Electronic supplementary information (ESI)

Analysis of the soybean metallothionein system under radical stress: protein modification connected to lipid membrane damages

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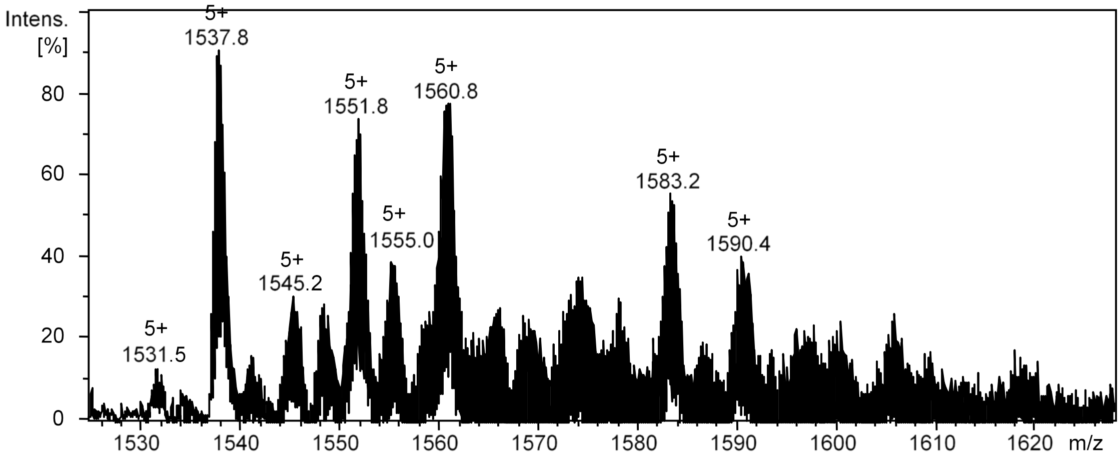
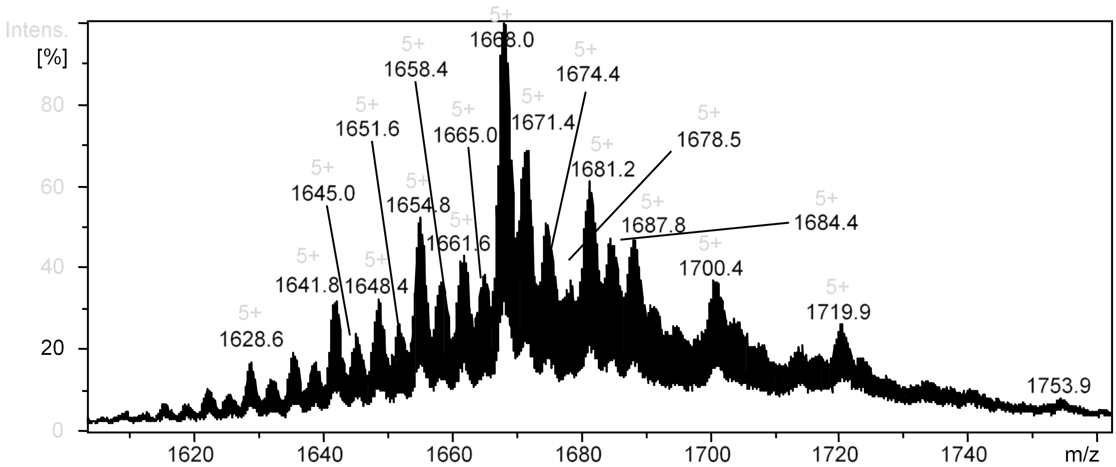
Table S1. Calculated and experimental molecular masses for the apo- and Zn-GmMT species detected by ESI-MS before γ-irradiation. The error associated with the exp. MW values was always lower than 0.1 %. The Zn per GmMT molar ratio calculated from the zinc and sulphur content measured by normal and acid ICP-AES was in agreement with the presence of these species and their abundances [1].

