Supplemental Data

Instructions for viewing ICP screen data of fast neutron (FN)mutagenized Arabidopsis

Ji-Ming Gong, Khush A Abid, Julian I Schroeder

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1): How are the data formatted?

All data for fast neutron mutagenized seeds are saved as Excel files, allowing user to either view the data online or download easily:

M2: Analyzed M2 ICP data

M2 data are split into 11 segments labeled by their Arabidopsis line numbers. For example: M2: FN001-080 contains files with line number from 1 to 80. M2 data can be viewed in 2 formats: the files are both organized such that for a given element (e.g.Ba2+) the data for all lines can be viewed or for a given line (e.g.FN565) the values for all elements can be viewed.

M3: Analyzed M3 ICP data

M3 data files are labeled by the date the data was collected. Each file was formatted by Arabidopsis line numbers.

2): How do we analyze data?

A): M2 data: For data from initial pilot experiments with lines FN001-FN366, we normalized the ICP-derived concentrations (PPM) to the concentration of Mg, and the results are shown in column "ppm/Mg".

For the remaining FN367-FN1191 data, we normalized to dry weight (DW), and the results are shown in column "ppm/DW". The <u>Ave and</u> <u>SD</u> values of each element (Ave: Average value; SD: Standard deviation value) are <u>calculated from</u> the <u>whole population</u> data of that element in one experiment.

B): M3 data: All data are normalized to dry weight, and shown in column "ppm/DW". The Ave <u>(Average) and SD</u> (standard deviation) values are <u>calculated from</u> only <u>wild type</u> data. And then the Ave and SD were applied to FN analyses.

3): How were putative mutants selected?

A): In screens of M2 fast neutron lines, Z values larger than 3(Z>3, i.e. 3 standard deviations larger or smaller than the average value) were used as a threshold to define a putative mutant. If the data

of a specific M2 plant (ppm/DW) was larger than "Ave+3SD" (i.e. Z>3), then it is shown as a "Putant", otherwise it is labeled as "-" (Fig. 1).

In addition, we list the "Ave+2SD" and "Ave-2SD" data, so the reader can evaluate borderline cases.

"Putant" or "-"

