

Improved mustard varieties in the making

06 January 2005 | News

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Extensive research to increase the productivity and quality of mustard oilseed is being carried out at the University of Delhi, South Campus. Soon Indian farmers will have access to new, hybrid and high yielding mustard varieties.

Hybrid mustard seeds showing more than 30 percent increase in yield are being tested at the Centre for Genetic Manipulation of Crop Plants (CGMCP) and the department of genetics, Delhi University South Campus. These potential hybrid varieties have been produced through the Cytoplasmic Male Sterility (CMS) approach both through conventional breeding as well as transgenic means.

The CMS approach

In mustard, a naturally occurring and suitable CMS-restorer system is not available. So compatible, high yielding parent plants are identified. Combiners having a high heterosis for yield and synchrony in flowering are chosen. Then the anthers or pollens of the seed bearing parent plant are made sterile to prevent self-fertilization. The corresponding pollinator parent plant contains a restorer gene in its nucleus, which restores fertility in all the hybrid seeds. A seemingly simple principle, but right from choosing the right parent lines to introduction of tissue specific sterility to the final production of fertile hybrid seeds is a complex interplay of genes, promoters and other associated factors.

Scientists at the CGMCP and department of genetics have exploited the use of barnase (a cytotoxic male sterile gene) and barstar (restorer gene) system for producing transgenic high yielding hybrid mustard seeds. The pollens of one parent plant are made sterile through the tissue specific expression of the barnase gene. The use of a spacer DNA fragment insulates the tissue-specific expression of the cytotoxic gene (barnase) and allows high-frequency generation of transgenic male sterile lines in *Brassica juncea*, a popular variety of mustard in the country. This cytotoxic gene produces a protein that destroys the

mRNA of the system. When coupled with the restorer protein (barstar gene product) the two complementary macromolecules form a complex, which inactivates the cytotoxic protein.

The barnase-barstar gene sequences have been isolated from the bacterium *Bacillus amyloliquefaciens*, a soil dwelling bacterium. The barnase (male sterile) and barstar (restorer) lines have been developed, which are now being utilized in the *B. juncea* breeding program at the CGMCP, University of Delhi South Campus, with the required permissions from the RCGM. The transgenic variety has shown more than 30 percent increase in yield in trial experiment as compared to the local varieties.

The researchers have also developed viable hybrids following the CMS-restorer approach through conventional breeding too. After extensive experimentation, last year, they were able to produce hybrid seeds on a commercial basis. "Presently the variety is being tested at 14 locations in the country like in Agra, Bharatpur, Delhi, Faridabad, Rewadi, etc. In each trial, the hybrid variety is being tested on a piece of one-acre land against the local popular variety. About 30 percent improvement in the yield of the resultant hybrid plants has been noted", said Dr AK Pradhan, head, genetics department, Delhi University, "Further extensive experimental trials need to be conducted to check for sustenance of the hybrid vigor", he added.

Better mustard oil

The Centre for Genetic Manipulation and the Genetics Department of the University are also working on a more nutritious mustard variety. The aim being to produce a variety that can compete with olive oil in nutritional value. And significantly, the scientists have not used any genes of foreign origin, instead, they have used biotechnology to suppress certain genes in mustard.

"A good quality oil should have high oleic acid (50 percent or more) and low linoleic and linolenic acid content (about 10 percent). As a high percentage of linoleic and linolenic acid reduces the keeping quality of the oil. The fatty acid desaturase-2 (FAD-2) gene isolated from *B. campestris* has been incorporated into *B. juncea* in an antisense orientation through transgenic approach to increase oleic and decrease the linoleic and linolenic acid fraction from the seed oil. This antisense RNA approach is used to block the activity of the FAD-2 gene and thus a biochemical pathway, which converts oleic acid to linoleic acid", explained Dr Deepak Pental, professor of genetics and director, University of Delhi, South Campus.

The preliminary evaluation of first generation transgenic plants has indicated increase of oleic acid (up to 73-76 percent) in *B. juncea* lines. Presently this potential line is being grown on a small scale in containment. Next year the scientists plan to take the necessary approvals from the government bodies and produce transgenic seeds for larger trials and toxicology and allergenicity testing.