MT	ESI-MS		
	Species	Cal. MW (Da)	Exp. MW (Da)
GmMT1	apo	7696.6	7693.0
	Zn_1	7760.0	7759.0
	Zn_3	7886.7	7885.2
	Zn_4	7950.1	7949.8
GmMT2	apo	8085.2	8082.6
	Zn_2	8212.0	8205.5
	Zn_3	8275.3	8271.0
	Zn_4	8338.7	8336.0
	Zn_5	8402.1	8400.8
GmMT3	apo	6878.7	6879.9
	Zn_2	7005.4	7003.2
	Zn_3	7068.8	7067.5
GmMT4	apo	8452.5	8450.4
	Zn_4	8706.0	8703.5
	Zn_5	8769.4	8769.8
	Zn_6	8832.8	8832.9



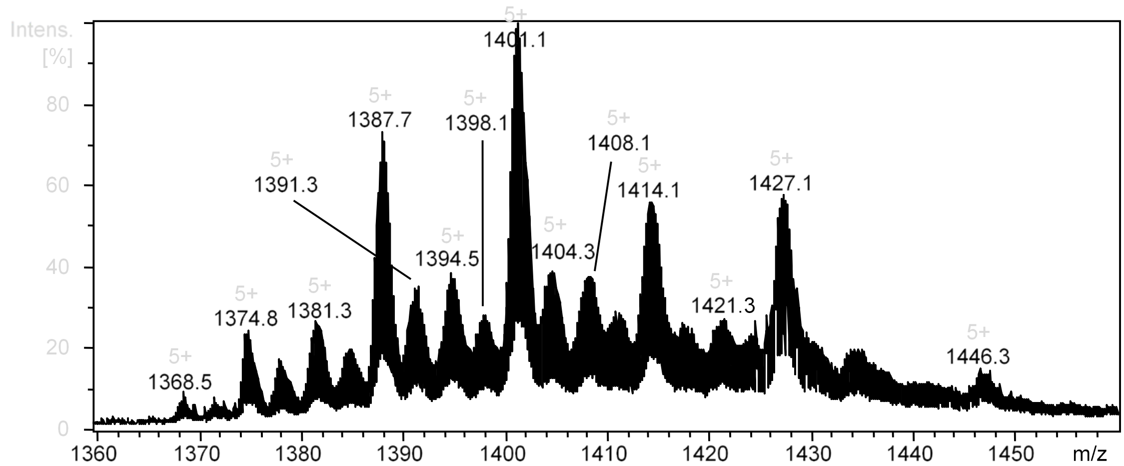
Species		m/z value	Cal. MW (Da)	Exp. MW (Da)
apo-GmMT1	$-1CH_3S$	1531.5	7649.5	7652.5
	-12H	1537.8	7684.6	7684.0
	-8H + 2O	1545.2	7720.6	7721.0
Zn_1-GmMT1	-4H-1HS/S	1545.2	7721.9	7721.0
	-6H	1551.8	7753.9	7754.0
	-6H +1O	1555.0	7769.9	7770.0
	-6H +3O	1560.8	7801.9	7799.0
Zn_4-GmMT1	-4H -1HS/S	1583.2	7912.0	7911.0
	-2H	1590.4	7948.1	7947.0
	-2H + 2O	1596.7	7980.1	7978.5

Figure S1. Representative charge-state for the ESI-MS spectra at pH 7.0 of the Zn-GmMT1 preparation after the γ-irradiation in N_2O-saturated aqueous solutions at 300 Gy. Calculated and experimental molecular masses corresponding to oxidised species or species that have lost sulphur-containing groups have been included for facilitating the interpretation of the experimental data: these species could have a weight gain because of the extra O atoms coming from sulphoxidation reactions (+*y*O) and/or a weight gain due to the extra O and H atoms coming from hydroxilation reactions (+*z*OH, which accounts for +*z*H +*z*O in the global assignment). The error associated with the exp. MW values was always lower than 0.1 %.



