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Original Research

Analysis on protein fingerprint, RAPD and fruit quality of tomato mutants by ion beam implantation

Authors:
Duan HY*, Wang CF, Yu YA, Li XW and Zhou YQ.

Institution:
College of Life Science, Henan Normal University, Xinxiang 453007, China.

ABSTRACT:

In this research, seeds of tomato were irradiated by ion beam or treated with ion beam and soybean DNA, and some tomato mutants with morphological variations were analyzed. Protein analysis in the leaves of mutants showed, changes of protein pattern in mutants were different as compared with the control, the main variation of protein pattern were darkening of bands, increase of protein bands were detected in mutant 12, mutant 14 and mutant 15 and lose of a band in mutant 15. Genomic DNA of mutants were analyzed by RAPD, and total number of amplification bands, number of differential bands and rate of differential bands were studied among mutants. Compared with the control, rate of differential bands was 100.0 % in mutant 9 and 15, also high in mutant 14 and 12, but was 20.0-50.0 % in other mutants except for mutant 3 and 11 without differential bands. In addition, content of vitamin C, soluble saccharide and protein were different, and fruit quality was multifarious in the fruit of mutants compared with the control; mutant 7 has better comprehensive nutritional quality of fruit, whereas mutant 12 and 14 stand second. The above results showed that effects of ion beam or soybean DNA on tomato genomic DNA would lead to the changes in gene expression, protein synthesis and fruit quality, moreover some tomato plants with better fruit quality or special characters were achieved, which would provide basis for the application of ion beam technology in tomato breeding.

Corresponding author:
Duan HY.

Keywords:
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Email Id:
dxdhy@126.com

Web Address:
http://jresearchbiology.com/documents/RA0454.pdf.

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INTRODUCTION

In recent years, mutation breeding has been a novel way in plant genetic improvement, especially low energy ion beam implantation which exhibits many advantages, such as low damage, high mutation rate, wide mutational spectrum, and so on (Yu, 2000). At present, ion beam mutation breeding technology has been successfully applied to a lot of crop breeding, such as rice, wheat, tobacco, cotton, soybean, rape and others (Zhou, 2009). In addition, the etching and sputtering effects of ion beam on cells would be very beneficial to foreign DNA entering into the cells (Wang *et al*., 2009, Li and Sun, 2011) and some transgenic plants have been achieved by ion beam implantation (Duan *et al*., 2012), thus the transgenic technology mediated by ion beam is a simple and feasible transgenic method.

Tomato is one of the most important vegetables and fruits that contain abundant nutrients, such as lycopene, vitamin C, trace elements and other nutrients (Xue *et al*., 2004, Wang *et al*., 2010). In order to meet the need of people, germplasm resources or genetic improvement breeding of tomato is required to be studied and new cultivar of tomato should be cultivated. In our laboratory, it was found that nitrogen ion (N^+) or argon ion (Ar^+) had obvious influences on cell mitosis and chromosome structure, and lead to various types of chromosome aberrations (Duan *et al*., 2013). Thus, dry seeds of tomato (tomato Zhongza No. 9) were irradiated by N^+ or Ar^+ ion beam and soak into soybean DNA after ion beam implantation to obtain a series of new germplasm and cultivars with important application value, and some tomato mutants with the variations of morphologic characters were found in M1 present generation. In this research, tomato mutants with morphologic variations were analyzed by SDS-PAGE and RAPD, and several indexes of fruit quality were also detected, which would provide foundation for new cultivars of tomato and theoretical basis for ion beam mutation breeding of tomato.

MATERIALS AND METHODS

Plant materials

In this study, seeds of tomato (tomato Zhongza No. 9) were provided by Vegetable Flower Institute of Agricultural Sciences, Beijing, P. R. China, and were respectively irradiated by N^+ or Ar^+ ion beam in the 30 kev energy conditions. Seeds of soybean (soybean Zaoshu No. 2) were preserved in our laboratory, soybean seedlings with single-leaf were used to extract genomic DNA with CTAB method, and DNA fragments of soybean genomic DNA were obtained by ultrasonication.

