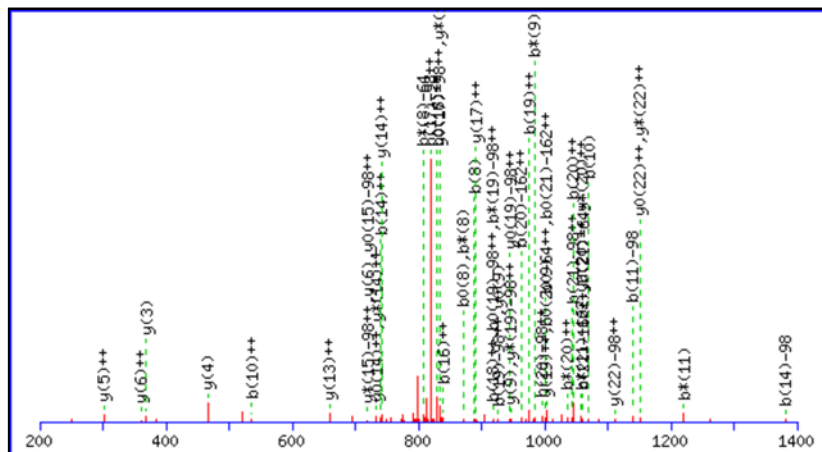
Match to Query 8: 2552.874723 from(851.965517,3+)

Title: PR1100_%_Salt.2750.2750.3.dta

Data file C:\Users\CooperB\Desktop\Ray\mgfs\MS3special.txt

Click mouse within plot area to zoom in by factor of two about that point

Or, Plot from 200 to 1400 Da Full range

Label all possible matches ☐ Label matches used for scoring ☒

Monoisotopic mass of neutral peptide Mr(calc): 2552.0388

Fixed modifications: Carbamidomethyl (C) (apply to specified residues or termini only)

Variable modifications:

M1 : Oxidation (M), with neutral losses 0.0000(shown in table), 63.9983

S10 : Dehydrated (S)

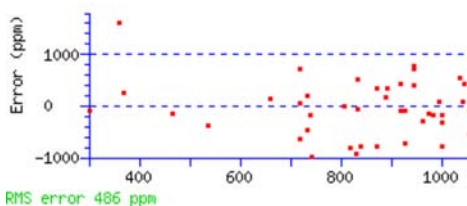
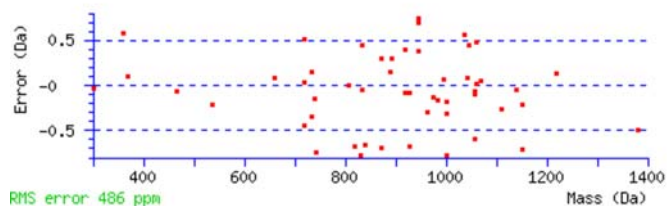
S11 : Phospho (ST), with neutral losses 0.0000(shown in table), 97.9769

Ions Score: 29 Expect: 3.2

Matches : 55/604 fragment ions using 56 most intense peaks ([help](#))