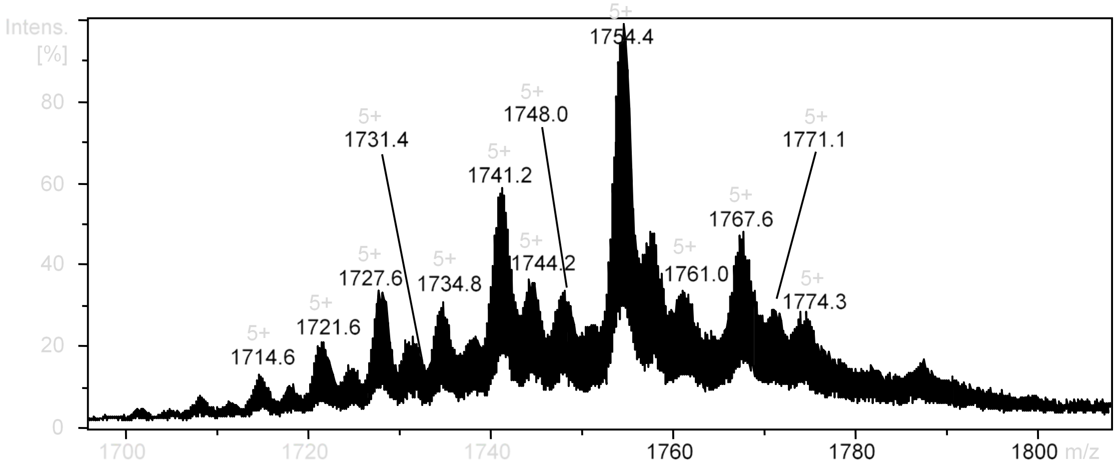
Species		m/z value	Cal. MW (Da)	Exp. MW (Da)
Zn_1-GmMT2	-10H	1628.6	8138.6	8138.0
	-8H	1641.8	8203.9	8204.0
	-8H +1O	1645.0	8219.9	8220.0
	-8H +2O	1648.4	8235.9	8237.0
	-8H +3O	1651.6	8251.9	8253.0
	-6H	1654.8	8269.3	8269.0
	-4H +1O	1658.4	8287.3	8287.0
	-4H +2O	1661.6	8303.3	8303.0
	-4H +3O	1665.0	8319.3	8320.0
Zn_4-GmMT2	-1HS/S	1661.6	8305.6	8303.0
	-1HS/S -1O	1665.0	8321.6	8320.0
	-4H	1668.0	8334.7	8335.0
	-3H +1O	1671.4	8351.7	8352.0
	-3H +2O	1674.4	8367.7	8367.0
Zn_5-GmMT2	-1CH_3S	1671.4	8355.0	8352.0
	-2H -1HS/S	1674.4	8367.0	8367.0
	-1HS/S +1O	1678.5	8385.0	8387.5
	-2H	1681.2	8400.1	8401.0
	-1H +1O	1684.4	8417.1	8417.0
	+2O	1687.8	8434.1	8434.0

Figure S2. Representative charge-state for the ESI-MS spectra at pH 7.0 of the Zn-GmMT2 preparation after the γ-irradiation in N_2O-saturated aqueous solutions at 300 Gy. Calculated and experimental molecular masses corresponding to oxidised species or species that have lost sulphur-containing groups have been included for facilitating the interpretation of the experimental data: these species could have a weight gain because of the extra O atoms coming from sulphoxidation reactions (+*y*O) and/or a weight gain due to the extra O and H atoms coming from hydroxilation reactions (+*z*OH, which accounts for +*z*H +*z*O in the global assignment). The error associated with the exp. MW values was always lower than 0.1 %. In the case of Zn-GmMT2 a particularly rich group of peaks has been obtained.