Culture of tomato plants

Tomato seeds implanted with N^+ or Ar^+ ion beam were treated as described in research (Ji *et al*., 2001), at first were respectively immersed into 0.1×SSC buffer solution or 300 μg ml^{-1} DNA working solution which was composed of soybean DNA and 0.1×SSC buffer solution, and then were separately washed several times with sterile water, but the control was only immersed into sterile water. The above seeds were sowed in the test field and cultured under the greenhouse conditions with 20°C light and 10°C dark temperature cycle. Seven days later, seeds germinated, seedlings with two leaves were transplanted in nutritive bowl and continued to be cultured. When cultured for two months, seedlings with five or six leaves were transplanted in the test field and cultured at the above culture condition.

In addition, the variations of morphologic characters in tomato plant were found, such as tall plant, fat leaves, thick stalk, and so on, moreover protein and DNA fingerprint of some tomato mutants were respectively analyzed by SDS-PAGE or RAPD, and several indexes of fruit quality were also detected.

SDS-PAGE of protein in leaves

Proteins were extracted from the fresh leaves of tomato plants with morphologic variations as described previously (Ji *et al*., 2001) with modifications. 1.0 g leaves were mixed together with 1ml sterile water and grinded in the mortar on ice-bath, and then the

homogenate of leaves were centrifuged for 20 min by 12000 rpm at 4°C. The supernatant in the centrifuge tube was transferred to 5 ml volumetric flask, furthermore, the precipitate in the centrifuge tube was extracted again with sterile water and then the supernatant was also transferred to the above 5 ml volumetric flask, in which the supernatant was diluted with sterile water to a constant volume, then the solution was mixed and preserved at -20°C. The content of soluble protein in the above solution was determined by Bradford colorimetric method (Bradford, 1976) at 595 nm, and the standard curve of soluble protein was drawn with Bovine Serum Albumin (BSA). In this research, SDS-PAGE of protein was performed under experiment conditions of 3 % stacking gel (pH6.8), 12 % separating gel (pH8.8) and Tris-Glycine buffer solution (pH8.3), and Coomassie Brilliant Blue method was used in this research.

RAPD amplification

In this study, leaves of tomato mutants were used to extract DNA by CTAB extraction procedure (Ausubel *et al*., 1987). RAPD amplification was performed as the method (Kangfu *et al*., 1994). Reaction system of RAPD amplification was 25 μl and composed of 20 ng DNA, 0.2 μmol L^{-1} primer, 0.2 μmol/L dNTPs, 2.0 mmol L^{-1} Mg^{2+}, 1U Taq DNA polymerase and double distilled water. RAPD amplification was performed as follows: initial denaturalization at 94°C for 5 min, followed by 35 cycles of 94°C for 1 min, 36°C for 1 min and 72°C for 1.5 min, with a final extension cycle of 72°C for 8 min. In addition, 100 primers were screened to obtain primers by which amplification bands are most distinctive, numbers of amplification bands are more and the repeatability is preferable.

Determination of soluble saccharide in fruit

Assay of soluble saccharide was performed by enthrone colorimetric method (Liu *et al*., 2013) with improvement. Mature fruit of tomato mutants was crushed with juicer, 0.5 g tomato juices were mixed together with 5 ml sterile water in test tube, subsequently the test tube was sealed with plastic film and put in boiling water for 30 min to extract soluble saccharide. The crude extract was filtered into 10 ml volumetric flask, simultaneously the text tube and residues were rinsed repeatedly with sterile water, and then the extract was diluted with sterile water to constant volume. The content of soluble saccharide was determined with spectrophotometry at 485 nm, and the standard curve of soluble saccharide was drawn with sucrose. In addition, determination of soluble saccharide content was repeated three times.

Determination of vitamin C and protein in fruit

Mature fruits of tomato mutants were crushed with juicer, 0.5 g tomato juices were diluted with sterile water to 100 ml volumetric flask, then extracted by vacuum extrusion machine and preserved for the determination of fruit protein and vitamin C. Determination of fruit protein was performed as determination of leaf protein in tomato, content of vitamin C was assayed by spectrophotometry (Chen *et al*., 2012) with modification and the standard curve of vitamin C was drawn with standard vitamin C. Moreover, determination of vitamin C and protein was repeated three times.