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| | | | | | | Fia | • 1 | м2 | data | | | | | $\langle \rangle$ | | | |
|-------|--------------|---------------------------------|-------------------------------|--------------|----------------|---------------------|------------|---------|---------|---------------|---------|----------|---------|-------------------|---------------|-------|---------|
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| | A1 | | olution Label | | | | | | | | | | | 1 | 1 | | |
| | A | В | C | D | E | F | G | Н | 1 | J | K | L | М | | N | 0 | |
| 1 | Solution La | bel Element | Corr Con | Units | DW | ppm/DW | Ave | SD | Z | Ave+2SD | Ave-2SD | Ave+3SD | M? >+2S | D M? < | -2SD M? | >+3SD | |
| 2 | col-0-1 | Ba 233.527 | 0.12839122 | ppm | 29.5 | 0.00435 | 0.00524 | 0.00327 | -0.2726 | 0.01179 | -0.0013 | 0.015063 | - | - | | / | <u></u> |
| 3 | col-0-2 | Ba 233.527 | 0.19896919 | ppm | 32.8 | 0.00607 | 0.00524 | 0.00327 | 0.25104 | 0.01179 | -0.0013 | 0.015063 | - | - | | | |
| 4 | JIS.fn367 | Ba 233.527 | 0.18174452 | ppm | 32.7 | 0.00556 | 0.00524 | 0.00327 | 0.09576 | 0.01179 | -0.0013 | 0.015063 | - | - | | | |
| 5 | JIS.fn368 | Ba 233.527 | 0.1610/86 | ppm | 28.9 | 0.00557 | 0.00524 | 0.00327 | 0.10056 | 0.01179 | -0.0013 | 0.015063 | - | - | | | |
| 5 | JIS.10369 | Ba 233.52/ | 0.04205671 | ppm | 1.2 | 0.00584 | 0.00524 | 0.00327 | 0.18231 | 0.01179 | -0.0013 | 0.015063 | - | - | | | |
| 6 | JIS.m370 | Da 233.52/ | 0.04659679 | ppm | 0.0 | 0.00552 | 0.00524 | 0.00327 | 0.00496 | 0.01179 | -0.0013 | 0.015063 | - | - | | | |
| | JIS.III371 | Da 200.027 | 0.06567971 | ppm | 3.7 | 0.00679 | 0.00524 | 0.00327 | 0.47274 | 0.01179 | -0.0013 | 0.015063 | - | - | | | |
| 10 | JIS fn373 | Bo 233.527 | 0.22347103 | ppm | 43.0 | 0.00024 | 0.00524 | 0.00327 | -0.0017 | 0.01179 | -0.0013 | 0.015063 | - | - | | | |
| 11 | IIS fn374 | Ba 233.527 | 0.0712181 | nnm | 14 | 0.00404 | 0.00524 | 0.00327 | -0.1041 | 0.01179 | -0.0013 | 0.015063 | _ | | | | |
| 12 | JIS fn375 | Ba 233.527 | 0.0712101 | nnm | 41.7 | 0.00505 | 0.00524 | 0.00327 | -0.0401 | 0.01179 | -0.0013 | 0.015063 | - | - | | | |
| 13 | JIS fn376 | Ba 233 527 | 0.21430300 | nnm | 19.3 | 0.00010 | 0.00524 | 0.00327 | -0.1423 | 0.01179 | -0.0013 | 0.015063 | | - | 1. | | |
| 14 | JIS fn377 | Ba 233.527 | 0.12359632 | nnm | 24.1 | 0.00513 | 0.00524 | 0.00327 | -0.0355 | 0.01179 | -0.0013 | 0.015063 | - | - | 1- | | |
| 15 | JIS fn378 | Ba 233 527 | 0.083856 | nnm | 17.1 | 0.0049 | 0.00524 | 0.00327 | -0.1041 | 0.01179 | -0.0013 | 0.015063 | - | - | - | | |
| 16 | JIS fn379 | Ba 233 527 | 0.12855527 | nnm | 19.5 | 0.00659 | 0.00524 | 0.00327 | 0.41189 | 0.01179 | -0.0013 | 0.015063 | - | - | - | | |