Species		m/z value	Cal. MW (Da)	Exp. MW (Da)
apo-GmMT3	-1CH_3S	1368.5	6831.6	6837.5
	-10H	1374.8	6868.6	6868.5
	-10H + 1O	1377.9	6884.6	6884.5
	-10H +2O	1381.3	6900.7	6901.5
Zn_1-GmMT3	-1CH_3S	1381.3	6895.0	6901.5
	-6H -HS/S	1381.3	6902.0	6901.5
	-8H	1387.7	6934.0	6934.5
	-7H +1O	1391.3	6951.0	6951.5
	-7H +2O	1394.5	6967.0	6967.5
	-7H +3O	1398.1	6983.4	6985.5
Zn_2-GmMT3	-1HS/S	1394.5	6972.3	6967.5
	-6H	1401.1	6999.4	7000.5
	-5H +1O	1404.3	7016.4	7016.5
Zn_3-GmMT3	-1HS/S	1408.1	7035.7	7035.5
	-2H	1414.1	7066.8	7065.5
Zn_4-GmMT3	-1HS/S	1421.3	7098.1	7101.5
	-2H	1427.1	7130.2	7130.5

Figure S3. Representative charge-state for the ESI-MS spectra at pH 7.0 of the Zn-GmMT3 preparation after the γ-irradiation in N_2O-saturated aqueous solutions at 300 Gy. Calculated and experimental molecular masses corresponding to oxidised species or species that have lost sulphur-containing groups have been included for facilitating the interpretation of the experimental data: these species could present a weight gain because of the extra O atoms coming from sulphoxidation reactions (+*y*O) and/or a weight gain due to the extra O and H atoms coming from hydroxilation reactions (+*z*OH, which accounts for +*z*H +*z*O in the global assignment). The error associated with the exp. MW values was always lower than 0.1 %.



Species		m/z value	Cal. MW (Da)	Exp. MW (Da)
Zn_2-GmMT4	-12H	1714.6	8567.3	8568.0
	-9H +2O	1721.6	8602.3	8603.0
	-6H -1S/SH	1721.6	8602.6	8603.0
	-10H	1727.6	8632.6	8633.0
	-7H +1O	1731.4	8651.6	8652.0
	-7H +2O	1734.8	8667.6	8669.0
Zn_4-GmMT4	-1HS/S	1734.8	8671.9	8669.0
	-6H	1741.2	8700.0	8701.0
	-5H +1O	1744.2	8717.0	8716.0
Zn_5-GmMT4	-1HS/S	1748.0	8735.3	8735.0
	-2H	1754.4	8767.4	8767.0
	-2H +2O	1761.0	8799.3	8800.0
	-1HS/S	1761.0	8798.8	8800.0
	+1O	1771.1	8848.7	8850.5
	+2O	1774.3	8864.7	8866.5

Figure S4. Representative charge-state for the ESI-MS spectra at pH 7.0 of the Zn-GmMT4 preparation after the γ-irradiation in N_2O-saturated aqueous solutions at 300 Gy. Calculated and experimental molecular masses corresponding to oxidised species or species that have lost sulphur-containing groups have been included for facilitating the interpretation of the experimental data: these species could present a weight gain because of the extra O atoms coming from sulphoxidation reactions (+*y*O) and/or a weight gain due to the extra O and H atoms coming from hydroxilation reactions (+*z*OH, which accounts for +*z*H +*z*O in the global assignment). The error associated with the exp. MW values was always lower than 0.1 %.