RESULTS AND DISCUSSION

Protein fingerprint in the leaves of tomato mutants

It is well known that, effects of ion beam on plant are very obvious and could cause versatility, such as stem diameter, flowering phase, plant height, quality characteristic, and so on (Phanchaisri *et al*., 2012). In this research, protein in the leaves of tomato mutants were analyzed by SDS-PAGE (Figure 1), and the electro photograph was drawn in Figure 2 to more clearly observe changes of the protein pattern. As compared with the control, the main variation of protein pattern in the mutants were some bands darkening, especially the band with 0.350 Rf value obviously darkened, however lose and increase of protein band was less found, only

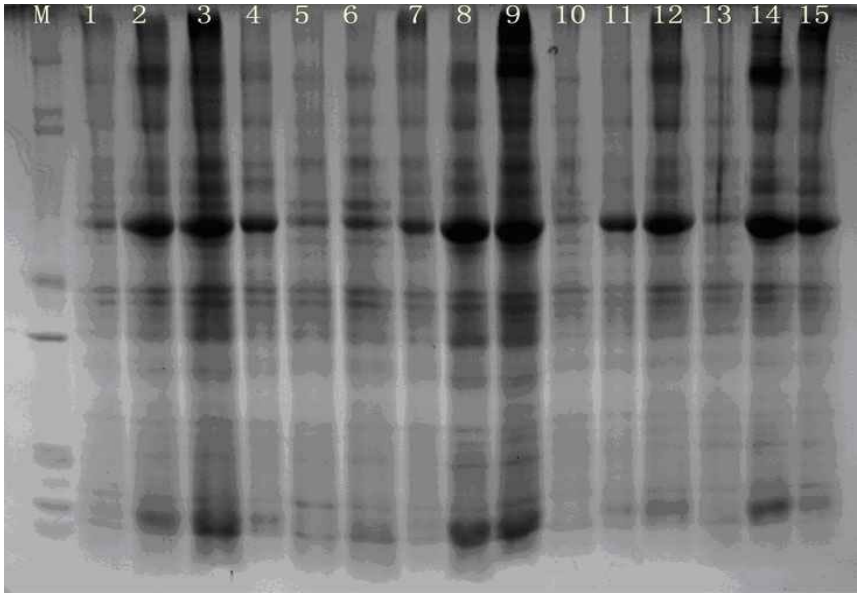

Figure 1: Protein pattern in the leaves of tomato mutants by SDS-PAGE

M: marker, 1: the control, 2-11: tomato mutant induced by ion beam and soybean DNA, 12: tomato mutant induced with $2\times10^{17}N^{+}/cm^{2}$ ion beam, 13: tomato mutant induced with $4\times10^{17}N^{+}/cm^{2}$ ion beam, 14: tomato mutant induced with $2\times10^{17}Ar^{+}/cm^{2}$ ion beam, 15: tomato mutant induced with $4\times10^{17}Ar^{+}/cm^{2}$ ion beam.

two bands increased in mutant 12, mutant 14 and mutant 15, and the Rf values were 0.05 and 0.083 respectively, furthermore mutant 15 lost one band (Rf=0.133) in comparison with the control and other mutants. The above results suggest the effects of ion beam or soybean DNA on leaf protein of tomato mutants were various, which was same to other researchers (Ji *et al*., 2001). Owing to the effects of ion beam on chromosome structure (Huang *et al*., 1994), we infer that variation of protein pattern in the leaves of tomato mutants might be caused by the changes of genomic DNA due to the damage of ion beam or integration of soybean DNA.

RAPD analysis of genomic DNA in tomato mutants

RAPD technology is actually PCR amplification, and any organism could be identified by RAPD markers (Williams *et al*., 1990, Welsh *et al*., 1991). Hither to, some plant mutants induced by ion beam implantation have been already analyzed by RAPD markers, such as *Nicotiana tabacum* (Zhang *et al*., 1998), *Cucumis melo* (Chen *et al*., 2002), *Arabidopsis thaliana* (Chang *et al*., 2003), *Dahlia pinnata* Cav. (Yu *et al*., 2008), *Jatropha curcas* (Pamidimarri *et al*., 2010), *Balsamine* (Gao *et al*., 2012), and so on. In this research, genomic DNA of tomato mutants was also analyzed with RAPD markers in order to explore changes in the genomic DNA.