| 17 | JIS.fn380 | Ba 233.527 | 0.0662686 | ppm | 9.8 | 0.00676 | 0.00524 | 0.00327 | 0.46368 | 0.01179 | -0.0013 | 0.015063 | - | - | - | | |
| 18 | JIS.fn381 | Ba 233.527 | 0.09070747 | maa | 17.1 | 0.0053 | 0.00524 | 0.00327 | 0.01834 | 0.01179 | -0.0013 | 0.015063 | - | - | 1 | | |
| 19 | JIS.fn382 | Ba 233.527 | 0.19359139 | ppm | 43.5 | 0.00445 | 0.00524 | 0.00327 | -0.2426 | 0.01179 | -0.0013 | 0.015063 | - | - | - | | |
| 20 | JIS.fn383 | Ba 233.527 | 0.13358292 | ppm | 28.9 | 0.00462 | 0.00524 | 0.00327 | -0.1901 | 0.01179 | -0.0013 | 0.015063 | - | - | | | |
| 21 | JIS.fn384 | Ba 233.527 | 0.12129249 | ppm | 26.1 | 0.00465 | 0.00524 | 0.00327 | -0.1825 | 0.01179 | -0.0013 | 0.015063 | - | - | | | |
| 22 | JIS.fn385 | Ba 233.527 | 1.12531281 | ppm | 30.4 | 0.03702 | 0.00524 | 0.00327 | 9.70777 | 0.01179 | -0.0013 | 0.015063 | Putant | - | Put | ant) | |
| 23 | JIS.fn386 | Ba 233.527 | 0.09990774 | ppm | 21.9 | 0.00456 | 0.00524 | 0.00327 | -0.2085 | 0.01179 | -0.0013 | 0.015063 | - | - | | / | |
| 24 | JIS.fn387 | Ba 233.527 | 0.11766249 | ppm | 23.1 | 0.00509 | 0.00524 | 0.00327 | -0.0461 | 0.01179 | -0.0013 | 0.015063 | - | - | - | | |
| 25 | JIS.fn388 | Ba 233.527 | 0.13077827 | ppm | 23.6 | 0.00554 | 0.00524 | 0.00327 | 0.09073 | 0.01179 | -0.0013 | 0.015063 | - | - | - | | |
| 26 | JIS.fn389 | Ba 233.527 | 0.07706961 | ppm | 17.1 | 0.00451 | 0.00524 | 0.00327 | -0.2253 | 0.01179 | -0.0013 | 0.015063 | - | - | - | | |
| 27 | JIS.fn390 | Ba 233.527 | 0.12387797 | ppm | 25.8 | 0.0048 | 0.00524 | 0.00327 | -0.1354 | 0.01179 | -0.0013 | 0.015063 | - | - | - | | |
| 28 | JIS.fn391 | Ba 233.527 | 0.10551994 | ppm | 28.4 | 0.00372 | 0.00524 | 0.00327 | -0.4672 | 0.01179 | -0.0013 | 0.015063 | - | - | - | | |
| 29 | JIS.fn392 | Ba 233.527 | 0.10899284 | ppm | 25.4 | 0.00429 | 0.00524 | 0.00327 | -0.2913 | 0.01179 | -0.0013 | 0.015063 | - | - | - | | |
| 30 | JIS.fn393 | Ba 233.527 | 0.09204909 | ppm | 16.8 | 0.00548 | 0.00524 | 0.00327 | 0.07168 | 0.01179 | -0.0013 | 0.015063 | - | - | - | | |
| 31 | JIS.fn394 | Ba 233.527 | 0.17429325 | ppm | 36.75 | 0.00474 | 0.00524 | 0.00327 | -0.1533 | 0.01179 | -0.0013 | 0.015063 | - | - | - | | |
| 32 | JIS.fn395 | Ba 233.527 | 0.13264874 | ppm | 22.7 | 0.00584 | 0.00524 | 0.00327 | 0.18303 | 0.01179 | -0.0013 | 0.015063 | - | - | - | | |
| 33 | JIS.10396 | Da 233.52/ | 0.1774611 | ppm | 3/ | 0.0048 | 0.00524 | 0.00327 | -0.137 | 0.01179 | -0.0013 | 0.015063 | - | - | - | | |
| 34 | | /03 03 imine / / 03 01 imine | 0.00012018 | hhui | 14.9 | 0.00000 | 0.00524 | 0.00327 | 0.04034 | 0.011/9 | -0.0013 | 0.010000 | | - | - | | - IC |
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| - | start | 🖻 (C 🕕 | - M2 An | alyzed A | Altered | E E Co | py of FN36 | 7-466al | Read | ime.doc - Mic | | | | - a | - Li (| - V 4 | 28 PM |