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                10        20        30        40        50        60        70
      ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....
QsMT  MSCCGGNCGCGTGCKCGSGCGGCKMFPDIS-SEKTTTETLIVGVAPQKTHFEGSEMGVGAEN-GCKCGSNCTCDPCNCK
GmMT2 MSCCGGNCGCGSACKCGNGCGGCKMYPDLSYTESTTTETLVMGVAPVKAQFESAEMGVPAENDGCKCGANCTCNPCTCK
      ***********:.****.*******:**:* :*.******::**** *::**.:**** *** *****:****:**.**
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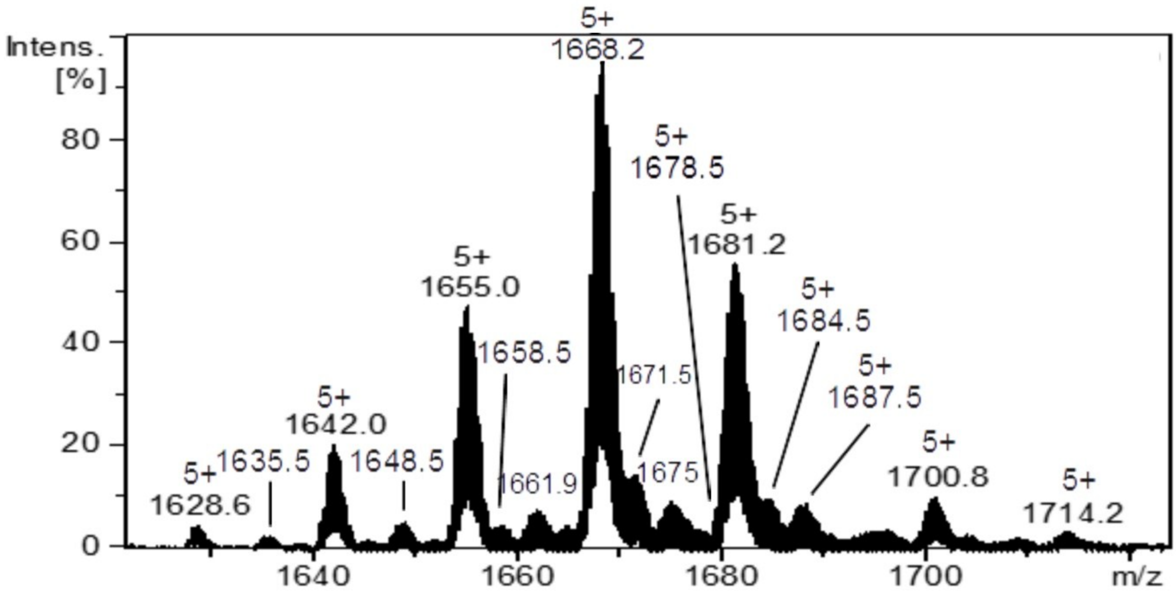
Figure S5. ClustalW alignment for the amino acidic sequences of the type 2 plant MT from *Quercus suber*, QsMT, *versus* the type 2 plant MT from *Glycine max*, GmMT2. The shaded boxes indicate the Cys residues, aromatic amino acids are in bold and Met are underlined. The asterisk (*) indicates positions which have a single, fully conserved residue, the colon (:) indicates conservation between groups of strongly similar properties and the period (.) indicates conservation between groups of weakly similar properties.



Species		m/z value	Cal. MW (Da)	Exp. MW (Da)
Zn_1-GmMT2	-10H	1628.6	8138.6	8138.0
	-6H -S	1635.5	8171.9	8172.5
	-6H	1642.0	8205.9	8205.0
	-4H -S	1648.5	8237.3	8237.5
	-6H	1655.0	8269.3	8270.0
Zn_4-GmMT2	-4H - CH_3S	1658.5	8287.7	8287.5
	-1HS/S	1661.9	8304.7	8304.5
	-2H	1668.2	8336.7	8336.0
Zn_5-GmMT2	-2H -1CH_3S	1671.5	8353.1	8352.5
	-1S	1675.0	8368.1	8370.0
	-2H	1681.2	8400.1	8401.0
Zn_6-GmMT2	- CH_3S	1684.5	8418.5	8417.5
	-1S	1687.5	8431.5	8432.5

Figure S6. Representative charge-state for the ESI-MS spectra at pH 7.0 of the Zn-GmMT2 preparation after the γ-irradiation of Ar-flushed aqueous solutions containing Zn-GmMT2 complexes (150 μM) and 0.2 M *t*-BuOH at 100Gy. Calculated and experimental molecular masses corresponding to species that have lost sulphur-containing moieties. Since all the Cys residues are coordinated to the metal ions, it is not possible to follow the formation of S-S bridges by this techniques since there is no change in the MW due to this process (there is the lack of SH moieties In the initial state of the compleses). The error associated with the exp. MW values was always lower than 0.1 %. In the case of Zn-GmMT2 a particularly rich group of peaks has been obtained.

References

[1]. M. Tomas, A. Tinti, R. Bofill, M. Capdevila, S. Atrian, and A. Torreggiani, *Journal of Inorganic Biochemistry*, 2016, **156**, 55-63.