100 random primers were used in the RAPD amplification, but only bands amplified by S11 primer (GTAGACCCGT) and S45 primer (TGAGCGGACA) could be variant between the control and tomato mutants, and numbers of amplification bands and length of amplification fragment were different in the mutants by different primer (Figure 3). As shown in the Figure 3 (a), only one DNA fragment with 550 bp was amplified by primer S11 in the control, mutant 3 and mutant 11. Compared with the control, DNA fragment with 850 bp increased in mutant 2, mutant 4-8, mutant 10 and mutant 13, DNA fragment with 550 bp lost in mutant 9, mutant 12, mutant 14 and mutant 15, and numbers of amplification bands and length of amplification fragment were same in mutant 12 and mutant 14. Furthermore, four DNA fragments were amplified in mutant 9, in which DNA fragment with 700 bp was also amplified in

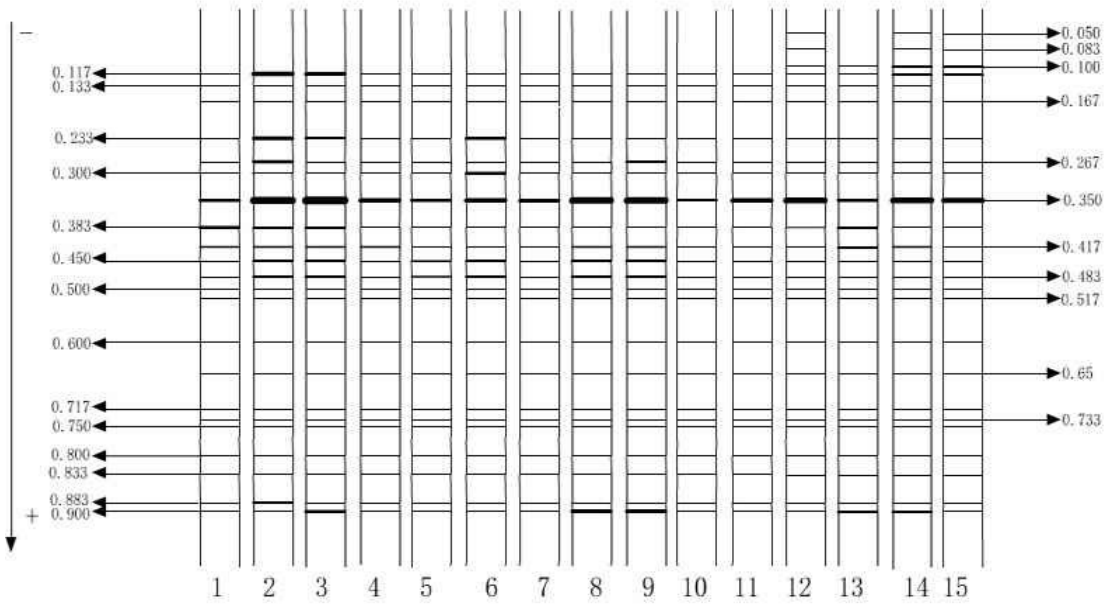

Figure 2: Protein ideograph in the leaves of tomato mutants

1: the control, 2-11: tomato mutant induced by ion beam and soybean DNA, 12: tomato mutant induced with $2\times10^{17}N^{+}/cm^{2}$ ion beam, 13: tomato mutant induced with $4\times10^{17}N^{+}/cm^{2}$ ion beam, 14: tomato mutant induced with $2\times10^{17}Ar^{+}/cm^{2}$ ion beam, 15: tomato mutant induced with $4\times10^{17}Ar^{+}/cm^{2}$ ion beam.

mutant 12 and mutant 14. On the other side, bands amplified by S45 primer were shown in Figure 3 (b); two bands were amplified from the control, mutant 2-6, mutant 11 and mutant 13, one special band was amplified in mutant 8, mutant 10, mutant 12 and mutant 15 compared with the control. Moreover, three bands were amplified in mutant 9, but their lengths were different from the control and other mutants. Meanwhile, there were two bands in mutant 14 in which one DNA fragment with 700 bp was also found in mutant 12 and mutant 15, yet other DNA fragment with 500 bp was only amplified in mutant 14.

In addition, RAPD amplification bands of tomato mutants by S11 and S45 primer were given in Table 1, total number of amplification bands, number of differential bands and rate of differential bands in tomato mutants were found to be different. Compared with the control, rate of differential bands were 100.0 % in mutant 9 and mutant 15, and number of differential bands were 7 and 3, respectively. Secondly, rate of differential bands in mutant 14 and mutant 12 were also high, the number of differential bands were 5 and 4, respectively. However, rate of differential bands in the mutant 3 and mutant 11 was 0.0 %, moreover rate of differential bands in other mutants was in the scope of 20.0-50.0 %. Further more, although rate of differential amplification bands was 100.0 % in mutant 9, some protein bands only darken and number of protein bands did not change in mutant 9. In addition, the variation of protein pattern in mutant 12, mutant 14 and mutant 15 were relatively large, and rate of differential amplification bands was respectively 66.7 %, 83.3 % or 100.0 %. Therefore, the differential DNA fragments amplified by RAPD might be related to the expression of some genes by encoding some proteins or regulating protein synthesis, but it is not clear whether differential DNA fragments could influence fruit quality.