#	b	b ⁺⁺	b [*]	b ^{*++}	b ⁰	b ⁰⁺⁺	Seq.	y	y ⁺⁺	y [*]	y ^{*++}	y ⁰	y ⁰⁺⁺	#
1	148.0427	74.5250					M							24
2	235.0747	118.0410			217.0641	109.0357	S	2406.0106	1203.5089	2388.9841	1194.9957	2388.0001	1194.5037	23
3	292.0962	146.5517			274.0856	137.5464	G	2318.9786	1159.9929	2301.9520	1151.4797	2300.9680	1150.9877	22
4	420.1547	210.5810	403.1282	202.0677	402.1442	201.5757	Q	2261.9571	1131.4822	2244.9306	1122.9689	2243.9466	1122.4769	21
5	549.1973	275.1023	532.1708	266.5890	531.1868	266.0970	E	2133.8986	1067.4529	2116.8720	1058.9396	2115.8880	1058.4476	20
6	636.2294	318.6183	619.2028	310.1050	618.2188	309.6130	S	2004.8560	1002.9316	1987.8294	994.4183	1986.8454	993.9263	19
7	773.2883	387.1478	756.2617	378.6345	755.2777	378.1425	H	1917.8239	959.4156	1900.7974	950.9023	1899.8134	950.4103	18
8	888.3152	444.6612	871.2887	436.1480	870.3047	435.6560	D	1780.7650	890.8861	1763.7385	882.3729	1762.7545	881.8809	17
9	1001.3993	501.2033	984.3727	492.6900	983.3887	492.1980	I	1665.7381	833.3727	1648.7115	824.8594	1647.7275	824.3674	16
10	1070.4207	535.7140	1053.3942	527.2007	1052.4102	526.7087	S	1552.6540	776.8306	1535.6275	768.3174	1534.6434	767.8254	15
11	1237.4191	619.2132	1220.3926	610.6999	1219.4085	610.2079	S	1483.6325	742.3199	1466.6060	733.8066	1465.6220	733.3146	14
12	1294.4406	647.7239	1277.4140	639.2106	1276.4300	638.7186	G	1316.6342	658.8207	1299.6076	650.3075	1298.6236	649.8155	13
13	1351.4620	676.2347	1334.4355	667.7214	1333.4515	667.2294	G	1259.6127	630.3100	1242.5862	621.7967	1241.6022	621.3047	12
14	1480.5046	740.7560	1463.4781	732.2427	1462.4941	731.7507	E	1202.5913	601.7993	1185.5647	593.2860	1184.5807	592.7940	11
15	1608.5632	804.7852	1591.5367	796.2720	1590.5526	795.7800	Q	1073.5487	537.2780	1056.5221	528.7647	1055.5381	528.2727	10
16	1679.6003	840.3038	1662.5738	831.7905	1661.5898	831.2985	A	945.4901	473.2487	928.4635	464.7354	927.4795	464.2434	9
17	1736.6218	868.8145	1719.5952	860.3013	1718.6112	859.8092	G	874.4530	437.7301	857.4264	429.2169	856.4424	428.7248	8
18	1835.6902	918.3487	1818.6637	909.8355	1817.6796	909.3435	V	817.4315	409.2194	800.4050	400.7061	799.4209	400.2141	7
19	1950.7171	975.8622	1933.6906	967.3489	1932.7066	966.8569	D	718.3631	359.6852	701.3366	351.1719	700.3525	350.6799	6
20	2087.7761	1044.3917	2070.7495	1035.8784	2069.7655	1035.3864	H	603.3362	302.1717	586.3096	293.6584			5

21	2184.8288	1092.9180	2167.8023	1084.4048	2166.8183	1083.9128	P	466.2772	233.6423	449.2507	225.1290			4
22	2281.8816	1141.4444	2264.8550	1132.9312	2263.8710	1132.4391	P	369.2245	185.1159	352.1979	176.6026			3
23	2378.9343	1189.9708	2361.9078	1181.4575	2360.9238	1180.9655	P	272.1717	136.5895	255.1452	128.0762			2
24							R	175.1190	88.0631	158.0924	79.5498			1



MS3: -98 Da triggered corroborating spectrum. Top match with positioning supports S10 and S11 as being phosphorylated. b ion for S10 [-18], S11 [+80] deduced.

NCBI **BLAST** search of [MSGQESHDISSGGEQAGVDHPPPR](#)
 (Parameters: blastp, nr protein database, expect=20000, no filter, PAM30)
 Other BLAST [web gateways](#)

All matches to this query

Score	Mr(calc):	Delta	Sequence
29.2	2552.0388	0.8359	MSGQESHDISSGGEQAGVDHPPPR
24.2	2552.3378	0.5370	RGHSQTTLHAISALLSLPTSVL
23.2	2552.0388	0.8359	MSGQESHDISSGGEQAGVDHPPPR
23.2	2552.0388	0.8359	MSGQESHDISSGGEQAGVDHPPPR
22.2	2552.0388	0.8359	MSGQESHDISSGGEQAGVDHPPPR
22.1	2553.0447	-0.1700	NSNAVTVSETSTMANQMVLKK
22.1	2553.0447	-0.1700	NSNAVTVSETSTMANQMVLKK
22.1	2553.0447	-0.1700	NSNAVTVSETSTMANQMVLKK
22.0	2551.9134	0.9613	VAATKAVQTQTFHCSSGDDPL
21.7	2554.0278	-1.1531	LEPGGPSTSSSTEEVSGQSGKLAK

S10 [-18]; no -98 neutral loss, S11 [+80]

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