3-2017

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INTRODUCTION

Over more than 100 years agricultural scientists all over the world have developed high yielding crop varieties to meet the energy demand of increasing population. However the nutritional qualities have not been given priorities as a result world is facing with serious malnutrition problem. Two of the most prominent deficiencies affecting the world are of iron (Fe) and zinc (Zn). Among others Fe is vital in building proteins of red blood cells, whereas Zn is essential in cellular growth and development. Epigenetic mechanisms such as DNA methylation, histone modification, and small interfering RNA (sRNA) regulate the transcription of DNA in living organisms (He et al. 2011). It has been reported that the abiotic and biotic stresses such as changes in temperature, pests, drought, disease, and the concentration of minerals and metals in the surrounding soil are involved with the changes in epigenetic and transcriptomic components (Hu et al. 2012) in plant. The long-terms goals of our work are to identify the epigenetic and transcriptomic components involved in the acquisition and translocation of micronutrients in common bean.

MATERIALS AND METHODS

In our work, we applied higher concentrations of Fe to a common bean genotype G122 which was previously identified as a genotype that is highly responsive to elevated Fe and other health-related minerals (Bauduin et al. 2014). The treated and control plants were planted in 8.5”x11” pots filled with Sunshine Mix. The sunshine mix was kept soaked with water until germination. After germination we kept the clear saucers beneath the treated pot filled with a solution of Fe (200 mg-^{1L}) until the leaves reached 50% senescence while the control plant continued to receive water. At 50% leaf senescence, the stems of the plants were harvested and chromatin and total histone were isolated using the Chromaflash Plant Chromatin Extraction Kit (P-2022) and Epiquik Total Extraction Kit (OP-0006) respectively (www.epigenetek.com). One hundred nanograms of total histone of treated and control samples were added into the wells of 20 H3 modification sites in duplication for identifying each of the 20 histone modification patterns. Following the procedure described in the EpiQuik Histone H3 Modification Multiplex Assay Kit (P-3100), the intensity of absorbance of the H3 modification sites was measured at 450 nm wavelength by a BioTek Microplate reader (Elx808). Using the formula (given below) provided by the EpiQuik Histone H3 Modification Multiplex Assay Kit, histone modifications for 20 of the 21 H3 modification sites were calculated in ng/µg of histone 3 protein and compared between treated and control stems and presented as fold change (Table. 1).

$$\text{H3 Modification or total H3 (ng/µg protein)} = \frac{(\text{Sample OD} - \text{Blank OD}) \div S}{(\text{Assay Control OD} - \text{Blank OD}) \div P} \times 1000$$

Where, blank OD was 0.063, assay control OD was 0.530 and **S** was the amount of input sample protein in ng (100 ng) and **P** is the amount of input assay control in ng (25 ng).

Table 1. H3 modification and fold of modification in Fe treated common bean Stem compared with control.

	3	4	5	6	7	8	9	10	11	12
A	0.184	0.186	0.182	0.278	1.98	0.241	0.248	0.258	0.309	0.553
B	0.20	0.168	0.199	0.287	1.99	0.266	0.225	0.227	0.283	0.435
H3 Modification (ng/µg) protein	68.984	60.963	68.182	117.38	1027.81	101.8717	92.7807	95.9893	124.5989	230.4813
C	0.116	0.087	0.18	0.135	0.248	0.194	0.159	0.235	0.243	0.389
D	0.156	0.102	0.099	0.158	0.214	0.199	0.177	0.237	0.278	0.392
H3 Modification (ng/µg) protein	39.037	16.845	40.909	44.6524	89.8396	71.39037	56.1497	92.51337	105.615	175.1337
Fold Change	1.7671	3.619	1.6667	2.62874	11.4405	1.426966	1.65238	1.037572	1.179747	1.316031
E	0.203	0.116	0.157	0.192	0.241	0.669	0.263	1.114	0.335	0.239
F	0.194	0.103	0.163	0.15	0.202	0.612	0.251	1.281	0.397	0.206
H3 Modification (ng/µg) protein	72.46	24.866	51.872	57.754	84.7594	308.8235	103.743	606.6845	162.0321	85.29412
G	0.144	0.146	0.132	0.135	0.22	0.292	0.265	0.668	0.269	0.368
H	0.154	0.068	0.155	0.142	0.205	0.277	0.245	0.659	0.281	0.301
H3 Modification (ng/µg) protein	45.9893	23.5294	43.0481	40.374332	79.946524	118.4492	102.6738	321.12299	113.36898	145.18717
Fold Change	1.57558	1.05682	1.20497	1.4304636	1.0602007	2.6072235	1.0104167	1.889259	1.4292453	0.587477

RESULTS AND CONCLUSIONS

We observed 2-11 fold increase in four H3Kme1, H3K27me2, H3K36me1, and H3K79me1 of the 20 H3 sites in common bean stems due to higher concentrations of Fe. Using different tissues (such as root, stem, pod, and seed) of selected varieties of treated and control samples, significance of the 21 sites of H3 modification can be further analyzed and antibody specific chromatin fragments can be identified with CHiP-Seq analysis. Analysis of the other histones sites, such as Histone 4, may also provide a better understanding in how higher concentrations of minerals and metals affect epigenetic changes.

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ACKOWLEDGEMENT

This research work was supported by an Institutional Development Award (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health under grant number P20GM103442.