Fruit quality of tomato mutants

As everyone knows, tomato is rich in nutrition, such as saccharide, vitamin C, protein, etc. (Xue *et al*., 2004, Wang *et al*., 2010). In this research, fruit quality of tomato mutants were assayed, content of vitamin C, soluble saccharide and protein in the fruit of tomato mutants were respectively listed in Table 2. As compared

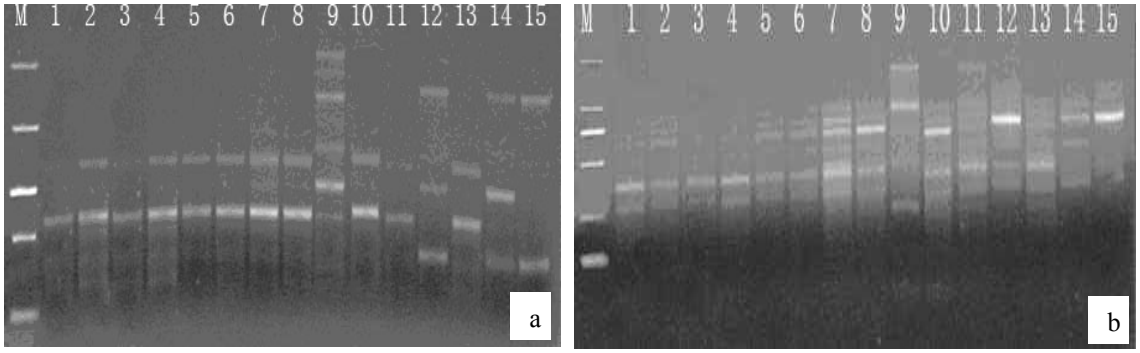

Figure 3: Results of RAPD amplification by S11 primer and S45 primer
(a) Results of RAPD amplification by S11 primer, (b) Results of RAPD amplification by S45 primer. M: DM2000, M: marker, 1: the control, 2-11: tomato mutant induced by ion beam and soybean DNA, 12: tomato mutant induced with $2\times10^{17}N^{+}/cm^{2}$ ion beam, 13: tomato mutant induced with $4\times10^{17}N^{+}/cm^{2}$ ion beam, 14: tomato mutant induced with $2\times10^{17}Ar^{+}/cm^{2}$ ion beam, 15: tomato mutant induced with $4\times10^{17}Ar^{+}/cm^{2}$ ion beam.

with the control, content of vitamin C in 50 % mutants was low, such as mutant 2-4, mutant 6, mutant 9, mutant 13 and mutant 15, especially lower in mutant 2, mutant 9 and mutant 4, and was 66.60 μg g^{-1}, 69.65 μg g^{-1} and 74.43 μg g^{-1}, respectively. However, content of vitamin C was high in mutant 5, mutant 7, mutant 8, mutant 10-12 and mutant 14, especially was higher in mutant 8 (152.03 μg g^{-1}), mutant 10 (167.09 μg g^{-1}) and mutant 12 (174.49 μg g^{-1}), moreover content of vitamin C was the highest in mutant 11 (242.24 μg g^{-1}). In addition, content of soluble saccharide in 64 % mutants was lower than the control, but was high in mutant 2, mutant 5, mutant 7, mutant 9 and mutant 10, particularly higher in mutant 7 (58.84 mg g^{-1}) and mutant 2 (46.96 mg g^{-1}). Furthermore, content of protein was high in 64 % mutants in comparison with the control, especially was the highest

Table 1: RAPD amplification bands of tomato mutants by S11 and S45 primer

Plants	Total number of bands	Number of differential bands	Rate of differential bands (%)
1	3	0	0.0
2	4	1	25.0
3	3	0	0.0
4	4	1	25.0
5	5	2	40.0
6	5	2	40.0
7	6	3	50.0
8	4	2	50.0
9	7	7	100.0
10	5	2	40.0
11	3	0	0.0
12	6	4	66.7
13	5	1	20.0
14	6	5	83.3
15	3	3	100.0

1: the control, 2-11: tomato mutant induced by ion beam and soybean DNA, 12: tomato mutant induced with $2\times10^{17}N^{+}/cm^{2}$ ion beam, 13: tomato mutant induced with $4\times10^{17}N^{+}/cm^{2}$ ion beam, 14: tomato mutant induced with $2\times10^{17}Ar^{+}/cm^{2}$ ion beam, 15: tomato mutant induced with $4\times10^{17}Ar^{+}/cm^{2}$ ion beam.

