ABTRACT

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Objectives : To understand the fundamental mechanisms involved in ion interaction with living organisms.

: To apply low energy ion beam to induce mutation in Thai jasmine rice and flower crops.

Methodology : Low energy bombardment of onion skin cells with both metallic and gaseous ion species, such as N, Ar, Mg, Ti, Fe, Ni, Cu at fluences of 1 – 5 x 10^{15} ions / cm^2 and energies of 25 – 30 keV were carried out. A scanning electron microscope and atomic force microscope (AFM) were used to observe the microcrater structure. Three different computer codes that provide a simulation of ion penetration in matter TRIM, T-Dyn and PROFILE have been used to investigate the expected depth profiles for the case of Ti bombardment, and compare with the experimental RBS results. The work also included the design and installation of an in-situ AFM system to the beam line of our bioengineering ion implantation facility and ongoing relevant experiments. The molecular dynamic simulations of Fe ion bombardment of onion skin cell wall were carried out. Cellular I – Beta surface was used as a model material for the cell wall.

Seeds of Thai purple rice and jasmine rice (*Oryza sativa incida* var purple rice and KDML 105, respectively) were bombarded with nitrogen ions at fluence of 2 – 8 x 10^{16} ions / cm^2 and energy of 60 keV number of rice mutants were obtained and characterized for 5 generations (MS). HAT – RAPD (high annealing temperature – RAPD) were used to determine genetic modification in the mutant genomes.

Rose, petunia and chrysanthemum were used in the studies of low energy ion beam induced mutation in flower crops. As for rose, nodal buds along the open flower stem were exposed to 60 keV nitrogen ions at a fluence of 1 x 10^{16} ions / cm^2. In petunia, seeds were bombarded with 60 keV nitrogen ions at fluences 1 – 10 x 10^{16} ions / cm^2. As for chrysanthemum, the variety Reagan Dark was used. The flower receptacle part was as optically precultured for 5 days to induce cell division before bombarding with 60 keV nitrogen ions and culturing in-vitro again.

Results : Ex-situ and in-situ Atomic Force Microscopy revealed the formation of microcrater – like structures with rim dimension

typically several several hundred nanometers ; we speculate that micrometers might provide channels for the transfer of exogenous macromolecules (such as DNA) through the cell wall into the cell interior. The RBS – measured implanted ion depth profile was compared to several computer simulations, and the similarities and difference seen can be interpreted as being due to severe sputtering of the cell wall material. The molecular dynamic calculations indicate that ion – bombarded cellulose molecules are broken into fragments by the collision, which fragments then initiate molecular collision cascades, leading to the ejection of intact molecules and molecular fragments from the surface.

Five ion bombarded KDML 105 rice mutants were obtained and characterized for 5 generations as listed below.

Rice Mutant	Phenotypic variation	Genotypic variation (HAT-RAPD)
BKOS6	- Photoperiod insensitive - Early flowering - Short in stature - Reddish- dark brown to black in leaf sheath - Dark brown to black in seed coat (hull) - Black rice seeds (brown rice)	Additional DNA band
TKOS4	- Photoperiod insensitive - Early flowering - Low tillering capacity - Low % filled-spikelets - Low number panicles/plant - Tall variety	Additional DNA band
PKOS1	- Photoperiod insensitive - Big and long panicles - high % filled spikelets - High number of seeds in panicle - Short in stature	Additional DNA band
PKOS2	- Photoperiod insensitive - Big and long panicles - high % filled spikelets - High number of seeds in panicle - Short in stature	Additional DNA band
PKOS3	- Photoperiod insensitive - Big and long panicles - high % filled spikelets - High number of seeds in panicle - Short in stature	Additional DNA band

In the studies on low energy nitrogen ion induced mutation of rose, petunia and chrysanthemum, following results were obtained. One rose variety, Ingrid Bergman shows changes in flower colour as well as wrinkle petal. The viability of the treated buds was 58.9 % in petunia. Wide arrays of mutated characteristics were obtained both in vegetative and reproductive parts. Various leaf changes were found, i.e. slim leaf, rolled leaf, large leaf, rough leaf surface, yellow leaf, and albino leaf. Up to 13-15% stunt growth were found in some doses. Concerning the flower, different patterns of changes were found on the petal, i.e. white strip, white spot, ununiformed colour, pale colour, and variegated flower. The changing percentage were interestingly high; especially the petal colour e.g. the variegated colour was 61.1 %. The ion beam 1-4 $x10^{16}$ ions / cm^2 could change the anther into small petal. The ion beam could also change the flower shape into round shape ,star shape as well as reduce in flower size .Fourteen attractive mutants were tissued cultured using shoot tip and propagated to test for their permanent characters. The tissue culture-derived plantlets appeared to be true to type, proving the mutants were caused by the change in the chromosome. Twenty four mutants were also selected for the identification at the molecular level using RAPD technique. Four (OPA 04,OPA 07,OPA 09 and OPA 10) out of eleven primers demonstrated bands in the range of 550-3120 base pairs. RAPD technique can be used to identify different banding patterns among petunia mutants result from ion beam bombardment. Each of the primers, however, was not able to set each mutant apart. Therefore, several primers were required. As for chrysanthemum : Nitrogen ion beam at 4x10 ions/cm^2 reduced survival percentage of the explants. The rooted shoot lets derived from the cultured explants were grown and forced to flower in a nursery. Flower colour changes in its intensity, paler (1.1-2.2%) and darker (20-33.3%) colour as well as variegated colour (1.5-5.6%) were observed. Only 1 plant (0.6%) changed the colour from pink to bronze. Low energy bombardment of onion skin cell with both metallic and gaseous ion species can induce the formation of microcrater – like structures on the onion skin cell walls.

Conclusion : Ex – situ and in – situ Atomic Force Microscopy revealed the formation of microcrater – like structures with rim dimension typically several hundred nanometers ; we speculate that the microcraters might provide channels for the transfer of exogenous macromolecules (such as DNA) through the cell wall into the cell interior.

Low energy nitrogen ions at a certain range of energy and fluence can induce mutation in Thai purple and jasmine rice and flower crops such as rose, petunia and chrysanthemum.

Recommendation : Understanding of the fundamental mechanisms involved in ion interaction with living cells is not yet well developed. A fundamental question about the mechanism is the possible formation of pathways due to ion bombardment that are responsible for the gene transfer.

This aspect of ion–biological cell interactions need further investigation.

This application of low energy ion beam to induce mutation in rice and other energy crops should be further explored to obtain mutants with specified phenotypes.

Keywords : ion bombardment ; micro-crater ; plant cell envelope ; included mutation, purple rice, jasmine rice, rose, petunia, chrysanthemum.