Note to Fig.1:

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"Column" # may change in different experiments, but the "Title" never

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| Column | Title | |
|--------|------------------|--|
| A | Solution Lable: | Sample name (of the FN lines) |
| в | Element: | Elements analyzed |
| С | Corr Con: | ICP derived concentrations of analyzed elements |
| D | Units: | The unit of concentration in column C (PPM) |
| E | DW: | Dry weight of each sample |
| F | ppm/DW: | ICP data normalized to DW(or ppm/Mg, if to Mg). |
| G | Ave: | Average value |
| н | SD: | Standard deviation value |
| I | Z: | =(Normalized data-Ave)/SD |
| J/K/L | Ave+2SD/Ave-2SD, | <pre>/Ave+3SD: Average value plus/minus 2SD, or plus</pre> |
| 3SD | | |

M/N/O <u>M? >2SD/M? <-2SD/M? >3SD</u>: If the normalized data of a specific sample was larger than Ave+2SD/Ave+3SD or smaller than Ave-2SD, then it is a putative mutant.

B): In M3, Z>2 was used as a threshold to define a mutant, in line with the analyses of D.Salt and colleagues. For each putative mutant, 7-12 M3 plants of each line were re-screened.

| Microsoft Excel - 10.14.02 jiming.xls | | | | | | | | | | | | |
|---------------------------------------|---|--------------------|-------------------------------|-----------------------|--------------------|--|------------|------------|--|--|--|--|
| 8 | <u>File E</u> dit <u>V</u> | jew <u>I</u> nsert | F <u>o</u> rmat <u>T</u> ools | <u>D</u> ata <u>W</u> | indow <u>H</u> elj | p | | | | | | |
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| | H2 - K =IF(OR(F2>G2), "Mutant", "-") | | | | | | | | | | | |
| | A | В | | | E | F | G | H I | | | | |
| 1 | Solution Lab | e Element | Corr Con | Units | DW | ppm/DW | Ave+2SD | Mutant? | | | | |
| 2 | FN492-1 | Ba 233.527 | 0.07205182 | ppm | 17.5 | 0.00411725 | 0.00615424 | ·]/ | | | | |
| 3 | FN492-2 | Ba 233.527 | 0.108595 | ppm | 25.6 | 0.00424199 | 0.00615424 | | | | | |
| 4 | FN492-3 | Ba 233.527 | 0.08880018 | ppm | 22.9 | 0.00387774 | 0.00615424 | - | | | | |
| 5 | FN492-4 | Ba 233.527 | 0.05282599 | ppm | 14.1 | 0.00374652 | 0.00615424 | - | | | | |
| 6 | FN492-5 | Ba 233.527 | 0.02755374 | ppm | 9.3 | 0.00296277 | 0.00615424 | - | | | | |
| 7 | FN492-6 | Ba 233.527 | 0.0381881 | ppm | 13.8 | 0.00276725 | 0.00615424 | - | | | | |
| 8 | FN492-7 | Ba 233.527 | 0.03650926 | ppm | 14.5 | 0.00251788 | 0.00615424 | - | | | | |
| 9 | FN492-8 | Ba 233.527 | 0.05455366 | ppm | 18.3 | 0.00298107 | 0.00615424 | - | | | | |
| 10 | FN492-9 | Ba 233.527 | 0.04788069 | ppm | 10.8 | 0.0044334 | 0.00615424 | - | | | | |
| 11 | FN492-10 | Ba 233.527 | 0.06021658 | ppm | 15.3 | 0.00393572 | 0.00615424 | - | | | | |
| • | EULUSZ M | | | • | (1 B) | | | • | | | | |
| 01Z 813 | EN1084-3 | S 190.669 | 43.9592443 | ppm | 21.0 | 1 60/315/9 | 3.40020702 | - | | | | |
| 814 | FN1084-10 | S 180 669 | 62.0366516 | nnm | 27.4 | 2 28075925 | 3 48026782 | | | | | |
| 815 | FN1084-17 | S 180 669 | 77 4481583 | nnm | 42.4 | 1.82660751 | 3 48026782 | _ | | | | |
| 816 | FN104-12 | S 180 669 | 60 70/9103 | nnm | 34.5 | 1.75956262 | 3 48026782 | _ | | | | |
| 817 | EN1044-7 | S 180 669 | 80 1395187 | nnm | 35.8 | 2 23853404 | 3 48026782 | - | | | | |
| 818 | EN492-1 | Zn 202 548 | 1.39071727 | nnm | 17.5 | 0.07946956 | 0.05141274 | Mutant | | | | |
| 819 | EN492-2 | Zn 202.548 | 2 10758638 | nnm | 25.6 | 0.08232759 | 0.05141274 | Mutant | | | | |
| 820 | FN492-3 | Zn 202,548 | 1.3519454 | ppm | 22.9 | 0.05903692 | 0.05141274 | Mutant | | | | |
| 821 | FN492-4 | Zn 202.548 | 1.04829609 | ppm | 14.1 | 0.07434724 | 0.05141274 | Mutant | | | | |
| 822 | FN492-5 | Zn 202.548 | 0.56869411 | ppm | 9.3 | 0.0611499 | 0.05141274 | Mutant | | | | |
| 823 | FN492-6 | Zn 202.548 | 0.74062204 | ppm | 13.8 | 0.05366826 | 0.05141274 | Mutant | | | | |
| 824 | FN492-7 | Zn 202.548 | 0.76037884 | ppm | 14.5 | 0.05243992 | 0.05141274 | Mutant | | | | |
| 825 | FN492-8 | Zn 202.548 | 1.15529728 | ppm | 18.3 | 0.063131 | 0.05141274 | Mutant | | | | |
| 826 | FN492-9 | Zn 202.548 | 0.75590283 | ppm | 10.8 | 0.069991 | 0.05141274 | Mutant | | | | |
| 827 | FN492-10 | Zn 202.548 | 0.9361406 | ppm | 15.3 | 0.06118566 | 0.05141274 | Mutant | | | | |
| 828 | FN493-1 | Zn 202.548 | 1.48812891 | ppm | 34.3 | 0.04338545 | 0.05141274 | - | | | | |
| | | | | | | | | | | | | |
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If most of the M3 plants passed (as shown in Fig.2 in green color), then the data were used to perform T-Tests (indicated by blue arrow in Fig.2).

Note to Fig.2

Z plot: Worksheet in which Z values of WT were plotted

 $\frac{\text{WT:}}{\& \text{SD.}}$ Worksheet in which data of WT were analyzed to calculate Ave a SD. Ave n SD: Worksheet in which Ave and SD of WT population were

listed <u>FN:</u> Worksheet in which data of FN was analyzed to confirm a putative mutant.

<u>T-Test</u>: Worksheet in which mutants passed the Z>2 criteria were chosen for the two tailed, heteroscedastic T-test.

In some cases, a putative mutant didn't show a statistically clear phenotype in the M3 screen for a specific element (e.g Cd accumulation in M2), but showed other apparent phenotypes (P<0.05) (e.g Zn accumulation in all M3s). In such cases, we went back to the M2 data of that line and checked if there was such a phenotype (> 2SD or >Ave). If it showed a positive result, we also listed it as a mutant.