Improved oilcake

Research is on to improve the quality of the oilcake also. Normally the oilcake left after extracting the mustard oil is very high in glucosinolates, which makes the oilcake pungent and unfit for poultry and animal feed. Presently, per gram of oilcake contains about 100 micromole of glucosinolate, which should be reduced to 30 micromole, if the oilcake is to be used. There is a high demand for such oilcake in the international market. Efforts are on at the genetics department to develop mustard seeds with low glucosinolate levels.

The East European mustard gene pool which contain a large number of useful characters like '0' erucic acid and low glucosinolate traits and shows significant levels of heterosis in hybrids with Indian varieties is being used. Since the production of glucosinolate is through a very complex biochemical pathway involving 6-7 genes, the scientists are using the doubled haploid microspore approach for producing such plants. And in order to retain the essential Indian characteristics in the hybrid variety recurrent back crossing is being done. It is expected that in the next 3-4 years low glucosinolate level plants will be ready for the farmers.

"Presently we are creating hybrid varieties having individual good traits and later all these traits will be converged into a single plant," elaborated Dr Pradhan.

Rolly Dureha

Stem cell therapy: Rekindling hopes

Stem cells may be the proverbial silver bullet offering a ray of hope for medical conditions, previously considered beyond cure.

For the first time ever, South Korean scientists are reported to have used stem cell therapy to make a paralytic women walk again after 20 years. Thirty seven year-old Hwang Mi Soon was bedridden since the past two decades after damaging her back in an accident. The researchers repaired her damaged spine using stem cells derived from umbilical cord blood. And Mi Soon was able to take tentative steps once again with the help of a walking frame. This is the world's first published case where treatment of spinal cord injuries was successfully done with stem cells from umbilical cord blood.

For the therapy, multipotent stem cells were isolated from cord blood, which had been frozen immediately after the birth of a baby and cultured for a period of time. Then these cells were directly injected to the damaged part of the spinal cord.

Of late, the scientists have coaxed these primordial cells to transform into a variety of specialized cells to treat a range of diseases. For instance, scientists at the University of Toronto have found that stem cells have the potential to restore vision in older people suffering from macular degeneration. The researchers found that human retinal stem cells regenerated when they were transplanted into the eyes of mice and chicks. They now plan to see if the same happens in diseased eyes in the hope of eventually treating humans. The researchers have said that stem cells taken from the back of the eye could eventually be used to restore normal vision in people with sight problems. These findings have appeared in Proceedings of the National Academy of Sciences.

Harvard scientists have manipulated stem cells already present in the brains of mice to induce the birth of new neurons, an advance once considered impossible by most scientists. They induced the birth of new cells by killing nearby neurons in mice, which set off a cascade of events that led to stem cells producing new neurons in the cerebral cortex. If scientists can turn this into a therapy for humans, it would mean that patients could literally heal themselves with stem cells already present in their brains. They hope experiments like this would lead to new ways to grow neurons.

Simultaneously, a team of Italian neurosurgeons and scientists are looking at stem cells from skin to fight brain tumors. They are looking into whether stem cells derived from a brain tumor patient's own skin can be used to fight the tumors. The researchers successfully grew stem cells from skin samples of adult patients with brain tumors. It is hoped that these cells can then be used as a new brain tumor therapy, which has been attempted successfully in mice. They found that mice with brain tumors that were treated with stem cells showed both increased blood supply and decreased tumor growth into the surrounding brain. These mice also lived on average 50 percent longer than mice that were not given this therapy.

Meanwhile, researchers at the Howard Hughes Medical Institute (HHMI) have isolated stem cells from the skin of mice and shown that they have the power to self-renew and differentiate into skin and functioning hair follicles when grafted onto mice. The findings mean that the human equivalent of these stem cells, which scientists are also trying to isolate, could ultimately be used to regenerate skin and hair.

In another unique discovery it has been found that embryonic stem cells have the capacity to influence neighboring defective cardiac cells and restore their capacity to function normally. Researchers at Memorial Sloan-Kettering Cancer Center have reported that 15 embryonic stem cells injected into early embryos of mice whose hearts were genetically predisposed to develop a lethal defect, rescued the heart from developing the disorder by not only producing normal daughter cells that were incorporated into the defective embryonic heart but also by releasing biological factors into the nearby vicinity. This prevented neighboring heart cells from developing into defective tissue.

These experimental findings indicate that the potential of stem cell therapy is slowly unfolding now and in the near future it may become the choice of treatment for many, if not all, difficult irreversible diseases.

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