Table 2: Content of vitamin C, soluble saccharide and protein in the fruit of tomato

Plant	Content of vitamin C (μg/g)	Content of soluble saccharide (mg/g)	Content of protein (mg/g)
1	111.95	19.18	18.88
2	66.60	46.96	13.98
3	95.07	13.17	20.51
4	74.43	17.09	26.44
5	114.28	21.37	18.48
6	95.66	14.19	20.12
7	116.91	58.84	29.19
8	152.03	16.35	17.45
9	69.65	37.48	46.57
10	167.09	40.51	6.17
11	242.24	11.06	18.58
12	174.49	16.46	25.48
13	92.36	13.65	21.19
14	122.95	19.12	24.86
15	99.96	12.62	20.35
The average content in mutants	119.71	23.87	21.88

1: the control, 2-11: tomato mutant induced by ion beam and soybean DNA, 12: tomato mutant induced with $2\times10^{17}N^{+}/cm^{2}$ ion beam, 13: tomato mutant induced with $4\times10^{17}N^{+}/cm^{2}$ ion beam, 14: tomato mutant induced with $2\times10^{17}Ar^{+}/cm^{2}$ ion beam, 15: tomato mutant induced with $4\times10^{17}Ar^{+}/cm^{2}$ ion beam.

in mutant 9 (46.57 mg g^{-1}), yet content of protein in mutant 2, mutant 5, mutant 8, mutant 10 and mutant 11 was lower than the control, and only 6.17 mg g^{-1} protein in mutant 10.

On the other side, content of vitamin C, soluble saccharide and protein were different in mutants, and fruit quality of mutants was multifarious. As shown in Table 2, compared with the control, content of vitamin C, soluble saccharide and protein in mutant 7 was all higher, so mutant 7 has better comprehensive quality of fruit, secondly were mutant 12 and mutant 14 because content of vitamin C and protein was both higher. Moreover, content of soluble saccharide and protein in mutant 9 was both higher, especially content of protein was the highest (46.57 mg g^{-1}). However content of vitamin C in mutant 11 was the highest (242.24 μg g^{-1}), and content of soluble saccharide and protein was only 11.06 mg g^{-1} and 18.58 mg g^{-1}. In addition, content of vitamin C and soluble saccharide was low in mutant 15 and mutant 3, one other thing to note is that nutritional quality of mutant 3 and mutant 11 are obviously different with the control, but rate of differential amplification bands was 0.0 % in mutant 3 and 11 which were treated with ion beam and soybean DNA, inferring some big insert segment of soybean DNA might be not amplified, perhaps there might be a more complicated relationship between nutritional quality of fruit and genomic DNA of tomato irradiated with ion beam or treated with ion beam and soybean DNA, moreover the effect mechanism of ion beam or foreign DNA was very complex and need to be further studied and explored.

CONCLUSION

This study shows that ion beam or soybean DNA could influence leaf protein, genomic DNA and fruit quality of tomato mutants, inferring the variation of leaf protein and fruit quality in tomato mutants might be caused by the changes of genomic DNA which would happen due to damage of ion beam or integration of soybean DNA. However the effects of ion beam or

soybean DNA were different, and the changes among protein, DNA and fruit quality was not consistent with each other, thus it is necessary to further study effect mechanism of ion beam or foreign DNA, which would contribute to provide foundation for ion beam mutation breeding of tomato.

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REFERENCES

Ausubel FM, Brent R, Kingston RE, Moore DD, Seidman JG, Smith JA and Struhl K. 1987. Current Protocols in Molecular Biology, John Wiley, New York.

Bradford MM. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72(1-2): 248-254.

Chang F, Liu X, Li Y, Jia G, Ma J, Liu G and Zhu Z. 2003. Changes in DNA base sequences in the mutant of *Arabidopsis thaliana* induced by low-energy N^+ implantation. Science in China (Series C). 46(5): 503-512.

Chen LZ, Zhai RR and Li J. 2012. Determination of vitamin C in green team from *Hainan Baisha*. Guangzhou Chemical Industry. 40(6): 103-104.

Chen RL, Song DJ, Li YF, Wu LJ and Yu ZL. 2002. Study in mutation of *Muskmelon* genome DNA due to low energy N^+ implantation using RAPD. Acta Laser Biology Sinica. 11(1): 75-78.

Duan HY, Yu YA, He YL, Zhou YQ and Lu LD. 2013. Mutagenic effects of low energy ions on root tip cells of tomato (*Lycopersicum esculentum*). Plant Omics. 6(5): 355-358.

Duan HY, Yu YA, Li XW and Duan ZQ. 2012. Summary of plant transformation mediated by low energy ion beam. Biology Teaching. 37(1): 12-13.

Gao WJ, Su JX, Xie L, Deng CL, Zhang T and Lu LD. 2012. The point mutation induced by the low-energy N^+ ion implantation in *impatiens balsamine* genome. Russian Journal of Genetics. 48(10): 1009-1014.

Huang WC, Fan SJ, Huang JN, Sang JL, Shi YF, Xia ZE, Wu WJ and Yao HL. 1994. Study on mutagenic effect of ion implantation into microorganism. Journal of Anhui Agricultural University. 21(3): 282- 285.

Ji SD, Li JX, Zhao JJ, Xu CS, Qin GY, Wang WD and Huo YP. 2001. Analysis of fingerprint of transgenic Wheat leaves irradiated with low energy. Journal of Triticeae Crops. 21(4): 52-55.

Kangfu Y and Peter PK. 1994. Optimization of DNA extraction and PCR procedures for Random Amplified Polymorphic DNA (RAPD) analysis in Plants PCR Technology. Current Innovation CRC Press. 193.

Li J and Sun SM. 2011. Review of plasma ion immersion implantation and deposition. China Science and Technology Review. 30: 76.

Liu HY, Wang HH, Cui CH, Wang M, Guo JJ, Wen ZP and Li AQ. 2013. Experiment improvement of the soluble sugar content determination by enthrone colorimetric method. Laboratory Science. 16(2):19-20.

Pamidimarri DV, Mastan SG, Rahman H and Reddy MP. 2010. Molecular characterization and genetic diversity analysis of *Jatropha curcas* L. in India using RAPD and AFLP analysis. Molecular biology reports. 37 (5): 2249-2257.

Phanchaisri B, Samsang N, Yu L, Singkarat S and Anuntalabhochai S. 2012. Expression of OsSPY and 14

-3-3 genes involved in plant height variations of ion-beam-induced KDML 105 rice mutants. Mutation research. 734(1-2): 56-61.

Wang T, Li W, Chen HW and Yu ZL. 2009. A research of the progress in low-energy ion beam bioengineering and its applications. Journal of Xuzhou Institute of Technology. 24(1): 6-10.

Wang XJ, Liang Y, Xu JX, Sun YD and Zuo JM. 2010. Multiple statistics analysis of the quality traits of tomato (*Solanum lycopersicum* L.). Acta Agriculturae Boreali-occidentalis Sinica. 19(9): 103-108.

Welsh J, Petersen C and Mcclelland M. 1991. Polymorphisms generated by arbitrarily primed PCR in the mouse application to strain identification and genetic mapping. Nucleic Acids Research. 19(2): 303-306.

Williams JGK, Kubelik AR, Livak KJ, Rafalski JA and Tingey SV. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Research.18(22): 6531-6535.

Xue J, Xia SY, Zhang YW, Jin FM and Liu ZQ. 2004. Study on diversity of quality characteristics in tomato. Acta Agriculturae Boreali-Sinica. 19(4): 7-10.

Yu LX, Li WJ, Dong XC, Zhou LB and Ma S. 2008. RAPD analysis on dwarf mutant of *Dahlia pinnata* Cav induced by 80 meV/u12 $C6^{+}$ ions. Nuclear Techniques. 31(11): 830-833.

Yu ZL. (2000). Ion beam application in genetic modification. IEEE Transaction on Plasma Science. 28 (1): 128-132.

Zhang ZH, Du LQ, Li YX, Li HJ, Guo BH and Zhu ZQ. 1998. The variations of M1 to the seeds of tobacco implanted with ion beam. Acta Biophysica Sinica. 14(4): 762-766.

Zhou GL. 2009. Research progress on the ion beam mutation technique. Modern Agricultural Sciences and Technology. 19: 342-343.